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Influence of Traditional Methods of Preparing Cassava Flour on the Content of Hydrogen Cyanide, Proteins and Aflatoxins in Burundi: Cases in Inyange, Ikivunde and Akambaranga in the Province of Makamba

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Abstract

In order to analyze the impact of traditional cassava flour preparation techniques, a study was conducted to determine the levels of hydrogen cyanide, protein and aflatoxins. A survey study was used to select three communes from the six that make up Makamba Province, which were then subjected to chemical analysis. The AOAC method was used to analyze HCN content. The ISO 16050 and AOAC methods were used to analyze aflatoxins. Thus, the results revealed that these three techniques of preparation of cassava flour (Inyange, Ikivunde and Akambaranga) globally reduce the HCN content below that set by FAO and WHO (10 mg/kg). Inyange flour was found to contain 1.36 ± 0.09 mg HCN/kg, Ikivunde flour 1.48 ± 0.12 mg HCN/kg and Akambaranga flour 1.43 ± 0.15 mg HCN/kg. The techniques used to produce Akambaranga and Ikivunde were characterized by a reduction in total protein following hydrolysis from 1.61% ± 0.21% to 1.46% ± 0.46% and from 1.45% ± 0.19% to 1.14% ± 0.18% respectively. However, the technique used to produce Inyange flour showed a slight increase (1.58% ± 0.24% to 2.19% ± 0.5% of dry matter) following the development of mould. Inyange flour was found to contain 1.36 ± 0.09 mg HCN/kg, Ikivunde flour 1.48 ± 0.12 mg HCN/kg and

Akambaranga flour 1.43 ± 0.15 mg HCN/kg. The techniques used to produce Akambaranga and Ikivunde were characterised by a reduction in total protein following hydrolysis from 1.61% ± 0.21% to 1.46% ± 0.46% and from 1.45% ± 0.19% to 1.14% ± 0.18% respectively. However, the technique used to produce Inyange flour showed a slight increase (1.58% ± 0.24% to 2.19% ± 0.5% of dry matter) following the development of mould. No aflatoxins were detected in any of the samples. These results will enable consumers and manufacturers to make decisions that promote good health.

Keywords

Cassava, Cyanide, Aflatoxins, Mould, Proteins, Burundi

1. Introduction

Cassava is one of the most important staple crops in Burundi [1]. According to URAM 2010 [2], Makamba province is the largest producer of cassava in Burundi, especially in the communes of Kayogoro, Kibago and in the natural region of Buragane and Moso. However, it contains toxic compounds, notably cyanogenic glycosides: linamarin (0.90%) and lotaustralin (0.10%) of total cyanogen [3] [4], which, once hydrolysed into hydrocyanic acid (HCN), cause acute or chronic poisoning due to cyanide [5]. A study by Diallo *et al.* showed that cassava varieties can be slightly toxic (containing less than 50 mg $HCN{\cdot}kg^{-1}$), moderately toxic (between 50 and 100 mg $HCN{\cdot}kg^{-1}$) and highly toxic (more than 100 mg $HCN{\cdot}kg^{-1}$) [6]. Hydrogen cyanide is a small, highly toxic chemical molecule which is highly soluble in lipids and water [7]. HCN causes several harmful effects. HCN causes delayed growth in children [8]. Calcifying pancreatitis, goiter, and optic neuropathy, with 100,000 cases in 2000 and 6788 cases in 2009 [9] and 3700 cases of Konzo in 1993 were reported in poor areas of Africa [10]. Very recently in Benin, seven deaths were recorded after consuming cassava flour paste [11]. In addition, long-term consumption of inadequately prepared cassava can lead to humans infertility [12]. Fortunately, several treatment methods, either individually or in combination, are traditionally used to reduce the cyanide level in cassava, including peeling, slicing, wet and solid fermentation, cooking, drying, pounding and grinding [13]. However, it has been demonstrated that a significant amount of cyanide exceeding the standards may remain depending on the technique used [14]-[17]**.** In Burundi, there are three traditional techniques for reducing HCN during flour preparation. These are retting (Ikivunde, ikirobeke in the local language), which is anaerobic fermentation; drying cassava in the sun (akambaranga in the local language); and fermentation (ubuzenge, ubunyange in the local language), which is aerobic fermentation. However, these techniques are criticized for not being able to eliminate all glucosides [18]. During the fermentation of cassava, mould grows, signalling the end of the detoxification process [19] [20]. However, these methods are suspected of causing aflatoxins infestation, which cause

health problems, especially in tropical and intertropical Africa, where production of cassava is high [20]. Aflatoxins also cause cancer, hepatitis B and liver cancer, and 1.5 million people die each year worldwide due to aflatoxins [21]. Consumption of food contaminated with aflatoxins exposes children to rickets and stunted growth [22], and hepatomegaly [23]. A study by Hamid *et al.* (2013) showed that more than 4.5 billion people worldwide are exposed to foods contaminated with aflatoxins, especially in developing countries [24]. In Kenya, the consumption of foods contaminated with aflatoxins also caused 317 cases of aflatoxicosis, resulting in the deaths of 125 people [25].

