

Full Length Research Paper

Effect of particulate materials on lactic fermentation of new local white variety cassava (“Bianbasse”) using both spontaneous and starter culture

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The effect of particulate materials on lactic fermentation of cassava using both spontaneous and starter culture - *Lactobacillus plantarum* was investigated. The mean value counts during spontaneous fermentation, the total dissolved loads in all the samples, the total reducing sugars of all samples, the microbial loads in all the samples, the contents of crude protein, crude fibre, ash contents, ether extract, phytic acid and tannin were determined. All samples had an increase in lactic acid bacteria counts through out the fermenting time of 72 h than total bacteria counts in all samples. Sample A had the highest counts of lactic acid bacteria but total bacteria counts in sample A showed decrease in their counts. Sample C had an increase in both lactic acid bacterial and total bacteria counts. Other samples with added particulate materials showed corresponding increase in lactic acid bacteria counts compare to total bacteria counts. There was corresponding increase in total dissolved solids of sample containing varying concentrations of particulate materials ranging from 300 - 2500 mg/l. Total reducing sugar content for the samples ranged from 3.0 - 6.4 mg/l. Sample A had the highest total reducing sugar of 6.4 mg/l at 72 h of fermentation while sample C had the lowest value of 4.8 mg/l. Sample A inoculated with starter culture and other samples with supplemented materials, sample A₁, A₂ and A₃ had an increase in crude protein, crude fibre, crude fat/ether, phytic acid and tannin content than sample C which was not inoculated.

Key words: Particulate materials, samples.

INTRODUCTION

In Africa, Cassava is very important to the people because fermented cassava products constitute a major part of the daily diets of many homes in Nigeria and most parts of West Africa (Oyewole and Odunfa, 1990). Cassava processing methods involve peeling, crushing, milling, slicing, dewatering, decanting sun-drying, smoke-frying, fermenting, heaping, stacking, sieving, cooking, boiling or steaming. Different combinations of these activities result in different products (Nweke, 1996). In „fufu“ production, the peeled cassava roots are retted for a period of 5 days, followed by a process of sieving and

dewatering. „Fufu“ is not subjected to any drying before being cooked for consumption.

Cassava tuber in itself considered as an unbalance feed-stock rich in starch but poor in protein and growth factors (Figuerola and Davila 1997). The low protein contents of cassava have been of major concern in its utilization (Brook and Stanton, 1969). Ngaba and lee (1979) implicated *Lactobacillus plantarum*, *L. buchneri*, *Leucostoc sp.*, *streptococcus sp* and yeast in cassava fermentation. *Lactobacillus sp* and *Leuconostoc sp* dominated the final stage of the fermentation. (Nwankwo et al., 1989). Lactic acid bacteria have been identified as the most useful micro-organism to the society with the possible future benefits and have been found to be beneficial in flavoring foods, inhibiting spoilage bacteria,

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and pathogens in intestinal health and other health benefits related blood cholesterol levels, immunocompetence and antibiotic production (Sandine, 1987). The development of *Lactobacillus* strains as started cultures for cassava fermentation could offer some advantages and could help in optimizing the processing. This process of fermentation with protein and legume enriching micro-organism improve protein contents of cassava.

Therefore the objective of this work is to study the effect of starter cultures, and varying concentrations of particulate materials on lactic fermentation of cassava in order to evaluating the proximate composition and nutritional analysis of fermented cassava.

MATERIALS AND METHODS

Materials

Cassava tubers of the new local white variety ("Bianbasse") about 12 months old obtained from savanna Agriculture Research Institute (SARI) farm at Nyankpala- Tamale, Ghana, particulate material-such as-prepared soybean husk and soy bean meal, brewer soluble (worts) and mash solid obtained from Ghana Brewery PLC Accra, and alumina obtained from the Department of Chemistry, University for Development Studies were used to study it effects on cassava fermentation using spontaneous and starter culture-*L. plantarum*. The Cassava tubers were selected such that no surface attack of pathogen or external wound was observed.

Experimental procedures

Fermenters were prepared using about 50 g of cut cassava tubers which were steeped into 250 ml of sterile water to form pulps in 500 ml sterile fermenter which was covered. This was fermented spontaneously for 3 days at laboratory temperature of $29 \pm 2^\circ\text{C}$. The process was monitored on 24 h basis for 3 days to observe any change in microfloral composition. Thus cassava was fermented by the traditional „fufu“ preparation method.

