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Variation in Toxicity and Physicochemical Parameters of Cassava Pulp (*Manihot esculenta*) during Storage

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Authors' contributions

This work was carried out in collaboration among all authors. Author DZAB designed the study, performed the statistical analysis of the data and drafted the first draft of the manuscript. Authors YJC and DKP wrote the study protocol and literature research. All authors read and approved the final manuscript.

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ABSTRACT

Background: A staple for about 800 million people in tropical countries, cassava contains a high content of hydrocyanic acid making it toxic.
Objective: This study was proposed to test the conservation effectiveness on tuber toxicity.
Materials and Methods: Tests were carried out on the varieties commonly known as Bonoua and Yacé. The conservation parameters studied were: the level of hydrocyanic acid, moisture content and hardness. The cassava tubers were stored at room temperature. The samples for analysis were taken on the pulp every 24 hours.
Results: Results showed that hydrocyanic acid levels reached their minimum levels between 96 and 120 hours of storage with 5.87 ± 0.46 and 5.66 ± 0.50 mg/kg in Bonoua, then 9.53 ± 0.78 and 9.85 ± 0.93 mg/kg in IAC. Water levels are positively correlated with those of hydrocyanic acid in both cassava types. Proteins reach reveal in their maximum concentrations between 48 and 72

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hours of storage (respectively to then drop. The ash and fat contents as well as the total carbohydrate contents generally decrease during storage. However, an increase is observed from 120 hours of storage, at the level of carbohydrate concentrations.
Conclusion: For a non-toxic cassava pulp, a storage period of at least 48 hours at the Bonoua and 96 hours at the IAC would be advisable with however a lower biochemical quality.

Keywords: Manihot esculenta; hydrocyanic acid; preservation; biochemical quality.

1. INTRODUCTION

The staple food of more than 800 million people [1], cassava is the most important tropical tuber cultivated. It is a major source of energy for millions of people. Cultivated for its tuberous roots rich in starch, it is known as the best producer of carbohydrates (sugars) among the staple crops. Its production is constantly increasing, bringing it to fourth (4) place in world food production with 277.9 million tonnes per year, of which 57% in Africa, 33% in Asia and 20% in Latin America. Cassava has become a staple food for people in developing countries. In Côte d'Ivoire, cassava ranks second (2) for food crops after yam [2] with a production of 5.3 million tonnes in 2017, placing the country in 3rd place in Africa. west. Several varieties of cassava exist, however, it is possible to group them into two large groups: sweet and bitter. The bitterness of each depends on the presence of hydrocyanic acid which, for its part, is largely dependent on climatic conditions [3]. The plant is indeed composed of cyanogenic molecules that are linamarin and to a lesser extent lotaustralin [4]. Under the effect of an endogenous enzyme, linamarase, cyanogenic glucosides can be hydrolyzed into hydrocyanic acid which is toxic to it [5]. This toxicity is believed to be at the origin, in populations that consume large amounts of cassava, of several visible manifestations, including goiter, cretinism, and ataxic neuropathy. Four types of toxicities are described according to the doses of cyanides ingested; acute toxicity at massive doses leads to rapid death, toxicity at very high doses can cause parkinsonian syndrome, subacute toxicity at high doses, responsible for KONZO disease which is spastic paraplegia, chronic toxicity at low doses, responsible for neuropathy tropical ataxic [6]. Cassava is usually eaten after processing. These different processes would reduce the toxicity of the tuber. However, the volatilization temperature of hydrocyanic acid is 27°C [7]. However, handling of the pulp during processing (traditional processing) is carried out at much higher temperatures, thus exposing to poisoning by inhalation and / or repeated ingestion of doses of hydrocyanic acid. It is to resolve this issue that this study was initiated. It aims to define a pre-treatment for detoxifying the pulp before processing. More specifically, it will be a question of following the evolution of the level of hydrocyanic acid during the storage of the pulps, then of determining the variation of certain physicochemical parameters of the cassava pulp [7].

2. MATERIALS AND METHODS

2.1 Materials

The plant material used in this study is cassava (Manihot esculenta). The tests were carried out on varieties commonly called Bonoua and IAC from the name of the institute that created it, (Agronomic Institute of Campinas) harvested on a cultivated plot located on the grounds of the Jean Lorougnon Guédé University in Daloa. (Ivory Coast).

