Effect Of Particulate Materials On Lactic Fermentation Of New Local White Variety Cassava ("Bianbasse") Using Both Spontaneous And Starter Culture

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ABSTRACT: Lactic acid bacteria isolated in the fermentation of cassava for 'fufu' were Lactobacillus plantarium, Lactobacillus sp and Leuconostoc mesenterodes. L plantarium was identified as the most predominant lactic acid bacteria and was used as a starter culture for the fermentation of 'fufu' production. 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 father, ash, crude fidbe, phytic acid and Tannin were determined. The mean value counts during spontaneous fermentation process from zero hour to 72hours were found to increase 0.67 x 1012cfu/ml to 3.56 x 1012 in lactic acid bacteria than total bacteria with an increase from 0.69 x 1012 to 2.94 x 1012cfu/ml and yeasts which increased from0.07 x 1012 to 2.06 x 1012cfu/ml. There was corresponding increase in total dissolved solids of sample from 600mg/l to 2500mg/l, when varying the concentration of particulate materials for 72 hours and from 500mg/l to 1400mg/l when varying the concentration of Osmoregulators. The total reducing sugar for all the samples ranged from 5.8mg/l to 5.7mg/l at zero hour. At 24 hours, it ranged from 3.0mg/l to 5.4mg/l, at 48 hours it ranged from 3.5mg/l to 6.2mg/l and 72 hours, it ranged from 4.8mg/l to 6.4mg/l. Sample A inoculated with starter culture highest counts of Lactic acid bacteria ranged from 3.35 to 5.50 x 109cfu/ml while total bacterial counts ranged from 1.23 to 1.32 x 109cfu/ml. Other samples with supplemented materials had lactic acid bacterial counts ranged from 2.60cfu/ml to 3.92 x 109cfu/ml while bacterial counts ranged from 3.15 to 3.80 x 109cfu/ml. Control had LAB counts ranged from 2.52 to 3.04 x 109cfu/ml while total bacterial counts ranged from 2.48 to 2.80 x 109cfu/ml.

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KEY WORDS: Lactobacillus planetarium,, Lactobacillus sp ,Leuconostoc mesenterodes, fufu, Osmoregulators, Lactic acid bacteria and fermentation.

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. Figueroa et al 1997). The low protein contents of cassava has been of major concern in its utilization (Brook et al., 1969). Ngaba and lee (1979) implicated *Lactobacillus plantarium, 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 has been found to be beneficial in flavoring foods, inhibiting spoilage bacteria, 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 form the Department of Chemistry, University for Development Studies were used to study it effects on cassava fermentation using spontaneous and starter culture- *Lactobacillus planetarium.* The Cassava tubers were selected such that no surface attack of pathogen or external wound was observed.

Experimental Procedures

Fermenter were prepared using about 50g of cut cassava tubers which were steeped into 250ml of sterile water to form pulps in 500ml sterile fermenter which was covered. This was fermented spontaneously for 3 days at laboratory temperature of $29^o \pm 2^oc$. The process was monitored on 24 hours 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^oC for 15 minutes after melted.

Isolation of Micro-organism in fermenting medium

Lactic acid bacteria were isolated from 5g of fermenting tuber. The samples were homogenized with 50ml 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^o C was poured on the plates (in duplicate), swirled and left to solidify. These plates were incubated at 30^oC for 2 days under anaerobic condition (BBL Gas pack, H_2 and $C0_2$ 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^oC for 15 minutes and adjusted to pH 5.5. The culture flasks were incubated an aerobically at 30^oC for 48 hours (using BBL Gas Pack, H_2 and $C0_2$ anaerobic system). At the end of incubation period, the broth was centrifuged at 5000g revolution per minutes for 10 minutes. 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 – 5cm length. 200g 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 A_1, A_2, A_3 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 = 200g Cassava + Lactobacillus plantrum

A_1 = 200g Cassava + 2.5g each of soybean husk, Plantarium, soybean meal, Alumina, mash solid and Brewer soluble.

A_2 = 200g Cassava + 2.5g each of soybean husk, soybean meal and Alumina 1.5g mash and 1.5ml brewer soluble.