Culture Media used for Isolation

De Mann Rogosa and Sharpe (MRS) Agar, De Mann Rogosa and Sharpe broth, peptone water, and Plate Count Agar (PCA) were used for the isolation of microorganisms. All media were autoclaved at 121°C for 15 min after melted.

Isolation of Micro-organism in fermenting medium

Lactic acid bacteria were isolated from 5 g of fermenting tuber. The samples were homogenized with 50 ml of sterile 0.1% peptone water. One-tenth and one-eleventh dilutions were poured on sterile plates and prepared sterile MRS agar cooled to 40°C was poured on the plates (in duplicate), swirled and left to solidify. These plates were incubated at 30°C for 2 days under anaerobic condition (BBL Gas pack, H_2 and CO_2 anaerobic system Becton Dickorison) representative colonies were picked randomly from the plates and purified by sub-culturing on fresh gar plates of MRS agar.

Preliminary identification

Preliminary tests were done according to Sharpe (1979). For lactic

acid bacteria, the organisms were tested for biochemical characterization according to the procedure described by Seeley and Van Demark (1972).

Cultivation of starter culture

The selected lactic acid bacteria isolated from cassava fermentation were separately cultivated on MRS broth that had been sterilized at 121°C for 15 min and adjusted to pH 5.5. The culture flasks were incubated an aerobically at 30°C for 48 h (using BBL Gas Pack, H_2 and CO_2 anaerobic system). At the end of incubation period, the broth was centrifuged at 5000 g revolution per minutes for 10 min. The supernatant were decanted while the pellets were washed with sterile distilled water and used as the inoculums. 1ml of the inoculums (starter culture) produced a concentration of approximately $10^5 - 10^6$ cfu/ml when grown on MRS agar. This test was done according to (Huang and Lin, 1993) procedure and (Burrows et al., 1986) method.

Preparation of cassava for fermentation

Cassava tubers were cut into small pieces of about 3 - 5 cm length. 200 g of cut cassava tubers were separately weighed into Eight (8) different fermenter. The cut cassava tubers were sterilized using 0.1% Hg CL in 70% ethanol followed by rinsing with sterile distilled water. One of the fermenter (A) was inoculated with starter culture. Three fermenters labeled A₁, A₂ and A₃ were used to determine the effect of particulate materials. These particulate materials were added in varying concentrations into the fermenter. Fermenter C contained only cassava, and it served as control.

Legends/sample codes

A = 200 g Cassava + *L. plantarum*

A₁ = 200 g Cassava + 2.5 g each of soybean husk, soybean meal, Alumina, mash solid and Brewer soluble.

A₂ = 200 g Cassava + 2.5 g each of soybean husk, soybean meal and Alumina 1.5 g mash and 1.5 ml brewer soluble.

A₃ = 200 g Cassava + 1.5 g each of soybean husk, soybean meal, and Alumina + 2.5 g mash solid and 2.5 g mash solid and 2.5 ml brewer soluble.

C = 200 g Cassava only (Uninoculated) control.

Fermentation of cassava samples using starter culture-*Lactobacillus plantarum*

Fermenter A was singly inoculated with 3 ml of starter culture, and it fermented for 72 h at room temperature. Fermenter C was fermented for 72 h at room temperature.

Effect of varying concentration of particulate materials on lactic fermentation of cassava spontaneously

Varying Concentration of particulate materials were added to each (3) three fermenters that contained 200 g of sterile cassava tubers in this order. Fermenter A₁ contained 2.5 g each of soy-bean husk, soy bean meal, alumina, 2.5 g mash solid and 2.5 ml brewer soluble. Fermenter A₂ contained 2.5 g each of soy bean husk, soybean meal and alumina; 1.5 g mash solid and 1.5ml brewer soluble. Fermenter A₃ contained 1.5 g each of soybean husk, soy bean meal and alumina; 2.5 g mash solid and 2.5 ml brewer soluble.

Evaluation of total dissolved solid

A method described by Frank and Watkins (1950) was used to evaluate the total dissolved solid contents. 50 ml of the sample was put in weighed crucible and heated to dryness in water bath. After heating the crucible was cooled in desiccators and reweighed.

Determination of the concentration of total reducing sugar

The DNSA reagent method of Miller (1959) was used to determine the concentration of total reducing sugar.

