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Assessment of Cassava (*Manihot esculenta* Crantz) Fermentation and Detoxification Using Previously Cassava-fermented Chips

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Authors' contributions

This work was carried out in collaboration between all authors. Author JJEN designed the study. Author NNZ performed the statistical analysis with author SSK, also wrote the protocol with authors HBA and FEM. Author NNZ wrote the first draft of the manuscript with authors SSK and JJEN. Authors JJEN, SSK and NNZ managed the analyses of the study. Author NNZ managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was aimed as the assessment of dried cassava-fermented chips (DFCC) efficacy in terms of their fermentation acceleration and cyanogens reduction potential.
Study Design: An experimental study design was employed in order to achieve the objective of the study.
Place and Duration of Study: The study was carried out in the *Laboratoire de Microbiologie* of the University of Yaoundé 1, Cameroon between October 2011 and August 2014.
Methodology: Commercial fermented cassava chips samples were analyzed for their performances to accelerate the retting of cassava roots. The performances of DFCC were determined by periodical measure of the degree of softening of the roots in retting process by penetrometry for 4 days. Further, cassava chips were produced at different fermentation times and

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assessed for the same properties. Finally, the best fermentation time to obtain cassava-fermented chips capable to reduce efficiently the retting time was determined and chips produced at this time were used after different pre-treatments in order to assess their impact on the fermentation performances and detoxification of cassava roots as compared to spontaneous retting.
Results: Results indicated that DFCC samples collected have high retting performances variability correlated to their fermentation time and microbial concentration. The best DFCC used as cassava retting accelerator (CRA) was obtained after 96h of fermentation (CRA-96). It permitted to reduce the retting time from 71.3±8.5 hours to 35.8±1.4 hours compared to the spontaneous fermentation; and final cyanides contain of 7.86±0.00 ppm corresponding to 98.8% reduction. Optimization assays showed that CRA-96 when soaked for 12 h before use, reduce retting time by 58.5% but did not modify cyanides reduction.
Conclusion: The adoption of the CRA-96h as cassava retting accelerator by cassava fermented by-products producers would be great advantage for them who often only ret for 1-2 days and then get into trouble with large cyanide levels in their flour.

Keywords: Cassava; cassava-fermented chips; retting time; cyanogens reduction; fermentation.

1. INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a plant of 1-2 m shrub which starchy roots provide food for over 500 million people in the developing world and up to about 60% of their daily calorific needs [1,2]. In spite of its importance, harvested cassava tubers cannot be stored without processing. Physiological deterioration occurs in cassava roots 2-5 days after harvest because of microbial activity [3]. In addition, cassava roots contains cyanogenic glycosides mainly linamarin and in less amount lotaustralin (or methyl linamarin) [4], which are the main nutritional drawbacks that must be avoided. One of this drawback, its toxicity [2,5], is minimised by traditional process during which, roots are transformed into many product. Among processing operations of cassava, retting, which is microbial fermentation of the root, is one of the most suitable methods to reduce the toxic and poisonous constituents (cyanogenic glycosides) of the raw fresh cassava [2,6-8]. During this fermentation, roots are softened, disintegration of the tissue structure results in contact of linamarine with linamarase, which is located in the cell walls and subsequent hydrolysis to cyanhydric acid soluble in water. This retting usually takes 3-5 days depending on the roots dimensions and age, ambient temperature and microbial population involved. The need to accelerate this process has arisen due to the rapid increase of population demand. For this reason, some attempt of starter production in order to enhance the initial microbial population and faster the retting process has been proposed by some authors [9-12]. Notwithstanding these efforts to propose microbial solutions to accelerate the cassava retting, the use of selected microorganisms have very low dissemination among the producers because of the difficulties to handle strains, their cost and the detoxification not always equal to the traditional product [7,13]. This single strain or selected strain approach to accelerate cassava retting has hence proved to be unsuccessful, indicating that microbial implication during this fermentation should be assessed in and holistic manner. It has been observed that, traditionally rural cassava producers use different empirical methods to accelerate the retting like adding, mild steel, using mother liquor of previous fermented cassava [14]. The use of cassava from previous fermentation is the one mostly adopted retting method in Cameroon even though with fluctuating results. The hypothesis behind the success of this technique is that these fermented chips contain different microbial organisms that constitute the initial flora of the fermentation with a holistic interaction. Currently, at the best of our knowledge, no study has assessed the reasons of this fluctuating results and the level of detoxification achieved when accelerating the process. It is in this perspective that the use of cassava-fermented chips (*starters*) for the acceleration of cassava retting was studied with the objective of assessing its impact on the cassava retting time and the total cyanogens reduction.

