Improvement of organoleptic quality of retted cassava products by alkali pretreatment of roots and addition of sodium nitrate during retting

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Abstract

Alkali pretreatment of cassava roots before retting and addition of sodium nitrate during retting were used to manipulate the metabolism of microorganisms involved in cassava (Manihot esculenta Crantz) retting, as a method for removing the characteristic offensive odour of retted cassava products. Odour was assessed by organoleptic methods. The characteristics of fermentation of cassava by the traditional method (control) were as follows; aerobic mesophilic count (APC) on nutrient agar (NA) at 30 °C/48 h, attained a maximum of $2.3 \times 10^7$/ml retting juice while counts on de Man Rogosa and Sharpe agar (MRS) at 30 °C/48 h were $1.6 \times 10^8$/ml. Maximum titrable acidity was 0.062% lactic acid by weight of retting juice. Cassava was retted in 3 days and the product exhibited characteristic offensive odour. Addition of NaNO3 into retting water effectively removed odour at a concentration of 0.3 g/l. Maximum APC on NA/30 °C/48 h was $6.8 \times 10^6$/ml. Counts on MRS/30 °C/48 h exceeded $2.4 \times 10^9$/ml. Retting was complete in 3 days with a final titrable acidity of 0.068% of retting juice. Removal of odour likely resulted from selection of homo-fermentative lactic acid bacteria, thus producing mostly odourless lactic acid. Alkali pretreatment of roots before retting was efficacious in removing odour at a concentration of 10 g/l for 30 min. This fermentation was characterized by APC on NA/30 °C/48 h of $5.4 \times 10^6$/ml; MRS/30 °C/48 h reached a maximum of only $10 \times 10^4$/ml and correspondingly low titrable acidity of 0.003%. Low counts of lactic acid bacteria correlate well with the absence of odour in this sample. Both treatments did not adversely affect the detoxification process, yielding “foo-foo” with HCN levels lower than 10 mg/kg. Residual nitrates and nitrites of 30 mg/kg in the sodium nitrate-treated sample were also within the safe limits of 156 mg/kg allowed in many countries. Organoleptically improved samples were acceptable to the public.

Keywords: Alkali; Nitrate treatment; Retting cassava; Removal; Offensive “foo-foo” odour

1. Introduction

A major problem with the utilization of cassava roots (Manihot esculenta Crantz) is the presence of toxic cyanogenic glycosides. The processing of cassava must therefore employ elaborate and sometimes expensive steps in order to detoxify products to levels safe for human consumption. The most popular method of utilizing cassava in Nigeria, West Africa is by processing it into garri, a partially gelatinized...
granular cassava meal with a pleasant aroma. For 4 to 5 days, several processing steps and expensive mechanical equipment are required to process cassava into garri. As the consumption of cassava increases, pressure on processing facilities and increased processing costs has forced some processors into using shortened procedures. This has increased the risk of consumption of improperly processed products and, correspondingly, has increased the possibility of health hazards.

The need has therefore arisen for the improvement of other safe traditional processing methods with lower processing costs. Cassava retting is one of these methods, which simply involves steeping of the roots in water for 3 to 4 days. During the period, the roots soften (rett). Gram-positive bacterial rods (GPR) including species of *Bacillus*, *Corynebacterium* and *Clostridium*, some of which produce pectinesterase, play a role in this (Okafor et al., 1984; Oyewole and Odunfa, 1992; Brauman et al., 1996). Retting is accompanied by a spontaneous lactic acid fermentation which produces organic acids and other volatiles that confer a characteristic odour (mainly offensive odour) on the product (Okafor et al., 1984; Kimaryo et al., 2000). Retted cassava is popular in Nigeria, and is known as “foo-foo” (Okafor et al., 1984), in Zaire as “Chikwuangue” (Jones, 1959) and in Tanzania as “Kivunde” (Kimaryo et al., 2000). Processing cassava by this method is cheap, not needing mechanical equipment and achieves a high level of detoxification. After preparation, “foo-foo” also has a pleasant smoothness and consistency, which is absent in garri. The major disadvantage of this method is that its products have a characteristic offensive odour, as has been noted by Okpokiri et al. (1984) and Kimaryo et al. (2000). This attribute has severely limited the acceptance of this food in urban societies.

