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Comparative Assessment of Fermentation Techniques in the Processing of Fufu, a Traditional Fermented Cassava Product

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Abstract: Fufu is a traditional Nigerian fermented cassava food product. Due to the production of objectionable odour that is disliked by many people, two improved techniques were used to ferment cassava and its performance compared with that of the traditional process. In one process, cassava fufu was produced involving the steeping of cassava tubers for 48h followed by grating and fermenting for another 48h. Another technique involved grating cassava tubers, dewatering/fermentation for 24h before re-steeping for another 48h. Quantitative determination of microbial, chemical and sensory changes that occurred during a 96h-fermentation period was studied. The dominant microflora was a mixed population of lactic acid bacteria, *Bacillus* spp and yeasts. The microflora was more diverse and with higher counts in the traditional product after 24h. Initial counts were 8.88log c.f.u./g whereas the respective counts in samples grated prior to fermentation or soaked and grated were 6.32 and 8.55. It then increased to 9.24log c.f.u/g after 48h fermentation. Enterobacteriaceae counts increased during the first 48h but fell below detectable levels after 72h in the traditional product and after 24h in the modified process. The pH decreased from 6.8 to 4.3 in the traditional process and from 6.6 to 4.2 in the modified process. The titratable acidity increased from 0.36 to 4.0% (w/w lactic acid) in the traditional product and from 0.24 to 1.0%, respectively, in the modified process. Grated mash fermentation reduced the cyanogenic glycosides content by 85.5% in 72h compared with 79.5% in the traditional fermented product. Odour and flavour ratings were significantly higher ($p< 0.05$) for the modified process. There was no difference in colour or texture due to the processing method. Fermentation of grated cassava produces a more acceptable product.

Key words: Cassava, lactic fermentation, fufu, sensory attributes, microbial flora

Introduction

Cassava (*Manihot esculenta* Crantz) is the staple food of more than 500 million people (Cock, 1982) and is a typical crop in developing countries. Cassava roots are potentially toxic due to the presence of cyanogenic glycosides especially linamarin (Butler, 1965). Physiological deterioration occurs in cassava roots 2-5 days after harvesting followed by microbial deterioration 3-5 days later (Rickard and Coursey, 1981).

Cassava farming populations have empirically developed several processing methods for stabilizing cassava and reducing its toxicity (Lancaster *et al*., 1982). Fermentation, which is part of almost all these processes, is widely used to transform and preserve it because of its low technology and energy requirements and the unique organoleptic qualities of the final product (Daeschel *et al*., 1987). Fermentation of cassava entails steeping roots in water for 3 to 4 days. During the consequent fermentation, roots are softened. Disintegration of the tissue structure results in contact of linamarin with linamarase which is located in the cell walls (Mkpong *et al*., 1990) and subsequent hydrolysis to glucose and cyanohydrins, which easily break down to ketone and HCN (Cooke, 1978). Fufu, one of the major food forms of cassava fermentation, is reconstituted by stirring in boiling water to form a dough and eaten with flavoured sauces. One potential problem in processed fufu is the flavour of the product, which may be undesirable to many people.

The fermentation process is initiated as a result of chance inoculation by microorganisms from the environment. Although, convenient there are concerns about its reliability the control of which is the basis of all technological measures that are used to obtain product at a defined quality. The presence of unspecified microorganisms complicates the control of the fermentation process and lead to the production of objectionable odours. Such problems have led to the development of several other processing techniques suitable for odourless fufu (Okpokiri *et al*., 1984; Ezeronye, 2003). Okolie *et al*. (1992) proposed a modification of the microbiological process in order to upgrade the cassava product but in practice is yet to receive great attention. This work is aimed at studying the impact of different fermentation techniques on fufu aroma as monitored by microbial population and the potential of replacing the traditional spontaneous fermentation with other techniques during production of fufu.

Materials and Methods

Cassava roots: Approximately 20kg of the roots of 12-month old NR8082 cassava cultivar was harvested from a farm at the National Root Crops Research Institute, Umudike, Nigeria. The roots were used immediately after harvest for the production of fufu.

Processing of cassava roots: Three different soaking procedures corresponding to the methods traditionally used in Nigeria were examined (Fig. 1). Fermentation experiments for each technique studied were prepared in triplicate. In all cases 20kg of cassava roots were soaked in a final volume of 5 liters of sterile tap water in glass jars covered with aluminum foil. In the standard procedure (TFF-traditional fermented fufu), the fresh and peeled cassava tubers were mixed directly with the tap water and allowed to ferment for 96h by the natural microflora.

