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Original Article

Comparative Nutritional Composition Analysis and Microbial Enumeration of Flour (*elubo*) from selected fermented indigenous tuber crops

[*1]Michael, T., [1]Idris, Mohammed., [2]Idowu, O. B. and [1]Adamu, S.

[1]Department of Biological Sciences, Federal Polytechnic, Bida
[2]Department of Hospitality Management Technology, Federal Polytechnic, Bida

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ABSTRACT

The composition of nutrition and microbial ecological safety of starch-based diets prevalent in the majority of developing African nations is crucial. The proximate, mineral, cultural, and enumeration of microorganisms interacting with tuber crop fermentation (sweet potatoes, yam and casava) in flour production were all examined in this study. The experiment was designed with two replicates, examining the effects of pre-treatment (peeled tubers), water temperature for steeping (cold water at 25°C $\pm$ 2°C and boiled water at 100°C), and fermentation duration (24 and 72 hours). Proximate and mineral contents were analyzed, microbial load during fermentation was also evaluated for safety and acceptability. Results showed that dry matter content ranged from 87.86% to 93.59%, with highest values observed in yam sample (BY 11: (peeled yam steeped in cold water for 24rs) was 93.59%. Energy content varied from 341.15 to 399.99 Kcal/100g, with cassava (CC 21: (peeled casava steeped in hot water for 24hrs.) was 399.99 Kcal/100g) and sweet potato samples providing the highest caloric density. Mineral analysis revealed that potassium content ranged from 0.13% to 0.55%, with sweet potato samples steeped in cold water for 24hrs. and yam sample in cold water for 24 hrs. (samples AP 11 and BY 11: 0.55%) showed a high potassium content. Calcium content varied from 0.26% to 1.46%, with cold water steeped sweet potato samples at 24 hrs. and72 hrs. (AP 11: 1.40% and AP 13: 1.46%) demonstrated high contents. Total aerobic bacteria count ranged from 3.0 to 68.0 ($\log_{10}$ Cfu/ml), total lactic acid bacteria count 9.8 to 69.0 ($\log_{10}$ Cfu/ml). Coliform bacteria were absent which showed the tuber crop flour was safe for consumption. The study provides valuable baseline data on nutritional dynamics during tuber crop fermentation, contributing to improved understanding of traditional processing methods, enhanced product quality and safety outcome in tuber crops flour.

Keywords: Fermentation: Bacteria count: Tuber crop flour: Proximate

***Corresponding author's email**: michaeltowobola1@gmail.com, +2348032895741

INTRODUCTION

Millions of people, notably in tropical and subtropical regions of Africa, Asia, and Latin America, rely on cultivated tubers as their main source of dietary energy, making them an essential part of international food security [1, 2]. Because of their high carbohydrate production, capacity to adapt to marginal soils, and cultural relevance, sweet potatoes (*Ipomoea batatas*), yams (*Dioscorea spp.*), and cassava (*Manihot esculenta*) are among the most important. These crops are extremely perishable after harvest due to their high moisture content, which makes them vulnerable to microbiological and physiological degeneration despite their agronomic resilience. This causes large losses, both quantitative and qualitative, which are estimated to be between 25 and 40 percent every year in developing countries. [3].

Traditional processing techniques have been established over ages to improve palatability and reduce these losses. Fermentation is one of the most widely used, affordable, and successful methods of biological preservation [4, 5]. Lactic acid bacteria (LAB), yeasts, and sometimes fungi are the main microorganisms that drive this natural process, which breaks down the sugars and starches that are naturally present in the tubers [6]. Organic acids, alcohols, and other antimicrobial compounds are produced as a result of this microbial activity. These chemicals increase shelf life, enhance safety by limiting pathogens, and alter the food's sensory characteristics, frequently producing distinctive flavors and textures valued in a variety of cuisines [7,8].

Microorganisms (such as bacteria (LAB) and yeasts) break down the carbohydrates in the tuber during the anaerobic biochemical process of fermentation, which results in the production of organic acids, alcohols, and gases that cause preservation and sensory changes [7]. Particularly in homes and small-scale artisanal settings, fermentation is a common technique with mostly uncontrolled and spontaneous processes. The type of tuber, the initial microbial load, pretreatment techniques (such peeling or chopping), the temperature of the soaking water, and the length of the fermentation all have an impact on the complex microbial ecology that drives these fermentations. [9]. The use of hot water, for instance, is a common practice believed to accelerate fermentation or soften the tubers, but its impact on the selection and succession of the microbial community is not well-documented scientifically. Similarly, the effect of peeling, which removes the natural outer barrier, on the introduction and proliferation of specific microorganisms remains unclear [10].