2. MATERIALS AND METHODS

2.1 Samples Collection

2.1.1 Fresh cassava roots

Twelve months old freshly cassava roots of the bitter variety, traditionally called *Six-mois,* were

always harvested, during the period of study, from Bafia region in Cameroon and transported directly in polyethylene back to the laboratory experience.

2.1.2 Cassava-fermented chips

Fifteen Gross-sellers from the main food distribution markets in Yaoundé-Cameroon were randomly selected and for each seller, samples were collected from top, middle and bottom of bags in order to have a weight of about 2 kg. All samples (15) were placed each, in sterile plastic bags, labelled (S1 to S15), then transported to the laboratory in an insulated box containing a freezing pack, and stored at 8℃.

2.2 Determination of Cassava Fermentation Time Necessary for Production of Starter with the Best Retting Performance

2.2.1 Production of starters

Cassava roots were peeled, cut into cylinder of approximately 4 cm length x 5 cm diameter, washed and divided in five set of 500 g each. Every set was submerged in 500 ml of sterilized tap water at the ambient temperature (between 28 and 32℃) in the laboratory and fermentation was stopped after 24 h, 48 h, 72 h, 96 h and 168 h respectively for the 5 set of experiment. At the end of each the fermentation time, cassava root were removed, dewatered and dried at 40℃ in a ventilated oven for two days, when water content of samples was inferior to 15% (w/w). The dried cassava chips obtained were crushed in a blender and flours got from each set, wrapped in labelled airtight plastic sachets and named CRA-24, CRA-48, CRA-72, CRA-96 and CRA-168 (constitute the starters with CRA for "Cassava Retting Accelerator") with respect to the length of fermentation, then stored at 4℃ for subsequent analyses.

2.2.2 Fermentation procedure with starters

Cassava tubers were peeled cut into cylinder like previously described, washed and divided into sets of 500 g each. Fermentation was performed by submerging 500 g of cassava pieces of each set in 500 ml of tap water and then, inoculated with 1% (w/v) of starters powder previously produced. One set representing the control (natural fermentation) were not inoculated. Retting (fermentation in water) was conducted at ambient temperature (between 28 and 32℃) in the laboratory and followed during 4 days by evaluation of cassava root softening. Three tests were performed for each essay.

2.2.3 Evaluation of cassava roots softening and determination of the retting performances of the starters

Retting performance was determined by periodical measure of the degree of cassava roots softening of the chunks with a penetrometry instrument (RPN10 Berlin). The more the softening was, the higher was the distance of penetration of the penetrometry-needle. The sigmoid nature of the softening kinetics made it possible for the data to be fitted to the Baranyi and Roberts [17] model. This fitting permitted to estimate the kinetics parameters (the time to initiate softening (TIS) and the softening rate (SR)) as shown in Fig. 1. The retting time (RT) was calculated with the expression below:

$$RT = TIS + 0.8^{*} / SR \quad (1)$$

*0.8 is the penetrometry index, which corresponds to the level softening most suitable for most transformed cassava foods chosen.

Each retting kinetics test was carried on three times and the results presented as mean plus standard deviation.

2.3 Improvement of Cassava Starter Activity

In order to further improve the performance of the cassava retting accelerator (CRA) used, the best starter obtained from previous experiment was subjected to the four series of pre-treatments described below before inoculation to 500 g of cassava pieces submerged in 450 ml of sterilised water.