Biochemical (proximate) analysis of the fermented cassava products in the fermenters

A method described by (AOAC 1984; Sidney 1984) was used to estimate crude protein, crude fat/ether, crude fibre contents and ash.

Nutritional analysis of the fermented cassava products in the fermenters

A method described by Maga (1982) was used to estimate phytic acid and a method described by Broadhurst and Jones (1978) was used to estimate tannin contents.

RESULTS

The predominant isolate during the spontaneous fermentation of cassava for 72 h was identified as *L. plantarum* and it was selected as starter culture for the fermentation. Effect of varying concentration of particulate materials on total dissolved solids (mg/L) using spontaneous fermentation and starter culture- *Lactobacillus plantarum* is shown in Table 1. Sample A and C had reduced total

dissolved sol, while sample A₁, A₂, and A₃ had highest total dissolved solids. Sample A and C had their total dissolved solids increased from 300 - 600 mg/L after 72 h

of fermentation. While sample A₁, A₂ and A₃ had their total dissolved solids ranged from 600mg/L to 2,500 mg/L after 72 h of fermentation. After 72 h of fermentation, sample A had total dissolved solid of 600mg/l, sample A₁ had 2500 mg/l, sample A₂ had 1100 mg/l, sample A₃ had 1050 mg/l and sample C had 600 mg/l.

Table 2 showed the effect of varying concentration of particulate materials on total reducing sugar. At zero hour, total reducing sugars increased for all samples, later at 24 h, it reduced for all samples and increased again after 24 h for all the samples, till 72 h of fermentation. Sample A had highest total reducing sugars of 6.4mg/L at 72 h, while sample C had lowest total reducing sugar of 4.8 mg/L at 78 h of fermentation. Other samples had their total reducing sugar contents with approximately 6.2 mg/L at 72 h of fermentation.

Table 3 showed the effect of varying concentration of particulate materials on microbial load (cfu/ml). Samples A and C had increase in total lactic acid bacterial counts throughout the fermentation than total bacterial counts. In sample A, total lactic acid bacteria increased from 3.35 x

Table 1. Effect of varying concentration of particulate materials on total dissolved solids (mg/l) during fermentation of cassava using both spontaneous and starter culture -*Lactobacillus plantarum*.

Samples	Fermentation time (h)		
	24	48	72
A	300 mg/l	540 mg/l	600 mg/l
A ₁	200 mg/l	2400 mg/l	2500 mg/l
A ₂	700 mg/l	960 mg/l	1,110 mg/l
A ₃	6000 mg/l	960 mg/l	1050 mg/l
C	300 mg/l	520 mg/l	600 mg/l

Legends/sample codes

A = 200 g Cassava + *Lactobacillus plantarum*

A₁ = 200 g Cassava + 2.5 g each of soybean husk, soybean meal, Alumina, mash solid and Brewer soluble.

A₂ = 200 g Cassava + 2.5 g each of soybean husk, soybean meal and Alumina 1.5 g mash and 1.5 ml brewer soluble.

A₃ = 200 g Cassava + 1.5 g each of soybean husk, soybean meal, and Alumina + 2.5 g mash solid and 2.5 g mash solid and 2.5 ml brewer soluble.

C = 200g Cassava only (Uninoculated) control.

Table 2. Effects of varying concentration of particulate materials and some osmoregulators on total reducing sugar (mg/l) using both spontaneous and starter culture.

Samples	Fermentation time (h)			
	0	24	48	72
A	5.8 mg/l	5.4 mg/l	6.2 mg/l	6.4 mg/l
A ₁	5.8 mg/l	5.2 mg/l	6.0 mg/l	6.3 mg/l
A ₂	5.8 mg/l	5.0 mg/l	6.0 mg/l	6.2 mg/l
A ₃	5.8 mg/l	5.2 mg/l	6.1 mg/l	6.2 mg/l
C	5.8 mg/l	3.0 mg/l	3.5 mg/l	4.8 mg/l

Legends/sample codes

A = 200 g Cassava + *Lactobacillus plantarum*

A₁ = 200 g Cassava + 2.5 g each of soybean husk, soybean meal, Alumina, mash solid and Brewer soluble.

A₂ = 200 g Cassava + 2.5 g each of soybean husk, soybean meal and Alumina 1.5g mash and 1.5 ml brewer soluble.

