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Microbial Succession and Effect of Fermentation on the Proximate Composition of Sweet Potato Tubers, Leaves and Vines

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study was aimed at determining the effect of fermentation on the proximate composition of sweet potato tubers, leaves and vines. The study involved the spontaneous fermentation of the substrates for five days (120h). Physico-chemical properties and microbial counts were determined during the fermentation period. Lactic acid bacteria and yeasts were isolated and characterized.

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Proximate composition of the substrates were determined at the end of the fermentation period. The results obtained show that the aerobic plate counts range from $6.80x10⁵ \pm 1.00x10⁵$ 8.40x10⁶±1.00x10⁵CFU/g, lactic acid bacteria count range from 1.70x10⁵±1.30x10⁵-1.60x10⁷±6.00x10⁶ CFU/g and fungal count range from $2.50x10^5 \text{+} 8.00x10^4$ - 7.75x10⁶±5.50x10⁵ CFU/g. The counts were significantly different in most cases (p≤0.05). The lactic acid bacteria and yeast isolated and identified from the study include *Lactobacillus plantarum*, *L. buchneri, L. delbrueckii, L. brevis, L. lactis, L. fructovorans, L. colloides, L. pentosus, Leuconostoc mesenteroides, Weisella confusa, Candida krusei, C. utilis, C. ciferii, C. spherical, C. rugosa, C. zeylanoides, C. guilliermondii, C. lipolytica, C. tropicalis, C. boidinii, Saccharomyces cerevisiae, Rhodoturulaminuta, R. glutinis, Kodamaeaohmeri, Kloekera*sp. The results of the proximate composition show that there was increase in both Nitrogen free extract and metabolisable energy and decrease in protein content and ash content after fermentation. Findings from this study imply that fermentation of these substrates is characterized by a wide variety of lactic acid bacteria and yeasts with positive effect on the proximate composition of the substrates.

Keywords: Microbial succession; proximate composition; antinutritional factors; effect; fermentation; sweet potato.

1. INTRODUCTION

Sweet potato is an important staple food crop in Africa in general and Nigeria in particular. Sweet potato [*Ipomoea batatas* L. (Lam.)] is among the world's most important, versatile and underexploited food crops. Nigeria is the leading producer of sweet potato (SP) in Africa with an estimated average production (1993–2013) of 3.45million metric tonnes[1]. In Nigeria, the two common local varieties are the purple skin–white fleshed and the yellow skin-yellow fleshed. However, improved varieties including orange-fleshed varieties, with varying genetic and agronomic characteristics are been developed in Nigerian research institutions and released to farmers [2].

The conventional energy feed sources constitute between 40-65% of formulated poultry diets and have high price tags as a result of their numerous alternative uses [3-7]. Among these sources, maize is the most widely employed for poultry feed formulation. In broiler production, corn accounts for approximately 55% of the feed[3,8]. In order to cut down on the high cost of poultry feed and ultimately cost of broilers and eggs, it is imperative to assess readily available and cheap alternatives to cereals and soyabeans for poultry feeds production. Sweet potato has the greatest potential as an alternative raw material to maize and soybean in poultry feed development.

Currently, sweet potato is of low economic value compared to other tubers such as arich potato and yams. Coupled with this it is easily perishable. Utilising sweet potato in animal feed

production will not only add economic value to it but serve as means of preserving it and that will free maize for human consumption.

Fermentation is known to improve the nutritional value especially protein content and amino acid profile of substrates. It is also known to remove anti nutritional factors and improve digestibility of substrates. Fermentation is also an important means of preservation. Lactic acid bacteria involve in the fermentation of substrates are known to have probiotics values and can improve animal health when present in animal feeds. Yeasts on the other hand which are also important microflora of fermentation contribute to the protein content (as single cell proteins) and amino acid and vitamin profile of the substrates. Fermentation can also improve the digestibility of sweet potato when consumed by animals thus making the nutrients readily available for the animals[9,10].

This research was aimed determining the microorganisms involved in the spontaneous fermentation of sweet potato tubers, leaves and vines and the effect of fermentation on the proximate composition of the substrates. It is also part of a preliminary research aimed at utilizing these substrates for broiler feed formulation.

