

Effect of single bacterial starter culture on odour reduction during controlled fermentation of cassava tubers for foofoo production

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ABSTRACT

Effects of single bacterial starter culture on odour reduction during controlled fermentation of cassava tubers for foofoo production were investigated. Pure cultures were used to ferment cassava tubers in water for 96 h. The cultures used include *Bacillus subtilis*, *Klebsiella sp.*, *Lactobacillus plantarum* and *Leuconostoc mesenteroides*. *L. plantarum* exhibited the highest acid producing ability, decreasing the pH of the Cassava tubers from 6.2 to 3.68 with a corresponding increase in total titratable acidity (TTA) from 0.082% to 0.290% during the 96 h fermentation period. The effected changes in pH and TTA by other organisms ranged respectively from 4.88 and 0.135% for *Klebsiella sp.*, 4.68 and 0.136% for *L. mesenteroides* to 4.90 and 0.139% for *B. subtilis* with in the period. All the cultures were found to contribute in varying degree to odour reduction in fermented cassava; *B. subtilis* effected the highest odour reduction followed by *L. plantarum*.

Keywords: bacterial starter culture, odour reduction, fermented cassava, foofoo

INTRODUCTION

Cassava (*Manihot esculent crantz*) is a dicotyledonous plant which originated from Latin America and has been cultivated in other parts of the world. It is an important staple food crop for millions of people in the tropics. Cassava roots are normally processed before consumption. Various fermented cassava products are available, this include garri, foofoo, lafun etc. (Oyewole, 1992).

Fermentation of cassava for 'foofoo' production entails peeling, washing and soaking submerge of cassava tubers in water for 3 - 4 days. During this period, the retted cassava tubers are softened. The softened pulpy mass is then disintegrated in water and passed through a coarse sieve. This separates the fiber from starch which is allowed to sediment then the water is decanted. It is then packed into cloth bags and excess water is squeezed out. The resulted excess meal is white and crumbly which is usually cooked before being eaten (Ihekoronye and Ngoddy, 1985). Foofoo is reconstituted by stirring in boiling water to form a dough and eaten with flavoured sauces (Pelczar *et al.*, 1993). Cassava farming population have empirically developed several processing methods for stabilizing cassava and reducing its toxicity (Coffey *et al.*, 1991). The single problem associated with foofoo consumption is the high offensive odour associated with it. The products of the breakdown of cyanogenic glucoside give-off this offensive odour and at the same time reduced the compound to safe level by traditional method of processing and preparing cassava for consumption by fermentation (Okafor, 1998).

Many studies have been carried out on cassava fermentation for the production of foofoo. Information is not available on the effects of bacterial starter culture on odour reduction during cassava fermentation for foofoo acceptability. Such information is necessary for commercializing traditional food processing method.

MATERIALS AND METHODS

Media used

The following media were used for growth studies of pure cultures. Nutrient agar (NA) (Oxoid) (Difco, MI, USA) for *B. subtilis* and *Klebsiella sp.*, Demann Rogosa and sharpe (MRS) agar (Oxoid) (Difco, MI, USA) for *L. plantarum* and Garvies(1986) medium (Oxoid) (Difco, MI, USA) for *L. mesenteroides*.

Source of cassava

Cassava tubers of varieties SRP 20424 and SLP 31411 were obtained from University farm of the University of Calabar, Nigeria. The tubers were from 9 – 11 months old plants.

Preparation of tuber

Peeled cassava tubers were cut into uniform sized piece (12 mm diameter x 42 mm) with a cork borer. The tuber pieces were sterilized with 0.1% mercury chloride in 80% alcohol by the method of Oyewole(1990). One hundred and thirty gram of the sterilized tubers were steeped in

150 mL of sterile distilled water contained in a previously sterilized beaker which was covered with sterilized aluminium foil.

Preparation and inoculation of starter culture

Pure cultures of bacteria which had earlier been isolated from fermenting cassava and confirmed by molecular technique using G + C ratio for identification according to (Coffey *et al.*,1991,Zhong *et al.*,1998) were obtained from the Industrial Microbiology Laboratory of the University of Calabar, Calabar. These include *B. subtilis* (RN02), *Klebsiella* sp. (RN29), *L. plantarum* (RR 182) and *L. mesenteroides* (Q247). The strains were selected base on an earlier finding as reported by Oyewole (1990; 1992). Pure culture inocula were made from suspension of bacteria cells on NA (Oxoid), Garvies (1956) (Oxoid) medium and de Mann Rogosa and Sharpe (MRS) (Oxoid) agar slants appropriate following the method of Oyewole(1990). Ten ml of sterile peptone water were added to 18-24 hold cultures on slants and shaken to make a suspension. Dilution of cultures were made so that 1 mL of inoculums would produce a concentration of approximately $10^6 - 10^7$ cfu/mL using a pre-fixed absorbance read at 660 nm against sterile peptone water. Three ml portions of the respective suspension were used as inocula for 130 g of sterilized cassava tubers steeped in 150 mL of sterile distilled water. Uninoculated sterilized, cassava tubers similarly steeped served as control.