2.2 Methods

2.2.1 Sampling

The parameters studied are: The rate of hydrocyanic acid, variations in humidity and loss of mechanical strength. The cassava tubers were stored in a ventilated room at room temperature. Samples for analysis were taken from the pulps of the tubers harvested at 0 hours and then every 24 hours and the shelf life was a function of the residual hydrocyanic acid content and the time the tubers deteriorate.

2.2.2 Analyzes

Determination of hydrocyanic acid

This analysis was carried out according to the method of Liebig Denige modified by FAO, 1956 which consists in hydrolyzing the heteroside contained in 27 grams of fresh cased pulp from the different samples were weighed and mixed with 200 ml of distilled water. The solution

obtained after 3 to 4 hours was distilled. A distillate (150 ml) has was collected in 20 ml of a solution containing 0.5 g of NaOH. 8 ml of 5% KI solution are added to 100 ml of distillate placed in a 250 ml Erlen Meyer vial. The distillate has was titrated with 10-2 M AgNO3 solution. The hydrocyanic acid content is determined using the following correspondence: 1 ml of AgNO3 is equivalent to 1.08 mg of HCN.

Determination of humidity

This determination method was inspired by BIPEA [8] where 100 grams of fresh, finely peeled pulp in thin layers were placed in an oven at 70°C until a constant weight was obtained. The weight differences will then be calculated.

$$\%\,\mathrm{MS} = \frac{\mathrm{m2}}{\mathrm{m1}}\,\mathrm{x100} \quad (1)$$

m1: mass of the sample before drying
m2: mass of the sample after drying

Determination of the loss of mechanical strength

The loss of mechanical strength of cassava pulps was measured by applying a crossbow type penetrometer to the middle part of the tuber [9]. It was calculated at each moment on the basis of the initial and final resistances.

Determination of protein content

The protein contents are obtained according to the AOAC method, [10] using KJELDHAL, based on the determination of the total nitrogen of the sample. It includes several steps including the evaluation of the percentage of dry matter, mineralization, distillation and determination of titrisol NaOH (0.1N). Knowing that 14 mg of nitrogen are captured by 1 ml of 1N HCl and that the protein / nitrogen ratio is 6.25; the following formulas made it possible to determine the protein contents.

$$\mathrm{M\,N\,(mg)} = 14\ \mathrm{mg\ N\ x\ 0{,}1\ x\ V\ HCl} \quad (2)$$

VHCl : Volume of hydrochloric acid
M N : Mass of nitrogen
0.1 : Normality of the acid

$$\%\,\mathrm{NITROGEN} = \frac{\mathrm{M\,N}}{\mathrm{PNe}}\,\mathrm{x}\,100 \quad (3)$$

PNe : Net weight of the sample

$$\%\,\mathrm{PROTEIN} = \%\,\mathrm{NITROGEN\ x\ 6.25} \quad (4)$$

Determination of the oil content

The oil content was determined according to the standardized Soxhlet method. This method consists of extracting the oil with an organic solvent (hexane) on a solid matrix (sample ground). The oil content relative to the dry matter after extraction with a giant Soxhlet was obtained from the following formula:

$$\mathrm{m3\ (oil)} = \mathrm{m1} - \mathrm{m2} \quad (5)$$

m1 : Cartridge pre-tared
m2 : The mass of the filters obtained after extraction and drying

$$\%\ \mathrm{oil\ dry\ mass} = \frac{\mathrm{m3}}{5}\ \mathrm{x}\ 100 \quad (6)$$

m3: Mass of the oil
5: Mass of the dried sample

Determination of crude ash content

Five grams (5 g) of dried and deoiled pulp samples were weighed and placed in a crucible. This is introduced into a muffle furnace set at 550°C. for 24 h, according to the BIPEA method [8]. The temperature is maintained until a white, light gray or reddish ash is obtained. The crucible is then cooled in a desiccator and weighed.

$$\%\,\mathrm{Ash} = \frac{\mathrm{m\ 2}}{\mathrm{m\ 1}}\ \mathrm{x}\ 100 \quad (7)$$

m 1: mass of sample before incineration
m 2: mass of ash obtained

Determination of total carbohydrate content

The total carbohydrate contents are calculated according to the calculation method recommended by the FAO [11] which takes into account the moisture, fat, protein and ash contents:

$$\mathrm{Total\ carbohydrates\ (\%)} = 100 - [\mathrm{Fat\ (\%)} + \mathrm{Ash\ (\%)} + \mathrm{Protein\ (\%)}] \quad (8)$$

