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Effect of Different Brine Concentration on the Quality of 'Fufu' Flour

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Abstract:
The study was carried out to establish the effect of brine at different concentration (10%, 15% and 20%) on the quality of 'fufu flour'. The tubers were peeled, washed and soaked in brine at different concentrations of 10%,15%,20% and water without brine. All the samples were allowed to ferment naturally for 96 hours at room temperature (25°C ± 1) and processed into "fufu" flour. Microorganisms were isolated and identified every 24 hours during fermentation using standard microbiological methods. During fermentation the changes in pH, hydrogen cyanide and total titratable acidity were determined. The proximate composition and selected mineral, functional properties of the fufu flour samples were determined. Sensory qualities of the fufu flour were determined. A total of nineteen (19) different microorganisms were isolated during the fermentation. The following microorganisms were isolated; Lactobacillus plantarum, Lactobacillus buchnerri, Lactobacilluscasei, Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus cellobiosus, Esherishia coli, Enterococcus aerogenes, Saccharomyces cerevisiae, Candida sp, Bacillus subtillus, Corynabacterium sp. and Bacillus coagulans. Fermentation reduced pH (5.98±0.01-4.87±0.01), hydrogen cyanide (75.50±0.01-22.17±0.06) and total titratable acidity (0.1±0.021-0.3±0.042). The quality of fufu fermented in 10% brine fermented sample had the best qualities and was generally acceptable.

Keywords: *Brine, different concentration, fermentation cassava, proximate composition, sensory qualities*

1. Introduction

Cassava *(Manihot esculenta* Crantz) is a very popular high energy root crop consumed in the tropical and many regions of the developing world. It is a shrubby, tropical, perennial plant that is not well known in the temperate regions. It is the seventh most important crop of the world and constitutes a staple food for about 12.50% of the world population (Hans, 1995). Cassava serves as a food security and income generation crop for millions of people in the developing world (Scott and Rosegrant, 2002). The cassava plant leaves are the same in shape but different in sizes. The cyanogenic glucosides content in the cassava roots also vary according to the studied varieties (Gomez *et al,* 1984).

The wild population of *Manihot esculenta* subspecies *flabellifolia*, shown to be the progenitor of domesticated cassava are centered in West Central Brazil where it was likely first domesticated not more than 10,000 years BC (Olsen and Schaal, 1999). By 6,000 BC, manioc pollen appears in the Gulf of Mexico low lands at the San Andres archaeological site (Pope *et al,* 2001). The oldest direct evidence of cassava cultivation comes from 1,400-year-old Maya site, Joya de Ceren in El Salvador, although the species *Manihot esculenta* likely originated further in South Brazil and Paraguay. With its high food potential, it has become a staple food of the population of northern south America, southern Meso-America and the west indies and it cultivation was continued by the colonial Portuguese and Spanish (Olsen and Schaal, 1999).

Cassava (*Manihot esculenta* Crantz) also called yucca, manioc and mandioca in various parts of the world has its origin in Brazil where it is the major staple food of the people (RMRDC, 2004). In 2010, Nigeria produced 45million tones making it the world's largest producer (Sobowale *et al*, 2007). Over 70% of production in Nigeria is consumed locally (Daramola and Osanyinhusi, 2006; Oyewole and Biola, 2006). Despite their higher cyanides content, the bitter cassava varieties are more predominantly utilized (RMRDC, 2004).

Brining is the application of salt in the fermentation of foods such as fish, vegetable and fruits. Brining has been used to control microbial proliferation and to inhibit the growth of putrefactive organisms (Hans, 1995).

During brining of vegetables, bacteria use up the natural sugar present in the vegetable to produce lactic acid also releasing carbondioxide gas, which bubbles to the surface of the brine with an agitating effect and fermentation stops when there is no further available sugar (Hans,1995). Brining of vegetable during lactic acid fermentation enhances flavour of the product and the salt provides a crisp texture (Steinkraus, 1996).

Vegetable fermentation results in an aerobic condition that promotes carbondioxide build up and due to bacterial activity (Steinkraus, 1996).

Fermentation has been reported to be responsible for product stability, flavour development, and cyanide elimination (Hans, 1995).

According to Oyewole and Biola (2006), organic acids produced during fermentation include lactic, acetic, butanoic and propanoic acids and these are believed to contribute to characteristics flavour of fermented cassava products'. The most important contribution of fermentation is the release from the plant tissue of the enzyme linamarase, which is involved in the breaking down of linamarin and lotuastraline (Cyanogenic glycosides) of cassava which releases HCN and thus detoxifies the product (Oyewole and Ogundele 2001).

1.1. The objectives of this research are to:

- determine the effects of spontaneous and brine (at different concentrations) on the composition of 'fufu' flour
- monitor the effect of brine at different concentrations on the microbial activities during the fermentation of cassava to produce "fufu''
- evaluate and compare the quality of ''fufu'' from two fermentation techniques in terms of their sensory evaluation, cyanide level, proximate composition, functional properties and mineral content.

2. Materials and Methods

2.1. Collection of Raw Materials

The raw cassava tubers (*Manihot esculenta* Crantz) were purchased from Oja Oba of Akure, Ondo State. The tubers were processed into 'fufu' immediately after harvesting.