Analysis

The extent of fermentation and the effectiveness of each inoculum on odour reduction were analyzed with the following parameters.

pH

A twelve gram portion of the fermenting tubers was removed aseptically at different times (0, 12, 24, 36, 48, 60, 72, 84 and 96 h) and homogenized in 100 mL of sterile distilled water. The resulting suspension was decanted and its pH determined by a Kent pH meter (Kent Industries Measurement Ltd, Surrey, England) model 7020 equipped with a glass electrode.

Total Titratable Acidity (TTA)

Total titrated acidity expressed as percentage of lactic acid of the fermenting Cassava tubers was determined by titrating 25 mL of the decanted homogenate samples used for pH determination against 0.2 N NaOH to pH 8.42.

The TTA was calculated with the equation below:

$$\% \text{ acid } (\frac{wt}{vol.}) = \frac{N \times V_1 \times E_{gut}}{V_2 \times 1000} \times 100$$

Where:

- N = Normality of titrant (NaOH)
- V₁ = Volume of titrant (NaOH)

- E_{qwt} = Equivalent weight of acid (mg/mEq)
- V₂ = Volume of sample (mL)
- 1000 = factor relating mg to gram (mg/g)

Sensory evaluation

A 10-man trained panel to determine the acceptability of fermented cassava product 'foofoo' was set up. A 5-point hedonic rating on the degrees of acceptability was conducted with score '5' having excellent odour reduction and score '1' for very low odour reduction for the attribute.

Each of the product 'foofoo' was considered against a standard commercial 'foofoo', and was presented to the panelists' one at a time. The final scores represent the means of all panelist impressions.

Growth studies

Growth of the pure inoculants on cassava tubers was monitored during the 96 h fermentation period. Eight grams of respectively inoculated tubers were homogenized with 50 mL of sterile 0.1% peptone waters to form pulp. The pulp was serially diluted and plated using the spread plate method under aseptic conditions. Total viable counts were made on NA for *Klebsiella* sp. and *B. subtilis*, Garvies (1986) medium for *L. mesenteroide* and MRS agar incubated under anaerobic condition (BBL Gas Pak. H₂ and CO₂ anaerobic system, Bectone Dickinson) for *L. plantarum* inoculant. Plates were incubated at 30 °C for 24 h and 37 °C for *L. plantarum* plates.

RESULTS

Changes in the pH of fermented cassava tubers inoculated with the various pure cultures are presented in Figure 1 and the statistical data is presented on Table 1. *L. plantarum* affected a decrease in the pH from 6.2 to 3.68 after 96 h of fermentation *B. subtilis* affect an initial slight increase in pH which later decreased to 4.90 at the end of the fermentation. *Klebsiella* sp. and *L. mesenteroides* effected reductions to pH 4.88 and 4.68 respectively.

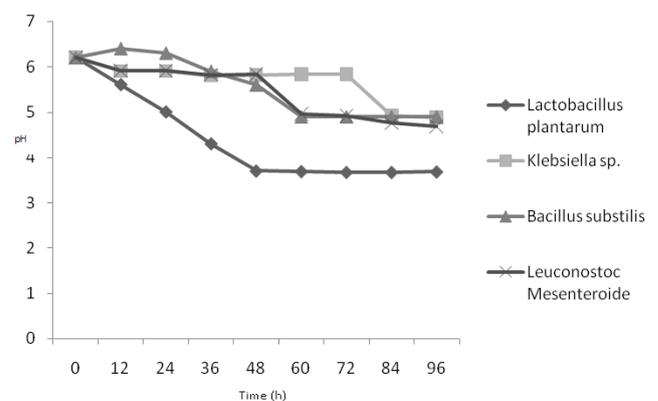


Figure 1: pH changes during fermentation with single pure cultures

Table 1: pH* changes during fermentation with single pure culture for different length of time (% lactic acid)

	Period of fermentation (h)									
	0	12	24	36	48	60	72	84	96	
<i>L. plantarum</i>	6.2	5.6	5.0	4.3	3.7	3.69	3.67	3.67	3.67	3.68
<i>L. mesenteroides</i>	6.2	5.9	5.9	5.82	5.83	4.95	4.92	4.75	4.75	4.68
<i>Klebsiella</i> sp.	6.2	5.9	5.9	5.8	5.81	5.83	5.83	4.92	4.92	4.88
<i>B. subtilis</i>	6.2	6.4	6.3	5.9	5.6	4.90	4.90	4.90	4.90	4.90