2. MATERIALS AND METHODS

2.1 Collection of Sweet Potato Tubers, Leaves and Vines

Sweet potato tubers, leaves and vines were obtained from sweet potato farms in parts of Plateau state. The white variety of sweet potato was used for this work. The tubers were washed with tap water and cut into slices. The leaves were removed from the vines. The cut tubers (chips), leaves and vines were sun-dried.

2.2 Fermentation of the Samples

Five kilogrammes (5 kg) each of the dried chips, leaves and vines were submerged in 5litres of sterile tap water in clean separate plastic buckets. All the buckets were covered and allowed to ferment spontaneously for 120 h. All fermentations were carried out in triplicates.

2.3 Analysis of the Samples During Fermentation

Samples were taken every 24 hfor analysis. The analysescarried out include microbial counts, pH, TTA [11,12] and Proximate composition [13].

2.4 Microbial Counts

A 10-6 serial dilution of each sample was carried out and the last two dilutions inoculated on appropriate media using the spread plate method.

2.4.1 Aerobic plate count

The last two dilutions were inoculated on Plate Count agar and incubated aerobically at 37°C 24h.

2.4.2 Lactic acid bacteria count

The last two dilutions were inoculated on de Man Rogosa Sharpes agar and incubated anaerobically at 35^oC for 24 - 72h.

2.4.3 Fungal counts

The last two dilutions were inoculated on Potato Dextrose agar and incubated aerobically atroom temperature for24 - 72h.

2.5 Identification of Lactic Acid Bacteria Isolates

The lactic acid bacteria isolates were identified based on Gram reaction, catalase test and the use of API 50 CHLkit as described by [14] and [15].

2.6 Identification of Yeast Isolates

Suspected yeast isolates on Potato Dextrose agar were subjected to Gram staining and lactophenol cotton blue staining. Large oval cells

that are blue in colour and Gram positive were further identified using API 20C AUX test kit^[16].

2.7 pH and Titratable Acidity (TTA) Determination

Ten grammes(10g) of the samples werehomogenised in 90ml of sterile distilled water and the pH values were taken using pH meter (HANNA HI 9025).

Ten milliliters (10ml) of the homogenate from each sample were titrated against 0.1N sodium hydroxide (NaOH) for the determination of TTA. The TTA was calculated using the formular below:

Percentage titratable acidity calculated as lactic acid:

 $TTA = \frac{Titre \times Normality \space of \space base \space x \space chemical \space factor \space (0.009018) \space x \space 100 \space s$ Weight of sample [11,12]

2.8 Proximate Analysis of the Samples

The fermented and unfermented sweet potato tubers, leaves and vines were analysed for crude fibre, lipids, ash, moisture, metabolisable energy and nitrogen free extract content [14].

2.9 Statistical Analysis

Results obtained were subjected to analysis of variance to establish significant differences.

3. RESULTS

The results of the count shows that the aerobic plate count for the tubers range from $2.42x10^6 \pm$ 3.80x10⁵ CFU/g at 0h to $6.80x10^5 \pm 1.00x10^5$ CFU/g at day 5, that of the leaves range from $1.90x10^{7} \pm 5.00x10^{6}$ CFU/g to 6.50x10⁶ ± 5.00x10⁵CFU/gwhile that of the vines range from $1.25x10^6 \pm 3.86x10^6$ CFU/g to 6.75x10⁶ \pm 5.50x10⁵CFU/g. The lactic acid bacteria counts range from 1.70x10⁵ \pm 1.30x10⁵CFU/g to $8.90x10^6$ \pm 4.00x10⁵CFU/g, 8.40x10⁶ \pm 8.00x10⁵CFU/g to 1.36x10⁷ ± 1.60x10⁶CFU/g, $4.90x10^5 \pm 6.00x10^4$ CFU/g to 5.50x10⁶ ± 3.00x10⁵CFU/g for the tubers, leaves and vines respectively. The fungal counts range from 4.00x10⁶ ± 5.00x10⁵ to $8.85x10^{6}$ \pm 5.00x10⁴CFU/g. In most cases there is no significant difference between the counts at different hours of fermentation (p≥0.05).At the end of the fermentation the fungi are the most dominant population with a count of $5.71x10^6 \pm$ 3.00x10⁵CFU/g which significantly differs from that of lactic acid bacteria count of $3.95x10^5 \pm$ 3.00x10⁵CFU/gand aerobic plate count of 6.75x10⁶ ± 5.50x10⁵CFU/g($p \le 0.05$).