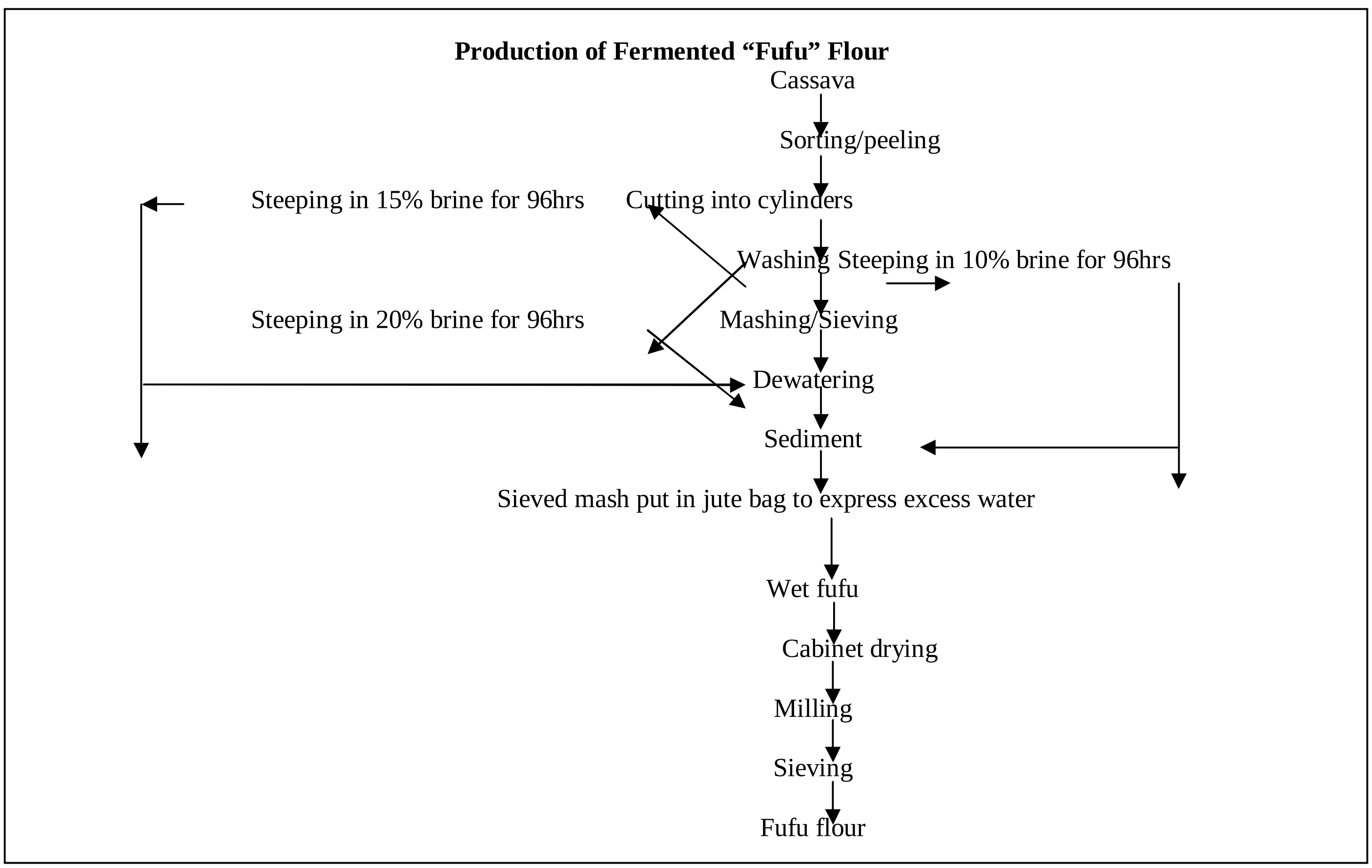

Figure 1: Sequence of operations employed in the processing of the fufu flour samples (Oyewole,1991)

2.1.1. Morphological and Biochemical Test

Microorganisms isolated from the fermenting cassava roots were generally subjected to the following morphological, biochemical and physiological tests following the procedures described in Bergey's manual of systematic Bacteriology (David*et al*,2012) Physico-Chemical Analysis

Changes in some physical, chemical, nutritional and organoleptic features of the unfermented cassava roots and samples collected at various stages of fermentation and the finished product were assessed.

2.1.1.1. Organoleptic (Sensory) Evaluation Test

A 15man trained panel which are familiar with "Fufu" flour was asked to assess the qualities of the fermented sample considering the appearance, odour (Level of presence of inherent odour), smoothens or texture in the mouth, flavour, overall acceptability. Nine points Hedonic scale was used from the evaluation with '9' having excellent acceptability for the attribute and '1' indicating high characteristic differences from normal or very low acceptability. The final scores represent the means of the entire panelist's impression. This was then analyzed statistically by analysis of variance (ANOVA) by using Duncans multiple rangeat 5% level of significance.

3. Results

3.1. Microorganisms Isolated During the Fermentation Process

A total of 19 microorganisms were isolated during the fermentation process. The coliforms isolated were; *Esherishia coli* and *Enterococcus aerogenes.* The total viable bacteria isolated were *Bacillus subtillis, Corynebacterium spp; Klebsiella aerogenes, Bacillus coagulans* while the lactic acid bacteria isolated were *Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacilluscellobiosus, Pediococcus spp; Lactobacillus casei and Lactobacillus brevis.* The fungi (yeast and mould) isolated were *Candida tropicalis, Saccharomyces cerevisiae, Geotricum klebahnii, Aspergillus niger.*

