

EFFECT OF FERMENTATION AGENTS ON THE pH, TTA AND MICROBIAL COMPOSITION OF *FUFU* DOUGH

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ABSTRACT

Fufu, a product of cassava has been adulterated by processors. They add toxic substances to the soaked roots to fasten the fermentation days and make quick money. There is need to evaluate the effect of these fermentation agents on the microbial, pH and total titratable acidity (TTA) of the *fufu* dough. *Fufu* dough was produced with fermentation agents; kerosene, detergent and palm ash and also with a control without agent. They were wrapped in polyethylene bags, stored at ambient temperature and evaluated for storage and microbial quality in the Biochemistry Laboratory of National Root Crops Research Institute, Umudike, Nigeria. These samples were assayed for chemical and microbial qualities which include pH and TTA. The results showed that the pH values ranged from 3.70 - 6.80, and Total Titratable Acidity (TTA) values ranged from 0.004 - 0.048 %. The microbial analysis showed increase in fungal (2.1×10^8 (cfu/g)) and bacterial (1.0×10^6 (cfu/g)) counts as the storage time increased with the control having the least microbial load. The fungal isolates from the samples are *Aspergillus niger*, *Aspergillus flavus* and *Penicillium* spp, while the bacteria isolates from the samples include *Bacillus* spp and *Staphylococcus aureus*. Statistically, there were significant differences ($p < 0.05$) in appearance of the *fufu* as storage time increased. The results from this study showed that the *fufu* with fermenting agents had higher microbial load than the control. It therefore encourages healthy practices among the *fufu* producers by stopping the use of fermenting agents to reduce the proliferation of pathogenic microorganisms in processed *fufu*.

Keywords: *Fufu*, microorganisms, pH, fermentation agents, total titratable acidity

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INTRODUCTION

About 800 million people in Africa, Asia as well as Latin America have been using cassava (*Manihot esculenta* Crantz) as a cheap source of carbohydrate (Montagnac *et al*, 2009). Because cassava is found all year round, and also have the capacity to survive in a dry and low moisture soils, it has been regarded as a crop that can be relied on to feed low income farmers and their household. Also, because the eatable roots can be left in the soil for about 3 years, it is usually used as a fallback crop for survival by many household. Therefore, it serves as a food reserve that may be used when unfavorable weather conditions restrict the production of other foods.

Cassava helps African nations' food security challenges due to its capacity to provide energy efficiently throughout the year, withstand harsh conditions by adapting the continent's current farming and food systems (Martin and Ejike, 2018).

Cassava roots may be eaten without being cooked or processed into different finished goods. Depending on the preferred mode of consumption and processing, different products are produced. There are different varieties which are bitter in taste, and these undergo intense grinding and fermentation before they can be consumed while the sweet varieties which contain low cyanide require little process before they can be eaten such as boiling, roasting or frying (Nweke, 1994). Different processing methods are employed to enhance the products' value, increase their shelf life, and remove the toxin contained in the roots, and this is achieved by eliminating the cyanogenic glucosides inherent in the crop (Westby, 2002, Cardoso *et al*, 2005). Flour, starch, and various fermented items are among the processed goods made from cassava roots. In Africa, the prominent and commonest products from cassava roots are *gari*, *fufu*, *tapioca*, *lafun* as well as *attieke* according to Ezemenari *et al*, (1998). Awoyale *et al*, (2021), also submitted that the final products of cassava can as well be prepared in form of thick paste, semolina – like particles and in form of flour.