1- With the view to obtain rapidly the vegetative form of microorganism generally present in the flour in spore forms 5 g of CRA called CRA-96 ThA, were diluted in 45 ml sterile water, heated at 80℃ for 10 min and immediately cooled down at 30℃
2- 5 g of CRA called CRA-96 ChA, was diluted in solution of ethanol 60%v/v, agitated at 60 rpm (rotation per minute) during one hour with the same aim as the firs pre-treatment.
3- To increase the initial charge of viable microorganism, 5g of CRA named CRA-96NB was inoculated in 45 ml of sterile

nutrient broth made of potatoes starch 2% w/v and 0.5% w/v of yeast extract, and then incubated at 37℃ for 12 h

4- In order the revivified spore flora of CRA before his utilization, 5 g of CRA called CRA-96W was simply soaked in 45 ml of sterile water and incubated at 37℃ for 12 h.

Two set of control were also used (CRA-96 and set without CRA).

2.4 Microbial Groups Cell Load Determination

Total mesophilic aerobic flora (TMAF); sulfito-reducing flora (SRF) and total *Bacillus* spp spores count were enumerated using the traditional plate count technique. For all the CRA produced or collected, 10 g of the product was mixed with 90 ml of 0.9%w/v NaCl sterile solution. The suspension obtained constituted the stock solution of the sample from which a series of decimal dilutions was performed and 0.1 ml of the appropriate dilution sowed on suitable solid medium surface. TMAF was enumerated at 30℃ for 24 hours on Plate Count Agar (PCA, Oxoid, Basingstoke, UK). Sulfito-reducing flora (SRF) was obtained by culturing in absence of oxygen in an anaerobic jar on TSN Tryptone Sulfite-Neomycin (TSN, Oxoid, Basingstoke, UK) at 45℃ for 24 hours. Total *Bacillus* spp spores (TBF) was enumerated on BCP (Bromocresol purple) agar supplemented with potatoes starch at 30℃ for 72 hours. Each sample was analysed three times and the results presented as mean plus standard deviation.

2.5 Determination of Total Cyanide Content

Cyanide content of cassava before and after processing, was determined using the picrate kit B2 for determination of the total cyanide content of cassava flour [15,16]. A 100 mg flour sample was placed in a plastic vial, a small filter paper impregnated with pH 6 buffers, and linamarase was added, followed by 0.5 ml of water and a yellow picrate paper. The vial was immediately closed and left at 30℃ overnight. The next day the yellow–brown picrate paper was separated from the plastic backing strip and placed in 5 ml of water. The absorbance of the solution was measured at 510 nm and the total cyanide content in ppm was calculated by multiplying by 396 [15].