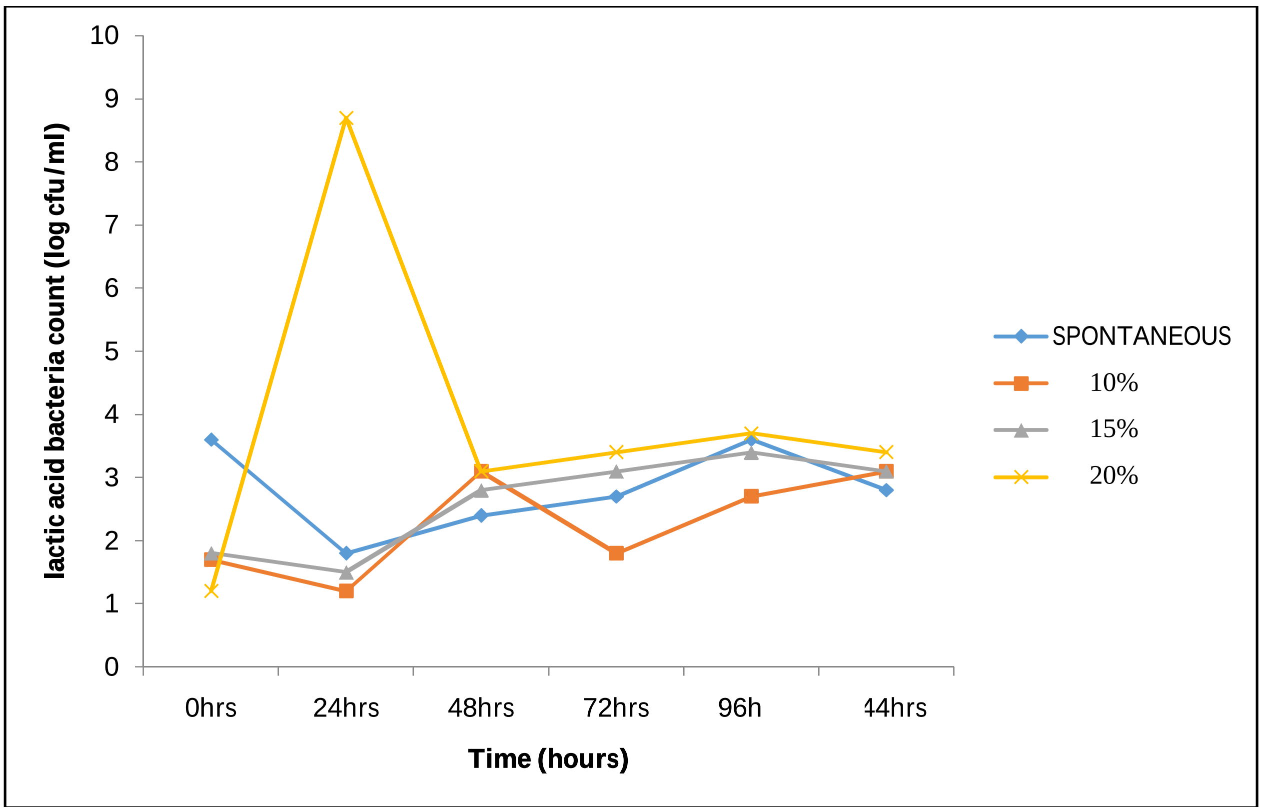

Figure 2: Total Lactic Acid bacteria counts during spontaneous, 10%, 15%, and 20% brine fermentation of cassava for fufu production

Keys:
10%: Fermented samples with 400g of brine
15%: Fermented samples with 600g of brine
20%: Fermented samples with 800g of brine

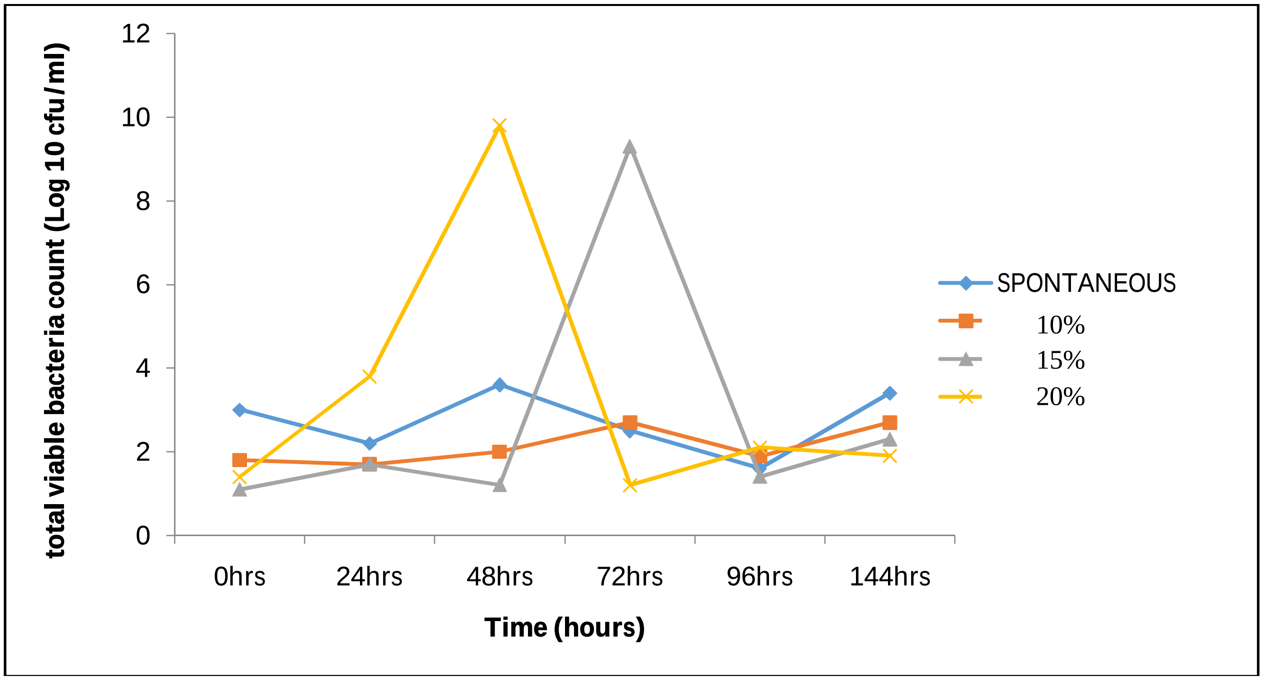

Figure 3: Total viable count during spontaneous, 10%, 15% and 20% brine fermentation of cassava for fufu production