In procedure 2 (SGF-soaked and grated fufu), the cassava roots were washed, peeled and soaked in sterile containers for 48h. The part-fermented tubers are removed and re-washed before pulping in a mechanized commercial grating machine. The grated pulp (mash) was put into a clean doth sack and left to ferment for another 48h at ambient temperature before dewatering. For procedure three (GFF-grated fermented fufu), peeled and washed cassava tubers were grated in a mechanized grater. The mash was left to ferment during dewatering for 24h at ambient temperature before sieving in a coarse sieve to remove fiber. The mash was re-steeped for 48h before pressing out the juice with a screw press. Samples were aseptically withdrawn at daily intervals for 4 days of fermentation (including the period of steeping). Microbial enumerations were carried out from freshly drawn samples while pH, total titratable acidity and reducing sugar were determined from samples stored at 5°C.

Microbiological analyses: Daily changes in the microbial population of the total viable bacteria, lactic acid bacteria coliforms and fungi were determined using plate count agar (Oxoid) deMan Rogosa and Sharpe (MRS) agar (Oxoid), Violet Red Bile (VRB) agar and malt extract agar (Oxoid), respectively Samples were enumerated by using appropriate sterile dilution and spread plate methods. The fungal plates were incubated at 25°C for 2-5 days while the bacterial cultures were incubated at 30 to35°C for 24-48h.

Identification of microbial isolates: After colony counting, several colonies were picked at random and differentiated based on morphology and motility. Three colonies for each morphological type was purified and maintained on appropriate agar plates. Systematic morphological and biochemical tests were conducted according to Cowan and Steel (1974) to classify the bacterial isolates into genera. Identification of bacterial isolates into species was done according to tests and descriptions given in Collins and Lyne (1984) and in Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984; Sneath *et al.*, 1986).

Chemical analyses: Triplicate samples of fermenting cassava mash (10g) were homogenized in distilled water (100ml) and the pH determined with a pH meter. The sample homogenate was then titrated to pH 8.0 with (O.IM) sodium hydroxide using phenolphthalein as the indicator (Speck, 1984). The titratable acidity was expressed as lactic acid equivalents on a wet weight basis. Cyanide content was determined using the alkaline pirate method as described by Balagopalan *et al.* (1988).The extract was also used for measurement of the total reducing sugar content(Miller, 1959).

Evaluation of sensory quality of cassava fufu: A semi-trained panel of 12 students and staff of the University of Agriculture, Umudike, who are familiar with cassava fufu, evaluated flavour, colour, odour, and texture and overall acceptability. The parameters were rated on a hedonic scale of 1-9 were 1 was disliked extremely and 9 was liked extremely. Cassava fufu samples (100g each) were prepared by stirring gently in 500 ml of boiling water. The paste was stirred continuously for about 6 min over low heat, and had a dough-like consistency.

Statistical methods: The whole experiment was replicated three times. Data obtained were analyzed using the analysis of variance to determine differences and Duncan's multiple range tests to separate the means.

Results

Quantitative determinations of the physicochemical changes and microbial counts produced by the different processing procedures were carried out. Table 1 shows the changes in microbial count, pH and titratable acidity that occur during the retting of cassava pieces. Microbial growth quickly commenced in the soaking waters and populations of 10^5 to 10^9 cfu/ml had developed by 12 to 24h. At 48h, the microbial counts in cassava fermented in the traditional way (procedure 1) were 9.24 $\log_{10}$ cfu. /g whereas in cassava grated prior to fermentation, 5.8 $\log_{10}$ cfu. /g. was obtained. The initial pH of the retted cassava samples was 6.8, followed by subsequent decreases to 4.3-3.8 at end of 96h fermentation.

Cassava fermented by procedure 1 produced lactic acid (4.0%) while samples grated prior to fermentation (procedure 3) was 0.20%. However, pulverizing whole tubers fermented for 48h and resoaking for another 48h showed a pattern of pH reduction consistent with increased microbial activity. Addition of lime juice in this batch result in rapid drop in pH and a titratable acidity of 3.8%.

Table 1: Changes in microbial counts and chemical attributes of fermenting cassava using different processing methods

	Processing methods/ Production stage	Time (h)	Total viable count (log_{10} cfu/g or ml)	PH	Titratable acidity (% lactic acid)
1	Steep Water	12	6.88	6.12	0.36
TFF	Steep Water	24	8.88	5.40	0.96
	Pulpy Water	48	9.24	5.06	2.20
	Pulpy Mass	72	10.60	4.64	3.40
	Pulpy Mass	96	12.32	4.30	4.00
	Steep Water	12	6.18	6.40	0.36
2	Steep Water	24	8.55	5.81	0.96
GGF	Steep Water	48	10.06	5.30	2.20
	Grated mash	72	7.24	3.83	3.30
	Grated Mash	96	8.40	3.80	3.80
3	Grated Mash	12	4.60	6.21	0.24
GFF	Grated Mash (dewatered)	24	6.32	5.55	0.36
	Re-steeped Mash	48	5.80	4.84	0.86
	,,	72	5.56	4.24	1.00
	,,	96	nd	nd	nd

1 = traditional fermented fufu (TFF); 2=soaked and grated fufu (SGF); 3= Grated and fermented fufu (GFF) n.d = not determined.