There was a progressive decrease in pH of the tubers with increase in length of fermentation, from an initial pH of 5.80 ± 0.20 to a final pH of 3.40 ± 0.40 . There was also progressive decrease in the pH of the leaves except at 48h. There was also a progressive decrease in the pH of the vines from 0h to 72h, a slight increase at 96h and a drop at 120h. There is no significant difference in the pH of the substrates at 0h, 72h and 96h (p≥0.05). Significant difference however exist at $24h$, $48h$ and $120h$ $p \le 0.05$). With respect to TTA, there was a progressive increase in TTA for both the tubers and vines with increase in the length of fermentation. The TTA values range between 0.154 ± 0.001 at 0h to 0.450 \pm 0.002 at 12h, 0.094 \pm 0.004 to 0.071 \pm 0.001, 0.099 ± 0.000 to 0.150 ± 0.000 for tubers, leaves and vines respectively. In all cases, there is a significant difference between the TTA values of the substrates ($p \le 0.05$)(Table 2).

Table 3 shows the results of the microbial succession with respect to lactic acid bacteria isolates. The result reveals that *Lactobacillus plantarum* was the most dominant organism during the fermentation of the three different substrates. *Lactobacillus fermentum* and *Lactobacillusplantarum* persisted through the fermentation period of the tubers and leaves. Other lactic acid bacteria associated with fermentation of the tubers include *Lactobacillusbuchneri* and *Lactobacillus brevis* which were isolated at 0h to 96h. *Lactobacillus delbrueckii* was isolated at 0h and 24h. *Lactobacillus lactis* was isolated from 0h to 48h. *Lactobacillus fructovorans* was not isolated at 0h but was isolated at 24h and 48h.
Lactobacilluscollonoides and Lactobacillus *Lactobacilluscollonoides* and *Lactobacillus pentosus* were only isolated at 48h and 96h respectively. *Weisella confuse* and *Leuconostocmesenteroides* were isolated at 24h to 48h of leaves fermentation. *Lactobacillusplantarum* was also isolated throughout the vines fermentation.

APC: Aerobic plate count; LABC: Lactic acid bacteria count; FC: Fungal count

Fermentation period (hrs)	Substrate	Ph	TTA	
0	Tubers	5.80 ± 0.20	0.154 ± 0.001 ^a	
	Leaves	5.85 ± 0.15	0.094 ± 0.004^b	
	Vines	6.10 ± 0.20	$0.099 \pm 0.000^{\circ}$	
	ANOVA	0.756	253.415	
	p-value	0.542	$< 0.001**$	
24	Tubers	$4.80 \pm 0.10^{\circ}$	0.264 ± 0.002 ^a	
	Leaves	5.30 ± 0.10^b	0.099 ± 0.000 ^c	
	Vines	5.90 ± 0.10^a	0.131 ± 0.004^b	
	ANOVA	30.333	1148.450	
	p -value	$0.010*$	$< 0.001**$	
48	Tubers	$4.70 \pm 0.00^{\circ}$	0.269 ± 0.003 ^a	
	Leaves	$5.45 \pm 0.05^{\circ}$	0.099 ± 0.000 ^c	
	Vines	5.15 ± 0.15^a	0.133 ± 0.003^b	
	ANOVA	17.100	1933.940	
	p-value	$0.023*$	$< 0.001***$	
72	Tubers	4.35 ± 0.01	0.383 ± 0.005^a	
	Leaves	5.20 ± 0.10	0.099 ± 0.000 ^c	
	Vines	4.70 ± 0.20	0.151 ± 0.002^b	
	ANOVA	3.174	2365.241	
	p-value	0.182	$< 0.001**$	
96	Tubers	4.10 ± 0.10	0.449 ± 0.002 ^a	
	Leaves	5.10 ± 0.50	0.077 ± 0.005 ^c	
	Vines	4.90 ± 0.00	0.150 ± 0.003^b	
	ANOVA	3.231	3512.173	
	p-value	0.179	$< 0.001**$	
120	Tubers	3.40 ± 0.40^b	0.450 ± 0.002 ^a	
	Leaves	5.55 ± 0.05^a	0.071 ± 0.001 ^c	
	Vines	4.85 ± 0.05^a	0.150 ± 0.000^b	
	ANOVA	21.864	239661.500	
	p-value	$0.016*$	$< 0.001**$	