However, it should be noted that no studies have been conducted on the impact of traditional cassava flour preparation technologies in Burundi. That is the reason this study is conducted on the influence of traditional methods of cassava flour preparation on hydrogen cyanide, protein and aflatoxin content. The overall objective is to assess the impact of traditional cassava flour preparation technology, specifically analyzing the reduction in HCN, protein content and aflatoxin contamination. These results allow producers and researchers to improve and innovate techniques aimed at significantly reducing HCN.

2. Material and Methods

2.1. Determination of the Three Specialized Municipalities for Each Type of Detoxification (Inyange, Ikivunde and Akambaranga)

Three municipalities for each type of flour preparation were selected after conducting a survey throughout the province to determine which ones consume and process more Akambaranga, Inyange or Ikivunde flour than others. To be more representative, the sample size was calculated based on the population of the municipality of Nyanza, which is more populous, and the same size was applied to other municipalities. According to the 2024 census, the municipality of Nyanza had a population of 225,893 inhabitants [26]. By applying Giezendanner's formula [27], we found a workforce of 383 after the application of the following formula.

$$n = \frac{t^2 \cdot N}{t^2 + (2e)^2 (N-1)}$$

n: sample size, t: risk of error linked to the confidence interval, which is equal to (1.96), N: parent population (total population) of the most populous former municipality of Nyanza: 225,893, e: desired absolute precision expressed as a function of 1 ($e = 0.05$). As the respondents were adults, 48% [26], we calculated those over the age of 18. This gave us 184 adult people. In order to achieve greater representativeness, we surveyed 200 people, based on one person per household. The same household shares preparation techniques. For six municipalities in the province, the survey was conducted on 1200 households, with 200 households per municipality.

To obtain representative samples, all communes in Makamba province were studied, and three hills per commune were randomly selected. For Makamba commune, these were the hills of Canda, Gisenyi, and Mihongo; for Nyanza commune,

the hills of Mugerama, Mugumure, and Nyabigina; for Mabanda commune, the hills of Musenyi, Mara, and Kayogoro; for Vugizo commune, the hills of Murinda, Kagege, and Mazuru; for Kayogoro commune, the hills of Butare, Gatabo, and Kabizi; and for Kibago commune, the hills of Rubimba, Nyarutuntu, and Kiyange. The results of this survey made it possible to identify three municipalities (Table 1) for each type of preparation on which sampling will continue for aflatoxin and hydrogen cyanide analysis. Thus, the municipalities of Kibago, Kayogoro and Makamba were selected for Akambaranga; the municipalities of Kibago, Mabanda and Makamba for Inyange; and the municipalities of Kibago, Vugizo and Nyanza for Ikivunde.

2.2. Collection of Samples from Inyange, Ikivunde and Akambaranga

After identifying the municipalities and hillsides that would be involved in the collection, samples of fresh cassava were harvested to obtain data before applying preparation or detoxification techniques. Cassava that had undergone traditional preparation techniques by the population was collected according to whether it was Akambaranga (sun-dried), Inyange (aerobic fermentation) or Ikivunde (an-aerobic fermentation) (Figure 1).