A_3 = 200g Cassava + 1.5g each of soybean husk, soybean meal, and Alumina + 2.5g mash solid and 2.5g mash solid and 2.5ml brewer soluble.

C = 200g Cassava only (Uninoculated) control.

Fermentation of Cassava samples using starter culture- Lactobacillus plantarium.
Fermenter A was singly inoculated with 3ml of starter culture, and it fermented for 72 hours at room temperature. Fermenter C was fermented for 72 hours 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 200g of sterile cassava tubers in this order. Fermenter A_1 contained 2.5g each of soy-bean husk, soy bean meal, alumina, 2.5g mash solid and 2.5ml brewer soluble.Fermenter A_2 contained 2.5g each of soy bean husk, soybean meal and alumina;1.5g mash solid and 1.5ml brewer soluble. Fermenter A_3 contained 1.5g each of soybean husk, soy bean mieal and alumina; 2.5g mash solid and 2.5ml brewer soluble.

Eavaluation of Total Dissolved Solid.
A method described by frank and Watkins (1950) was used to evaluate the total dissolved solid contents. 50ml of the sample was put in weighed crucible and heated to dryness in water bath. After heating the crucible was cooled in desiccator 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 (A. O.A.C 1984) was used to estimiated 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 hours was identified as *Lactobacillus plantarium* 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 Table1. Sample A and C had reduced total dissolved sol, while sample A_1, A_2, and A_3 had highest total dissolved solids. Sample A and C had their total dissolved solids increased from 300mg/L to 600mg/L after 72 hours of fermentation. While sample A_1, A_2 andA_3 had their total dissolved solids ranged form 600mg/L to 2,500mg/L after 72 hours of fermentation. After 72 hours of fermentation, sample A had total dissolved solid of 600mg/l, sample A_I had 2500mg/l, sample A_2 had 1100mg/l, sample A_3 had 1050mg/l and sample C had 600mg/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 24hours, it reduced for all samples and increased again after 24hours for all the samples, till 72 hours of fermentation. Sample A had highest total reducing sugars of 6.4mg/L at 72hours, while sample C had lowest total reducing sugar of 4.8mg/L at 78hours of fermentation. Other samples had their total reducing sugar contents with approximately 6.2mg/L at 72 hours 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 3.35×10^9 at 24 h to 5.50×10^9cfu/ml after 72 h of fermentation while that of total bacteria counts reduced from 1.32×10^9 at 24h to 1.23×10^9 cfu/ml after 72h of fermentation. For sample C, total lactic acid bacteria and total bacteria increased from 2.52×10^9 at 24h to 3.04×10^9cfu/ml after 72h and from 2.48×10^9 to 3.80×10^9 cfu/ml after 72h respectively.Other samples A_1,A_2,and A_3 had an increase in their total lactic acid bacteria counts ranging from 2.82×10^9 at 24h to 3.92×10^9cfu/ml after 72h of fermentation while their total bacteria counts ranging from 3.30×10^9 to 3.80×10^9 after 72h of fermentation.

Proximate composition of the entire sample at various 24, 48, 72 hours of fermentation are shown in Table 4, 5 and 6 respectively. Table 4 showed that, at 24 hours sample 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% and0.08% respectively. Sample A_1, A_3, had the highest crude protein ranging from 6.13% to 6.56%;. Sample A_2 had highest crude fibre and ash contents of 4.18% and 2.13% respectively

In table 5 at 48 hours 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 A_1,A_2 and A_3 ranged from 2.19% to 4.63%.Sample A_3 had highest crude fibre of 4.16%.

In table 6, at 72 hours of fermentation, sample A, A_1, had highest crude protein content of 9.19% and 8.38% respectively while Sample C had least crude protein content of 1.33% Sample A_2 and A had highest crude fibre content of 8.30% and 7.90% respectively..

From table 4, 5 and 6, sample A and A_1 had highest crude protein. Sample A and A_1 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.

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 plantarium*.