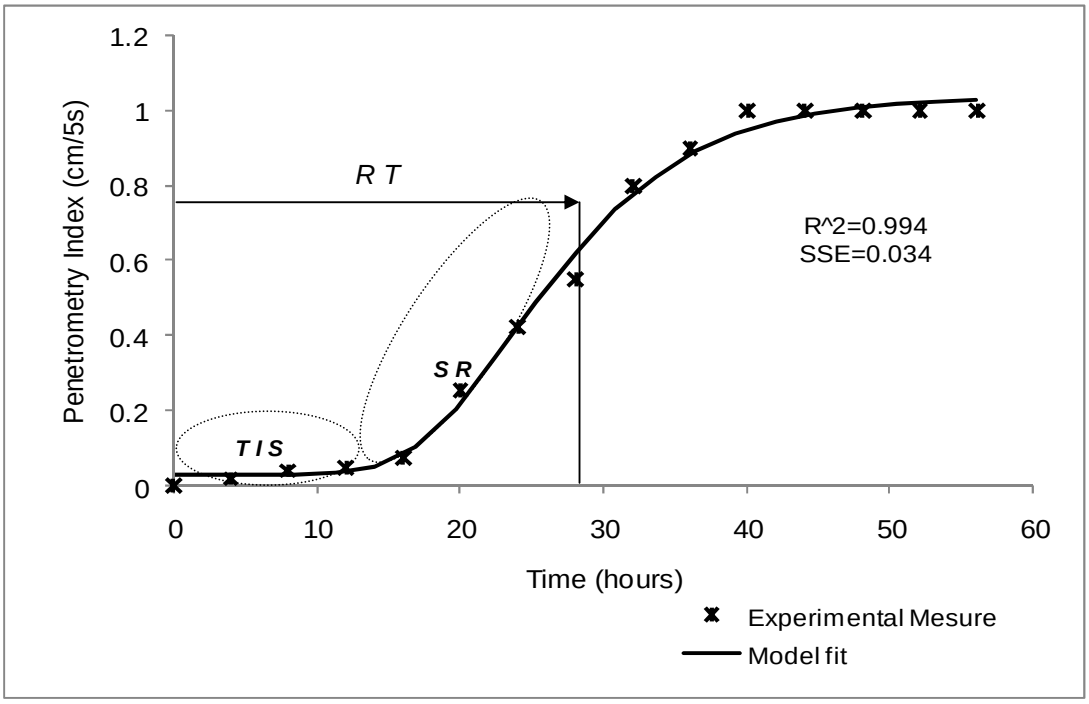

Fig. 1. Evolution of penetromrtry index with time fitted with Baranyi and Robert model

Kinetics parameters are shown in italic. TIS= Time to initiate softening; SR= Softening rate; RT= Retting time

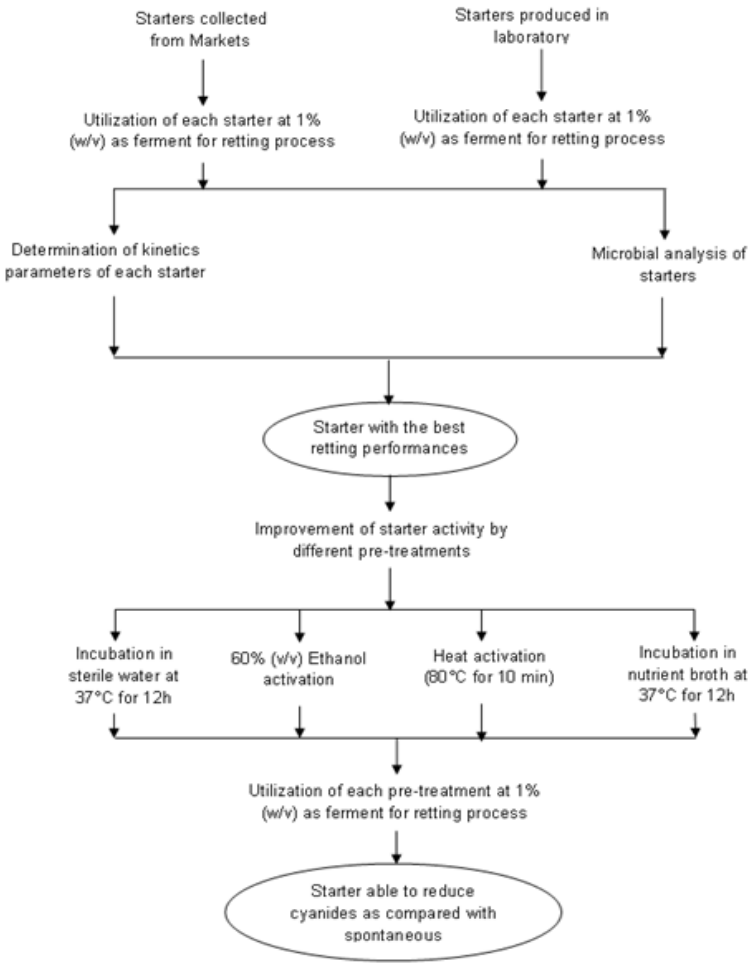

Fig. 2. Overall experimental description

2.6 Statistical Analysis

The analysis of the data of the profile of softening according to time was made by adaptation to the model of Baranyi and Robert [17] by using *DM-fit* software made free available on the www.combase.cc site. SPSS version 16.0 Chicago, IL, USA was used for analysis of variance (ANOVA), and statistical differences between means ($p < .05$) were tested by the Fisher's least significant difference (LSD) test.