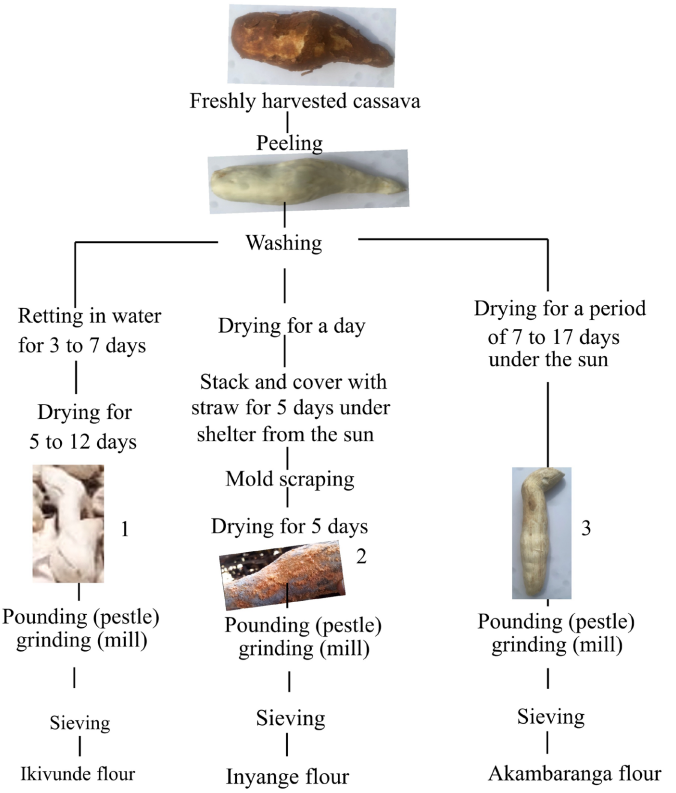

Figure 1. Traditional method of making cassava flour.

2.3. Determination of Total HCN Content

The transport, pre-treatment and measurement of HCN in Inyange, Ikivunde and Akambaranga cassava at the ISABU laboratory was determined using the AOAC method [28]. 20 g of each type of flake was transferred to a Kjeldahl flask. 200 ml of water was added and mixed with the sample. After 2 hours, the solution was then distilled. The distillate was collected in a flask containing 20 ml of a 2.5% NaOH solution (0.5 g in 20 ml of H_2O) until it was distilled to a defined volume. 8 ml of 6 M NH_4OH and 2 ml of 5% KI solution were added to the distillate before titration with 0.02 M $AgNO_3$ using the microburette. HCN was calculated as follows: HCN (mg) = ml of 0.02 M $AgNO_3$ × 1.08.

2.4. Protein Content

Crude protein is determined from the total nitrogen content, using the Kjeldahl method [28]. After sulphuric mineralisation, in the presence of a selenium catalyst, the nitrogen content is multiplied by 6.25: nitrogen to protein conversion coefficient.

$$\text{Protein}(\%) = \text{Nitrogen}(\%) \times 6.25$$

$$\text{Nitrogen}(\%) = \frac{(S-B) \times N \times 14 \times f \times 100}{1000 \times M}$$

where S: volume in ml of 1N H_2SO_4 used to titrate the sample; B: volume in ml of 1N H_2SO_4 used to titrate the blank; 14: atomic mass of nitrogen; f: correction factor; M: mass of the sample; N: normality of the H_2SO_4 solution (N = 0.05).

2.5. Determination of Aflatoxin Content

Aflatoxins were measured using the ISO 16050:2003 method (ISO, 2003). The key steps in these methods are extraction, purification and quantification [29]. High-performance liquid chromatography (HPLC) with UV detection as described by Hussaini *et al.* [30] was used for the separation and quantification of aflatoxins, after using the sampling, sample preparation and analysis methods described in AOAC (1990); and by Matsiko *et al.* [28] [31]. The samples of Inyange, Ikivunde and Kambaranga chips were finely grounded using a grinder (Ramtons, model: RM/161 China). A quantity of 20 g of the grounded material from each type of cossette was put into a 200 ml flask, then 100 ml of a mixture of methanol and double-distilled water (80/20: v/v) was added. The resulting solution was then homogenized away from light for 30 minutes. After centrifuging the homogenate at 4000 rpm for 5 minutes, the supernatant obtained was filtered under vacuum on Whatman No. 4 filter paper in an Erlenmeyer flask [32]. Fourteen (14) ml of this filtrate were collected and then diluted using 86 ml of phosphate-buffered saline (PBS) solution. 50 ml of the diluted solution was placed in the immunoaffinity column, which had been pre-conditioned using 10 ml of PBS buffer added drop by drop. After rinsing with 1.5 ml of PBS buffer, the aflatoxins were eluted with 1.5 ml of methanol and then diluted using 1.5 ml of double-distilled water [32] to obtain the solution ready for injection into the chromatographic system, whose protocol for the injection and quantification of aflatoxins has been detailed by

Kpan *et al.* [29]. Elution was performed in isocratic mode with an elution flow rate of 1 ml·min^{-1} at an oven temperature set at 40˚C with excitation and emission wavelengths of 330 nm and 460 nm, respectively, in accordance with the conditions for analysis and validation of the HPLC method for aflatoxin determination described by Fofana-Diomande *et al.* [32].