MATERIALS AND METHODS

Fresh tubers of sweet potato (*Ipomoea batatas*), yam *(Dioscorea rotundata*) and cassava (*Manihot esculenta*) were used in this study. They were purchased from a local market in Bida, Bida local Government area of Niger State (Nigeria). All bench work was carried out in an aseptic condition, at the Department of Biological Sciences, Microbiology Laboratory, Federal Polytechnic Bida, Niger State. Nigeria.

Production of Tuber crop flour (*elubo*)

The tuber crops were washed, peeled and steeped in hot water (100^{o}C) and cold water (25^{o}C) for 24 hours and 72 hours respectively.

Drying of the fermented tuber

After fermentation for 24 and 72 hours of the samples, the fermented tubers were dried in the sun for 3 days to completely remove moisture and grinded using grinder (Marlex Excella, Daman, India) to obtain fine flour.

Microbiological analysis

Each steeping water was repeatedly diluted in ten (10) instances; 1 mL of the sample was pipetted and diluted up to 10 mL with sterile distilled water in a test tube, yielding a dilution of 10^1. The process was repeated until the dilutions reached 10^5 [11]. MacConkey agar (HIMEDIA) was used for the total coliform count, Man Rogosa Sharpe agar (HIMEDIA) for the isolation of lactic acid bacteria, and Nutrient agar (HIMEDIA) for the total viable bacteria. Following the manufacturer's instructions, all media were prepared and autoclaved for 15 minutes at 121°C to sterilize them. Using 1 mL of 10^4 dilutions of the material with sterile molten media at 45 °C, the pour plate technique was used for inoculation. The MRS plates were anaerobically incubated at 35°C for 48 hours, whereas the NA, MacConkey petri dishes were incubated inverted aerobically at 37°C for 24 hours. The logarithm colony forming unit per millimeter was used to compute, count, and expressed the characteristic's observed colonies. (Log Cfu/mL) [12].

Proximate analysis

The tuber crop flour's moisture content, crude protein, crude fat ash levels, and total carbohydrate content were measured using conventional procedures as outlined by [13]. Every analysis was done in duplicate.

Data analysis

The experimental data were analyzed using the Analysis of Variance (ANOVA). Duncan multiple range test was used to compare mean and to determine the significant difference at confidence level of ($p<0.05$). Statistical Package for Social Sciences (SPSS) version 15.0 was used [14].

RESULTS AND DISCUSSIONS

Proximate and minerals

The proximate composition of tuber crop flour as shown in Table 1 indicates that peeled sweet potatoes sample steeped at 100C for 72 hrs. (sample AP 23) had the highest carbohydrate content of 85.60 ± 0.026 (%) while peeled casava sample steeped at 25C for 24 hrs. (sample CC 11) had the lowest content of 76.99 ± 1.701 **(%) t**his result is in similar trend with the findings of [15, 16]. The caloric contents of the tuber flour shows that sample CC 21 (peeled casava steeped at 100^oC for 24 hrs.) had the highest energy content of 399.99 ± 8.948^g (Kcal/100g) while peeled sweet potatoes sample steeped at 25^oC for 24 hrs. had the least energy content of 352.72 ± 0.028^b (Kcal/100g). The crude fiber and ash contents of potatoes flour is significantly ($p<0.05$) different from yam and casava flour. However, casava flour had significantly ($p<0.05$) high moisture and fat contents. The crude protein content of potatoes and yam are in similar trend but casava got significantly ($p<0.05$) higher contents of 6.39 ± 0.062^h.

The mineral analysis of the flour produced revealed that potatoes had a significant ($p<0.05$) high potassium, calcium and phosphorus content with sample AP 11 at 0.55, sample AP 13 at 1.46 and sample AP

23 at 990.11 respectively. This result is similar to the findings of [17] who reported that potatoes flour contains a reasonable content of potassium, calcium and phosphorus.

