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Cassava Retting: Optimisation of a Traditional Fermentation by an Experimental Research Methodology

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Abstract: Retting, a spontaneous and traditional fermentation of cassava roots in Central Africa, was optimised in terms of time and the quality of the end-product. Optimal conditions were achieved by using an experimental research methodology. Temperature is the most influential factor, with an optimum of 34°C for quicker retting. The roots should be peeled and soaked in water immediately after harvesting to increase the quality of cassava foods. Inoculation of water with juice from a prior retting helps in cassava detoxification but has no influence on the time or the quality of foo-foo. Foo-foo samples had the most favourable organoleptic quality when an incubation temperature of between 28 and 37°C was used. Using optimal conditions, retting time was reduced 3-fold, and foo-foo of high and constant quality could be processed.

Key words: cassava, fermentation, foo-foo, optimisation.

INTRODUCTION

Retting is a traditional fermentation of cassava that is common throughout Central Africa. This major step in the preparation of most indigenous cassava-based foods is performed to soften the roots, yield specific flavour by the production of organic acids and decreasing the pH, and degrade the endogenous cyanogenic compounds (Ogunsa 1980; Ayernor 1985).

The two main products associated with fermented cassava (*Manihot esculenta*, Crantz) are foo-foo and chikwangue. The former is a flour obtained from crushed, sun-dried cassava mash. This flour may be mixed with boiling water, and served with sauce and fish or meat. Chikwangue (cassava bread) is obtained after multiple post-retting steps, including defibring, pugging, kneading and several cooking steps. There are significant differences in retting processes throughout Central Africa making the quality and taste of cassava foods somewhat variable (Trèche and Massamba 1989). Several varieties of cassava can be used, peeled or unpeeled roots are retted in rivers, standing water, large barrels of water or even buried in the soil. The fermentation temperature varies with the season and the location. Cassava roots may be stored for a few days before fermentation (Trèche pers comm).

Several of these factors have been shown to influence the retting process. Peeling the roots before retting decreases the amount of cyanide in the mash (El Tinay *et al* 1984), and increases the end-product yield (Ayernor 1985). Furthermore, small pieces of cassava allow retting to be completed more quickly than when whole roots are used (Okafor *et al* 1984), and the quality of retting water can be improved by using barrels (Regez *et al* 1987). However, in these studies, factors were considered apart from one another and, to our knowledge, no general and systematic study on retting has yet been reported

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An experimental research methodology has been used with success to study the influence of a great number of factors on a single phenomenon with a reduced number of experiments (Sergent *et al* 1985; Dumenil *et al* 1988; Lamothe *et al* 1988; Kemp *et al* 1989). In this study, an experimental research methodology was used to study cassava retting with a view to optimising the process in terms of product quality and fermentation time. The influence of several factors on cassava retting could be defined taking the possible interactions into account. Recommendations on the conditions to give a better and safer product could then be given to small cassava-processing units in urban areas.

EXPERIMENTAL

Methodology

A two-step design method was designed and optimal conditions for retting were defined.

The first step was designed to evaluate the influence of the following factors on traditional retting:

(1) *Retting temperature*—three different temperatures (24, 28 and 32°C) were used.

(2) *Inoculation of the water*—rettings were performed either in inoculum-free water or with a 10% (v/v) inoculum. Inoculum was prepared as follows: a 50 litre barrel was filled with a mixture (1 : 1) of peeled roots from Ngansa and Mpembé varieties, topped up with well water to 50 litres and left at ambient temperature until retting was completed. The juice was collected and six rettings of the experimental design were immediately inoculated each with 2 litres of this juice.

(3) *Cassava varieties*—two varieties, Mpembé and MM86 (Ngansa), were tested. They were chosen because of their commercial significance: the former is widely spread throughout the Congo and the latter, selected by agrofood companies, is the main cultivar used for the industrial production of foo-foo and chikwangue in the Congo. Both have a high cyanide content (total cyanide content in the fresh mash without peel between 200 and 400 mg kg^{-1}), and can therefore serve as a good model to follow detoxification. Fields of both varieties in the Brazzaville area were harvested 18 months after planting.

(4) *Post-harvest storage*—roots were soaked in water either immediately after harvesting or after 48 h storage at ambient temperature in dry and dark conditions.

(5) *Peeling*—roots were peeled either before or after retting.

(6) *Root size*—two sizes of roots were tested: one of a circumference below 17 cm (small) and the other over 22 cm (large).