2.6. Statistical Analyses

Statistical analyses of the results obtained were performed using IBM SPSS Statistics 20. An analysis of variance (ANOVA) was performed to calculate significant differences in the data at a threshold of $\alpha = 0.05$. The ANOVA was supplemented by Duncan's multiple comparison test to detect levels of difference, and the results were expressed as mean values ± standard error (SE).

3. Results and Discussion

3.1. Lever of Consumption of Akambaranga, Inyange, Ikivunde in Makamba Province

The province of Makamba consumes cassava flour prepared in three ways. The most commonly consumed throughout the province is called Inyange (49%), followed by akambaranga (30%) and finally ikivunde (21%) (Table 1). However, preferences vary from one municipality to another. Akambaranga flour (sun-dried) is much more widely consumed in the municipality of Kayogoro (57.5%), followed by Kibago (35.3%) and then Mabanda (29.6%). Inyange (prepared by aerobic fermentation away from the sun) is much more widely consumed in the municipalities of Makamba, Mabanda and Kibago, with respective frequencies of 85.7%, 66.7% and 58.8%.

Table 1. Frequency of consumption of Akambaranga, Inyange, Ikivunde in each municipality of Makamba Province.

Commune	Akambaranga (%)	Inyange (%)	Ikivunde (%)
Kibago	35.3	58.8	5.9
Mabanda	29.6	66.7	3.7
Kayogoro	57.5	40.2	2.3
Vugizo	3.8	38.5	57.7
Makamba	9.5	85.7	4.8
Nyanza	9.1	18.2	72.7
TOTAL	**30**	**49**	**21**

3.2. Effect of Preparation Technique on HCN and Protein Content in Inyange Flour

The Inyange preparation method significantly reduces cyanide levels. Table 2 shows that, regardless of the production site, the cyanide mean levels ranged from 87.94 ± 0.84 to 1.36 ± 0.09 mg HCN/kg. Differences, not very significant ($p < 0.05$), in cyanide mean levels were detected in the following order: 1.51 ± 0.44 >

1.35 ± 0.17 > 1.24 ± 0.32 respectively in the flour produced in Mabanda, Kibago and Makamba. However, the reduction rate is as follows: Makamba (87%) > Mabanda (86.77%) > Kibago (85%). According to the FAO and WHO (1991), only a maximum concentration of 10 ppm, or 10 mg/kg of cassava flour, is considered acceptable after treatment [4] [6]. This preparation technique has been shown to improve the total protein content due to the molds that develop during fermentation (**Figure 1**). The content increased from 1.58% ± 0.24% to 2.19% ± 0.5% (**Table 2**). However, this content remains far below human requirements. Protein requirements for adults are 0.8 g/kg/day and 1 g/kg/day for the elderly [33]. This shows that a 70 kg adult needs 70 g of protein per day. However, a person consuming 500 g of flour per day will consume 10 g of protein from Inyange flour. Consuming this flour should be accompanied by protein-rich foods. Regarding aflatoxin levels, nothing was detected (**Table 2**). This shows that during preparation or storage, whether it be cassava or flour, consumers do not need to worry about the effects of aflatoxins in inyange flour.

Table 2. HCN (mg/Kg), protein (%) and aflatoxin contents in Inyange flour.

Communes	HCN before	HCN after	Protein before	Protein after	Aflatoxin
Kibago	89.09 ± 6.21^{a}	1.35 ± 0.17^{b}	1.5 ± 0.35	2.15 ± 0.12^{b}	Nd
Makamba	86.71 ± 3.33^{b}	1.24 ± 0.32^{c}	1.31 ± 0.08	1.48 ± 0.01^{c}	Nd
Mabanda	88.12 ± 7.07^{a}	1.51 ± 0.44^{a}	1.95 ± 0.15	2.94 ± 0.22^{a}	Nd
Average	87.94 ± 0.84	1.36 ± 0.09	1.58 ± 0.24	2.19 ± 0.5	Nd

In each column, values with letters a, b, c, mean that they are significantly different ($p < 0.05$) while those with the same letter in the column are not significantly different ($p > 0.05$); nd: not detected; HCN before = hydrogen cyanide content before applying the technique, HCN after = hydrogen cyanide content after application of the technique; Protein before = protein content before applying the technique, and Protein after = protein content after application of the technique.