A₃ = 200 g Cassava + 1.5 g each of soybean husk, soybean meal, and Alumina + 2.5 g mash solid and 2.5 g mash solid and 2.5 ml brewer soluble.

C = 200 g Cassava only (Uninoculated) control.

10^9 at 24 h to 5.50×10^9 cfu/ml after 72 h of fermentation while that of total bacteria counts reduced from 1.32×10^9 at 24 h to 1.23×10^9 cfu/ml after 72 h of fermentation. For sample C, total lactic acid bacteria and total bacteria increased from 2.52×10^9 at 24 h to 3.04×10^9 cfu/ml after 72 h and from 2.48×10^9 to 3.80×10^9 cfu/ml after 72 h respectively. Other samples A₁, A₂, and A₃ had an increase in their total lactic acid bacteria counts ranging from 2.82×10^9 at 24h to 3.92×10^9 cfu/ml after 72 h of fermentation while their total bacteria counts ranging from 3.30×10^9 to 3.80×10^9 after 72 h of fermentation.

Proximate composition of the entire sample at various 24, 48 and 72 h of fermentation are shown in Table 4, 5 and 6 respectively. Table 4 showed that, at 24 h sample

Table 3. Effect of varying concentration of particulate materials on micro bila loads (cfu/ml) during fermentation of cassava using both spontaneous and starter culture - *Lactobacillus plantarum*

Samples	Time (h)					
	24		48		78	
	Total bacteria count on PCA (cfu/ml)	Lactic acid bacteria counts on MRS (cfu/ml)	Total bacteria PCA (cfu/ml)	Lactic bacteria counts on MRS (cfu/ml)	Total bacteria counts PCA	Lactic acid counts on MRS (cfu/ml)
A	1.32 X 10 ⁹	3.35 X 10 ⁹	1.28 X 10 ⁹	4.26 X 10 ⁹	1.32 X 10 ⁹	5.50 X 10 ⁹
A ₁	3.82 X 10 ⁹	3.51 X 10 ⁹	3.88 X 10 ⁹	4.26 X 10 ⁹	1.32 X 10 ⁹	5.50 X 10 ⁹
A ₂	3.31 X 10 ⁹	2.82 X 10 ⁹	3.35 X 10 ⁹	3.06 X 10 ⁹	3.28 X 10 ⁹	3.28 X 10 ⁹
A ₃	3.30 X 10 ⁹	2.87 X 10 ⁹	3.23 X 10 ⁹	3.34 X 10 ⁹	3.35 X 10 ⁹	3.40 X 10 ⁹
C	2.48 X 10 ⁹	2.52 X 10 ⁹	2.60 X 10 ⁹	2.89 X 10 ⁹	2.80 X 10 ⁹	3.04 X 10 ⁹

Table 4. Effect of particulate on proximate composition fermented cassava using both spontaneous and started culture- (*L. plantarum*) at 24 h fermentation.

Samples	Proximate analysis				Nutritional analysis	
	%crude protein	% crude fibre	% ether extract	% ash content	5 phytic content	Tannins mg/g
A	2.63	2.84	0.77	1.80	0.001	0.18
A ₁	6.13	4.16	1.11	1.14	0.015	0.08
A ₂	3.06	4.18	1.24	2.13	0.001	0.13
A ₃	6.56	2.94	.83	1.83	0.004	0.12
C	2.46	1.40	0.44	1.52	0.004	0.08

Table 5. Effect of particulate materials and some osmoregulators on proximate composition of fermented cassava using both spontaneous and starter culture (*L. plantarum*) at 48 h of fermentation.

Samples	Proximate analysis				Nutritional analysis	
	%crude protein	% crude fibre	% ether extract	% ash content	5 phytic content	Tannins mg/g
A	8.75	1.91	0.96	0.57	0.007	0.17
A ₁	3.50	1.81	1.42	3.95	0.012	0.28
A ₂	2.19	4.05	0.88	3.82	0.008	0.22
A ₃	4.63	4.16	1.01	3.78	0.006	0.21
C	1.86	2.60	0.72	1.64	0.006	0.10

A had low crude protein, crude fibre, ether extract and phytic acid of 2.63, 2.82, 0.77 and 0.001% respectively compare with other sample. Sample C that had lowest crude protein, fibre, ether extract, ash and tannins of 2.46, 1.40, 0.44, 1.52 and 0.08% respectively. Sample A₁, A₃, had the highest crude protein ranging from 6.13% to 6.56%; Sample A₂ had highest crude fibre and ash contents of 4.18 and 2.13% respectively.