Efforts are already being made towards the production of “Kivunde” with a fruity aroma using a *Lactobacillus plantarum* starter confirms the possibility of using this method for the retting of cassava. The present work was carried out to reduce the offensive odour of retted cassava by manipulating the metabolism of microbes naturally involved in its fermentation. Various pretreatments of roots and additives to retting water were also used.

2. Materials and methods

2.1. Cassava samples and retting into “foo-foo”

Fifteen-month-old cassava roots of the Nwugo variety were used. Roots were peeled and cut into cylinders of approximately 10-cm length × 5-cm diameter. The pieces were washed. Samples for each set of experiments were drawn from a single pool of washed pieces. Retting was performed by completely submerging approximately 2 kg of the cassava pieces in 2 l of tap water at 30 °C until retted or for a maximum of 7 days. Retted roots were mashed, sieved and dewatered as described by Okafor et al. (1984). All experiments were replicated thrice.

2.2. Preliminary experiments

Two approaches were used. The first approach was to try and eliminate the probable occurrence of the stringent response phenomenon (cassava is poor in nutrients), which may encourage the production of unusual odourous substances. The following additives were employed during retting as described in Section 2.1; 2 g/l Lab lemco powder (Oxoid, London), 2 g/l NaNO3 (May & Baker, Dagenham, England), 1 g/l Na2SO4 (M & B), 1 g/l glucose (Sigma, USA) and 2 g/l dry soya bean, waste powder.

The second approach was to selectively inhibit different groups of microorganisms known to be involved in the fermentation of cassava. This was done by addition of 30 g/l NaCl (M & B), 1 g/l NaNO2 (M & B) and pretreatment of peeled cassava roots in 25 g/l NaOH (M & B) for 30 min.

The odour of the retted cassava samples from the various treatments was compared organoleptically with control samples by a 20-member panel
using the triangle test (Watts et al., 1989). Reduction of offensive odour was determined by reference to probability tables for the one-tailed binomial test at $P < 0.05$.

Two treatments, i.e. the addition of NaNO$_3$ and alkali pretreatment, gave favourable results and were thus investigated further.

### 2.3. Minimum effective concentrations of NaOH for alkali pretreatment and NaNO$_3$ as additive

For alkali pretreatment, roots were submerged in 0, 5, 10 and 15 g/l solutions of NaOH for 30 min. They were then washed clean of NaOH by repeated rinses in fresh tap water and retted. Sodium nitrate was added to retting water at the rate of 0, 0.2, 0.3, 0.4 and 0.5 g/l, respectively.

### 2.4. Analytical methods

#### 2.4.1. Determination of retting of cassava pieces

This was done by hand feel.

#### 2.4.2. Organoleptic analyses

To determine the odour of cassava “foo-foo” samples, trained panelists were asked to score the intensity of offensive odour in coded samples using a 15-cm line scale (Watts et al., 1989). The ends of the scale were labeled “odorless” and “odoriferous”, respectively. Panelists’ marks were converted to numerical scores by measuring distance in centimeters and equating 0.5 cm to a 1-unit score. The numerical scores were tabulated and analysed by two-way analysis of variance (ANOVA). Tukey’s multiple comparison test was used to determine the difference between the samples at $P \leq 0.01$.

Acceptance of organoleptically improved “foo-foo” was determined by separately cooking and preparing each sample for consumption as described by Okafor et al. (1984). Coded samples of “foo-foo” were served to an untrained panel of 40 members drawn randomly from staff and students of the Faculty of Agriculture, Ebonyi State University Abakaliki. Panelists were asked to rank the samples from 1 to 3 in order of most acceptable to least acceptable quality based on their odour, colour and textural qualities. Data were analysed using the Friedman test (Watts et al., 1989).

#### 2.4.3. Effect of treatments on population of microorganisms and organic acid production

The population of microorganisms in retting juice was determined daily on de Man Rogosa and Sharpe agar (MRS) (Difco Laboratories, Detroit, MI, USA) and nutrient agar (NA) (Oxoid). Inoculated MRS plates were overlaid with approximately 20 ml of agar. All plates were then incubated aerobically at 30 °C and colonies counted after 48 h. Production of organic acids was determined by the measurement of titrable acidity as percent lactic acid by weight (AOAC, 1990) and the pH of the retting juice (Corning pH meter 220).