Table 1: Proximate Composition of tuber crops flour

Sample	Dry Matter (%)	Moisture (%)	Ash (%)	Crude Fat (%)	Crude Protein (%)	Crude Fiber (%)	CHO (%)	Energy (Kcal/100g)
AP 11	92.61±0.005^{d}	7.39±0.005^{a}	3.415±0.003^{g}	0.99±0.006^{a}	4.73±0.062^{f}	2.25±0.002^{g}	81.22±0.067^{bc}	352.72±0.028^{b}
AP 13	89.65±0.047^{ab}	10.35±0.047^{cd}	3.40±0.002^{g}	0.99±0.000^{a}	3.85±0.000^{d}	2.24±0.001^{g}	79.21±0.050^{ab}	341.15±0.200^{a}
AP 21	93.07±0.005^{d}	6.93±0.005^{a}	1.49±0.002^{de}	1.47±0.001^{ab}	3.94±0.310^{e}	0.98±0.001^{de}	85.19±0.311^{de}	369.77±0.025^{de}
AP 23	93.58±0.028^{a}	6.44±0.028^{a}	0.99±0.005^{a}	0.97±0.002^{a}	5.34±0.013^{g}	0.65±0.004^{a}	85.60±0.026^{e}	372.64±0.025^{de}
BY 11	93.59±0.004^{d}	6.41±0.004^{a}	1.98±0.002^{f}	1.97±0.002^{abc}	4.64±0.006^{f}	1.31±0.001^{f}	83.70±0.016^{cde}	371.09±0.021^{de}
BY 13	91.84±1.069^{c}	8.91±1.061^{ab}	1.49±0.032^{d}	1.49±0.002^{ab}	4.12±0.000^{e}	0.99±0.021^{d}	83.01±1.112^{cde}	361.87±0.021^{de}
BY 21	92.82±1.745^{d}	5.94±1.746^{a}	0.98±0.003^{a}	3.94±0.004^{de}	4.12±0.000^{e}	0.65±0.002^{a}	84.38±1.748^{cde}	389.43±6.951^{e}
BY 23	90.69±0.139^{a}	9.31±0.139^{bc}	0.99±0.002^{a}	2.99±0.006^{cd}	3.59±0.000^{e}	0.65±0.002^{a}	82.58±1.748^{cde}	371.53±0.566^{de}
CC 11	88.42±1.651^{a}	12.75±1.651^{d}	1.50±0.008^{e}	1.48±0.007^{ab}	6.39±0.062^{h}	0.99±0.005^{e}	76.99±1.701^{a}	346.45±6.500^{b}
CC 13	87.86±1.033^{a}	12.87±1.033^{d}	1.17±0.004^{c}	2.48±0.004^{bc}	5.17±0.062^{g}	0.77±0.003^{c}	77.54±0.981^{a}	353.11±1.038^{bc}
CC 21	93.31±0.392^{d}	6.97±0.392^{a}	1.03±0.005^{b}	6.93±2.097^{f}	2.80±0.062^{a}	0.68±0.003^{b}	81.60±2.419^{cde}	399.99±8.948^{g}
CC 23	90.58±0.053^{bc}	9.45±2.067^{cd}	0.98±0.022^{a}	4.41±0.014^{ef}	3.24±0.093^{b}	0.65±0.014^{e}	81.27±0.010^{bc}	377.74±0.139^{ef}

Data are means of two independent experiments ± standard deviation (n=2). Key**:** AP 11 (Peeled sweet potato steeped in cold water for 24 h), AP 13 (Peeled sweet potato steeped in cold water for 72 h), AP 21 (Peeled sweet potato steeped in boiled water for 24 h), AP 23 (Peeled sweet potato steeped in boiled water for 72 h), BY 11 (peeled yam steeped in cold water for 24 h), BY 13 (peeled yam steeped in cold water for 72 h), BY 21 (peeled yam steeped in boiled water for 24 h), BY 23 (peeled yam steeped in boiled water for 72 h), CC 11 (Peeled Casava steeped in cold water for 24 h), CC 13 (Peeled Casava steeped in cold water for 72 h), CC 21 (Peeled cassava steeped in boiled water for 24 h), CC 23 (Peeled cassava steeped in boiled water for 72 h).

