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 Culture

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Abstract: The effect of particulate materials and osmoregulatators on lactictic 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 % crude protein contents, crude fibres, crude fat/ether, ash, phytic acid and tannin. Sample A_1 inoculated with varying 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 varying concentrations of osmoregulator. There was corresponding increase in sample A_2 and A_3 compare to sample B_2 and B_3. Sample C which serves as control had the lowest value in all at 24h, 48h and 72h of fermentation. Most of samples that contained varying concentration particulate materials had higher values in their proximate analysis and nutritional analysis than samples that contained varying concentrations of osmoregulators. Sample C with neither particulate materials nor osmoregulator had the least values in all analysis. [Academia Arena, 2009;1(6):8-14]. (ISSN 1553-992X).

Key Words: Particulate materials, Osmoregulatators, Lactictic 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 show that it is made up of water, 62%; carbonhydrate, 35%; protein, 1% and mineral salt, 1%. The major organic acids produced during cassava fermentation has been identified as including 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 the possible future benefits and has 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 varying the 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") for 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 and some osmoregulators- such as tryptone,

lysine, glycine and proline obtained from the Department of Biochemist, University for Development Studies and ash were used to study their effects on cassava fermentation using spontaneous and starter culture-Lactobacillus on cassava fermentation using 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 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 C$\pm$ 2^{o}C. The process was monitored on 24 hours 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^{o}C for 15 minutes 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 – 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. 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. Another three fermenters labeled B_1, B_2, B_3 were used to determine the effect of osmoregulations. These also were added in varying 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.

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, mash solid and 2.5ml of brewer soluble. Fermenter A_2 contained 2.5g of soy bean husk, soybean meal and alumina; 1.5g each mash solid and 1.5ml brewer soluble. Fermenter A_3 contained 1.5g each of soybean husk, soy bean meal and alumina; 2.5g mash solid and 2.5ml brewer soluble.

Effect of Varying Concentration of Osmoregulators on lactic fermentation of cassava spontaneously.

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

Legends/sample codes

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

Enumeration of Lactic Acid Bacteria and Total Bacteria

1ml 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

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. C 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

Effect of varying concentration of particulate materials and some osmoregulators on total dissolved solids (mg/L) using spontaneous fermentation were shown in Table 1 and 2 respectively. In Table 1, sample C had reduced total dissolved solids, while sample A_1, A_2 and A_3 had highest total dissolved solids. Sample 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.

In Table 2, sample B_1 B_2 and B_3 had their dissolved solids ranged from 500gg1h to 1,400mg1h. After 72 hours of fermentation.

Table 3 showed the effect of varying concentration of particulate materials and some osmoregulators 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 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 4 showed the effect of varying concentration of particulate materials and some 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 bacterial counts. Proximate composition of the entire sample at various 24, 48, 72 hours of fermentation are shown in Table 5, 6 and 7 respectively. Table 5, at 24 hours, Sample C that had lowest crude protein, fibre, ether extract, ash and tannins. Sample $A_1$and A_3, had the highest crude protein, and tannins. Sample B_3 had highest crude fibre and ash.

In table 6 at 48 hours of fermentation, sample A_3 B_1 and B_3 had highest crude protein content, while sample C had the least protein contents. A_3 had highest crude fibre while B_3 had least crude fibre.

In table 7, at 72 hours of fermentation, sample $A_1$and B_3 had highest crude protein while C had least crude protein Sample A_2, and B_1 had highest crude fibre.

From table 5, 6 and 7, sample $A_1$and B_3 had highest crude protein. Sample A_1 showed highest crude fibre. Sample 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 during Fermentation of Cassava (mg/ml)

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

Table 2: Effect of Varying Concentration of Some Osmoregulators on Total Dissolved Solids during Adetunde, I. A **Fermentation of Cassava (mg/ml)**

Samples	Fermentation Time (Hours)		
	24	48	72
B_1	100	1350	1400
B_2	540	640	700
B_3	500	660	700
C	300	520	600

Table 3: Effects of Varying Concentration of Particulate Materials and Some Osmoregulators on Total Reducing Sugar (mg/ml)

Samples	Fermentation Time (Hours)			
	0	24	48	72
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

Table 4: Effect of Varying Concentration of Particulate Materials and Some Osmoregulators on Micro Bila Loads during Fermentation of Cassava (cfu/ml X 10^9)

Samples	Time (Hours)					
	24		48		78	
	Total bacteria count on PCA	Lactic Acid Bacteria counts on MRS	Total bacteria PCA	Lactic bacteria counts on MRS	Total bacteria counts PCA	Lactic acid counts on MRS
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
B1	3.44	2.60	3.50	3.20	3.52	3.32
B2	3.34	2.62	3.42	3.22	3.40	3.38
B3	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

Table 5: Effect of Particulate and Some Osmoregulators on Promixate Composition Fermented Casssava at 24hrs Fermentation

Samples	Proximate Analysis				Nutritional Analysis	
	%crude protein	% crude fibre	% ether extract	% ash content	5 phytic content	Tannins mglg
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
B1	2.63	4.18	1.18	233	0.013	0.06
B2	3.94	3.41	1.21	2.48	0.012	0.16
B3	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

Table 6: Effect of Particulate and Some Osmoregulators on Promixate Composition Fermented Casssava at 48hrs Fermentation

Samples	Proximate Analysis				Nutritional Analysis	
	%crude protein	% crude fibre	% ether extract	% ash content	5 phytic content	Tannins mglg
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
B1	4.38	3.28	0.92	2.82	0.011	0.09
B2	3.68	3.16	0.97	0.75	0.016	0.14
B3	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

Table 7: Effect of Particulate Materials and Some Osmoregulators on Proximate Composition of Fermented Cassava at 72hrs of Fermentation

Samples	Proximate Analysis				Nutritional Analysis	
	%crude protein	% crude fibre	% ether extract	% ash content	5 phytic content	Tannins mglg
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
B1	3.50	6.54	1.12	3.80	0.500	0.31
B2	5.69	4.52	0.92	1.92	0.026	0.26
B3	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

Legends/ Sample codes

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.

B_1 = 200g Cassava + 1.0g each of glycine, lysine, proline, tryptone and ash.

B_2 = 200g Cassava + 0.5g each of tryptone, ash and lysine 0.25g of proline and glycine.

B_3 = 200g + 0.25g each of trytone, ash and lysine + 0.5g each of proline and glycine.

C = 200g Cassava only (Uninoculated) control.

Discussion of Results and Conclusion

The majority of the Lactic Acid bacteria encountered belongs to Lactobacillus Plantarium and has 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 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 hours 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 is a confirmation of starch degrading potential. Initially, total reducing sugar was high, but reduced within 24 hours. 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 hours 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 abilities of lactic acid bacteria, L. plantarium to improve /or enrich protein contents of the cassava products. Other fermenters except fermenter C had increased 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 interim of nutritional value.

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