3. RESULTS AND DISCUSSION

3.1 Evaluation of the of Cassava-fermented Chips Collected from Market to Accelerate the Retting

Retting parameters presented on Table 1, show that Retting Time (RT) and the time to initiate softening (TIS) varied with the commercial chips used, which confirms the observations made during traditional processing. It appears from the land survey that the use of these dry fermented cassava samples for new fermentations permitted to obtain a variety of retting kinetics differing for their TIS and SR. This result can be attributed to the different origins of the samples and the nature of the microbial communities responsible for the fermentation. Microbial analysis (Table 2) shows that, Total mesophilic aerobic flora (TMAF) value ranged from 3.6±0.2 log CFU/g to 7.4±0.0 log CFU/g while total *Bacillus* spp spores (TBS) cell loads, they varied between 2±0.0 log CFU/g and 5.6±0.2 log CFU/g (Table 2). The lowest microbial group content was that of SRF, which did not exceed 1.4±0.3 logs CFU/g and were under the detection value in some samples. The reduction of the TIS and retting time (RT) by most of the samples used as compared to the control indicates that the initial

microorganism pool of dry fermented cassava samples have contributed in different ways in initiating the softening of the cassava roots. Microbial enzymes like cellulases, xylanases, pectin methylesterases, polygalacturonases, and lyases are known to be responsible of cell walls disruption, facilitating the microbial actions leading to cassava softening [18,6]. The fact that dry fermented samples with good performances on the TIS are not always the same with good performances on the softening rate (SR) indicates that different microbial communities are involved in these phases and the retting is just the combination of their actions. This also explains why it was at the level of the retting time that the significant correlations were observed with the microbial group cell loads (Table 3).

Table 1. Retting kinetics parameters of spontaneous fermentation and fifteen dried cassava chips samples (S)

Dry fermented cassava samples	Retting kinetic parameters		
	TIS	SR	RT
Control	53.7±12.5	1.8±0.7	58.8±10.2
S1	42.8±2.2	0.6±0.3	56.5±2.8
S2	42.3±2.8	0.6±0.1	55.7±0.4
S3	50.7±7.5	2.5±0.8	54.2±6.2
S4	34.2±3.9*	0.8±0.1	44.5±3.8*
S5	44.3±3.5	4.7±1.9*	46.2±2.6*
S6	41.1±6.2	2.9±0.2	46.8±6.0*
S7	48.2±3.9	2.5±0.7	51.6±3.0
S8	40.1±2.5*	0.3±0.0	63.2±0.2
S9	39.0±1.8*	0.8±0.2	61.1±4.2
S10	36.9±1.9*	1.4±0.2	42.8±1.1*
S11	32.8±0.4*	0.8±0.1	42.3±1.2*
S12	28.7±3.6*	0.7±0.1	39.9±2.3*
S13	37.8±2.4*	1.5±0.3	43.4±1.2*
S14	35.4±4.0*	0.8±0.1	44.9±2.7*
S15	34.9±1.9*	0.9±0.1	44.1±1.3*

*NB: those results are results from 15 processors sampled once but analyzed in triplicate. TIS: Time to initiate softening. SR: Softening rate; RT: Retting time. Mean ± SD = Mean values ± standard deviation of error of means of three experiments. * indicates values significant different statistically with control at (P <.05)*