* Values are means of triplicate determination

Table 2: Total titratable acidity(%)* as lactic acid for different length of time (% lactic acid)

	Period of fermentation (h)									
	0	12	24	36	48	60	72	84	96	
<i>L. plantarum</i>	0.082	0.12	0.15	0.17	0.20	0.23	0.25	0.27	0.29	
<i>L. mesenteroides</i>	0.082	0.082	0.084	0.085	0.085	0.130	0.131	0.134	0.136	
<i>Klebsiella</i> sp.	0.082	0.082	0.130	0.120	0.120	0.131	0.133	0.134	0.135	
<i>B. subtilis</i>	0.082	0.082	0.083	0.089	0.11	0.131	0.132	0.137	0.139	

* Values are means of triplicate determination

Table 3: Growth of single pure cultures on cassava tubers for different length of time

(log ₁₀ × 10 ⁷ cfu/g)	Period of fermentation (h)									
	0	12	24	36	48	60	72	84	96	
<i>L. plantarum</i>	8.2±0.12	9.5 ±0.05	10±0.11	10.9±0.1	11.1±0.1	12.2±0.11	12.9±0.11	13.1±0.11	13.3±0.11	
<i>L. mesenteroides</i>	7.0± 0.11	7.2 ±0.5	7.8±0.5	7.9±0.5	8.1±0.11	8.8±0.12	9±0.5	9.3±0.05	10.0± 0.11	
<i>Klebsiella</i> sp.	8.2± 0.12	9±0.11	9.2± 0.11	8.8±0.5	9±0.5	9.2±0.5	9.3 ±0.05	9.2±0.05	9.2±0.05	
<i>B. subtilis</i>	8.1±0.11	8.9±0.12	9±0.5	8.8±0.11	8.9±0.12	9.1±0.5	8.1±0.11	8.2±0.11	7.9±0.05	

* Values are the mean ± S.E of three replicate

The TTA changes of the cassava tubers inoculated with the culture are shown in figure 2 and statistical data is presented on table 2. *L. plantarum* produced the highest increase in TTA content from 0.082% at 0 h to 0.290% after 96 h. The abilities of other inocula to effect acid production are relatively low. *B. subtilis*, *Klebsiella* sp. and *L. mesenteriods* increased the TTA from 0.082% at 0 h to 0.139%, 0.135% and 0.136% respectively after 96 h fermentation period.

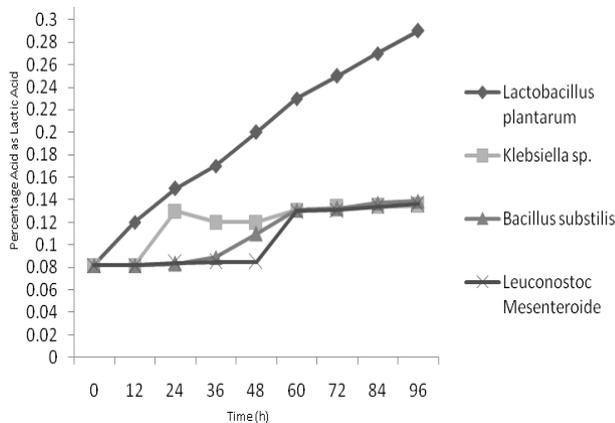


Figure 2: Total titratable acidity change during fermentation using bacterial pure culture

The growth patterns of the pure inocula on the cassava tubers are shown in figure 3 and the statistical data is presented in table 3. *L. plantarum* grew relatively better than others. The population of *B. subtilis* remained almost constant for most part, with a slight decreased in population towards the end of the fermentation. *Klebsiella* sp. population increased slightly within the first 24 h, remained constant for the remaining period, while *L. mesenteroides* showed the same pattern as in *Klebsiella* sp.

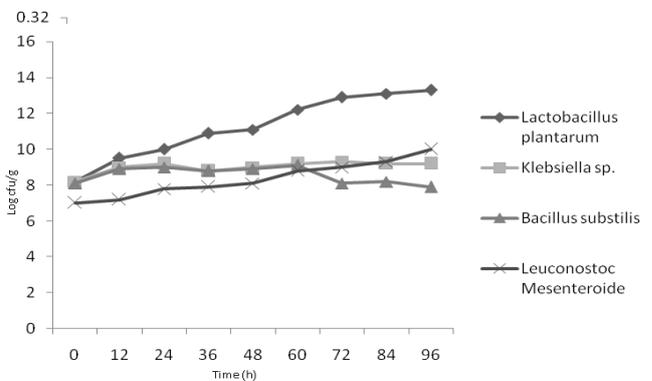


Figure 3: Growth of single bacterial pure culture on fermenting cassava tubers.