These conditions—five factors with two possibilities and one with three—allowed $(3 \times (2)^5) = 96$ possible experiments. An experimental matrix was built using Nemrod software (Mathieu and Phan-Tan-Luu 1980) with the intention that 10–16 experiments might be performed. The resulting 12 × 6 matrix (Table 1) gave the conditions for 12 experiments. The efficiency coefficient (G) was 87.5%. Retting time and product quality were then optimised.

For each retting, roots were washed, peeled when needed, and 10 kg of 5 cm slices were steeped in well

TABLE 1
Experimental matrix for first step optimisation

Experiment number	*Level of each factor*[a]					
	Temperature	*Inoculation*	*Variety*	*Storage*	*Peeling*	*Root size*
I	1	1	1	1	1	1
II	1	1	2	1	2	2
III	3	1	1	1	1	2
IV	2	1	1	2	2	2
V	2	1	2	2	1	1
VI	3	1	2	2	2	1
VII	2	2	1	1	2	1
VIII	3	2	2	1	2	1
IX	2	2	2	1	1	2
X	1	2	1	2	1	1
XI	3	2	1	2	1	2
XII	1	2	2	2	2	2

[a] Factors studied were temperature (1, 24°C; 2, 28°C; 3, 32°C); inoculum (1, without, 2, with use of an inoculum); variety (1, Ngansa; 2, Mpembé); storage (1, no storage; 2, 48 h storage); peeling (1, before retting; 2, after retting); and root size (1, circumference < 17 cm; 2, circumference > 22 cm).

water; the volume was adjusted to 20 litres. Roots retted unpeeled were peeled after retting and the mash was used for any further assay.

The second step was designed to study in detail the influence of temperature in a range from 26 to 45°C. Other factors were kept at their optimal level according to the first step of optimisation.

Analytical methods

Penetometry index

Penetrometry was used as an indicator of root softening during retting. A previous study showed that a penetrometry index of 15 mm s^{-1} corresponded to the end of a retting as it is traditionally evaluated (Brauman *et al* 1991). A penetrometer (PNR 10-SUR, Berlin, Germany) was used to measure the consistency of the roots. For each experiment, six root sections were randomly chosen every 10 h. Penetrometry depth was estimated with six repetitions for each root section. Retting time was defined as the time for the penetrometry index to be equal to 15 mm s^{-1}, and retting velocity as the inverse of retting time (expressed in h^{-1}).

pH of retting juice

A 50 ml retting juice sample was tested for pH (measured with CG 838 pH-metre from Schott, Geräte, Germany).

Microbial population

Lactic acid bacteria (LAB) have been shown to be the predominant microorganisms from the second day of retting until the end (Brauman *et al* 1991). LAB were, therefore, enumerated as an indicator of total microflora. Sampling was carried out at $t = 0$, 48 h and at the end of retting by random selection of six root sections cut into 0·5 cm cubes and mixed. A 60 g sample was diluted in 540 ml of sterile peptonised water. The suspension was then mixed in a Blender (Turnmix ME 88, Sofraca, France), and 0.1 ml of decimal solutions (dilutions ranged from 10^{-4} to 10^{-10}) were spread in triplicate on MRS medium (De Man *et al* 1960). Colonies were counted after 48 h incubation at 30°C.

Cyanide content

Total cyanides in the mash were assayed using Cooke's method (Cooke 1978) to check for proper detoxification of the roots. Assays were performed in duplicate at $t = 0$, 48 h and at the end of retting.

pH and acidity of the roots

Distilled water (120 ml) was added and mixed with 20 g of the sample in a Blender (Waring Products Division) for 15 s at low speed and 1 min at high speed. The mixture was then filtered in a GF/A filter and the volume was made up to 200 ml with distilled water. Extraction was performed in duplicate initially, after 48 h and at the end of retting. The pH of the roots was measured in the mixture and acidity was titrated with 0·01 M NaOH.

Organic compounds

Volatile fatty acids (VFA), lactate and ethanol concentrations in the roots were assayed by HPLC (LDC Analytical) as previously described (Giraud *et al* 1992).

Organoleptic tests

Foo-foo was chosen for organoleptic testing because only a few post-fermentation steps are required for its preparation. Foo-foo was prepared by drying retted cassava from each experiment at 45°C for 72 h. These conditions were similar to that of traditional sun-drying. The cassava was then crushed with an electric grinder (type BB3, Roucaire, Velizy, France) to make flour. Foo-foo was prepared by incorporating 100 g of flour into boiling water and homogenised.