Keys:
10%: Fermented samples with 400g of brine
15%: Fermented samples with 600g of brine
20%: Fermented samples with 800g of brine

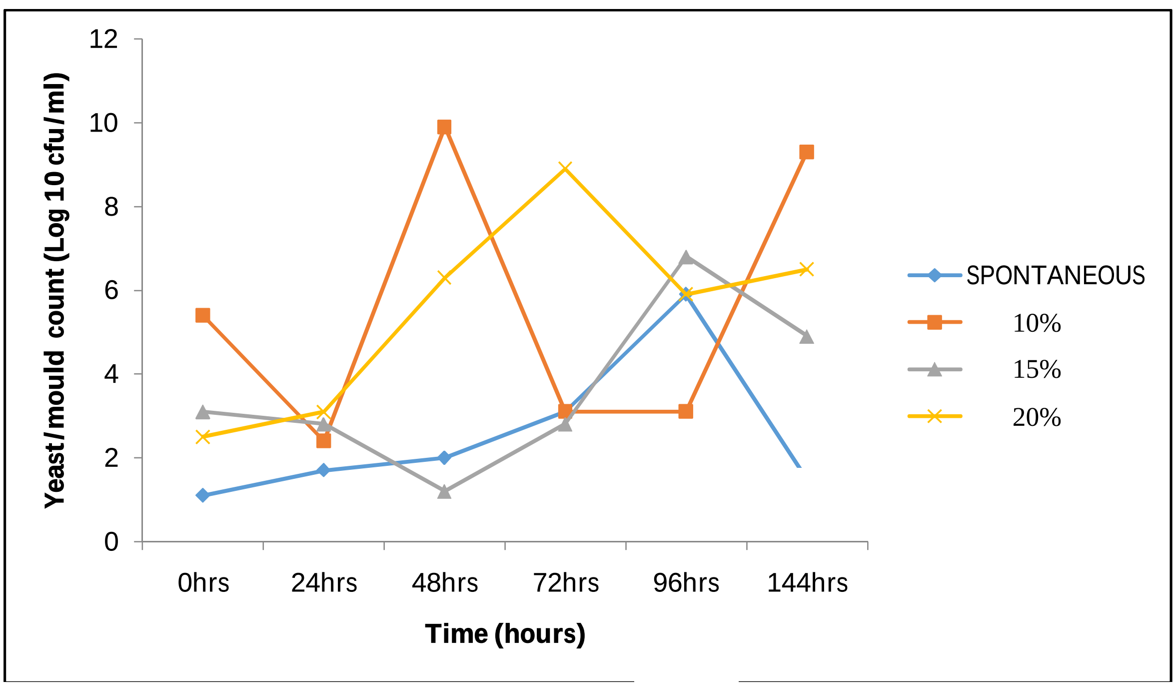

Figure 4: Total Yeast/Mould count during spontaneous, 10%, 15% and 20% brine fermentation of cassava for fufu production

Keys:
10%: Fermented samples with 400g of brine
15%: Fermented samples with 600g of brine
20%: Fermented samples with 800g of brine

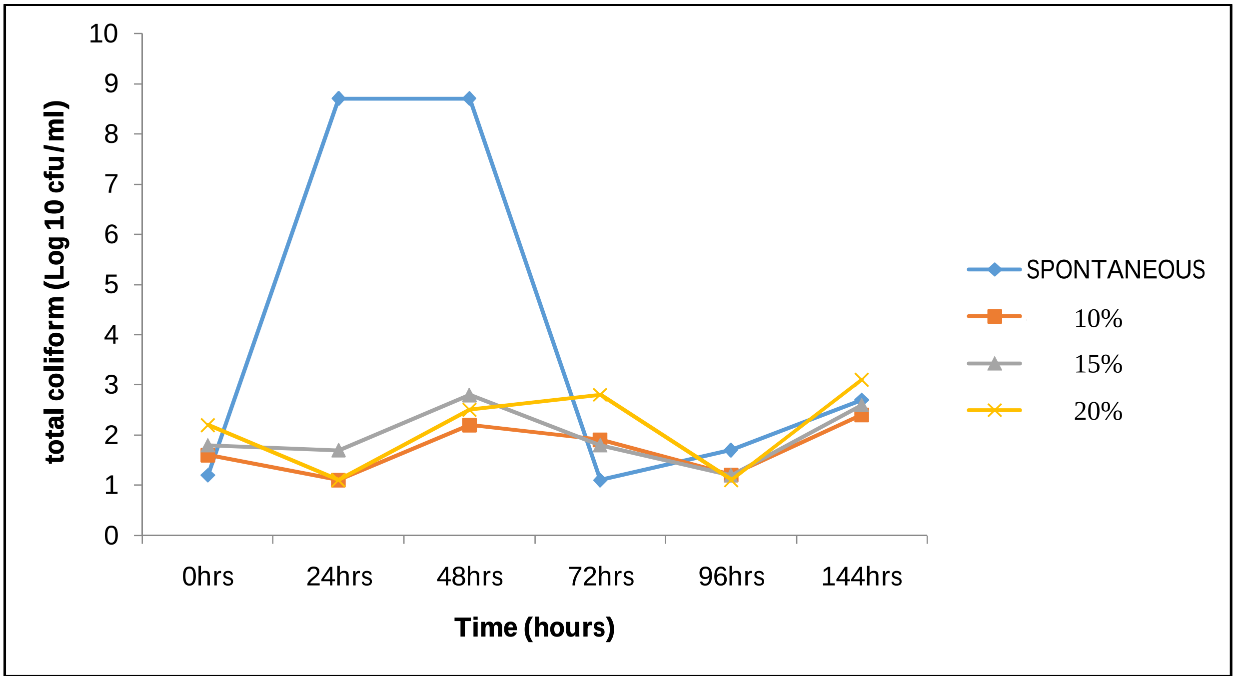

Figure 5: Total coliform count during spontaneous, 10%, 15% and 20% brine fermentation of cassava for fufu production