2.2.3 Statistical analysis

Each of the tests described in this work was repeated 3 times, and the results subjected to an analysis of variance using Statistica 6.0 software, at a threshold of 5%. Newman-Keuls

comparative tests were used, if there was a significant difference.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Hydrocyanic acid level

The results obtained show that the level of hydrocyanic acid in the *Bonoua* type drops significantly from 40.13 ± 2.91 to 3.45 ± 0.8 mg / kg throughout storage. This variation reaches a level of hydrocyanic acid less than or equal to 10 mg / kg from 48 hours (two days) of storage. The levels are however statistically constant from 72 hours (three days) to 120 hours of storage with respective values of 6.11 ± 0.32 to 5.66 ± 0.45 mg / kg and then drop significantly beyond (Fig. 1).

The *IAC* type shows very high concentrations of hydrocyanic acid of up to 140 mg / kg. These concentrations decrease during storage to reach their minimum values at 96 hours (4 days) with 9.53 ± 0.95 mg / kg. However, these hydrocyanic acid levels increase beyond 96 hours of storage with values of 9.85 ± 0.88 mg / kg (120 hours) and 24.90 ± 2.01 mg / kg (144 hours) (Fig. 2).

3.1.2 Humidity level

The results obtained during storage revealed that the moisture content of *Bonoua* type pulps drops significantly to stabilize between 120 and 144 hours of storage. The values are respectively 60.10 ± 3.43 and 51.22 ± 5.31% (from 0 to 120 hours), then 52.50 ± 4.01% (144 hours) (Fig. 3).

Cassava type *IAC* shows moisture concentrations which decrease significantly in the pulps also up to the 120th hour of storage from 62.52 ± 2.04 to 48.60 ± 3.77%. The Rates then go up to 144 hours with a value of 52.96 ± 5.10% (Fig. 4).

3.1.3 Loss of mechanical strength

Fig. 5 shows the results of the effect of storage time on the mechanical strength of the pulp of *Bonoua* type cassava. The loss of firmness of the pulp is observed throughout storage. The loss rates are higher between the 48th (2 days) and 96th hour with respectively 3.15 ± 1.26 and 17.30 ± 1.55%. This loss continues, but less so reaching at 144 hours (6 days) 22.18 ± 1.86%.
The evolution of the rate of loss of mechanical strength in the *IAC* type shows almost similar variations to those of the *Bonoua* type. They rise significantly from 0 to 10.00 ± 0.78% (0 to 72 hours), to remain statistically constant up to 120 hours (16.11 ± 1.83%). The loss of firmness subsequently reaches its maximum value up to 30.05 ± 1.40% at the 144th hour (Fig. 6).

3.1.4 Determination of protein content

The variations reveal in the two types of cassava (Bonoua and IAC) that the pulps between 48 and 72 hours of maximum values (respectively 5.21 ± 0.22 and 4.89 ± 0.31% then 5.83 ± 0 , 12 and 4.70 ± 0.33%) above the protein concentrations fall Table 1. The sweet variety of cassava (*Bonoua*) is generally richer in protein than the bitter variety (*IAC*).

3.1.5 Determination of fat content

In minimal quantity in the cassava pulp, the fat shows contents which generally decrease from harvest to the end of storage. In the *Bonoua* variety, the values fluctuate between 0.76 ± 0.03 and 0.20 ± 0.01%, when they vary from 0.58 ± 0.12 and 0.15 ± 0.01%. *Bonoua* pulps contain statistically more fat than *IAC* (Table 1).

3.1.6 Determination of crude ash content

The crude ashes determined throughout the storage reveal statistically higher concentrations in IAC than in *Bonoua* (Table 1). Mineral contents also generally decrease throughout storage. They vary from 2.10 ± 0.15 to 1.33 ± 0.10% and from 2.20 ± 0.15 to 1.53 ± 0.12 respectively in *Bonoua* and *IAC*.

3.1.7 Determination of total carbohydrate content

A major component of cassava pulps, total carbohydrates are found to be more concentrated at the 120 th and 144 th hours of storage. The values are 94.34 ± 1.13 and 96.05 ± 1.99% (*Bonoua*) and 94.48 ± 1.01 and 95.19 ± 0.88% (*IAC*). The variations in content are however heterogeneous with decreases and increases by storage time (Table 1).