Table 2. pH and TTA values during the fermentation of the substrates

The result of the fungal succession shows that *Candida utilis* and *Saccharomyces cerevisiae* were present from the beginning to the end of the sweet potato tubers fermentation. *Rhodoturula minuta* and *Rhodoturula glutinis* were isolated at 0h to 48h but were not recovered at 72h to 120h. *Candida ciferii, Candida rugosa* and *Kodamaeaohmeri* were isolated at 48h, *C. rugosa* persisted to 96h, *C. ciferii*was also isolated at 72h.*Candida spherical* was isolated at 72h and 96h but not at 120h. From the sweet potato leaves *Lactobacillus fermentum, Lacto bacillus plantarum* and *Lactobacillus pentosus*were the dominant yeast since they were isolated from 0h to 72h, with *Lactobacillus fermentum* and *Lactobacillusplantarum* persisting to the end of the fermentation. *Candida guilliermondii* and *Candida tropicalis* were present from the beginning of the fermentation of sweet potato vines to the end, while *Kloekera*sp and *Candida boidinii* were isolated at 72h and 96h (Table 4).

The result of the proximate composition is as shown in Table 5. The moisture content of the tubers increased while that of the leaves and the vines decreased after fermentation. In all cases, there was a decrease in the protein content after fermentation. The protein content of the tubers decreased from 26.10% to 6.48% (75.2% decrease), that of the leaves decreased from 18.64% to 17.06% (8.5% decrease) and vines from 7.34% to 7.07% (3.7% decrease). The crude fibre content of the leaves and vines increased marginally from 20.50% to 30.10% and 32.90% to 39.00% respectively. However, the crude fibre content of the tubers decreased considerably from 30.00% to 6.40%. The lipid content of the tubers and vines increased marginally while that of the leaves decreased. The ash content of all the substrates decreased following fermentation, but there was increase in the free nitrogen extract and metabolisable energy after fermentation.

Table 3. Lactic acid bacteria succession during the spontaneous fermentation of the substrates

Table 4. Fungal succession during the spontaneous fermentation of the substrates

Samples	Moisture	Crude protein	Crude fibre	Lipids	Ash	Nitrogen Free Extract	Metabolisable Energy (cal)
Unfermented Sweet potato tuber	8.20	26.10	30.00	0.65	2.15	32.90	241.84
Fermented Sweet potato tuber	11.00	6.48	6.40	0.75	1.45	73.92	328.35
Unfermented Sweet potato leaves	10.65	18.64	26.50	2.40	14.50	27.31	205.40
Fermented Sweet potato leaves	10.10	17.06	30.10	1.40	8.05	33.29	214.00
Unfermented Sweet potato vines	9.05	7.34	32.90	1.20	10.05	39.46	198.00
Fermented Sweet potato vines	7.25	7.07	39.00	1.60	4.05	41.03	206.80

Table 5. Proximate composition of the sweet potato tubers leaves and vines

Values based on 100g of sample. Cal = calories

4.DISCUSSION

The dominance of fungi during the spontaneous fermentation of the substrates may be due to the fact the fungal isolates obtained from this study are cellulolytic and amylolytic thus giving them a competitive advantage over the aerobic mesophilic bacteria and the lactic acid bacteria. In addition, the moulds isolated during the fermentation are spore formers and can survive during the fermentation. The low pH during the fermentation also favoured the growth of the fungi. The aerobic plate count and fungal count obtained during the fermentation of sweet potato tubers in this study is similar that reported by [9] who reported counts of $9.0x10⁵$ to $8.6x10⁶$ cfu/g and $1.5x10^6$ to $7.4x10^6$ cfu/b respectively.

