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Comparison of the effect of particulate materials and some osmoregulators on lactic fermentation of new local white cassava variety "Bianbasse" using both spontaneous and starter cultures

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The effects of particulate materials and osmoregulatators on lactic fermentation of cassava were determined on total dissolved loads of all the samples; the total reducing sugars of all samples, the microbial loads in all the samples, the percentage crude protein contents, crude fibres, crude fat/ether, ash, phytic acid and tannin. Sample A_1 inoculated with varied concentrations of particulate materials had the highest total dissolved solid, total reducing sugar, lactic acid bacteria counts and total bacteria counts than sample B_1 with varied concentrations of osmoregulator. There was corresponding increase in sample A_2 and A_3 compared to sample B_2 and B_3. Sample C which served as control had the lowest value in all at 24, 48 and 72 h of fermentation. Most of the samples that contained varied concentration particulate materials had higher values in their proximate analysis and nutritional analysis than samples that contained varied concentrations of osmoregulators. Sample C with neither particulate materials nor osmoregulator had the least values in all analysis.

Key words: Particulate materials, osmoregulatators, lactic fermentation, samples.

INTRODUCTION

Cassava is one of the major root crops grown in the tropics. It provides much of the food for nearly 500 million people around the world (Oke, 1982; Cook, 1985). Cassava is relatively rich in vitamin C and calcium but poor in protein, minerals and other vitamins (Lancaster et al., 1982). The chemical composition of fresh cassava roots showed that it is made up of water (62%), carbohydrate (35%), protein (1%) and mineral salt (1%). The major organic acids produced during cassava fermentation have been identified as lactic, acetic, propanoic, butanoic, oxalic, pyruvic, succinic and formic acids (Cerede et al., 1985; Dougan et al., 1983; Akinrele, 1964) . The low protein contents of cassava has been of major concern in its utilization (Brook et al., 1969).The methods of enriching cassava and its products with protein include processes of fermentation with protein enriching microorganisms (Daubresse et al., 1987; Opoku and Adoga, 1980) and the mixed fermentation of cassava with legumes (Bassir and Bababunmi, 1971; Akinrele, 1967).

Lactic acid bacteria have been identified as the most useful microorganisms to the society with possible future benefits and have been found to be beneficial in flavoring foods, inhibiting spoilage bacteria and pathogens in intestinal health and antibiotic production (Sandine, 1987) . Therefore the objective of this work is to study the effect of varied concentration of particulate materials and osmoregulators on lactic fermentation of cassava in order to evaluate the proximate composition of the fermented cassava products.

MATERIALS AND METHODS

Materials

Cassava tubers of the new local white variety "Bianbasse" of about

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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 and some osmo-regulators such as tryptone, lysine, glycine and proline obtained from the Department of Biochemistry, University for Development Studies and ash were used to study their effects on cassava fermentation by the use of spontaneous and starter culture-*Lactobacillus-plantarium*. The cassava tubers were selected such that no surface, attack of pathogen or external wound was observed.

Experimental procedures

Fermented cassava was 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 ± 2°C. The process was monitored on 24 h basis for 3 days to observe any change in micro floral 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 autoclaved at 121°C for 15 min after melted. MRS agar was used to isolate lactic acid bacteria, and PCA was used to isolate other bacteria.

Preparation of cassava for fermentation

Cassava tubers were cut into small pieces of about 3 - 5 cm long. 200 g of cut cassava tubers were separately weighed into 8 different fermenters. The cut cassava tubers were sterilized using 0.1% HgCl in 70% ethanol followed by rinses with sterile distilled water. Three fermenters labeled A_1, A_2 and A_3 were used to determine the effect of particulate materials. These particulate materials were added in varied concentrations into the fermenter. Another three fermenters labeled B_1, B_2 and B_3 were used to determine the effect of osmoregulations. These also were added in varied concentration into fermenter. Fermenter C contained only cassava, and it served as control.

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

Varied concentrations of particulate materials were added to each three (3) fermenters that contained 200 g of sterile cassava tubers in this order: fermenter A_1 contained 2.5 g each of soy-bean husk, soy bean meal, alumina, mash solid and 2.5 ml of brewer soluble. Fermenter A_2 contained 2.5 g of soy bean husk, soybean meal and alumina, 1.5 g of mash solid and 1.5 ml brewer soluble. Fermenter A_3 contained 1.5 g each of soybean husk, soy bean meal and alumina, 2.5 g mash solid and 2.5 ml brewer soluble.