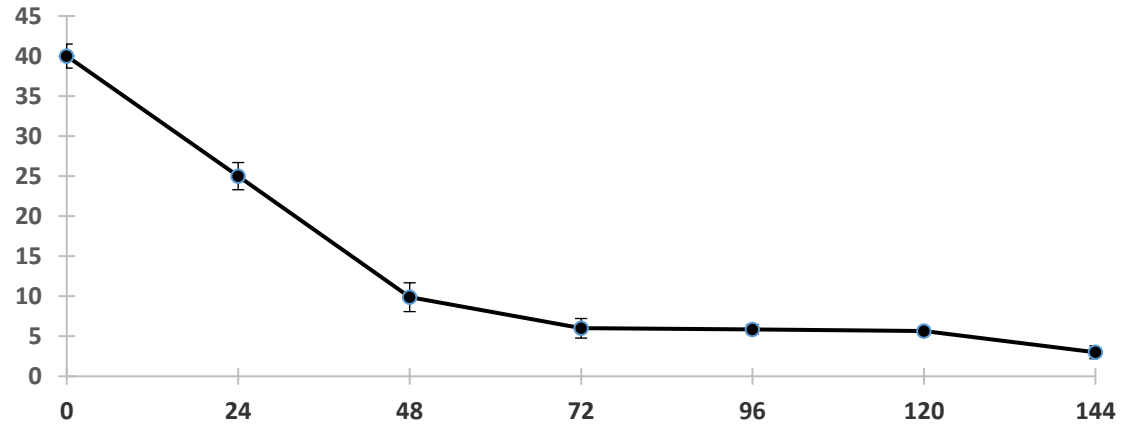

Fig. 1. HCN level (mg / kg) of the *Bonoua* type as a function of time (hour)

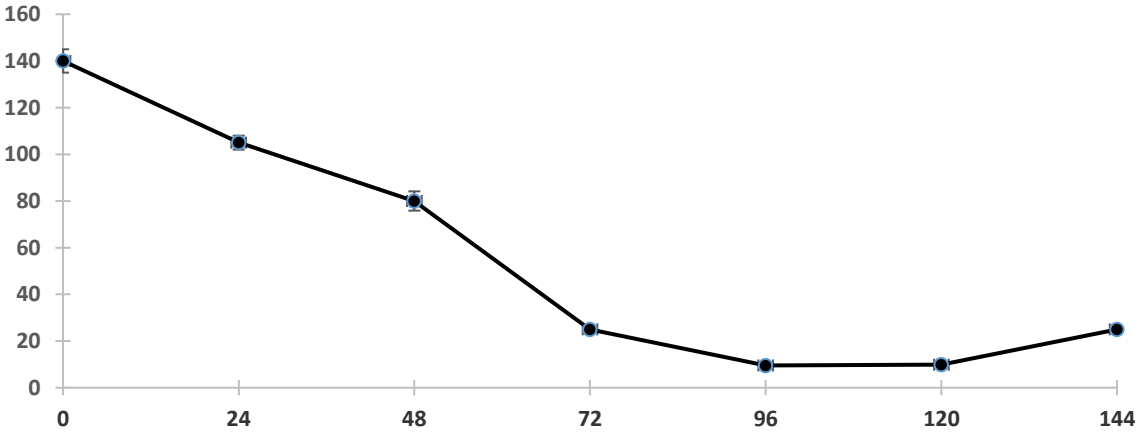

Fig. 2. HCN level (mg / kg) of the *IAC* type as a function of time (hour)

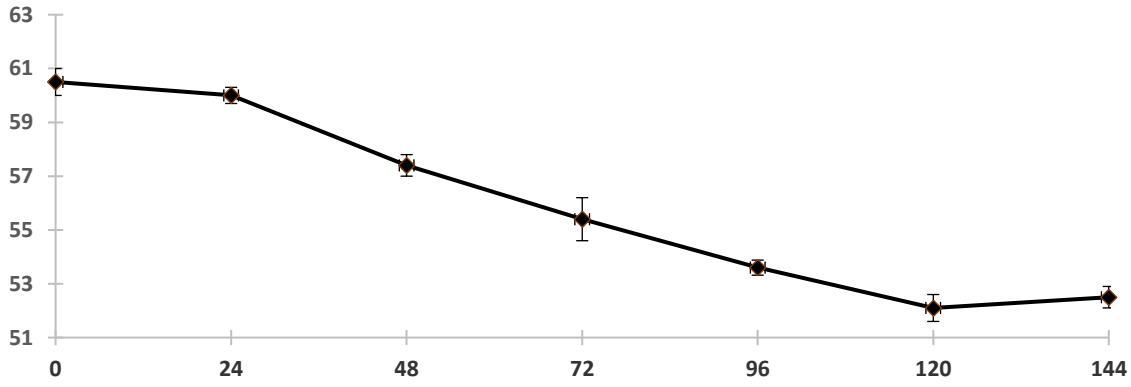

Fig. 3. Humidity rate (%) of the *Bonoua* type as a function of time (hour)