Keys:
10%: Fermented samples with 400g of brine
15%: Fermented samples with 600g of brine
20%: Fermented samples with 800g of brine

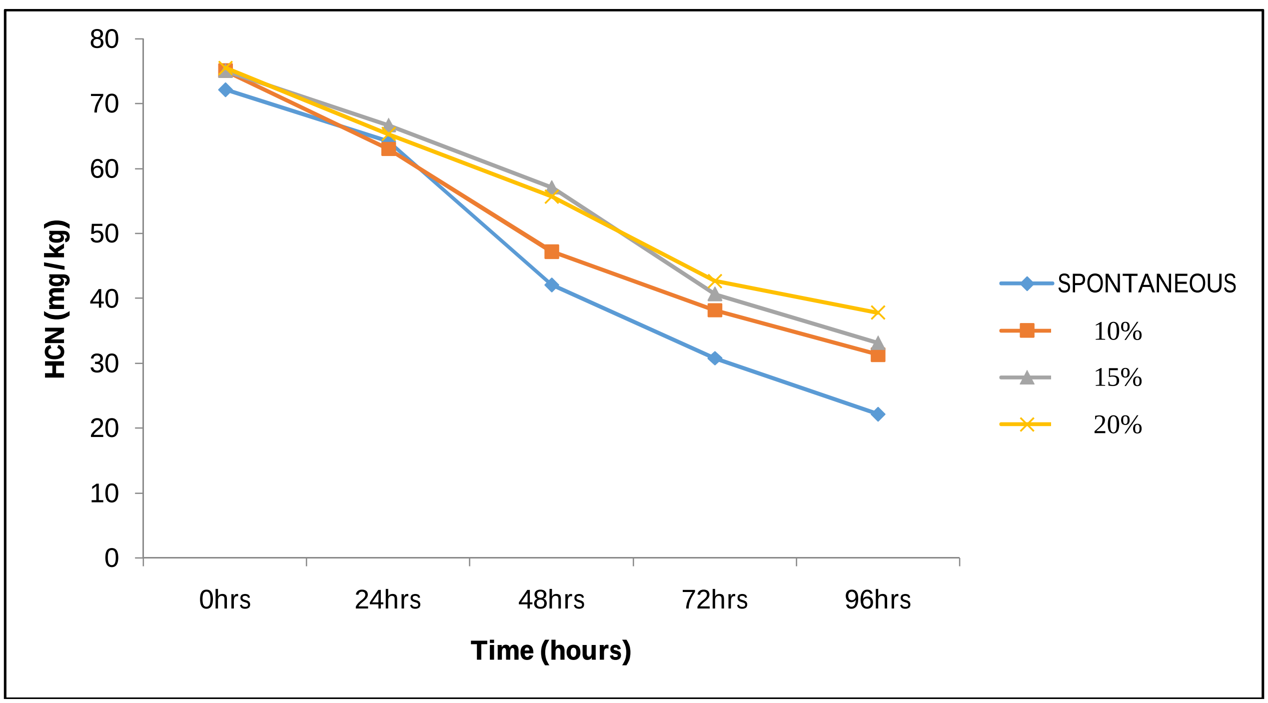

Figure 6: changes in hydrogen cyanide content of cassava root during fufu production by spontaneous, 10%, 15%, and 20% brine fermentation

Keys:
10%: Fermented samples with 400g of brine
15%: Fermented samples with 600g of brine
20%: Fermented samples with 800g of brine