Effect of varied concentrations of osmoregulators on lactic fermentation of cassava spontaneously

Fermenter B_1 contained 1 g each of tryptone, ash, glycine, lysine and proline, Fermenter B_2 contained 0.5 g each of tryptone, ash, glycine, lysine and proline and Fermenter B_3 contained 0.25 g of tryptone, ash, glycine, lysine and proline.

Enumeration of lactic acid bacteria and total bacteria

1 ml of 10^9 dilution of fermented medium was used to enumerate lactic acid bacteria and total bacteria by pour plate method using MRS agar and PCA, respectively.

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 a 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 AOAC (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

The 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

Effect of varied concentration of particulate materials and some osmoregulators on total dissolved solids (mg/l) using spontaneous fermentation are shown in Tables 1 and 2, respectively. In Table 1, sample C had reduced total dissolved solids, while sample A_1, A_2 and A_3 had high total dissolved solids. Sample C had their total dissolved solids increased from 300 to 600 mg/l after 72 h of fermentation, while sample A_1, A_2 and A_3 had their total dissolved solids range from 600 to 2,500 mg/l after 72 h of fermentation. In Table 2, sample B_1, B_2 and B_3 had their dissolved solids range from 500 to 1,400 mg/ml. The effect of varied concentration of particulate materials and some osmoregulators on total reducing sugar after 72 h of fermentation is shown in Table 3. 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 the 72nd hour of fermentation. Sample C had the lowest total reducing sugar of 4.8 mg/ml at 72 h of fermentation. Other samples had their total reducing sugar contents with approximately 6.2 mg/ml at 72 h of fermentation. Table 4 shows the effect of varied concentration of particulate materials and some

Table 1. Effect of varied concentration of particulate materials on total dissolved solids (mg/ml) during fermentation of cassava.

Samples	Fermentation time (h)		
	24 (mg/ml)	48 (mg/ml)	72 (mg/ml)
A_1	200	2400	2500
A_2	700	960	1,110
A_3	6000	960	105
C	300	520	600

A_1 = 200 g Cassava + 2.5 g each of soybean husk, plantarium, soybean meal, alumina, mash solid and brewer soluble. A_2 = 200 g Cassava + 2.5 g each of soybean husk, soybean meal and alumina, 1.5 g mash and 1.5 ml brewer soluble. A_3 = 200 g Cassava + 1.5 g each of soybean husk, soybean meal and alumina and 2.5 g mash solid and 2.5 ml brewer soluble. C = 200 g cassava only (uninoculated) control.

Table 2. Effect of varied concentration of some osmoregulators on total dissolved solids (mg/ml) during fermentation of cassava.

Samples	Fermentation time (h)		
	24 (mg/ml)	48 (mg/ml)	72 (mg/ml)
B_1	100	1350	1400
B_2	540	640	700
B_3	500	660	700
C	300	520	600

B_1 = 200 g Cassava + 1.0 g each of glycine, lysine, proline, tryptone and ash. B_2 = 200 g Cassava + 0.5 g each of tryptone, ash and lysine, 0.25 g of proline and glycine. B_3 = 200 + 0.25 g each of trytone, ash and lysine + 0.5 g each of proline and glycine. C = 200 g cassava only (uninoculated) control.

Table 3. Effects of varied concentrations of particulate materials and some osmoregulators on total reducing sugar (mg/l).

Samples	Fermentation time (h)			
	0 (mg/ml)	24 (mg/ml)	48 (mg/ml)	72 (mg/ml)
A_1	5.8	5.2	6.2	6.3
A_2	5.8	5.0	6.0	6.2
A_3	5.8	5.2	6.1	6.2
B_1	5.8	5.0	5.8	6.2
B_2	5.8	4.4	5.4	6.2
B_3	5.8	4.0	6.1	6.1
C	5.8	3.0	3.5	4.8

A_1 = 200 g Cassava + 2.5 g each of soybean husk, plantarium, soybean meal, alumina, mash solid and brewer soluble. A_2 = 200 g Cassava + 2.5 g each of soybean husk, soybean meal and alumina, 1.5 g mash and 1.5 ml brewer soluble. A_3 = 200 g Cassava + 1.5 g each of soybean husk, soybean meal and alumina and 2.5 g mash solid and 2.5 ml brewer soluble. B_1 = 200 g Cassava + 1.0 g each of glycine, lysine, proline, tryptone and ash. B_2 = 200 g Cassava + 0.5 g each of tryptone, ash and lysine, 0.25 g of proline and glycine. B_3 = 200 g + 0.25 g each of trytone, ash and lysine + 0.5 g each of proline and glycine. C = 200 g cassava only (uninoculated) control.