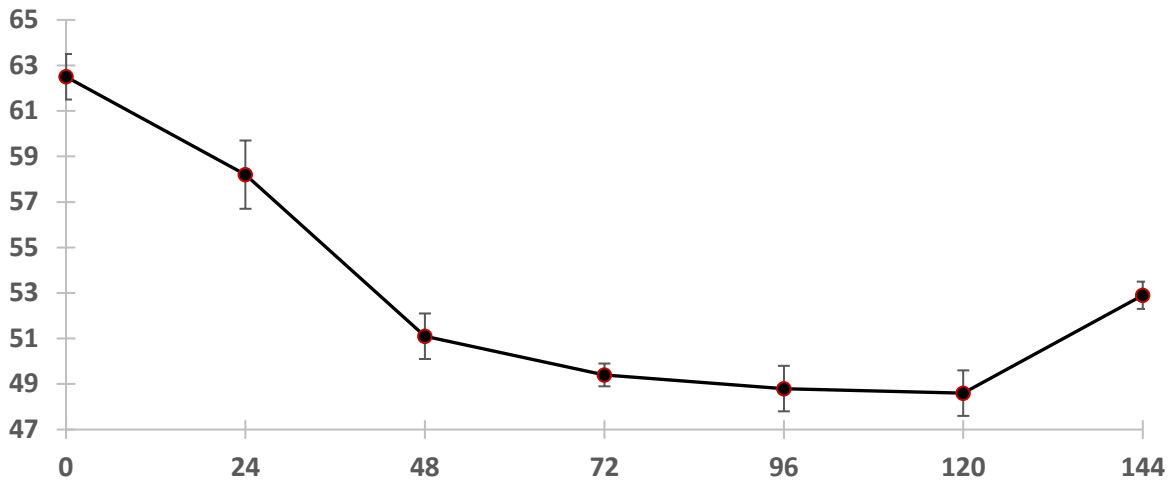

Fig. 4. Humidity rate (%) of the *IAC* type as a function of time (hour)

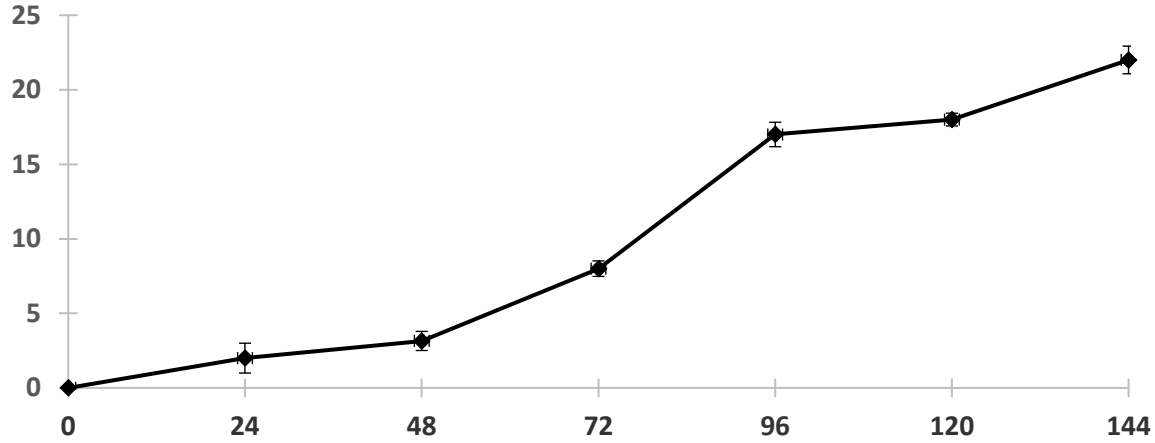

Fig. 5. *Bonoua* type mechanical resistance loss as a function of time (hour)