Table 2: Mineral Analysis of tuber crops flour

Sample	K (%)	Na (%)	Ca (%)	Mg (%)	P (mg/Kg)
AP 11	0.55^{j}	0.10^{a}	1.40^{e}	1.16^{a}	752.21^{d}
AP 13	0.38^{h}	0.07^{a}	1.46^{e}	1.57^{a}	651.96^{abcd}
AP 21	0.34^{h}	0.07^{a}	0.55^{a}	2.32^{a}	686.27^{abcd}
AP 23	0.27^{e}	0.06^{a}	0.44^{ab}	1.84^{a}	990.11^{e}
BY 11	0.55^{i}	0.10^{a}	1.43^{de}	1.70^{a}	760.01^{cd}
BY 13	0.34^{h}	0.10^{a}	0.91^{cd}	2.21^{a}	728.92^{bcd}
BY 21	0.28^{f}	0.07^{a}	0.66^{bc}	1.91^{a}	684.80^{abcd}
BY 23	0.23^{d}	0.05^{a}	1.01^{d}	1.75^{a}	647.06^{abcd}
CC 11	0.32^{g}	0.07^{a}	0.64^{bc}	2.47^{a}	730.39^{bcd}
CC 13	0.20^{c}	0.05^{a}	0.26^{bc}	2.63^{a}	598.04^{a}
CC 21	0.18^{b}	0.05^{a}	0.55^{ab}	2.01^{a}	610.29^{ab}
CC 23	0.13^{a}	0.05^{a}	0.43^{ab}	2.63^{a}	622.55^{abc}

Data are means of two independent experiments ± standard deviation (n=2); Key**:** AP 11 (Peeled sweet potato steeped in cold water for 24 h), AP 13 (Peeled sweet potato steeped in cold water for 72 h), AP 21 (Peeled sweet potato steeped in boiled water for 24 h), AP 23 (Peeled sweet potato steeped in boiled water for 72 h), BY 11 (peeled yam steeped in cold water for 24 h), BY 13 (peeled yam steeped in cold water for 72 h), BY 21 (peeled yam steeped in boiled water for 24 h), BY 23 (peeled yam steeped in boiled water for 72 h), CC 11 (Peeled Casava steeped in cold water for 24 h), CC 13 (Peeled Casava steeped in cold water for 72 h), CC 21 (Peeled cassava steeped in boiled water for 24 h), CC 23 (Peeled cassava steeped in boiled water for 72 h).

Microbial analysis

Tuber crops are typically fermented into a variety of products to extend its shelf life, make packaging simple, and facilitate cost-effective distribution. According to [18], processing tubers by submerged state fermentation procedures using conventional methods typically results in mash, which has an unpleasant odor due to uncontrolled fermentation and storage methods. A drop in the overall viable count of aerobic mesophiles was noted during the retting phase, and this can be connected to the fermentation medium's reduced pH [19]. Additionally, pathogenic-like microbes may grow less when the medium becomes more acidic [20]. There was aerobic plate count of between 3.0-20.0 (CFU/mL x 10^4) this is presumably as a result of the consortium of the LAB in the fermenting media, and total lactic acid count ranged from 9.8-69 (Cfu/mL x10^4). However, there was no coliform count which indicated no fecal contamination as reported by [21].

Table 3: Total aerobic bacteria count of the fermenting steep water

Sample Code	Dilution Factor	Colony Count (CFU/mL x 10^4)
AP 11	10^4	18.0
AP 13	10^4	20.0
AP 21	10^4	7.0
AP 23	10^4	3.0
BY 11	10^4	12.0
BY 13	10^4	18.0
BY 21	10^4	10.0
BY 23	10^4	13.0
CC 11	10^4	30.0
CC13	10^4	20.0
CC 21	10^4	17.0
CC23	10^4	42.0

Key: NG; no growth. AP 11 (Peeled sweet potato steeped in cold water for 24 h), AP 13 (Peeled sweet potato steeped in cold water for 72 h), AP 21 (Peeled sweet potato steeped in boiled water for 24 h), AP 23 (Peeled sweet potato steeped in boiled water for 72 h), BY 11 (peeled yam steeped in cold water for 24 h), BY 13 (peeled yam steeped in cold water for 72 h), BY 21 (peeled yam steeped in boiled water for 24 h), BY 23 (peeled yam steeped in boiled water for 72 h), CC 11 (Peeled Casava steeped in cold water for 24 h), CC 13 (Peeled Casava steeped in cold water for 72 h), CC 21 (Peeled cassava steeped in boiled water for 24 h), CC 23 (Peeled cassava steeped in boiled water for 72 h).