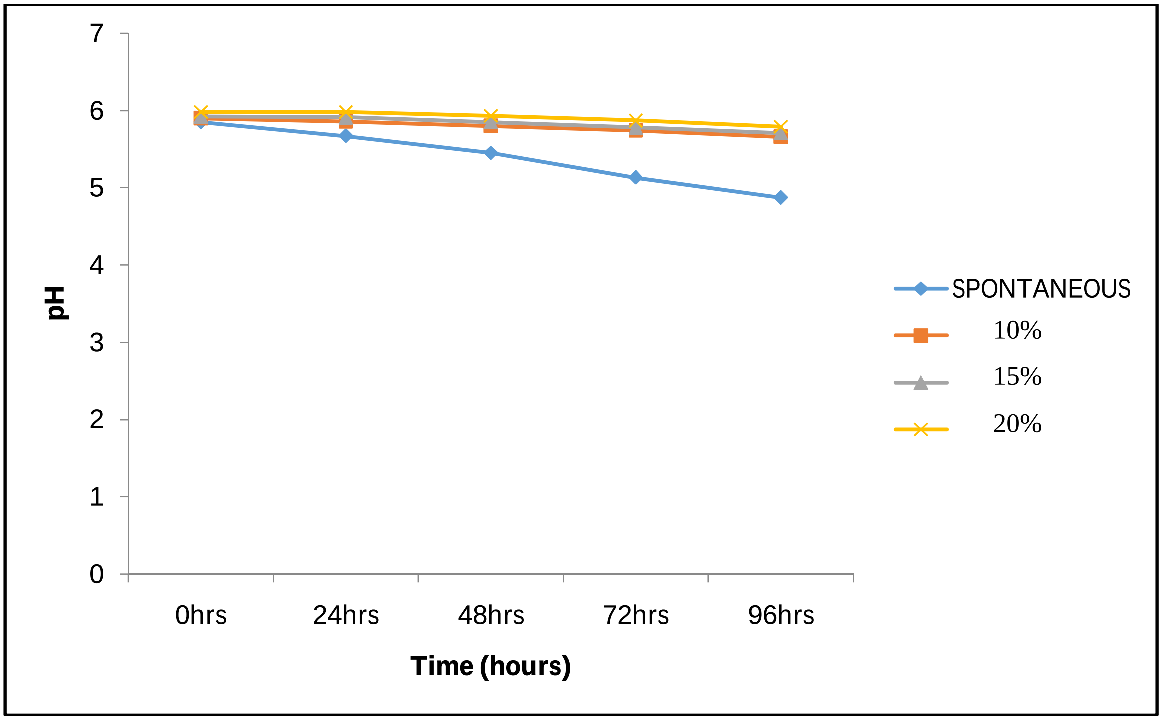

Figure 7: pH Changes during the fermentation of Cassava root for fufu production by spontaneous, 10%, 15% and 20% brine fermentation

Keys:
10%: Fermented samples with 400g of brine
15%: Fermented samples with 600g of brine
20%: Fermented samples with 800g of brine

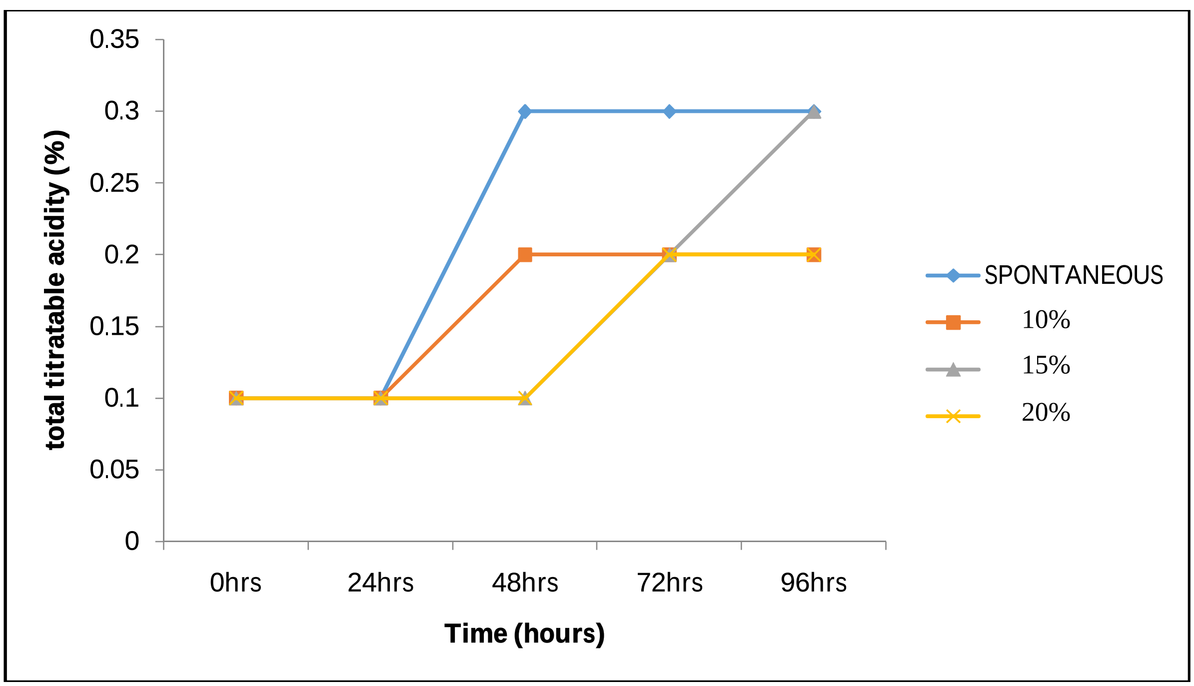

Figure 8: Change in the Total Titratable Acidity levels during the fermentation of Cassava root for fufu production by spontaneous 10%, 15% and 20% brine fermentation

Keys:
10%: Fermented samples with 400g of brine
15%: Fermented samples with 600g of brine
20%: Fermented samples with 800g of brine

Samples Parameters	Spontaneous	10%	15%	20%	LSD
Moisture content (%)	12.98±0.049^{a}	11.24±0.010^{b}	12.98±0.049^{a}	8.67±0.021^{d}	0.1109
Protein (%)	1.13 ±0.010^{d}	1.65 ±0.055^{c}	1.78 ±0.010^{b}	1.99 ±0.058^{a}	0.0760
Carbohydrates	87.37±0.021^{a}	85.5±0.027^{b}	84.47±0.021^{c}	82.37±0.040^{d}	0.0421
Fat (%)	0.26±0.010^{a}	0.26 ±0.08^{a}	0.24±0.0058^{b}	0.22±0.0058^{c}	0.0154
Ash (%)	1.12±0.014^{b}	1.13±0.006^{b}	1.11±0.0057^{c}	1.15±0.01^{a}	0.0109
Fibre (%)	1.45±0.01^{a}	1.43±0.006^{b}	1.4±0.0058^{c}	1.39±0.001^{d}	0.0154

Values are Mean±Standard Deviation(SD) of triplicate determinations. Means with different superscripts within the same row are significantly different ($p<0.05$). LSD: Least significant difference

Table 1: proximate compositions of traditionally, 10%, 15% and 20% brine fermented "fufu" flour samples.