Table 2: Microbial counts of predominant microflora of fermenting cassava using different processing methods

		Incubation Period				
Processing Method[a]	Predominant Flora	12	24	48	72	96
1	Lactic acid bacteria	3.04	5.32	7.86	8.56	9.81
TFF	*Bacillus* spp	2.82	3.41	4.44	5.26	5.64
	Enterobacteriaceae	1.10	1.80	1.68	1.02	-
	Yeasts	-	-	1.14	2.56	2.92
2	Lactic acid bacteria	3.61	4.34	7.12	5.64	6.24
SGF	*Bacillus* spp	3.90	4.28	6.42	2.83	3.20
	Enterobacteriaceae	1.86	2.12	1.08	-	-
	Yeasts	-	-	0.80	1.04	2.26
3	Lactic acid bacteria	3.34	4.62	3.84	5.64	nd
GFF	*Bacillus* spp	1.82	2.42	2.80	3.52	nd
	Enterobacteria ceae	-	1.20	0.44	-	nd
	Yeasts	-	-	1.30	2.46	nd

TFF, traditional fermented fufu; SGF, soaked and grated fufu; GFF, grated and fermented fufu. n.d. = not determined.
Each value represents the mean of three determinations.

Table 3: Cyanogenic glucoside changes of fermenting cassava using different processing methods

	HCN gkg (dwb)		
Processing method	Before	After	%HCN Reduction
TFF	0.3875	0.3084	79.7
SGF	0.4125	0.2607	63.3
GFF	0.3625	0.3095	85.5

Each value represents the means of three determination.

The microflora of cassava samples fermented by the traditional method were dominated mainly by lactic acid bacteria, *Bacillus* spp coliforms and yeasts (Table 2). The yeasts appeared only after 48h and their numbers increased to a maximum of 2.92 log_{10} cfu/g at the end of the fermentation process. All the strains found belonged to the genus *Candida*. When the cassava roots were grated before fermentation, the microbial populations were mainly lactic acid bacteria and *Bacillus* spp. The Enterobacteriaceae were not detected in these fermentations (procedure 2 and 3).

Determination of cyanide compounds responsible for toxicity of cassava roots was also carried out. Linamarin, the main cyanogenic glycoside, was highly degraded (79.7%) after 96h of retting by the traditional method. (Table 3) A higher decrease in cyanogens content was found after 72h in samples grated before fermentation 85.5.

Sensory analysis of traditional fermented fufu (procedure 1), fufu produced by grating prior to fermentation and the samples produced by addition of limejuice were

Table 4: Mean sensory scores of cassava fufu samples prepared by three processing methods

Treatment	Sensory attribute				
	Colour	Texture	Odour	Flavour	Overall acceptability
TFF	6.27[b]	5.53[b]	4.67[b]	4.93[b]	4.87[c]
2SGF	7.40[a]	5.33[b]	6.33[a]	7.07[a]	7.00[b]
3GFF	7.20[a]	7.20[a]	7.43[a]	7.47[a]	7.80[a]

Mean scores with the same letter are significantly different ($p<0.05$) n=12. scores are 1-9 on a hedonic scale.

compared. Obviously, it was observed that the fufu prepared from fermented cassava prepared in the traditional way had unacceptable characteristic putrid odour. On the other hand, samples prepared from cassava grated prior to fermentation had a higher overall acceptability in terms of the aroma. There were no significant difference ($p<.0.05$) in colour and texture of the fufu samples (Table 4).

Discussion

The fermentation of cassava roots, called retting, allows softening for further processing and the reduction of potentially toxic cyanogenic glucosides present in the roots (EL-Tinay *et al.*, 1984).