Table 4. Effect of varied concentrations of particulate materials and some osmoregulators on micro bila loads (cfu/ml) during fermentation of cassava.

Samples	24 h		48 h		78 h	
	Total bacteria count on PCA (cfu/ml) × 10^9	Lactic acid bacteria counts on MRS (cfu/ml) × 10^9	Total bacteria PCA (cfu/ml) x 10^9	Lactic bacteria counts on MRS (cfu/ml) × 10^9	Total bacteria counts PCA × 10^9	Lactic acid bacteria counts on MRS (cfu/ml) × 10^9
A_1	3.82	3.51	3.88	4.26	1.32	5.50
A_2	3.31	2.82	3.35	3.06	3.28	3.28
A_3	3.30	2.87	3.23	3.34	3.35	3.40
B_1	3.44	2.60	3.50	3.20	3.52	3.32
B_2	3.34	2.62	3.42	3.22	3.40	3.38
B_3	3.15	2.68	3.22	3.22	3.25	3.30
C	2.48	2.52	2.60	2.89	2.80	3.04

A_1 = 200 g Cassava + 2.5 g each of soybean husk, plantarium, soybean meal, alumina, mash solid and brewer soluble. A_2 = 200 g Cassava + 2.5 g each of soybean husk, soybean meal and alumina 1.5 g mash and 1.5 ml brewer soluble. A_3 = 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. B_1 = 200 g Cassava + 1.0 g each of glycine, lysine, proline, tryptone and ash. B_2 = 200 g Cassava + 0.5 g each of tryptone, ash and lysine, 0.25 g of proline and glycine. B_3 = 200 + 0.25 g each of trytone, ash and lysine + 0.5 g each of proline and glycine. C = 200 g cassava only (uninoculated) control.

Table 5. Effect of particulate and some osmoregulators on promixate composition fermented casssava at 24 h fermentation.

Samples	Proximate analysis				Nutritional analysis	
	% Crude protein	% Crude fibre	% Ether extract	% Ash content	Phytic acid content	Tannins (mg/g)
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
B_1	2.63	4.18	1.18	233	0.013	0.06
B_2	3.94	3.41	1.21	2.48	0.012	0.16
B_3	3.06	5.15	1.03	3.07	0.003	0.11
C	2.46	1.40	0.44	1.52	0.004	0.08

A_1 = 200 g Cassava + 2.5 g each of soybean husk, plantarium, soybean meal, alumina, mash solid and brewer soluble. A_2 = 200 g Cassava + 2.5 g each of soybean husk, soybean meal and alumina 1.5 g mash and 1.5 ml brewer soluble. A_3 = 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. B_1 = 200 g Cassava + 1.0 g each of glycine, lysine, proline, tryptone and ash. B_2 = 200 g Cassava + 0.5 g each of tryptone, ash and lysine, 0.25 g of proline and glycine. B_3 = 200 + 0.25 g each of trytone, ash and lysine + 0.5 g each of proline and glycine. C = 200 g cassava only (uninoculated) control.

osmoregulator on microbial load (cfu/ml). Samples C had increase in total lactic acid bacterial counts throughout the fermentation than total bacterial counts. Other samples had an increase in both total lactic acid and total bacterial counts. Proximate composition of the entire sample at 24, 48 and 72 h of fermentation are shown in Tables 5, 6 and 7, respectively. In Table 5 at 24 h, sample C had the lowest crude protein, fibre, ether extract, ash and tannins. Samples A_1 and A_3 had the highest crude protein and tannins. Sample B_3 had the highest crude fibre and ash.

In Table 6, at 48 h of fermentation, sample A_3, B_1 and B_3 had high crude protein content, while sample C had the least protein contents. A_3 had the highest crude fibre while B_3 had the least crude fibre. In Table 7, at 72 h of fermentation, sample A_1 and B_3 had the highest crude protein while C had the least crude protein. Sample A_2 and B_1 had highest crude fibre. From Tables 5, 6 and 7, sample A_1 and B_3 had the highest crude protein. Sample A_1 showed highest crude fibre. Sample C had least value in crude protein, fibre, ether, phytic acid and tannins.