Keys:
10%: Fermented samples with 400g of brine
15%: Fermented samples with 600g of brine
20%: Fermented samples with 800g of brine

Samples Parameters	Spontaneous	10%	15%	20%	LSD
Swelling Index	28.73±0.012^{d}	29.14±0.001^{c}	30.74±0.006^{b}	31.64±0.02^{a}	0.0243
Bulk Density	1.73±0.021^{d}	1.81±0.012^{c}	2.31±0.01^{b}	2.44±0.023^{a}	0.0326
WAC	1.0±0.0058^{d}	1.16±0.026^{c}	1.23±0.01^{b}	1.37±0.006^{a}	0.0277

Values are Mean±Standard Deviation(SD) of triplicate determinations. Means with different superscripts within the same row are significantly different ($p<0.05$). LSD: Least significant difference

Table 2: The function properties of the traditional, 10%, 15% and 20% brine fermented 'Fufu' flour samples

Keys:
10%: Fermented samples with 400g of brine
15%: Fermented samples with 600g of brine
20%: Fermented samples with 800g of brine

Samples Parameters	Spontaneous	10%	15%	20%	LSD
Calcium	0.97±0.01^{d}	2.24±0.011^{c}	2.76±0.01^{b}	3.44±0.055^{a}	0.0546
Mg	0.60±0.047^{d}	0.68±0.006^{c}	0.95±0.006^{b}	1.46±0.0306^{a}	0.0535
K	0.33±0.0058^{d}	0.47±0.050^{c}	0.61±0.006^{b}	0.69±0.010^{a}	0.0130
Phosphorus	0.08±0.010^{a}	0.088±0.001^{a}	0.093±0.0006^{a}	0.097±0.001^{a}	0.0095
Fe	3.10±0.1^{d}	3.52±0.005^{c}	3.81±0.01^{b}	4.25±0.017^{a}	0.0962
Na	0.27±0.0058^{d}	0.56±0.016^{c}	0.63±0.01^{b}	0.71±0.007^{a}	0.0154

Values are Mean±Standard Deviation(SD) of triplicate determinations. Means with different superscripts within the same row are significantly different ($p<0.05$). LSD: Least significant difference

Table 3: The mineral contents (mg/100g) of the traditional, 10%, 15% and 20% brine fermented 'fufu' flour samples

Keys:
10%: Fermented samples with 400g of brine
15%: Fermented samples with 600g of brine
20%: Fermented samples with 800g of brine

Samples Parameters	Spontaneous	10%	15%	20%	LSD
Taste	6.93^{b}	7.07^{a}	7.07^{a}	6.87^{b}	0.970
Appearance	8.13^{a}	7.60^{b}	7.07^{c}	7.80^{b}	0.653
General Acceptability	7.87^{a}	7.40^{a}	7.33^{a}	7.67^{a}	0.938
Aroma	7.47^{a}	7.27^{a}	6.87^{b}	7.20^{a}	0.921
Texture	7.73^{a}	7.33^{a}	7.27^{a}	7.73^{a}	0.886

Means with different superscripts within the same row are significantly different (p<0.05). LSD: Least significant difference

Table 4: Sensory properties of traditionally, 10%, 15% and 20% brine fermented "fufu" flour samples.

Keys:
10%: Fermented samples with 400g of brine
15%: Fermented samples with 600g of brine
20%: Fermented samples with 800g of brine

4. Discussion

All the Lactic acid bacteria isolated during the fermentation process were species of *Lactobacillus* except for the *Pedioicoccus.* This is similar to the findings of Kobawila et al; (2005) who found out that most of the lactic acid bacteria involved in cassava fermentation were of the *Lactobacillus* species. There was increase in the total viable bacteria Counts form 0 – 96 hours of the fermentation and apparent increase in the lactic acid bacteria counts throughout the fermentation period this is similar to the findings of Fagberii *et al*: (2006) who reported that the total viable counts increase with increasing fermentation time while the counts of the lactic acid and fungi increased at the later stage of fermentation due to the acidity of the medium. It is believed that these lactic acid bacteria that were implicated in brine fermentation of cassava roots may be as a result of these bacteria having the ability to withstand the different concentrations of brine (10%, 15% and 20%) employed in the fermentation process.

Non-involvement of *Lactobacillus acidophilus*, L. *brevis* and *L. cellobiosus* in the brine at different fermentation techniques employed is probably due to intolerance of these organisms to the fermenting medium whereas all the lactic acid and bacteria isolated where implicated in the spontaneous techniques. Occurrence of *Saccharomyces cerevisiae and Candida* species as the prevalent yeasts during the fermentation process has been reported by Oyewole (1990). Yeast was not discovered during the brine fermentation of cassava roots to produce "fufu" flour until the 48 hours of the fermentation process this may be due to intolerance of the yeast to the brine. This has not been reported. The two Coliforms bacteria isolated as(*E. coli and Enterobacter aerogenes)* at the 0 hour in brine at different concentrations and spontaneous fermentations were later eliminated as the fermentation progressed could have found their way into it during the preparation and handling of the sample for fermentation (Adegore and Babalola, 1998). During the fermentation process, the pH reduced gradually as the fermentation progressed in all the fermentation techniques employed. This is in accordance with the findings of Mahingu *et al;* (1867) who reported that during steeping of cassava roots, fermentation decreases pH, softens the roots and helps to reduce potentially toxic cynogenic compounds. Fleming, (1982) had also reported that during brine fermentation of vegetable, bacteria of *Lactobacillus* group convert the natural sugar present in the vegetable to lactic acid thereby decreasing the pH with the liberation of carbon dioxide on the surface of the brine solution.Gradual increase in total titratable acidity (TTA) observed as the fermentation progressed is in accordance with the finding of Oyewole and Ogundele (2001) who attributed the increase to the production of organic acids during the fermentation. Also, Adenye and Ogunjobi (1998) reported that the production of organic acid compounds due to the activities of microorganisms during fermentation of cassava roots are responsible for the increase acidity during the fermentation process. Oyewole and Odunfa (1990) also attributed the increasing acidity during the fermentation to the activities of the lactic acid bacteria on the carbohydrate of cassava roots.