Submerged fermentation over 4 days by traditional methods usually produce mash which contain a foul odour resulting from uncontrolled fermentation and storage techniques (Okpokiri *et al.*, 1984; Okolie *et al.*, 1992). This type of fermentation, although the simplest way to achieve cassava retting, involves a complex microbial process (Daeschel *et al.*, 1987; Ogumbawo *et al.*, 2004), and may result in variations in the quality of the product. In addition, the number and types of microorganisms involved in each fermentation process influence the quality of the final product. The population and composition of the microbiota as well as the reduction of cyanogenic glucosides at various stages of the present study is similar to that reported in earlier studies of fufu fermentation (Okafor *et al.*, 1984; Brauman *et al.*, 1996; Giraud and Rainbault, 1992). However, our use of pulverized cassava mash for the fermentation (e.g as in procedure 3) accelerated pH decrease coupled with increase in the production of organic acids (Table 1). Natural fermentation of cassava roots may involve undesirable microorganisms resulting in a delayed decrease in pH (Daeschel *et al.*, 1987) and the production of off-odours. This indicates that the loss in structural integrity caused by grating is of great importance for cell wall degradation, which perhaps enhance contact with endogenous enzymes. Therefore, increased surface area as a result of grating may also increase contact between microbial enzymes and linamarin (Essers *et al.*, 1995; Mkpong *et al.*, 1990) thereby reducing the effect of innocuous microorganisms. Brauman *et al.* (1996) observed a slow drop in pH during cassava root retting, but results

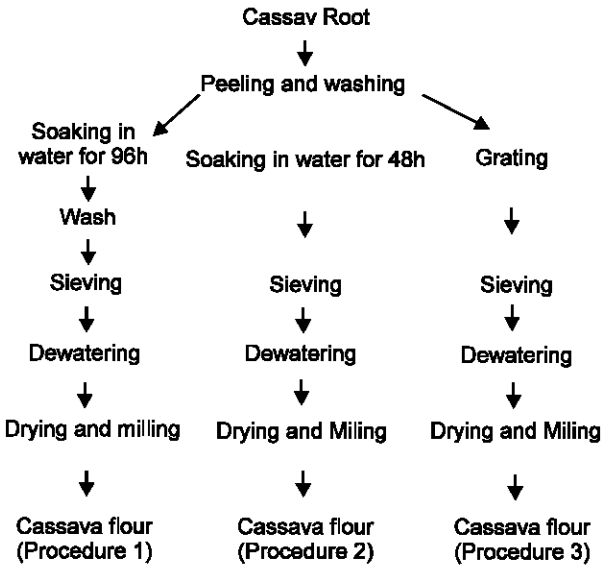

Fig 1: Sequence of operations in cassava fufu production.

obtained on gari production (a similar cassava based fermented product) confirm the results of the present study that a rapid drop in pH can be obtained when cassava is grated prior to fermentation (Ogunsua, 1980; Oteng-Gyank and Anuonye, 1987). Interestingly, cassava fermentation used for gari production does not possess the objectionable odours associated with cassava root fermentation for fufu production.

The addition of lime juice into the fermenting medium apparently has an additional effect of inhibiting growth of undesirable microflora (Tassou *et al.*, 1996) apart from reducing the pH. The inactivation of undesirable microflora especially coliforms which are considered to be contaminants will obviate extensive microbial activities which also led to accumulation of foul odour usually associated with complex fermentations (Okpokiri *et al.*, 1984). The interactions of microorganisms in the fermenting mash are likely to be affected by the moisture content of the cassava mash. Thus it is important to ensure that as much water as possible is expressed during the dewatering process to prevent microbial proliferation in the mash.

Clearly, it is evident that production of cassava fufu is

initially mediated by a diverse microflora, which eventually is dominated by the lactic acid bacteria. This pattern of microbial succession is a general feature of fermenting plant materials (Daeschel *et al*., 1987; Achi and Akubor, 2000; Obilie *et al.,* 2004). Results obtained from procedure 2 and 3 illustrate that successful cassava fermentation is achievable with the lactic acid bacteria. Strong starter cultures may reduce fermentation time and further minimize the risk of incidental microflora causing off-flavour. Desirable innocuous organisms such as *Lactobacillus* spp. were found to influence the characteristics of fermented cassava during fufu production (Adegoke and Babalola 1988; Oyewole, 1990; Oyewole and Odunfa, 1990).

Enterobacteriaceae were detected, but only in low numbers and then only in samples from procedure I. This might be due to the high numbers of competing microorganisms especially lactic acid bacteria. The wild yeast species of the genus *Candida* appeared towards the end of fermentation in samples from procedure I. According to studies by Oyewole (1990) the occurrence of wild yeast at high numbers is more alarming than high numbers of Enterobacteriaceae during cassava processing, due to their more immediate effect regarding sensory characteristics.

Mean sensory ratings for dough prepared from cassava flour produced by the different processing methods are shown in Table 4. Grating prior to fermentation enhanced the consumer acceptability of the cassava fufu. Acceptability scores for odour was significantly higher for procedure 2 and 3 than for procedure I. This is thus an indication of the possible potential use of grating prior to fermentation for the production of odourless fufu.

Conclusion: Based primarily on sensory, but also on biochemical and microbiological parameters determined, GFF samples was the most effective for achieving overall acceptability.

Since this method impacts on the odour and flavour of cassava products, the method may provide benefits, which can off-set the higher cost of the process.

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