DISCUSSION

The majority of the lactic acid bacteria encountered belongs to *Lactobacillus plantarium* and had been identified as predominant species (Okafor et al., 1984) in cassava product ('fufu'). Reduction in total bacteria counts when *L. planetarium* was inoculated into the

Table 6. Effect of particulate materials and some osmoregulators on proximate composition of fermented cassava at 48 h of fermentation.

Samples	Proximate analysis				Nutritional analysis	
	% Crude protein	% Crude fibre	% Ether extract	% Ash content	Phytic acid content	Tannins (mg/g)
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
B_1	4.38	3.28	0.92	2.82	0.011	0.09
B_2	3.68	3.16	0.97	0.75	0.016	0.14
B_3	4.38	1.42	0.98	1.80	0.014	0.24
C	1.86	2.60	0.72	1.64	0.006	0.10

A_1 = 200 g Cassava + 2.5 g each of soybean husk, plantarium, soybean meal, alumina, mash solid and brewer soluble. A_2 = 200 g Cassava + 2.5 g each of soybean husk, soybean meal and alumina 1.5 g mash and 1.5 ml brewer soluble. A_3 = 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. B_1 = 200 g Cassava + 1.0 g each of glycine, lysine, proline, tryptone and ash. B_2 = 200 g Cassava + 0.5 g each of tryptone, ash and lysine, 0.25 g of proline and glycine. B_3 = 200 + 0.25 g each of trytone, ash and lysine + 0.5 g each of proline and glycine. C = 200 g cassava only (uninoculated) control.

Table 7. Effect of particulate materials and some osmoregulators on proximate composition of fermented cassava at 72 h of fermentation.

Samples	Proximate analysis				Nutritional analysis	
	% Crude protein	% Crude fibre	% Ether extract	% Ash content	Phytic acid content	Tannins (mg/g)
A_1	3.38	4.63	0.76	2.10	0.009	0.15
A_2	2.63	8.30	1.01	1.93	0.041	0.38
A_3	3.06	4.06	1.26	1.91	0.048	0.34
B_1	3.50	6.54	1.12	3.80	0.500	0.31
B_2	5.69	4.52	0.92	1.92	0.026	0.26
B_3	6.06	5.87	0.79	2.00	0.006	0.18
C	1.33	3.44	0.74	1.70	0.004	0.12

A_1 = 200 g Cassava + 2.5 g each of soybean husk, plantarium, soybean meal, alumina, mash solid and brewer soluble. A_2 = 200 g Cassava + 2.5 g each of soybean husk, soybean meal and alumina 1.5 g mash and 1.5 ml brewer soluble. A_3 = 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. B_1 = 200 g Cassava + 1.0 g each of glycine, lysine, proline, tryptone and ash. B_2 = 200 g Cassava + 0.5 g each of tryptone, ash and lysine, 0.25 g of proline and glycine. B_3 = 200 + 0.25 g each of trytone, ash and lysine + 0.5 g each of proline and glycine. C = 200 g cassava only (uninoculated) control.

fermenter A may be due to the antimicrobial effect created by *L. planetarium*. Oyewole and Odunfa (1988) reported that reduction of other bacteria strain within 36 h of natural fermentation may be due to high acidity of the fermenting medium. Increase in total bacteria counts in other fermenters may be due to available particulate materials and osmoregulators utilized by them.

Increase in total reducing sugar content observed was a confirmation of starch degrading potential. Initially, total reducing sugar was high, but reduced within 24 h. This may be due to the utilization of available simple sugar for metabolic activities of lactic acid bacteria.

Afterwards, there was an increase in total reducing sugar. Longe (1980) reported similar reduction in reducing sugar within 24 h of fermentation. Ejiofor and Okafor (1981) reported that increase in total reducing sugar was due to the amylase enzymes that break down starch to sugar which are necessary for the growth of lactic acid bacteria.

Proximate composition of fermenter A with highest crude protein, crude fibre and lowest in ash contents and others may be due to the abilities of lactic acid bacteria *L. plantarium* to improve or enrich protein contents of the cassava products. Other fermenters except fermenter C had increase in proximate composition of crude protein and crude fibre. This may be due to the added particulate materials and osmoregulators as well.

The use of starter culture can be employed to control fermentation, improve odour and flavor and nutritional value of cassava product – 'fufu'. Moreso, addition of appropriate concentration of particulate materials and some osmoregulators to fermenting medium of cassava

can produce better acceptable cassava product.

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