The progressive decrease in pH and increase in TTA during the fermentation of the substrates has been reported by other workers in this field [17,5]. The decrease in pH is due to the hydrolysis of the complex carbohydrates (starch and cellulose) in the substrates into simple sugars and the fermentation of the simple sugars into lactic and acetic acid by lactic acid bacteria and citric acid by *Aspergillus niger.* According to [18] rapid acidification is advantageous for the process because it creates unsuitable environment for spoilage and pathogenic organisms thus improving the safety of the substrates for poultry feed production.

Findings from the microbial succession revealed that *Lactobacillus plantarum* was the most predominant organism during the fermentation of the three different substrates. *Lactobacillus fermentum* and *Lactobacillus plantarum* persisted throughout the fermentation period of the tubers and leaves. This is attributed to their acid tolerant

ability. *Lactobacillus plantarum* has been reported as the predominant microorganism implicated in several natural and spontaneous lactic acid fermentation of food-related ecosystem [19,20]. According to [21]*Lactobacillusplantarum* is more acid tolerant and oftendominate fermentation processes of vegetablesand cereals in particular because of its ability totransport and metabolize different carbohydrates.According to [22] lactic acid bacteria fermented feeds can protect the intestinal health of broilers and promote the growth of broilers as well as increase relative weight, villi and the small intestine. They further reported that a large number of lactic acid bacteria in fermented feeds can protect the feed from contamination by microorganisms that may be detrimental to animal health.

The presence of yeast during the fermentation may have positive influence on the quality of the formulated feeds. It has been reported that feed supplementation with live yeast cells improve feed efficiency, enhance feed digestibility, increase animal performance, reduce the number of pathogenic bacteria and generally improve animal health [23].

The decrease in crude protein content observed in this study may be due to the proteolytic activity of the microorganisms involved in the fermentation. This differs from the findings of [17] who reported increase in protein content of sweet potato leaves after 3 weeks of fermentation. The observed decrease in protein content may be due to the proteolytic activity of the microorganisms involve in the fermentation. This proteolytic activity may increase the digestibility of the substrates when use in animal feed production [24].

There observed increase in Nitrogen Free Extract implies higher levels of soluble or near soluble carbohydrates such as sugars resulting from the degradation of cellulose^[17]. This can be seen in the increase in metabolizable energy of the fermented substrates.

The increase in metabolizable energy and decrease in fibre content is particularly important, according to [25] poultry birds can derive energy from simple carbohydrates, fat and protein. They cannot digest and utilize some complex carbohydrates, such as fiber, so feed formulation should use a system based on available energy. Metabolizable energy (ME) is the conventional measure of the available energy content of feed ingredients and the requirements of poultry. This takes account of energy losses in the faeces and urine. Birds eat primarily to satisfy their energy needs, provided that the diet is adequate in all other essential nutrients. The energy level in the diet is therefore a major determinant of poultry's feed intake. When the dietary energy level changes, the feed intake will change, and the specifications for other nutrients must be modified to maintain the required intake. For this reason, the dietary energy level is often used as the starting point in the formulation of practical diets for poultry. Different classes of poultry need different amounts of energy for metabolic purposes, and a deficiency will affect productive performance. To sustain high productivity, modern poultry strains are typically fed relatively high-energy diets. The dietary energy levels used in a given situation are largely dictated by the availability and cost of energy-rich feedstuffs.

Amongst the wide range of factors affecting the efficiency of production, of greatest significance is an adequate and balanced supply of energy and nutrients. Dietary energy represents the major quantitative and costliest component in poultry feed formulations, and is the first component to be considered when the diets are being balanced. Dietary energy also controls the feed consumption, which is the major driver of bird growth. The increase in metabolizable energy as a result of fermentation sweet potato tubers, leaves and vines is therefore an important aspect of the positive effect of fermentation on the proximate composition of the substrates for animal feed formulation [26].

5. CONCLUSION

It can be concluded from the findings of this research that the spontaneous fermentation of sweet potato tubers, leaves and vines is characterized by a wide range of lactic acid bacteria and fungi.

Fermentation had a positive effect on the proximate composition of the substrates such as increased in metabolisable energy, ash content and also increase digestibility of protein content of the substrates.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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