Fufu is a local fermented food that is being consumed in Nigeria, especially in the Southern, Eastern and Western regions even in other West African nations (Rosales-soto *et al*, 2016). In the southern region of Nigeria, it is listed right behind *gari* as a typical native fermented food for many families (Egwim *et al*, 2013). Due to different cultures obtainable in different regions, many families and processors produce *fufu* using diverse techniques (Chijioke *et al*, 2020). In recent times, *fufu* has been faced with safety challenges as the processors who engage in its production now adulterate the food product so as to get enough money. They accomplish this by reducing the number of allowable fermentation days required for the correct retting of the soaked roots. To speed up the fermentation process and ret faster than it should be, they add toxic substances like kerosene, detergent, and palm ash to the already fermenting, soaked roots (Ogbete *et al*, 2022). The idea that these fermentation agents serve to boost the *fufu* yield is another justification for their use. According to them, the cassava would not produce high *fufu* mash yield if the fermentation isn't done properly (Ogbete *et al*, 2022). Therefore, the aim of this research work is to analyse and evaluate the effect of the substances they add to the fermenting or soaked cassava roots on its microbial load, pH as well as the total titratable acidity (TTA) of the *fufu* dough produced. The knowledge of microbiological quality of these *fufu* will help to ascertain the level of safety involved with the use of the fermentation agents in *fufu* production thereby make better recommendation to the processors. The aim of this research work was to analyse and evaluate the effect of fermenting substances (powdered detergent, kerosene and ash) on the microbial load, pH and total titratable acidity (TTA) of *fufu* dough.

The specific objectives of this research work were to:

- i. produce *fufu* using different fermentation agents (detergent, kerosene and palm ash).
- ii. determine the microbial load of the *fufu* dough.

- iii. determine the pH and TTA of the *fufu* dough.

MATERIALS AND METHODS

Source of Experimental Materials

Fresh roots from cassava (TME 419) were sourced from Cassava Programme in National Root Crops Research Institute (NRCI), Umudike Nigeria. The cassava roots were of 12 months of age when they were harvested.

Preparation of the *fufu* materials

Sixty kilogramme (60 kg) of TME 419 cassava variety was used to produce the *fufu* samples. The fresh roots of the cassava were peeled, washed, and divided to four equal portions of fifteen kilograms each. The first portion was added 5 ml of kerosene, the second portion was added 5 g. of powdered detergent (Omo) which contains sodium silicate, sodium tripolyphosphate, sodium hydroxide, sulphonic benzene acid of the linear alkyl group as well as lauryl ether sulfate of sodium; the third portion was added 4 g. of palm ash while the fourth portion had nothing added to it which thereby served as the control. They were sliced to small chunk sizes of 7 cm, washed and soaked in a two different bowls which were poured 30 litres of clean water and had equal volume and diameter of 30 litre and 64cm respectively. They were properly labeled as K (*fufu* with kerosene), D (*fufu* with detergent), P (*fufu* with palm ash) and C (control), left to ferment and ret for 72 hrs (3 days) at ambient temperature. After the 72 hr retting, the soft root pulp was sieved to give resulting sediment; the wet *fufu* mash (Ogbete *et al*, 2022). To make *fufu* dough, the procedure outlined by Ogbete *et al*, (2022) was adopted. 100g of the dewatered, sieved mash was formed into balls, cooked in a 700 ml of water using a modern gas cooker for 15 minutes at 100 °C, then taken out and pounded to make the *fufu* dough. To finish the final *fufu* dough making, the previously pounded dough was re-molded, cooked for another 10 minutes at 180 °C, and then pounded again. It was pounded finally and then given 45 minutes to cool. The *fufu* dough samples were collected using containers that have been previously sterilized, promptly covered, and brought in the lab to be conducted analysis on. 50 g of each of the cooked *fufu* was packaged in clear polyethylene material typically involved in the packaging and selling of cooked *fufu*. The *fufu* samples were properly wrapped, put in a dried clean trays, allowed for an 8-day storage while maintaining an ambient temperature of 28±2°C. After the 8-day storage, analysis of pH, total titratable acidity (TTA) and also the microbial count were carried out on the *fufu* samples daily throughout the period of their storage. Duplicates of the samples were made while they were being collected for the analysis.