#### 2.4.4. Safety of organoleptically improved cassava “foo-foo”

Total cyanides in “foo-foo” sample were analysed by Cooke’s method (Cooke, 1978). To determine residual nitrates, and nitrite total nitrogen was determined twice, first by the routine macro-Kjeldhal method (Pearson, 1976) and then by the modified method. This enabled determination of nitrates and nitrites additionally. This modification comprised the addition of 25-ml ice-cold conc. H$_2$SO$_4$ and 1-g salicylic acid, after which the flask was shaken at intervals for 10 min. Subsequently, 8 g of Na$_2$SO$_4$ and 5 g Na$_2$S$_2$O$_4$ were added. Determination of nitrogen by macro-Kjeldahl method was then repeated (Pearson, 1976). Residual nitrate and nitrite levels were obtained by calculating the difference between the two determinations.

### 3. Results and discussion

Two treatments, i.e. the use of NaNO$_3$ as an additive and alkali pretreatment, yielded retted cassava with less offensive odour than the control. The failure of Lab lemco powder, soy waste and glucose additives to influence odour production indicated the absence of a role for nutritional stress. NaCl and NaNO$_2$ prevented retting, probably by inhibition of growth of GPR, which plays a vital role in the initiation of retting and acid production (Table 1).

Statistical analysis of scores obtained from organoleptic tests on odour of “foo-foo” samples retted in the presence of NaNO$_3$ showed that these were significantly different from the odour of the control
sample (treatment $F_{\text{cal}} 4221.44>F_{\text{tab}} 2.51$). Multiple comparison of treatment means obtained with different concentrations also revealed that 0.3 g/l NaNO₃ was the minimum efficacious concentration for producing “foo-foo” without the offensive odour.

The addition of NaNO₃ to retting cassava probably stimulated nitrate reductase activity which is widely distributed in bacteria. This elicited the initial rapid microbial growth observed during the first day of fermentation. The APC of retting juice (NA/30 °C) was $6.9 \times 10^6$ cfu/ml as compared with $9 \times 10^5$ cfu/ml for the control. However, the reduction of nitrates usually results in the accumulation of nitrites. This soon became inhibitory for much of the bacterial flora, which consequently declined in population (Fig. 1).

On the other hand, growth and acid production by lactobacilli, which are resistant to nitrites (Castellani and Niven, 1995), were stimulated by the absence of competition and the favourable pH conditions created (Fig. 2 and Table 2). These changes in the characteristics of the fermentation likely removed the offensive odour in two ways. First, by rapid selection of nitrite-resistant homo-fermentative LAB and, second, by production of mainly lactic acid which is odourless. This may have been complemented by a mechanism that has been reported in cured meats. According to Hammes and Knauf (1994), nitrates and nitrites can prevent the formation of off flavours from compounds such as formate by their inhibition of pyruvate formate lyase activity in lactobacilli.

There is a risk in the use of nitrates and nitrites in foods (Buckenhuskes, 1997). However, this risk is not expected to be significant in organoleptically im-

Table 1
Screening of various additives and pretreatments for effect on retting and offensive odour reduction

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Retting time (days)</th>
<th>Odour (probability of correct judgements in 20 trials, $P \leq 0.05$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Addition to retting water of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 g/l Lab Lemco Powder</td>
<td>3</td>
<td>0.848</td>
</tr>
<tr>
<td>2 g/l dry soy waste</td>
<td>3</td>
<td>0.940</td>
</tr>
<tr>
<td>1 g/l glucose</td>
<td>3</td>
<td>0.521</td>
</tr>
<tr>
<td>1 g/l NaNO₃</td>
<td>3</td>
<td>0.004</td>
</tr>
<tr>
<td>1 g/l Na₂SO₄</td>
<td>3</td>
<td>8.848</td>
</tr>
<tr>
<td>30 g/l NaCl</td>
<td>not retted</td>
<td>–</td>
</tr>
<tr>
<td>1 g/l NaNO₂</td>
<td>not retted</td>
<td>–</td>
</tr>
</tbody>
</table>

| Pretreatment of roots in:                |                     |                                                               |
| 25 g/l NaOH for 30 min                   | 4                   | 0.001                                                         |

Fig. 1. Effect of alkali (10 g/l NaOH for 30 min) and NaNO₃ (0.3 g/l) treatments on aerobic mesophilic plate counts of microorganisms in retting juice (nutrient agar/30 °C/48 h).
proved “foo-foo”. Concentrations of nitrates and nitrites in the finished product of approximately 30 mg/kg (Table 3) fall well below the statutory maximum of 156 mg/kg nitrites allowed in many countries. A major factor responsible for this would be dilution and leaching away of these substances during the subsequent steps of retted cassava processing, which involves sieving, repeated washing and dewatering. Significant levels of nitrosoamines are also not expected to accumulate within the short 3-day fermentation.