The reduction observed in the Hydrogen Cyanide (HCN) content of the "fufu" "wetcake" after 96 hours of fermentation and processing may be as a result of microbial enzymes activities during fermentation process. This is in agreement with the finding of Kobawila*et al*; (2005), who reported that fermentation process effectively reduce the Cyanogenic potential of different cassava varieties to safe level through microbial enzymes activities which is usually release during fermentation.

The reduction in HCN of brine fermented "fufu" (wet cake and flour) sample despite the fact that roots did not ret after 96 hours of fermentation unusual but this reduction could probably be as a result of fermentation of the cassava roots. This is consistent with the finding of Westly *et al;* (2007) that microorganisms play little or no role in Cyanogens reductions during grating fermented cassava roots.

The significantly ($0 \leq 0.05$) lower moisture content obtained in the brine fermented "fufu" flour samples are an indication of a good stable shelf life than the traditionally fermented sample. The four flour samples have values of moisture content that are within the recommended standard of 13% (m/m) for edible cassava four (Sanni *et al*; 2005).

The significantly ($0 \leq 0.05$) lower ash content values observed in the spontaneous, 15% and 20% brine fermented samples may probably be as a result of inability of the cassava roots to ret very well after 96hrs of fermentation thereby preventing leaching of mineral nutrient during brine fermentation process as proved by Aderiye (1998). There was a reduction in the crude fibre content of the 20%, 15% and 10% brine fermented "fufu" flour samples compared with traditional fermentation. This may probably due to the higher microbial activities recorded during brine fermentation of cassava roots as reported by Odunfa (1992).

The crude protein of the 10%, 15%, and 20%fermented brine "fufu" flour samples were significantly ($0 \leq 0.05$) higher than that of the flour sample obtained from the traditional fermentation. This could be as the result of the NaCl which prevent the cassava from losing it nutrients also the protein in the lactic acid bacteria in the brine fermented samples can increase the protein content of the fufu flour. The carbohydrate content of the 10%, 15% and 20% brine fermented samples were lower than that of the traditionally fermented sample. This can be as the result of high lactic acid bacteria activities which took place in the brine fermented samples that may have reduce the carbohydrate content (Odunga, 1998).

Generally, variations in proximate composition could be attributed to the fermentation and processing techniques employed (Etudaye *et al;* 2009).

The results of the functional properties show that 10%, 15% and 20% brine fermented samples have the highest water absorption capacity observed in the samples could be as a result of the Nacl used for the fermentation. This is in accordance with the findings of Etudaiye *et al.,* (2009) who reported that fermentation increases the water absorption capacity of 'fufu' flour because during fermentation, proteolytic activity takes place which causes increase in the number of polar groups and this would increase hydrophilicity of the flour proteins. The increase in water absorption capacity may also be as a result of the higher protein content observed in these three fermented samples which increase the water imbibing capacity (Etudaiye *et al;*2009). The significant ($P \leq 0.05$) highest Water absorption capacity observed in the brine fermented flour samples could be as a result of the 10%, 15%,20% NaCl (common salt) used for the fermentation. This is similar to the report of Fagbemi and Ijah (2004) that attributed an increase in Water absorption capacity of seed flour at salt concentration of 0.5-1% to electrostatic repulsion at low salt.

The lower bulk density observed in spontaneous "fufu" flour samples may be due to the utilization of part of the carbohydrate and its hydrolysis during fermentation and processing of the "fufu wet cake" to flour (Etudaiye *et al;* 2009)

The values of CA, Mg, K, P, Fe and Na for all four samples were higher than the values reported by Sobowale *et al*; (2007). High values in brine fermented samples could be due to NaCl used for the fermentation of the cassava root. This implies that 'fufu' flour can be fortified with Na and K through brine fermentation.

The general preference for the 10% brine fermented sample may be due to the addition of brine which resulted in changes in the taste that made it more acceptable. This is similar to the findings of Ranken (1988), who proved that brine fermentation encourages both physical and chemical changes which eventually affect the appearance, texture and flavour during brine fermentation of vegetable. Steinkraus (1996) also reported that brining of vegetables during lactic acid fermentation enhances flavour of the product and the salt provides a crisp texture. Tandon *et al.* (1986) also found out that the carbonates found in common salts used for brining result in pickles that have a soft texture.

5. Conclusion

This research was carried out on the effect of brine at different concentrations on the fermentation of cassava for fufu flour production.

The results of the research showed that some species of yeast, Bacillus and Lactic acid bacteria can tolerate brine fermentation of cassava tubers at 10%,15% and 20% brine concentration respectively. There was decrease in the hydrogen cyanide in all the three (3) brine fermented samples of cassava than in the traditional fermented samples, although processing of the cassava can further decrease the hydrogen cyanides of the cassava., Proximate composition such as protein, fat, carbohydrate contents were all increased in brine fermented samples (10%, 15% and 20%) than in the usual traditional fermentation.

There were also increase in Na, K, Ca and other minerals in the brine fermented fufu flour (10%, 15% and 20%) than the traditional sample.

More so, the swelling index obtained in all the brine fufu flour were lower than the traditional fufu flour and bulk density of the fermented brine samples were higher to that of the traditional sample. 20% concentration of brine has the highest water absorption capacity than the traditional samples.

Sensory evaluation shows that 10% brine fermented fufu flour sample was generally acceptable than 20%, 15% brine fermented fufu as well as the traditional samples.

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