Microbiological Analysis

One gram (1 g) of the *fufu* samples each were individually homogenized in nine ml of already distilled water. Each sample was diluted serially ten times until a dilution level of 10⁻⁴ was reached. For the purpose of determining the load of microbes present in each of the agar media,

the lowest dilution of 1ml as well as other dilutions were poured on the plates of nutrient agar with Sabouraud dextrose agar inclusive. Thereafter, at ambient temperatures for 48 and 72 hrs for the proper growth of bacteria and fungi, laboratory incubation were done followed by the determination of the total counts of bacteria and fungi which are viable. This was done by counting the units that form the colony (cfu/g) by the end of the time of incubation with this formula by Jideani and Jideani, (2006):

$$c = n/vd$$

Where,

c: colony forming unit per gram

n: number of the colonies formed

d: dilution blank

v: volume of colonies transferred to the plate

Until pure cultures were obtained, the isolated microorganisms were sub-cultured by streaking them onto sterile nutritional agar in a repeated manner, MacConkey agar slants were for bacteria, while Sabouraud dextrose agar slants were for fungi.

Characterizing and identifying the microbial isolates.

To characterize and identify the isolated microorganisms, the procedure as described by Ogburie *et al*, (2005) was used. The morphology of colonies based on their characteristics as well as their chemical tests (such as gram stain, oxidase etc.) were used to define and identify bacterial isolates. On the other hand, the morphological observations made with a low-power objective lens and cultural characteristics stained with cotton-blue lacto phenol solution were used to identify the fungal isolates.

pH of the *fufu* samples

This was determined according to the method of Ogiehor and Ikenebomeh (2005). 10 g each of the *fufu* dough was mixed together in distilled water (10 ml) while the pH of the mixture was read with a reference glass electrode; a HANNA pH meter (made by HANNA Instruments, model HI96107, Italy).

Titrateable acidity

This was determined according to Obilie *et al* (2004). Exactly 10 grams of sample was mixed in 200 ml of distilled water that was filtered with Whatman filter paper. A 0.1 M NaOH titration of 80 ml of the filtrate was performed with 1% phenolphthalein serving as the indicator. Equation (1) was used to calculate the titrateable acidity based on the fact that lactic acid was the predominant fermentation product.

$$\text{Titrateable acidity} = \frac{V_b \times N_b \times 0.09}{V_s} \times 100\%$$

Where:

V_b = volume of the base;

0.09 = milli-equivalent factor of the lactic acid;

N_b = Normality of the base

V_s = volume of the sample

RESULTS AND DISCUSSION

The pH and Total Titrateable Acidity (TTA) of the *fufu* dough samples

The values for the pH of the *fufu* dough samples after an 8 day storage are shown in table 1. The values down of the column ranged from 3.60 (Day 0) of sample K to 6.80 (Day 7) of sample C. No significant ($p > 0.05$) difference was observed on pH value of Day 0 (3.60) to Day 7 (4.40) of *fufu* sample K down the column while there was significant ($p < 0.05$) difference with the rest of the days of the other samples down the column. The *fufu* samples had pH values that are significantly ($p < 0.05$) different from each other across the row for all of the days except for Day 2 where no significant ($p > 0.05$) difference was seen among them. This pH ranges agrees with the values from Odo *et al*, (2016), who got the pH range of 3.70 - 6.80 for 'Cassava *Fufu* Sold in Abakaliki Metropolis', as well as Omafuvbe *et al*, (2007) who got the pH range of 3.65 – 5.12 for 'Ready-to-eat *Fufu* and *Lafun* sold in Ile-Ife, Nigeria'. The raising pH of food during storage has been associated with the removal of ammonia by spoiling bacteria as reported by Olawepo and Akoma, (2001). The results indicates that the added fermentation agents had no effect on the pH of the *fufu* samples.