Samples	Fermentation	Time (Hours)	
	24	48	72
A	300mg/l	540mg/l	600mg/l
A_1	200mg/l	2400mg/l	2500mg/l
A_2	700mg/l	960mg/l	1,110mg/l
A_3	6000mg/l	960/mg/l	1050mg/l
C	300mg/l	520mg/l	600mg/l

Legends/sample codes
A = 200g Cassava + Lactobacillus plantrum
A_1 = 200g Cassava + 2.5g each of soybean husk, Plantarium, soybean meal, Alumina, mash solid and Brewer soluble.
A_2 = 200g Cassava + 2.5g each of soybean husk, soybean meal and Alumina 1.5g mash and 1.5ml brewer soluble.
A_3 = 200g Cassava + 1.5g each of soybean husk, soybean meal, and Alumina + 2.5g mash solid and 2.5g mash solid and 2.5ml 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 (Hours)			
	0	24	48	72
A	5.8mg/l	5.4mg/l	6.2mg/l	6.4mg/l
A_1	5.8mg/l	5.2mg/l	6.0mg/l	6.3mg/l
A_2	5.8mg/l	5.0mg/l	6.0mg/l	6.2mg/l
A_3	5.8mg/l	5.2mg/l	6.1mg/l	6.2mg/l
C	5.8mg/l	3.0mg/l	3.5mg/l	4.8mg/l

Legends/sample codes
A = 200g Cassava + Lactobacillus plantrum
A_1 = 200g Cassava + 2.5g each of soybean husk, Plantarium, soybean meal, Alumina, mash solid and Brewer soluble.
A_2 = 200g Cassava + 2.5g each of soybean husk, soybean meal and Alumina 1.5g mash and 1.5ml brewer soluble.
A_3 = 200g Cassava + 1.5g each of soybean husk, soybean meal, and Alumina + 2.5g mash solid and 2.5g mash solid and 2.5ml brewer soluble.
C = 200g Cassava only (Uninoculated) control.

TABLE 3:- EFFECT OF VARYING CONCENTRATION OF PARTICULATE MATERIALS ON MICRO BILA LOADS (cfu/ml) DURING FERMENTATION OF CASSAVA USING BOTH SP0NTANEOUS AND STATER CULTURE – Lactobacillus plantarum

Time	24 Hours		48 Hours		78 Hours	
Samples	Total bacteria count on PCA (cfu/ml)	Lactic Acid Bacteria counts on MRS (cfu/ll)	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 X10^9
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 PROMIXATE COMPOSITION FERMENTED CASSSAVA USING BOTH SPONTANEOUS AND STARTED CULTURE- (*L.plantarum*) AT 24HRS FERMENTATION

SAMPLES	Proximate Analysis				Nutritional Analysis	
	%crude protein	% crude fibre	% ether extract	% ash content	5 phytic content	Tannins mglg
A	2.63	2.84	0.77	1.80	0.001	0.18
A1	6.13	4.16	1.11	1.14	0.015	0.08
A2	3.06	4.18	1.24	2.13	0.001	0.13
A3	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 ATARTER CULTURE (*L. plantarum*) AT 48 HOURS OF FERMENTATION.

SAMPLES	Proximate Analysis				Nutritional Analysis	
	%crude protein	% crude fibre	% ether extract	% ash content	5 phytic content	Tannins mglg
A	8.75	1.91	0.96	0.57	0.007	0.17
A1	3.50	1.81	1.42	3.95	0.012	0.28
A2	2.19	4.05	0.88	3.82	0.008	0.22
A3	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

Table 6: EFFECT OF PARTICULATE MATERIALS AND SOME OSMOREGULATORS ON PROXIMATE COMPOSITION OF FERMENTED CASSAVA USING BOTH SPONTANEOUS AND ATARTER CULTURE (*L. plantarum*) AT 72 HOURS OF FERMENTATION

SAMPLES	Proximate Analysis				Nutritional Analysis	
	%crude protein	% crude fibre	% ether extract	% ash content	5 phytic content	Tannins mglg
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

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 *Lactobacillus plantarum* is the predominant amongst lactic acid bacteria.(Ngaba and Lee,1979,Okafor et al.,1984, Oyewole and Odunfa 1990).In this study, *Lactobacillus plantarum* was used as a starter culture in the fermentation of cassava.

Increase in total dissolved solids in sample A_1 and A_2,A_3 in table 1 may be due to the added materials that dissolved in the medium inspite of 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 24h of spontaneous fermentation. However increase in the total reducing sugar contents till the end of 72h 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 Ejiofor 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 72h 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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