3.3. Effect of Preparation Technique on HCN Content and Protein in Ikivunde Flour

Ikivunde is prepared from cassava roots by peeling, soaking for 3 - 5 days, drying, pounding/grinding and sifting the flour called Ikivunde [34]. That is called the retting technique.

A significant reduction in HCN was found in the flour prepared in the municipality of Mabanda, followed by that prepared in the municipality of Vugizo and finally that of Nyanza. Thus, the reduction rate calculated between the initial content and that after application of the Ikivunde technology preparation (**Table 3**) was 86.23%, 86.57% and 83.63% for Mabanda, Vugizo and Nyanza, respectively. Even though there is a slightly significant difference in reduction ($p < 0.05$), the remaining levels of 1.37 ± 0.13, 1.4 ± 0.28 and 1.67 ± 0.12 respectively in ikivunde flour from Mabanda, Vugizo and Nyanza are far below the value set by the 1991 FAO and WHO standard, which is 10 ppm, or 10 mg/kg of cassava flour [35]. However, attention must be paid to the initial levels. Some varieties have levels as

high as 211 mg/kg [36]. With the reduction mean obtained in Ikivunde production technique, 86.95% (**Table 3**), the content of 211 mg/kg will be reduced by 181 mg/kg and it would remain 30 mg HCN/kg in cassava flour. This content is three times the value set by the FAO and WHO.

Table 3. HCN (mg /kg), protein (%) and aflatoxin content in Ikivunde flour.

Commune	HCN before	HCN after	Protein before	Protein after	Aflatoxins
Mabanda	85 ± 2.04^{b}	1.37 ± 0.13^{b}	1.75 ± 0.75^{a}	1.28 ± 0.01^{a}	Nd
Nyanza	87.9 ± 0.91^{a}	1.67 ± 0.12^{a}	1.21 ± 0.33^{c}	0.87 ± 0.00^{b}	Nd
Vugizo	87.97 ± 1.21^{a}	1.4 ± 0.28^{b}	1.41 ± 0.63^{b}	1.28 ± 0.32^{a}	Nd
Average	86.95 ± 1.30	1.48 ± 0.12	1.45 ± 0.19	1.14 ± 0.18	Nd

In each column, values with letters a, b, c, mean that they are significantly different ($p < 0.05$) while those with the same letter in the column are not significantly different ($p > 0.05$); nd: not detected; HCN before = hydrogen cyanide content before applying the technique, HCN after = hydrogen cyanide content after application of the technique; Protein before = protein content before applying the technique, and Protein after = protein content after application of the technique.

In terms of protein content, the Ikivunde production technique is not favorable. Even the small amount that exists is lost as a result of the hydrolysis of water-soluble proteins [37]. Before applying the technique, we had 1.75% ± 0.75%, 1.41% ± 0.63%, and 1.21% ± 0.33% mg protein/kg flour respectively for flour from Mabanda, Vugizo and Nyanza, which became 1.28% ± 0.01%, 0.87% ± 0.00% and 1.28% ± 0.32% mg protein/kg flour respectively. The Ikivunde preparation technique involves important steps of retting the cosettes for 4 to 7 days depending on whether the climate is hot or cold (**Figure 1**). Cassava is not a good source of protein [38] [39].

3.4. Influence of Preparation Technique on HCN Content and Protein in Akambaranga Flour

Unlike Ikivunde and Inyange flour, Akambaranga is prepared by peeling and sun-drying fresh cassava for 7 - 14 days without fermentation.

Table 4. HCN, protein and aflatoxin content in Akambaranga flour.

Commune	HCN before	HCN after	Protein before	Protein after	Aflatoxins
Kayogoro	88.44 ± 2.55^{a}	1.67 ± 0.16^{a}	1.94 ± 0.55	1.86 ± 0.02^{a}	nd
Kibago	88.71 ± 1.32^{a}	1.35 ± 0.23^{b}	1.41 ± 0.13	1.05 ± 0.58^{b}	nd
Mabanda	84.92 ± 4.02^{b}	1.29 ± 0.15^{c}	1.5 ± 0.09	1.24 ± 0.02^{b}	nd
Average	87.35 ± 1.63	1.43 ± 0.15	1.61 ± 0.21	1.46 ± 0.46	nd

In each column, values with letters a, b, c, mean that they are significantly different ($p < 0.05$) while those with the same letter in the column are not significantly different ($p > 0.05$); nd: not detected; HCN before = hydrogen cyanide content before applying the technique, HCN after = hydrogen cyanide content after application of the technique; Protein before = protein content before applying the technique, and Protein after = hydrogen cyanide content after application of the technique.