The total titrateable acidity (TTA) shown in Table 2 ranged from 0.008 (Day 7) in samples K, P and C respectively to 0.058 (Day 0) in sample D. Down the column, a significant ($p < 0.05$) difference was observed in the *fufu* dough samples with the exception of sample C which was not seen any significant ($p > 0.05$) difference among the days. Across the row, there was no significant ($p > 0.05$) difference observed in days 4 and 5 as well as in day 6 of all the samples while significant difference ($p < 0.05$) was observed in days 0, 1, 2, 3 and 7 of all the samples. The TTA obtained were within the range 0.004 to 0.063 reported by Odo *et al*, (2016) for Cassava *Fufu* Sold in Abakaliki Metropolis. From the results, it could be seen that the TTA values of all the *fufu* dough varies across the days. This may be caused by microbial activity, metabolic processes, and environmental factors such as temperature variation, the effect exerted by of carbon (iv) oxide, the absence or effect of oxygen, and humidity under which the samples were stored (Odo *et al*, 2016).

Total Fungal and bacterial Count of the *fufu* dough samples

Table 3 shows the fungal count (cfu/g) of the *fufu* dough samples. The values of the samples down the column ranged from 2.0×10^8 cfu/g as seen in Day 0 of sample P to 4.9×10^8 cfu/g in Day 6 of sample D. No significant ($p > 0.05$) difference was seen on the total fungal count value of Day 0 (4.0×10^4 cfu/g) to Day 6 (4.7×10^6 cfu/g) of *fufu* sample K down the column with corresponding significant ($p < 0.05$) difference seen with the first three days of the other samples down the column. Across the row was seen no significant ($p > 0.05$) difference in the total fungal count at days 0 and 9, while there was seen a significant difference ($p < 0.05$) in the remaining days for all of the samples. As the number of days increased, the results showed a rise in microbial count among the samples especially with the *fufu* samples with fermentation agents. The overall fungal count increased from the third day until the ninth day, when there were too many counts to tally.

Table 4 shows the total count for the bacteria. The values for the total bacterial count of the *fufu* dough down the column ranged from 1.0×10^6 cfu/g (Day 0) of sample C to 8.6×10^6 cfu/g (Day 9) of sample D. Significant ($p > 0.05$) difference was observed on the total bacterial count of the *fufu* dough for all the days down the column. No significant ($p > 0.05$) difference was observed in the total bacterial count of the *fufu* dough at day 0 across all the samples, while a significant difference ($p < 0.05$) was seen for the remaining days for all the samples. From the result, it showed that as storage duration increased, the number of bacteria increased. The high bacterial count found in the *fufu* samples especially with those that contains fermentation agents indicates that the fermentation agents could have aided the proliferation of the bacteria which helped create a conducive environment for the microorganisms.

CONCLUSION

Fufu that has been kept at room temperature for longer than two days is more likely to develop microorganisms and lose its acceptability. From this study, it was found that after the third day of storage, the microorganisms changed the *fufu* sample's pH and titratable acidity (TTA), thereby rendering it unfit for ingestion. Prominently observed, the *fufu* samples with fermenting agents contained larger loads of moulds and bacteria, as was evident from the result. Due to this, using these agents of fermentation should be discouraged totally. Additionally, the sort of microorganisms on the preserved *fufu* may pose some health and food safety risks due to the production of mycotoxins and bacterial toxins. As a result, *fufu* meant for consumption should be adequately prepared without the introduction of fermentation agents and stored for no longer than two days. To reduce the possibility of infection, the *fufu* must be processed in hygienic circumstances to increase its quality.

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Table 1: pH of the *fufu* dough under 8 days storage

SAMPLES		pH		
Days	K	D	P	C
0	3.70 ^b ±0.01	3.80 ^a ±0.01	3.90 ^a ±0.01	3.80 ^a ±0.01
1	3.80 ^b ±0.01	3.80 ^a ±0.01	3.80 ^b ±0.01	3.90 ^a ±0.01
2	3.90 ^b ±0.01	3.90 ^b ±0.01	3.70 ^b ±0.01	4.80 ^b ±0.01
3	4.00 ^b ±0.1	3.90 ^b ±0.1	3.60 ^a ±0.1	4.60 ^b ±0.01
4	4.10 ^b ±0.1	3.95 ^c ±0.1	4.80 ^c ±0.1	5.90 ^c ±0.1
5	4.20 ^b ±0.1	4.10 ^b ±0.1	4.90 ^d ±0.1	5.70 ^d ±0.1
6	4.20 ^b ±0.1	4.15 ^d ±0.1	5.50 ^e ±0.1	6.70 ^e ±0.1
7	4.40 ^b ±0.1	4.20 ^d ±0.1	5.80 ^e ±0.1	6.40 ^f ±0.1

Note: values are means of duplicate determinations. Means with different superscripts along the column are significantly different at ($p < 0.05$).