3.2 Influence of Retting Time on the Performances Cassava-fermented Chips

In order to understand the origin of the variability observed with commercial samples, cassava chips were produced at different fermentation (retting) times. Those chips were compared for their microbial loads and their kinetics parameters when for retting acceleration. It can be noted from the 5 Cassava Retting Accelerator (CRA) produced, that three of them: CRA-72; CRA-96 and CRA-168) considerably reduce the retting duration with respect to the control experiment where fermentation was spontaneous with respective retting times of 34.3±4.6 hours, 30.9±3.1 hours and 28.1±4.5 hours. While retting time of control was 71.3±8.5 hours (Table 4). Although the RT of those three CRA was not significantly different, the data of this parameter and those of TIS obtained with CRA-96 and CRA-168 were much closed and slightly better than that of CRA-72. From these results, because of the time, saving with respect of CRA-168, 96 hours appears, as the time require for cassava retting accelerator production. For microbial load (TBS and SRF), it can be observed that, SRF did not increase with the fermentation time chosen for the CRA production as it was the case for TBS, or TBF (Table 4). The variability and the quantity of microbial groups are result of the different fermentation length of the samples used as cassava retting accelerators. CRA obtained after 48 h of cassava fermentation (CRA-72; CRA-96 and CRA-168) proved to have statistically the same performances in accelerating the retting process. Generally, before 48 hours of fermentation, all microorganism groups that contribute to the retting of cassava are not well developed [7,19]. After the 48 hours, Abriba et al. [20] and Kostinek et al. [8] shown that *Bacillus subtilis* and *Latobacillus plantarum* are the most dominant flora throughout the cassava retting. The choice of the CRA-96 was hence a balance between the attribute obtained with CRA-72 and CRA-168. The process of drying in order to produce CRA favours the survival of spore forming bacteria that are mainly *Bacillus* spp and *Clostridium* spp. Fermentation of cassava roots using *Bacillus* spp rich dough as inoculums showed to permit the achievement of the disintegration of the cassava tissue due to the production of wide range of pectinolytic enzymes [21].

The presence of SRF generally less than 1 log cfu/g in dry fermented sample and 4 log CFU/g at the end of fermentation or in the pre-incubated CRA, conducted us to an experiment where pre-incubated CRA in water was tenfold diluted and all the dilutions used as inoculums for a new cassava retting. As result, all the fermentations were accelerated, but the butyrate odour perception was very low in the retting with the inoculums of the fifth decimal dilution and hence,

with low probability of SRF. This experiment, which data are not presented in this work, indicated the sulfito-reducing spores were the most responsible of the butyrate odour and were not capital in the retting process.

Table 2. Selected microbial group cell loads of dry fermented cassava samples obtained from different markets in the centre region Cameroon

Dry fermented cassava samples	Microbial groups (Log CFU/g)		
	TAMF	TBS	SRF
S1	4.4±0.2	3.3±0.2	0.0±0.0
S2	4.9±0.1	4.6±0.1	0.3±0.0
S3	4.1±0.2	3.0±0.0	0.0±0.0
S4	3.6±0.2	3.1±0.2	0.7±0.0
S5	4.3±0.3	3.3±0.0	1.4±0.3
S6	6.3±0.1	4.9±0.0	1.0±0.0
S7	6.2±0.0	4.1±0.1	0.0±0.0
S8	5.8±0.3	5.6±0.0	1.0±0.0
S9	7.3±0.0	4.4±0.2	0.0±0.0
S10	7.4±0.0	5.6±0.1	0.0±0.0
S11	5.5±0.1	2.0±0.0	0.7±0.0
S12	7.4±0.0	5.0±0.4	0.7±0.0
S13	7.4±0.2	4.6±0.3	1.0±0.0
S14	7.1±0.1	3.9±0.0	0.4±0.0
S15	7.2±0.3	3.0±0.0	0.0±0.0

CFU: Colonny forming units; TMAF: Total mesophylic aerobic flora; TBS: Total Bacillus spp spores

Table 3. Correlation results between microbial groups' cell loads from different dried cassava chips and the kinetics parameters of the retting

Microbial groups	Retting kinetic parameters	
	TIS	RT
TMAF	0.091	-0.390*
TBS	-0.005	-0.547*
SRF	-0.107	-0.322*

**All correlation significant at P<.01. TMAF: Total mesophylic aerobic flora; TASF: Total aerobic spore flora; TBS: Total Bacillus* spp. *spores; SR: Softening rate; TR: Retting time*