Table 4: Sensory* rating of pure inocula for odour reduction for different length of time

(% lactic acid)	Period of fermentation (h)								
	0	12	24	36	48	60	72	84	96
<i>L. plantarum</i>	0	1.9	2.1	2.3	2.5	3.2	3.3	3.4	3.4
<i>L. mesenteroides</i>	0	1.7	1.8	1.9	2.1	2.3	2.5	2.5	2.6
<i>Klebsiella sp.</i>	0	1.5	1.6	1.6	1.8	1.9	2.0	2.2	2.5
<i>B. subtilis</i>	0	3.5	5.0	5.0	5.0	5.0	5.0	5.0	5.0

*Values are means of triplicate determination

Table 5: Sensory* rating of pure inocula for texture for different length of time

(% lactic acid)	Period of fermentation (h)								
	0	12	24	36	48	60	72	84	96
<i>L. plantarum</i>	0	1.2	2.4	2.5	3.0	3.1	3.4	3.4	3.4
<i>L. mesenteroides</i>	0	1.0	1.3	1.5	1.6	1.8	2.5	2.6	3.0
<i>Klebsiella sp.</i>	0	3.2	4.0	4.3	4.5	4.5	4.6	4.6	4.6
<i>B. subtilis</i>	0	1.2	1.5	1.9	2.4	3.5	3.6	3.6	4.3

* Values are means of triplicate determination

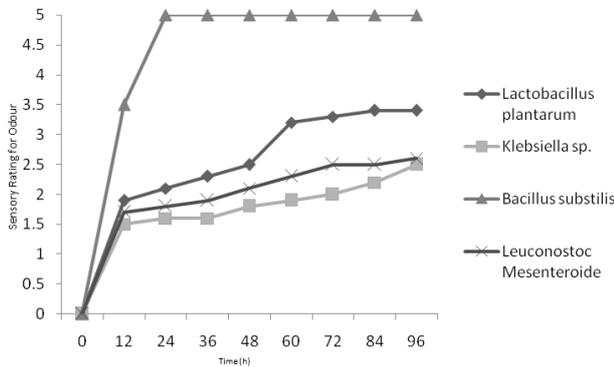


Figure 4: Characteristic fofofo odour reduction by single bacterial pure cultures during cassava fermentation.

The sensory panel rating for odour reduction and texture in fermenting cassava by the single culture inoculants is shown in figure 4 and 5 and the statistical data are presented in table 4 and 5 respectively. *B. subtilis* scored the highest rating for odour reduction ability followed by *L. plantarum*. None often pure cultures could singly cause satisfactory retting of the tubers (scale = 5.0), but *B. subtilis* showed great odour reduction to an acceptable level.

DISCUSSIONS

Four pure culture inocula were investigated. *L. plantarum* was found to give the highest acid producing ability on the fermenting cassava tubers. Oyewole (1990) had earlier reported that *L. plantarum* strain is associated with high acid production during cassava fermentation for the production of fofofo. It appears that *L. plantarum* and possibly other strains of lactic acid bacteria are responsible for acid production during cassava fermentation process. This view is supported by the

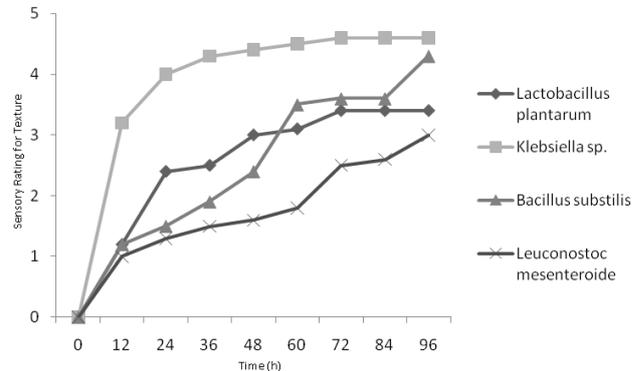


Figure 5: Effect of single bacterial pure culture on the retting juice of fermented cassava

The sensory ratings showed that all the pure culture inocula investigated had varying contributions to odour reduction with *B. subtilis* playing a major role in odour reduction followed by *L. plantarum*. This may be due in part to the fact that *B. subtilis* and *L. plantarum* occur naturally and produces ethanol and high concentration of lactate which strongly acidify the retting juice (Asiedu, 1992). The volatile fatty acid along with lactate produced is responsible for the typical odour in fermented cassava tubers (Brauman *et al.*, 1996). Also the cyanogenic glucoside are been reduced to hydrocyanogenic acid thereby giving off a characteristics odour (Odufina, 1985) relatively low acid producing abilities of other major strains implicated in the fermentation.

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