In Table 5 at 48 h of fermentation, sample A had highest crude protein content of 8.75%, while sample C had the least protein contents of 1.86% Crude protein contents for samples A₁, A₂ and A₃ ranged from 2.19 - 4.63%. Sample A₃ had highest crude fibre of 4.16%.

In Table 6, at 72 h of fermentation, sample A, A₁, had highest crude protein content of 9.19 and 8.38% respectively while Sample C had least crude protein content of 1.33% Sample A₂ and A had highest crude fibre content

of 8.30 and 7.90% respectively.

From Tables 4, 5 and 6, sample A and A₁ had highest crude protein. Sample A and A₁ showed highest crude fibre. Sample A showed lowest phytic acids and Tannins than other samples. Only C had least value in crude protein, fibre, ether, phytic acid, and Tannins.

DISCUSSION OF RESULTS AND CONCLUSION

Fermented products of cassava constitute a major part of the daily diets of many homes in most part of West Africa countries. The most predominant bacteria in cassava fermentation processes is the Lactic acid bacteria of which *L. plantarum* is the predominant amongst lactic acid bacteria. (Ngaba and Lee, 1979; Okafor et al., 1984; Oyewole and Odunfa, 1990). In this study, *L. plantarum*

Table 6. Effect of particulate materials and some osmoregulators on proximate composition of fermented cassava using both spontaneous and starter culture (*L. plantarum*) at 72 h of fermentation.

Samples	Proximate analysis			Nutritional analysis		
	%crude protein	% crude fibre	% ether extract	% ash content	5 phytic content	Tannins mg/g
A	9.19	7.90	0.18	1.95	0.007	0.21
A1	3.38	4.63	0.76	2.10	0.009	0.15
A2	2.63	8.30	1.01	1.93	0.041	0.38
A3	3.06	4.06	1.26	1.91	0.048	0.34
C	1.33	3.44	0.74	1.70	0.004	0.12

was used as a starter culture in the fermentation of cassava.

Increase in total dissolved solids in sample A₁ and A₂, A₃ in Table 1 may be due to the added materials that dissolved in the medium despite the ones consumed by the fermenting organisms. Decrease in total dissolved solids in samples A and C may be as a result of non added materials to the medium.

Increase in total reducing sugar content in sample A in Table 2 after 24h is a confirmation of starch degrading potential and the added materials. Decrease in total reducing sugar in sample C as compare to other samples may be due to the utilization of available simple sugar for metabolic activities of the fermenting bacteria. Longe (1980) reported similar reduction in the total reducing sugar with in 24 h of spontaneous fermentation. However increase in the total reducing sugar contents till the end of 72 h of fermentation may be due to the action of other bacteria species which produces amylase necessary for breakdown of starch to sugar which are used for the growth of the lactic acid bacteria. Olatunji (1986) and Ejiyor and Okafor (1981) confirmed the activities of amylase for initial breakdown of cassava starch to simple sugar increase.

Increase in lactic acid bacteria counts recorded in all samples in Table 3 may be due to their acid tolerant. Decrease in total bacteria counts in all the samples in Table 3 may be due to the high acidity of the fermenting medium created by lactic acid bacteria, which they cannot tolerate. Though varying concentration of particulate materials on the medium provide medium of growth for lactic acid bacteria which enable them to produce more acid that suppress the growth of other bacteria.

Increase in crude protein contents recorded in sample A and other samples may be as a result of the contributing protein content of the added materials and lactic acid bacteria involved or added as a starter culture in the fermentation. Decrease in crude protein contents in sample C may be as a result of non added particulate materials and the starter culture. Increase in crude fibre contents, ether extract and ash contents in all samples till 72 h of fermentation, may be due to the added particulate materials while non inclusion of particulate materials in samples A and C reduce their proximate composition.

The use of starter culture therefore can be employed to

control the acid content of the fermenting medium to inhibit and discourage undesirable bacterial from the medium, to control fermenting time, improve odour and flavor and nutritional value of cassava fermenting products. Moreover addition of appropriate concentration of particulate materials to the fermenting medium of cassava can increase the growth of lactic acid bacterial and this in addition can improve and better produce acceptable nutritional value of cassava products than naturally fermented cassava products.

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