3.3 Influence of Some Pre-treatment of CRA-96 on the Reduction of Cassava Retting Time and Cyanide Contain

In Table 5, the addition of 1% of CRA-96 during fermentation or the same accelerator previously treated at 80℃ for 10 min did not improve significantly the TIS as it was the case with the other pre-treatments (Chemically activated; water soaked and pre-culture in nutrient broth). The best performance in the reduction of the retting time of chemical activation with respect to the thermal activation may be due to the low damage induced to the vegetative cells, which could easily regenerate after the pre-treatment. Regarding the softening rate, only two pre-treatments of CRA-96 (CRA-96W and CRA-96NB) induced a significant increase with respect to the control and other pre-treatments. The calculation of the retting time indicated that, it ranged from 54.9±9.3 hours in the control to 25.7±1.9 hours to the fermentation accelerated by the addition of CRA-96 previously pre-incubated in water (Table 5). It can be observed that the use of accelerators after incubation in nutrient broth (CRA-96 NB), in water (CRA-96 W) and activated with ethanol (CRA-96 Ch A) significantly improved de the retting time with respect to the control, the use of thermally activated CRA and CRA without pre-treatment. The best gain in retting time was observed with the use of CRA-96 W, which permitted to reduce the retting time of the control experiment, by 58.5%. Using CRA-96 without any pre-treatment only permitted to reduce the time of the control experiment by 42.6%. When the pre-treatment involved an incubation of the CRA in a solution before the use, enriching the solution with nutrients (CRA-96 NB), reduced the softening rate of the accelerator. This may be explained by the fact that nutrient rich solution favours the growth of all microorganisms in the CRA, reducing the cell load advantage of spores that have to initiate the softening. On the other hand, pre-incubating CRA-96 in simple water aerobically or in anaerobic condition, results in the hydration of the spores that favours their germination and the growth of micro flora present in a less pronounced way due to nutrient limits.

Retting independently of the method always resulted in cyanide content reduction (Table 6). The use of pre-treated CRA-96 did not result in higher detoxification compared to the direct use of CRA-96. Moreover, the use of CRA-96 and CRA-96 NB produce a cassava flour with HCN content of 7.86±0.00 ppm and 36.3±0.0 ppm respectively that was lower than that of control (52.3±0.0 ppm) (Table 6). Using CRA-96 brought to a 98.8% reduction of total cyanide contain compared to 92% of the spontaneous retting; this reduction using the cassava retting accelerator should be associated the 33.5% reduction of the retting time achieved, while CRA-96W which induced a 53.2% reduction of the retting time only brought to a 85.8% reduction of the cyanide

content (Table 6). The initial pH of the retting environment did not specifically influence the detoxification based on the present data. Cyanide reduction is known to be combination of endogen enzyme and microbial enzyme action [7,11,22-24]. The best cyanide content reduction was obtained when CRA-96 without any pre-treatment was used to accelerate the retting. In fact, it can be observed that pre-incubation of CRA-96 leads to inoculums with

Table 4. Retting kinetics parameters and microbial groups cell loads of cassava retting accelerator (CRA) compare to spontaneous fermentation (control)

Retting conditions	Retting kinetic parameters			Microbial groups cell loads (log CFU/g)	
	TIS (h)	SR (mm/h)	RT (h)	TBS	SRF
Control	42.2±8.3^{b}	0.3±0.1^{a}	71.3±8.5^{e}	Nd	Nd
CRA-24	38.1±11.8^{ab}	0.4±0.2^{a}	62.1±6.1^{d}	2.9±0.0^{a}	0.7±0.0^{a}
CRA-48	39.5±6.1^{ab}	1.0±0.1^{ab}	47.2±5.1^{c}	3.5±0.8^{a}	1.4±0.2^{b}
CRA-72	34.3±4.6^{b}	1.7±0.7^{b}	40.4±1.7^{ab}	4.9±0.6^{b}	4.0±0.1^{d}
CRA-96	30.9±3.1^{ab}	1.8±0.6^{b}	35.8±1.4^{a}	5.7±0.1^{b}	3.2±0.2^{c}
CRA-168	28.1±4.5^{a}	1.6±0.5^{b}	33.3±2.7^{a}	5.4±0.1^{b}	3.2±0.2^{c}