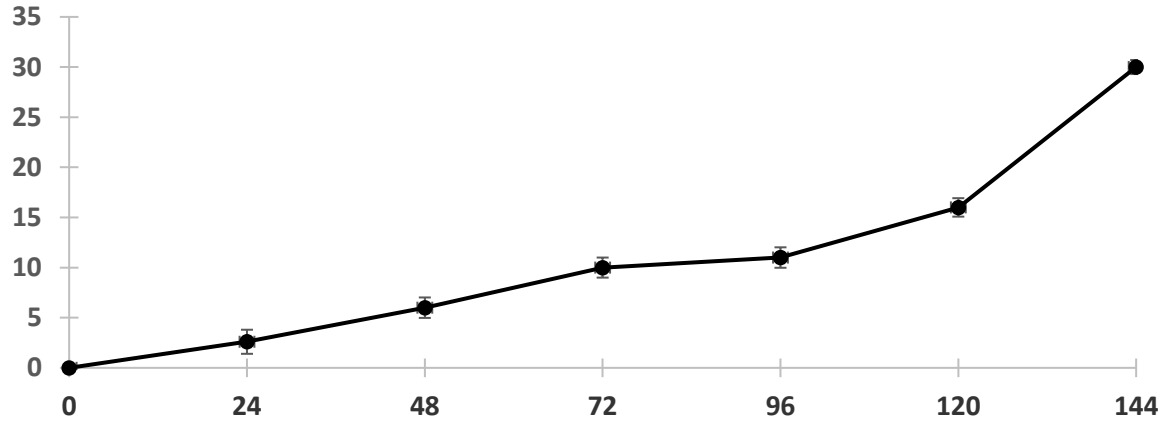

Fig. 6. Loss of mechanical strength of the *IAC* type as a function of time (hour)

Table 1. Variation in protein, fat, ash and total carbohydrate (dry matter) contents

Parameter	Time	Bonoua	IAC	P intra processing
Protein (%)	0	3.91 ± 0.33 ^{c}*A*	3.55 ± 0.05 ^{d}*B*	0.043
	24	3.77 ± 0.24 ^{c}*A*	3.23 ± 0.22 ^{d}*B*	0.032
	48	5.21 ± 0.22 ^{b}*A*	4.89 ± 0.31 ^{a}*A*	0.058
	72	5.83 ± 0.12 ^{a}*A*	4.70 ± 0.33 ^{a}*AB*	0.003
	96	3.68 ± 0.33 ^{c}*A*	4.01 ± 0.18 ^{b}*A*	0.067
	120	3.79 ± 0.25 ^{c}*A*	3.36 ± 0.20 ^{cd}*A*	0.098
	144	2.42 ± 0.11 ^{d}*B*	3.13 ± 0.15 ^{d}*A*	0.011
P intra material		0.022	0.008	
Fat (%)	0	0.76 ± 0.03 ^{a}*A*	0.58 ± 0.12 ^{a}*B*	0.003
	24	0.60 ± 0.01 ^{b}*A*	0.46 ± 0.05 ^{b}*B*	0.001
	48	0.43 ± 0.02 ^{c}*A*	0.46 ± 0.10 ^{ab}*A*	0.060
	72	0.36 ± 0.01 ^{d}*A*	0.30 ± 0.05 ^{c}*A*	0.113
	96	0.29 ± 0.00 ^{e}*A*	0.26 ± 0.11 ^{c}*A*	0.076
	120	0.33 ± 0.02 ^{d}*A*	0.28 ± 0.05 ^{c}*A*	0.058
	144	0.20 ± 0.01 ^{f}*A*	0.15 ± 0.01 ^{d}*B*	0.009
P intra material		<0.001	0.003	
Ash (%)	0	2.10 ± 0.15 ^{a}*A*	2.20 ± 0.15 ^{a}*A*	0.482
	24	1.88 ± 0.10 ^{ab}*B*	2.20 ± 0.10 ^{a}*A*	0.001
	48	1.88 ± 0.06 ^{b}*B*	2.10 ± 0.08 ^{a}*A*	0.014
	72	1.69 ± 0.09 ^{c}*A*	1.78 ± 0.11 ^{bc}*A*	0.199
	96	1.73 ± 0.13 ^{bc}*A*	1.90 ± 0.05 ^{b}*A*	0.083
	120	1.54 ± 0.05 ^{d}*B*	1.88 ± 0.05 ^{b}*A*	<0.001
	144	1.33 ± 0.10 ^{e}*A*	1.53 ± 0.12 ^{c}*A*	0.176
P intra material		<0.001	0.032	
Total carbohydrates (%)	0	93.13 ± 2.33 ^{b}*A*	93.67 ± 1.00 ^{b}*A*	0.201
	24	93.75 ± 1.00 ^{b}*A*	94.11 ± 0.56 ^{ab}*A*	0.114
	48	92.48 ± 1.18 ^{b}*A*	92.55 ± 1.16 ^{b}*A*	0.077
	72	92.12 ± 1.09 ^{b}*A*	93.19 ± 0.73 ^{b}*A*	0.540
	96	94.30 ± 1.10 ^{ab}*A*	93.83 ± 0.58^{b}*A*	0.115
	120	94.34 ± 1.13 ^{ab}*A*	94.48 ± 1.01 ^{ab}*A*	0.286
	144	96.05 ± 1.99 ^{a}*A*	95.19 ± 0.88 ^{a}*A*	0.113
P intra material		0.041	0.028	