Key: K = *Fufu* with kerosene, D = *Fufu* with detergent, P = *Fufu* with palm ash, C = *Fufu* Control

Table 2: TTA of the *fufu* dough under an 8 days storage

Days	Samples		TTA	
	K	D	P	C
0	0.038 ^d ±0.00	0.058 ^c ±0.00	0.048 ^d ±0.00	0.042 ^b ±0.16
1	0.028 ^c ±0.00	0.046 ^c ±0.00	0.040 ^c ±0.00	0.052 ^b ±0.16
2	0.017 ^b ±0.00	0.042 ^c ±0.00	0.038 ^c ±0.00	0.018 ^b ±0.16
3	0.012 ^b ±0.00	0.034 ^b ±0.00	0.038 ^c ±0.00	0.021 ^b ±0.16
4	0.010 ^b ±0.00	0.030 ^b ±0.00	0.026 ^b ±0.00	0.028 ^b ±0.16
5	0.009 ^b ±0.00	0.026 ^b ±0.00	0.020 ^b ±0.00	0.016 ^b ±0.16
6	0.009 ^b ±0.00	0.020 ^b ±0.00	0.018 ^b ±0.00	0.010 ^b ±0.16
7	0.008 ^a ±0.00	0.010 ^a ±0.00	0.008 ^a ±0.00	0.008 ^b ±0.16

Note: values are means of duplicate determinations. Means with different superscripts along the column are significantly different at ($p < 0.05$).

Key: K = *Fufu* with kerosene, D= *Fufu* with detergent, P = *Fufu* with palm ash, C = *Fufu* Control

Table 3: Total count of the fungi in the stored *fufu* dough (cfu/g)

Days	Total fungal count of stored <i>fufu</i> (cfu/g)			
	K	D	P	C
0	4.0x10 ^{4a}	4.0x10 ^{4 a}	2.0x10 ^{8 a}	2.1x10 ^{8 a}
3	4.0x10 ^{5 a}	4.5x10 ^{6 b}	3.0x10 ^{5 a}	3.0x10 ^{6 a}
6	4.7x10 ^{6 a}	4.9x10 ^{8 c}	4.5x10 ^{6 b}	4.0x10 ^{8 b}
9	TNTC	TNTC	TNTC	TNTC

Note: values are means of duplicate determinations. Means with different superscripts along the column are significantly different at (p < 0.05).

Key: K = *Fufu* with kerosene, D = *Fufu* with detergent, P = *Fufu* with palm ash, C = *Fufu* Control

Table 4: Bacterial count for the stored *fufu* dough (cfu/g)

Days	Total bacterial count of stored <i>fufu</i> (cfu/g)			
	K	D	P	C
0	3.0x10 ^{6 a}	4.0x10 ^{8 a}	2.0x10 ^{6 a}	1.0x10 ^{6 a}
3	4.8x10 ^{5 b}	5.8x10 ^{6 a}	4.8x10 ^{5 b}	2.8x10 ^{5 ab}
6	5.6x10 ^{5 c}	6.8x10 ^{5 b}	6.6x10 ^{6 c}	4.6x10 ^{6 b}
9	7.8x10 ^{6 d}	8.6x10 ^{6 b}	7.8x10 ^{6 d}	6.8x10 ^{4 c}

Note: values are means of duplicate determinations. Means with different superscripts along the column are significantly different at (p < 0.05).

Key: K = *Fufu* with kerosene, D = *Fufu* with detergent, P = *Fufu* with palm ash, C = *Fufu* Cont