Table 4: Total Lactic acid bacteria (LAB) count of the fermenting steep water

Sample Code	Dilution Factor	Colony Count (CFU/mL x 10^4)
AP 11	10^4	69.0
AP 13	10^4	15.6
AP 21	10^4	17.6
AP 23	10^4	9.8
BY 11	10^4	13.2
BY 13	10^4	12.0
BY 21	10^4	TNC
BY 23	10^4	12.0
CC 11	10^4	16.7
CC13	10^4	TNC
CC 21	10^4	TNC
CC23	10^4	TNC

Key: AP 11 (Peeled sweet potato steeped in cold water for 24 h), AP 13 (Peeled sweet potato steeped in cold water for 72 h), AP 21 (Peeled sweet potato steeped in boiled water for 24 h), AP 23 (Peeled sweet potato steeped in boiled water for 72 h), BY 11 (peeled yam steeped in cold water for 24 h), BY 13 (peeled yam steeped in cold water for 72 h), BY 21 (peeled yam steeped in boiled water for 24 h), BY 23 (peeled yam steeped in boiled water for 72 h), CC 11 (Peeled Casava steeped in cold water for 24 h), CC 13 (Peeled Casava steeped in cold water for 72 h), CC 21 (Peeled cassava steeped in boiled water for 24 h), CC 23 (Peeled cassava steeped in boiled water for 72 h).

Table 5: Total Coliform Count of the fermenting steep water

Sample Code	Dilution Factor	Colony Count (CFU/mL x 10^4)
AP 11	10^4	NG
AP 13	10^4	NG
AP 21	10^4	NG
AP 23	10^4	NG
BY 11	10^4	NG
BY 13	10^4	NG
BY 21	10^4	NG
BY 23	10^4	NG

CC 11	10^4	NG
CC13	10^4	NG
CC 21	10^4	NG
CC23	10^4	NG

Key: NG; no growth. AP 11 (Peeled sweet potato steeped in cold water for 24 h), AP 13 (Peeled sweet potato steeped in cold water for 72 h), AP 21 (Peeled sweet potato steeped in boiled water for 24 h), AP 23 (Peeled sweet potato steeped in boiled water for 72 h), BY 11 (peeled yam steeped in cold water for 24 h), BY 13 (peeled yam steeped in cold water for 72 h), BY 21 (peeled yam steeped in boiled water for 24 h), BY 23 (peeled yam steeped in boiled water for 72 h), CC 11 (Peeled Casava steeped in cold water for 24 h), CC 13 (Peeled Casava steeped in cold water for 72 h), CC 21 (Peeled cassava steeped in boiled water for 24 h), CC 23 (Peeled cassava steeped in boiled water for 72 h).

There was a decline in the pH readings of the fermenting substrates as show in figure 1. The activities of Lactic acid bacteria during fermentation may be attributed to the pH drop observed throughout the fermentation period. A number of studies [9, 19] have reported a rise in acidity and a drop in the pH readings of the tuber fermenting media.

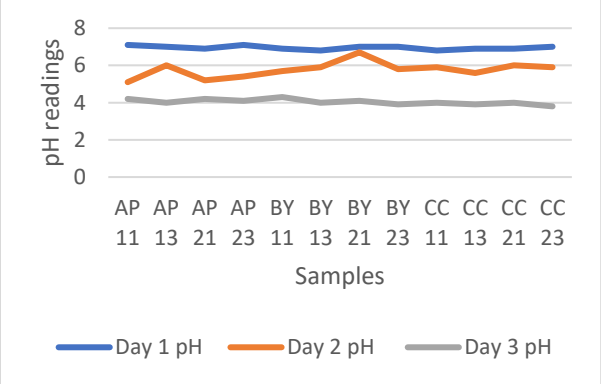

Figure 1: pH readings of tuber crops during fermentation

CONCLUSION

This study revealed that potatoes can be used as an alternative flour as well as can compete favourably in nutrient quality with its other tuber crop contemporaries like yam and casava in the production of flour (*elubo*) to minimize postharvest losses. Since it is highly perishable because of its moisture content, it has a shorter shelf stability than yam and casava after harvest. Its use in the production of flour (*elubo*) thereby serves as preservation method as well as in the prevention of both qualitative and quantitative postharvest loss of the crop (potatoes). Consequently, there are several microbial ecologies at the onset of fermentation during tuber crop flour production. However, as fermentation progresses and the pH of the medium reduces, the microbial succession of other pathogenic bacteria is inhibited as a result of the organic acid which is produced by the population of Lactic acid bacteria (LAB) in the fermenting medium thereby ensuring food safety during fermentation.

Authors' Contribution

MT conceptualized the study. MT and IM designed the study. MT, IM, IOB and AS participated in benchwork and data collection. MT and IM performed the data analysis; MT and IM interpreted the data. MT prepared the first draft of the manuscript, reviewed by IM, and AS. All authors contributed to the development of the final manuscript and approved its submission.

Disclosure of Conflict of Interest

None

Ethics Approval and Informed Consent

This study did not use human or animal subjects. Therefore, ethical consideration was not applicable.

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