Sensory analysis was performed on flour and foo-foo using 12 trained panellists. Appearance and aroma were evaluated in duplicate for flour and foo-foo. Foo-foo was evaluated in duplicate with respect to flavour, aroma, texture and appearance. Notation ranged from 1 (very bad) to 7 (very good). Data were analysed with Statitcf software.

RESULTS AND DISCUSSION

Experimental design

Retting time, organoleptic qualities and total cyanide content of the 12 foo-foo samples prepared according to the constraints shown by the experimental matrix (Table 1) were evaluated (Table 2). The effects of the six factors (root size, peeling, storage, variety, inoculation and temperature) on the three responses were computed (Fig 1).

Retting time (Fig 1a)

Retting times ranged from 25 to 200 h depending upon the experiments (Table 2). Temperature had a very strong effect on retting time. At 32°C, the retting time was considerably diminished. At this temperature mesophilic fermentative bacteria, such as LAB and clostridia that have been shown to be involved in retting (Brauman *et al* 1991) reach their maximal concentration. Also the enzyme pectinesterase, which is partly responsible for cassava cell wall disruption (Ampe *et al*

TABLE 2
Retting time, foo-foo quality and cyanide content for the 12 experiments

Experiment number	*Retting time*[a] *(h)*	*Foo-foo quality*[b]	*[CN] mg kg*$^{-1}$
I	74	4·8	7·5
II	74	4·0	4·4
III	28	6·1	6·9
IV	55	3·7	6·9
V	42	5·7	5·8
VI	40	3·0	5·2
VII	60	5·7	3·2
VIII	25	4·2	2·8
IX	28	5·8	2·7
X	200[c]	3·2	2·6
XI	28	5·2	2·4
XII	80	3·0	3·6

[a] Time needed to reach a penetrometry index of 15 mm s^{-1}.
[b] Evaluated on a scale ranging from 1 (very bad) to 7 (very good).
[c] Eliminated data.

1991; Oyewele and Odunfa 1992), has its optimal activity at this temperature (Dahodwala *et al* 1974; Baron *et al* 1980; Oteng-Gyank and Anuonye 1987; Sakellaris and Evangelopoulos 1989; Tsuyumu *et al* 1989).

Inoculation did not diminish the retting time. The expected 'starter effect' might have been balanced by a partial inhibition of some enzymic reactions due to a large pH decrease of the retting water at the beginning of fermentation. The initial pH of the retting juice for inoculated media was between 4 and 5 (versus 6·5 and 7 in inoculum-free fermentations).

Contrary to what was suggested by Okafor *et al* (1984), tuber size had no significant effect on retting time. Storage time also had no effect.

Others factors such as peeling, cassava variety and storage appeared to have little or no influence on retting time (Fig 1a).

Organoleptic qualities of foo-foo (Fig 1b)

The most influential factors effecting the organoleptic qualities of foo-foo were temperature, storage and peeling.

As seen previously for retting time, temperature had a strong effect on foo-foo taste, flavour and aspect. The optimal temperature was 28°C, followed by 32°C. These temperatures may enhance the production of organic compounds that are appreciated by local consumers.

Peeling cassava roots immediately after harvesting before soaking them in water also greatly enhanced foo-foo quality. The adverse effect of storing cassava roots could be linked to the synthesis of antinutritional compounds such as polyphenols and tannins (Swain 1979; Rickard 1986). Moreover, foo-foo prepared with roots peeled after retting was light brown whereas peeled roots gave white foo-foo. The dark coloration was probably due to the presence of tannins or phenolic compounds in cassava peels (Rickard 1986). Panelists prefered white foo-foo, so peeling of roots before retting is preferable.

Other factors (root size, variety and inoculum) had little or no influence on foo-foo quality.

Cassava detoxification (Fig 1c)

All the experimental conditions studied produced flours that had a total cyanide content below the limit of tolerance (10 mg kg^{-1}) (Table 2). As the flours were soaked in boiling water to prepare foo-foo, we assumed that free CN^- evaporated to yield a final cyanide content that was even lower in the products that were eaten. A previous study (Ayernor 1985) concluded that peeling helps the degradation of cyanide compounds; however the present work clearly shows that peeling had no significant influence on the detoxification process (Fig 1a). Roots peeled before or after retting were all detoxified to an extent greater than 90% (initial cyanide content of