P: probability of the test (at the 5% threshold)

NB: the values assigned the same letter in lower case and in bold in the same column are not significantly different for each parameter. Values assigned the same uppercase and italic letter on the same line are not significantly different for each parameter

3.2 Discussion

The results of this study showed that throughout storage, cultivar *IAC* is more concentrated in hydrocyanic acid than *Bonoua*. These results are consistent with those of Abbor-Egbe and Mbone, [12]. During the different storage periods, the levels generally decrease in *Bonoua* and *IAC*. These variations would be the consequences of phenomena of volatilization of hydrocyanic acid molecules. In fact, the tuber harvest is accompanied by scratches where hydrolysis reactions of cyanogenic glucosides, linamarin and lotaustralin into hydrocyanic acid [13] occur. Volatilization is then triggered as soon as exposure of tubers to temperatures above 27°C [7]. The unearthed root finishes its ripening and then begins its deterioration process. rich enzymatic reactions are then produced. These reactions would be rather less in the *Bonoua* type than in the *IAC*. This difference could be linked to the lower humidity level in *Bonoua* which would thus limit during storage, hydrolysis and evaporation reactions in the pulps, unlike IAC cassava. Hence the increase in the rate of hydrocyanic acid in the latter beyond five (5) days of storage, while the decline continues in the Bonoua. The results of the effect of storage time on the mechanical strength of the pulps reflect the loss of firmness in the two types of cassava. Hardness is a function of the mechanical resistance of the organ [14]. The loss of this character in the pulp would be linked to the process of deterioration of the tuber. Thus, a

lower water composition would slow down the process of deterioration and the loss of the hardness of the organ [15]; which would explain the greater variation in *IAC* cassava which is richer in water than in *Bonoua* which has a weaker composition. The protein variations in the two types of cassava (*Bonoua* and *IAC*) reveal that the pulps reach their maximum values between 48 and 72 hours of storage and then decrease. These decreases are due to the use of sulfur compounds (thiocyanates), amino acids (cystine and leucine) in the detoxification of cyanide [16]. The fat contents of the pulps studied show that they do not constitute the main energy reserve because of the low levels observed in the two cultivars. Their variations during storage must be linked to their uses in catabolic activities leading to the formation of carbohydrates, ie in lipogenesis [17]. Like fat, mineral contents generally decrease during storage. The different variations observed are due to the use of the different minerals of the ash in biochemical activities which occur there during conservation [18]. Cassava pulp mainly contains carbohydrate complexes in the form of starch (amylose and amylopectin). The variations in total carbohydrates observed would be the result of their use in the form of energy in the body. Carbohydrates are indeed the crossroads of several biochemical syntheses that take place during storage [19].

4. CONCLUSION

Cassava is one of the staple foods of third world countries. Its toxicity is one of the brakes on its use. This study was therefore initiated with the aim of making the tubers less toxic before any processing. It appears that the tolerated quantity of hydrocyanic acid which is 10 mg / kg of product is obtained in the pulps, after 48 hours (2 days) of storage in the Bonoua type cassava and 96 hours (4 days) in the IAC. Apart from the protein concentrations which reach their maximum values between 48 and 72 hours of storage, the different tubers see their firmness and certain biochemical qualities decrease throughout storage. In addition, additional studies on organoleptic tests on products from preserved tubers would be essential to complete this work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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