The HCN levels remaining after drying in the cosettes from the municipalities of Kibago, Kayogoro and Vugizo differ significantly ($p < 0.05$) between them. They vary from 1.35 ± 0.03; 1.67 ± 0.06; 1.29 ± 0.05mg HCN/kg flour (**Table 4**), representing reductions in HCN of 86.77%; 87.36%; and 83.63% respectively. These results are comparable to those of Kalakuko *et al.* [40] who worked on dried cassava and sold it at the Muhanzi beach market in Bukavu. Studies have shown that climatic conditions, size and thickness of cassava roots could also be responsible for these differences in residual HCN content observed [41]. Overall, it can be said that the method used to obtain Akambaranga reduced HCN from 1.43 ± 0.15 to 1.61 ± 0.21 mg HCN/kg. As with Ikivunde and Inyange flours, the HCN reduction level is below the 10 mg/kg limit set by the 1991 FAO and WHO standard and recognized by the Burundi Institute of Standardization [35]**.** However, a detrimental reduction was observed for proteins. The protein content, which was already very insufficient in relation to human needs, was reduced from 1.61 ± 0.21 to 1.46 ± 0.46. Differences that were not highly significant ($p < 0.05$) were noted depending on the harvest sites. Furthermore, moulds that could develop, as in the case of Inyange flour, cannot do so in Akambaranga flour preparation because of continuous drying.

3.5. Aflatoxin Content in Akambaranga, Ikivunde and Inyange Flour

Despite the kind of the three cassava flour preparation, no aflatoxin was detected (**Tables 2-4**), while the maximum acceptable level in flour or cassava chips is 10 µg/kg [42]. It could be stated that there is no aflatoxin danger from cassava flour produced in Burundi. This shows that the preparation was done carefully so as not to contaminate the cassava chips with aflatoxin-producing microorganisms. Studies have reported that there are types of fungi that produce these toxins, such as Aspergillus *flavus* (*LINK*, *A. flavus oryzae*, *A. niger*, *A. fumigatus* and *A. glaucus chevalieri*) [19]**.** According to the same author, this production begins around the third day and continues until the sixth day if the process is not stopped in time by drying. Based on these latest studies, the techniques used to prepare cassava flour in Makamba province, which takes at least five days, are carried out in accordance with all the necessary health and safety requirements. Furthermore, the province of Makamba has a temperature that would favor the development of fungi. It has been reported that the growth of fungi producing aflatoxins is optimal in the temperature range of 27˚C to 35˚C. Thus, the temperature varies from 17.3˚C to 23˚C in the Buragane depressions (Makamba, Mabanda, Kayogoro and Kibago) and varies from 23.3˚C to 24.5˚C in the Imbo plain (Nyanza and part of Vugizo) and from 13.9˚C to 15.7˚C at altitude (part of Vugizo) [43]. The lactic acid bacteria that develop during the fermentation of Inyange, Ikivunde and Akambaranga corn cobs are also believed to be responsible for the absence of aflatoxins in these cobs. It has been demonstrated that the bacteria produce antifungal metabolites such as organic acids, bioactive antimycotics, peptides, carbox-

ylic acids, hydrogen peroxide and alcohols [44]. Other factors are likely to have contributed to the fact that the flakes were not damaged by insects. Insects also contribute to a certain level of aflatoxin contamination by making the flakes more vulnerable to invasion by Aspergillus [45].

4. Conclusion

During this study, it was found that the three traditional methods of cassava flour preparation (Ikivunde, Inyange and Akambaranga) have a positive impact on reducing HCN content. They reduce this content to levels below those set by the FAO/WHO. These techniques make cassava edible and protect consumers from all diseases caused by HCN. It was also revealed that three of these methods do not promote the growth of toxin-producing fungi or moulds. However, they have almost no positive effect on protein content, except for the Inyange method, which increases it by a very small amount. Instead, these techniques cause the small amount of protein contained in the cosettes to be lost, especially in the Ikivunde and Akambaranga preparation methods. This means that when consuming these products, they must be accompanied by other protein-rich foods like legumes and fish, given that the region is coastal on Lake Tanganyika, and the meat of small domestic animals, which are accessible to many people. During the period of our study, when these three methods were applied, no traces of aflatoxins were observed, but particular attention should be paid during storage. Further studies could delve deeper into varietal differences and localities.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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