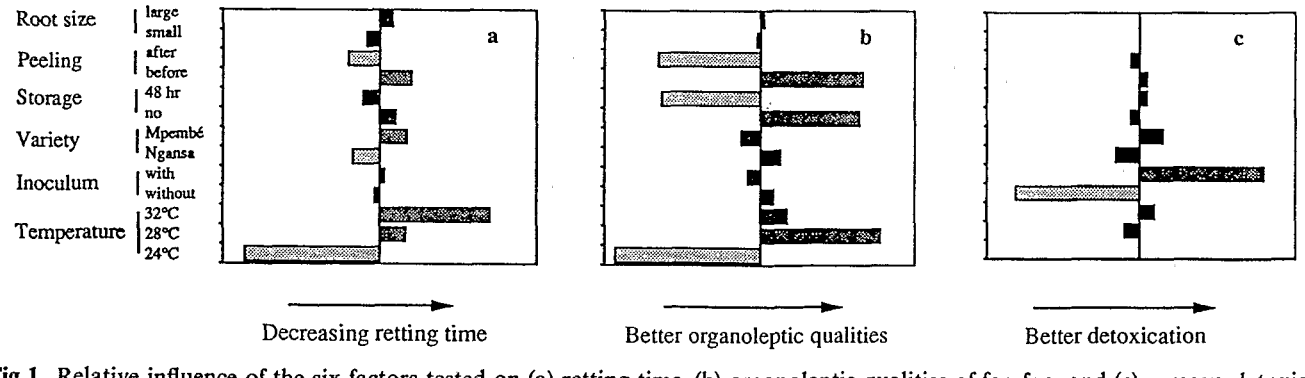

Fig 1. Relative influence of the six factors tested on (a) retting time, (b) organoleptic qualities of foo-foo, and (c) cassava detoxication. ▒, positive influence; ▒, negative influence; ▒, no significant influence. The sizes of the bars are proportional to the effect of each factor at the level on the response: a significant and positive influence is represented by a large bar on the right of the histogram. These histograms were obtained by computing the raw data from Table 2 with Nemrod software. Mathieu and Phan-Tan-Luu (1980).

cassava mash—without peel—for Mpembé and Ngansa varieties were 230 and 303 mg kg^{-1}, respectively), and no difference between roots peeled before or after retting could be found by statistical treatment. Previous results have shown that endogeneous linamarase found in cassava mash—the peeled part of the roots—is the major enzyme responsible for detoxification (Brauman and Ampe 1992). Therefore, enzymes and microflora of the peel do not interfere with the action of this endogenous linamarase.

However, we showed that the use of an inoculum significantly decreased the total cyanide content. The native linamarase activity of lactic bacteria (Okafor and Ejiofor 1985; Giraud *et al* 1992) might, in the case of a massive inoculation, supplement the activity of the endogenous enzyme in cassava detoxification. Moreover, after the addition of an inoculum, the retting pH was close the optimal pH (5·5–6) of endogenous linamarase (Cooke *et al* 1978).

Effects of the factors on physico-chemistry, biochemistry and microbiology of retting

Inoculation

Using an inoculum considerably decreased the variability associated with spontaneous fermentations: LAB in the retted mash represented between 8·6 and 8·85 log (cfu) in inoculated samples and between 6 and 9 log (cfu) in samples without an inoculum (data not shown). Inoculation with water from a previous fermentation may help in the standardisation of the fermentation. Inoculation allowed better control of the microflora, and the drastic pH decrease (4·5 in the inoculated retting compared to 6·5 in the uninoculated one) might help to prevent pathogenic microorganisms from invading the retting media.

Production of lactate (Table 3), which is the main organic compound of retting, was higher when an inoculum was used. The lactate concentration reached 59·7–240·6 mM in inoculated rettings (experiments VII–XII) compared with 3·5 and 35·9 mM in uninoculated ones (experiments I–VI). However, the acetate and ethanol concentrations were not significantly different. Inoculation seems to stimulate homo-fermentative transformation of sugars by lactic bacteria. On the other hand, butyrate slightly decreased, the clostridia responsible for this VFA production (Brauman *et al* 1991) being supplanted by LAB. Surprisingly, butyrate seemed to be an important VFA for foo-foo quality. However, no conclusive correlation could be established between VFA content of the mash and foo-foo quality.

Further experiments (data not shown) showed that an inoculum in excess (over 1 : 1 (v/v)) from a previous fermentation, resulted in incomplete retting. The pH was probably too low for the retting microflora to develop.