CFU: colony forming unit; RT: retting time SR: Softening rate; TBS: Total Bacillus spp *spores; SRF: Sulfito-reducing flora; TIS: Time to initiate softening. Nd: No detected. Mean ± sd = Mean values ± standard deviation of error of means of three experiments. The means in the same column followed with different letters are significantly different at (P <.05)*

Table 5. Variation of retting kinetics parameters and percentage reduction of retting time with respect to the spontaneous retting (control) obtained using different pre-treatments modalities of CRA-96

Different pre-treatments modalities of CRA	TIS (h)	SR (mm/h)	RT (h)	%RT reduction
Control	39.8±6.6^{b}	0.5±0.3^{a}	62.0±11.5^{c}	0
CRA-96	27.4±6.3^{a}	1.2±0.6^{ab}	35.6±2.3^{b}	42.6
CRA-96 ThA	38.1±9.8^{b}	0.7±0.2^{ab}	50.3±6.1^{a}	18.9
CRA-96 ChA	20.4±2.5^{a}	0.8±0.0^{ab}	30.3±2.1^{a}	51.1
CRA-96W	19.2±0.7^{a}	1.2±0.0^{b}	25.7±0.4^{a}	58.5
CRA-96NB	19.5±1.7^{a}	0.9±0.1^{ab}	27.9±1.71^{a}	50

CRA-96: ThA= thermal activation in water at 80°C fo r 10 min; ChA = Chemical activation using ethanol 60%. BN= Pre-culture in nutrient broth. W= soaked 12h in water; RT: Retting time SR: Softening rate; TIS: Time to initiate softening. Mean ± sd = Mean values ± standard deviation of error of means of three experiments. The means in the same column followed with different letters are significantly different at (P <.05)

Table 6. Cyanide levels of peeled root and fermented cassava flour obtained after retting using different CRA-96 pre-treatments and initial pH of the retting medium

Peeled root/Different CRA pre-treatments	Cyanide levels (ppm)	cyanide levels reduction %	RT (h)	Initial pH of the retting medium
Peeled root	655±0.9^{g}	0	0	-
Spontaneous retting	52.3±0.0^{c}	92.0	54.9±9.3^{a}	6.3
CRA-96ThA	110.7±0.0^{f}	83.1	52.5±9.6^{a}	5.2
CRA-96ChA	90.4±0.0^{d}	86.2	30.3± 2.8^{c}	5.2
CRA-96	7.8±0.0^{a}	98.8	36.5±2.8^{b}	5.3
CRA-96W	92.7±0.0^{e}	85.8	25.7±1.9^{c}	4.8
CRA-96NB	36.3±0.0^{b}	94.4	27.7±2.7^{c}	5.0

CRA-96: ThA= thermal activation in water at 80°C fo r 10 min; ChA = Chemical activation using ethanol 60%. BN= Pre-culture in nutrient broth. W= Soaked 12h in water; RT: Retting time Mean ± sd = Mean values ± standard deviation of error of means of three experiments. The means in the same column followed with different letters are significantly different at (P <.05)

a low pH that also lowers the initial pH of the cassava fermentation batch. This may result in the reduction of linamarase activity obtains by cassava cell disintegration or by microbial growth. Ogbonnaya and Oyebuchi, [25] observed between pH 4 and 5 more than 60% of linamarase relative activity is expressed with a maximum at around pH 4.5. The fact that the initial pH does not have a direct impact on the level of cyanide reduction suggests that the endogen enzyme activity alone is not enough for the retting and the action of each group of the microorganism with their enzymatic diversity are fundamental. Although, the cyanogens residues obtained in the cassava flour were higher than the limit of 10 ppm propose by WHO in all cases except for none pre-treated CRA-96. Agbor and Mbome [26] demonstrated that steam cooking could further reduce these values to about 12% of the dry material concentration.

4. CONCLUSION

This study has shown that the use of dry fermented cassava chipsat 1% (w/v) can contribute in the acceleration of the retting time of cassava. This acceleration can also provide up to 98.8% of cyanogens content reduction as compare to the fresh root and about 85% when compared to the traditional spontaneous fermentation. These results can boots cassava based foods production as this CRA can be easily implemented compared to the management of pure strains based starters.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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