Statistical analysis of scores obtained from organoleptic tests on alkali-treated samples shows these to be significantly different from the control (treatment $F_{cal} > F_{tab}$, with a minimum efficacious concentration of 10 g/l NaOH for 30 min.

Alkali treatment may alter the pH and cause loss of nutrients from the material treated (Buckenhuskes, 1997). During this study, initial pH of retting juice was elevated to 9 (Table 2). This initial unfavourable pH slowed down the rate of growth of all microorganisms in the juice, during the first 3 days (Fig. 1) and also delayed retting (4 days) in this sample (Table 1). Organisms growing on MRS agar, including LAB, were particularly inhibited. MRS counts on retting juice were $10^4$ cfu/ml after 4 days of fermentation compared with $1.6 \times 10^8$ cfu/ml in the control after 3 days (Fig. 2). Poor growth and acid production of LAB appear to correlate with the absence of offensive odours, confirming their assign-

Table 2
Influence of various treatments on the production of organic acids in retting juice

<table>
<thead>
<tr>
<th>Days of steeping</th>
<th>Control pH (%)</th>
<th>Control TA (%)</th>
<th>Treatment with 0.3 g/l NaNO$_3$ pH (%)</th>
<th>Treatment with 0.3 g/l NaNO$_3$ TA (%)</th>
<th>Treatment with 10 g/l NaOH for 30 min pH (%)</th>
<th>Treatment with 10 g/l NaOH for 30 min TA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6</td>
<td>6.1</td>
<td>6.1</td>
<td>0.001</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>5.5</td>
<td>5.2</td>
<td>5.2</td>
<td>0.012</td>
<td>6</td>
<td>0.001</td>
</tr>
<tr>
<td>2</td>
<td>4.5</td>
<td>4.2</td>
<td>4.2</td>
<td>0.033</td>
<td>5.8</td>
<td>0.002</td>
</tr>
<tr>
<td>3</td>
<td>4.1</td>
<td>3.8</td>
<td>3.8</td>
<td>0.068</td>
<td>5.2</td>
<td>0.002</td>
</tr>
<tr>
<td>4</td>
<td>3.9</td>
<td>3.9</td>
<td>3.9</td>
<td>0.062</td>
<td>4.6</td>
<td>0.003</td>
</tr>
</tbody>
</table>

TA = titrable acidity (percent lactic acid by weight).

Table 3
Influence of various treatments on safety of retted cassava

<table>
<thead>
<tr>
<th>Sample/treatment</th>
<th>Total cyanide (mg/kg)</th>
<th>Total nitrogen (mg/kg)</th>
<th>Residual NO$_3$ and NO$_2$ (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.4</td>
<td>3.536</td>
<td>–</td>
</tr>
<tr>
<td>Pretreatment of roots in 10 g/l NaOH for 30 min</td>
<td>5.8</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Addition to retting water of 0.3 g/l NaNO$_3$</td>
<td>9.4</td>
<td>3.566</td>
<td>30</td>
</tr>
</tbody>
</table>
ment (Okafor et al., 1984; Kimayo et al., 2000) with this role.

The level of detoxification of cyanogenic glycosides achieved in both samples (Table 3) falls below the tolerable limit of 10 mg/kg HCN. This indicates that both treatments did not adversely interfere with the detoxification process.

Both samples of organoleptically improved “foo-foo” did not show significant changes in colour and texture. Statistical analysis of results of acceptance tests showed that differences between rank total pairs were as follows: control and NaNO₃-treated samples, 52; control and alkali-treated samples, 32; NaNO₃ and alkali-treated samples, 20. The tabulated critical value of $p < 0.01$ for 40 panelists and 3 samples is 27. These results show that the improved samples were not only acceptable, but also preferred by most panelists for their odourlessness. An additional advantage is that nitrate-treated samples which attain higher acidity will be expected to exhibit a better shelf life than the control samples.

References