Storage

Storage of roots prior to retting slightly increased the production of lactate as well as that of ethanol during retting (experiments IV–VI and X–XII). This could explain partially the decrease in acceptability of foo-foo.

Other factors

The organic acid contents of fresh cassava roots from the two varieties were not significantly different. Peeling cassava roots before retting decreased acetate production and root size had no significant influence on the examined factors.

Determination of optimal temperature

The effect of temperature both on retting time and on the organoleptic qualities of foo-foo, was significant and

TABLE 3

VFA, lactate and ethanol content (mM) of cassava mash at the end of each of the 12 retting

Experiment number	*Lactate*	*Acetate*	*Propionate*	*Isobutyrate*	*Butyrate*	*Isovalerate*	*Valerate*	*Ethanol*
I	10·6	8·3	1·1	2·4	13·1	3·	0	33·9
II	3·5	13	5·6	4·3	16·1	1·9	0	29·1
III	6·2	10·2	0·8	5·1	20	3	0	24·1
IV	39	19·7	3·3	1·9	16·7	0·8	0	53·6
V	20·5	11·5	4·9	2·4	10·1	0	0	28·7
VI	35·9	21·1	14·3	9·1	16·5	1·6	0	59·9
VII	152·4	11·5	2·8	1·4	9·9	0·3	0·2	39·7
VIII	137·4	12	11·8	6·2	17·1	0·9	0	33·4
IX	59·7	9·8	3·8	0	5	0	0	27·8
X	152·1	6·5	0·1	0·8	2·5	0	0·1	24·7
XI	147·6	12·8	1·3	2·6	8·8	0·4	0	39·5
XII	240·6	24·6	9·8	4·4	3·2	0·3	0	37·3

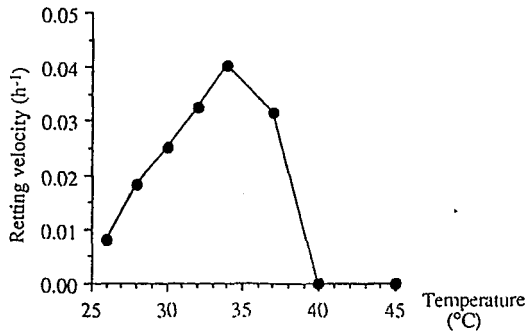

Fig 2. Influence of temperature on retting velocity.

needs further exploration. There are no published data on the influence of temperature on cassava fermentation. Consequently, this factor was studied in detail, the other factors being kept at their optimal levels. Mpembé roots were peeled, soaked in water immediately after harvesting, large or small roots were used, no inoculum was added.

The influence of temperature on retting time and the organoleptic qualities of foo-foo was studied in the 26–45°C range (Fig 2). High retting velocities were obtained in the range 32–37°C, with a peak at 34°C at which temperature retting was completed in 24 h. A temperature of 40°C was found to be the upper limit for retting to occur. This profile closely matches that of mesophilic bacterial growth.

Multiple comparison revealed that the qualities of the six foo-foo were not significantly different (however, no foo-foo could be prepared from experiments at 40 and 45°C as no softening was observed for the roots). All foo-foo were evaluated to be 'good' to 'very good' by the panellists. This corroborates the results obtained for the first optimisation step.

Total cyanide content was below 10 mg kg^{-1} for all foo-foo. This confirms that detoxification was not a limiting factor for retting optimisation.

As previously found, lactate, acetate, ethanol and butyrate were produced (Table 4). The butyrate content increased slightly between 32 and 37°C, indicating that this range of temperature was more suitable for butyrate producers such as *Clostridium butyricum* previously found in the retting juice (Miambi E, pers comm).

TABLE 4
Organic compounds production during retting as a function of temperature

Temperature (°C)	*Lactate* (mM)	*Acetate* (mM)	*Butyrate* (mM)
26	3·0	2·3	0
28	9·1	3·5	7·3
30	13	2·8	4·3
32	6·4	2·8	9·9
34	9·9	2·7	11·9
37	8·7	6	9·9

CONCLUSIONS

Optimisation showed that retting should be performed with freshly harvested and peeled roots for a better taste and in a range of temperature going from 32 to 37°C for a quicker process. Detoxification was not a limiting factor as the final cyanide content was always below 10 mg kg^{-1}. A scaling up of these results will be carried out and technological solutions for their application will be studied.

The study of the influence of inoculation with retting juice showed that no microbiological starter is required to decrease the retting time or to improve the organoleptic qualities of foo-foo. However, inoculation allows a better detoxification and control of the microflora.

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