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Effect of Fermentation on Chemical Composition of Cassava Peels

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ABSTRACT

The bioconversion of some agro-wastes resulting from microbial activities in biotechnological relatedness has greatly enhanced the nutritional composition and reduction in its anti-nutritional content for useful end-products formation. In this present study, cassava peels were fermented for 96 h at room temperature 28 ± 2°C. Seven species of bacterial (Lactobacillus plantarum, Bacillus subtilis, B. megatarum, L. fermentum, L. bulgaricus, L. casei, L. delbrueckii) and five species of fungi (Aspergillus flavus, Mucor mucedo, Penicillium citricum, A. flavus, Rhizopus racemosus) were identified. The pulverized samples were pretreated with lactic acid before pasteurization, and then inoculated with a loopful bacterial isolate prior fermentation. The proximate composition and total cyanide content of the fermented cassava peels were determined. The result of the proximate analysis revealed that there was an increase in the protein content of the natural and pretreated fermented cassava peels from 4.80 to 6.59 and 6.24 to 10.46. There was no considerable difference in the ash content while there was a decrease in fibre content from 16.91 to 11.20 and 13.34 to 10.21. Anti-nutrient such as cyanide decreased in the natural and pretreated fermented sample from 10.0 to 5.59 and 0.02 to 0.01. However, the improvement in the nutritional component and reduction in cyanide content of cassava peels occurred with increase in fermentation time.

Keywords: Cassava waste, Fermentation, Cyanide content, Proximate composition, Degradation

INTRODUCTION

The global increase in wastes generated majorly from agro-industry has generated more concern basically on the menace pose by indiscriminate disposal of these wastes into the environment. Generally, waste generation could either cause havoc to the ecosystem or recycling for desirable product formation [1]. Waste could either classified as domestic, agricultural, refuse and sewage [2]. The amount of wastes generated from different environment might permeate serious health challenges if not properly managed.

Contamination of our environment arising from indiscriminate disposal of cassava peels has resulted in release of obnoxious smells, surface and ground water pollution and contamination of soils [3]. Adequate food processing methods can help modifying these wastes into various forms; prolong shelf life and improvement in the nutritional content [4]. Despite the use of cassava peels, its utilization is limited due to its low protein, high fibre and anti-nutrients content. Fermentation process as an approach could be more efficient in improving the nutritional quality and reduction in its cyanide content for both domestic and industrial use. The reduction in the toxicity attributed to the cassava and cassava peels can be averted through fermentation by microorganisms capable of secreting endogenous linamarase enzyme. Fermentation technology employed in industries in developed and underdeveloped countries because of the less required has improved the organoleptic properties and preservation of final products. Fermentation of cassava peels involved the enzymatic degradation mechanism by microorganisms in breaking down the cyanogenic glycosides (linamarin and loutastralin) into glucose and acetohydrins which spontaneously broken down to acetone and hydrogen cyanide that can easily soluble in water [5]. Biodegradation of waste materials through fermentation

process involving different microorganisms could help in improving the shelf life, texture, aroma, nutritional quality, digestibility and reduction in antinutrient content under different conditions [6]. The objectives of this study are to determine the effect of fermentation on nutritional composition and cyanide degradation level of fermented cassava peels that could be used in formulation of animal feed.

MATERIALS AND METHODS

Sample collection

The peel from fresh cassava tubers obtained from Teaching and Research Farm, the Federal University of Technology, Akure, Nigeria was used for this study.

Microbial analyses

The different microorganisms associated with the fermentation of the cassava peels were isolated. The bacterial and fungal counts of the substrates were determined using pour plate techniques [7]. One millilitre (1 ml) of cassava peels were collected and aseptically transferred into 9 ml of sterile distilled water and serially diluted to give suspension range of 10^{-1} to 10^{-6}. From these dilutions 0.1 ml was aseptically plated out using pour plate method for total viable counts on Nutrient agar, lactic acid bacteria on de Man Rogosa and Sharpe (MRS) agar and total fungal counts on Czapek dox agar. The colonies were observed and counted using colony counter. Representatives of the different purified colonies were subjected to various cultural, morphological and biochemical analyses. Identification was based on Bergey's Manual of Determinative Bacteriology. For fungi identification, wet mount method was used.

Proximate composition analyses

Proximate analysis of the fermented cassava peels was carried out to determine its nutritional compositions. Ash content was determined using the method of Pearson, crude fibre content was determined by the tricyclic acid TCA method (IITA) [8], moisture content was determined according to the oven method of AOAC [9], fat content was determined using the Soxhlet extraction, protein content was determined by the Micro-Kjeldahl method as described by Pearson and modified and carbohydrate content was given as total carbohydrate by difference.

Total cyanide determination

The cyanide contents of the fermented cassava peels were also determined using the method [10].

RESULTS

From this present study, the microbial load from fermented blanched and unblanched cassava peels increase with increase in fermentation time (Table 1). The unbleached cassava peels showed higher in microbial load when compared with blanched fermented cassava peels. For blanched fermented cassava peels the microbial load increased from 7.01 to 18.01 (bacterial), 6.01 to 58.01 (Lactic acid bacteria) and 0.00 to 4.01 (fungi) while un-blanched fermented cassava peels increased from 56.01 to 128.01 (bacterial), 35.01 to 135.01 (Lactic acid bacteria) and 0.00 to 7.01 (fungi).

The microorganisms isolated and the most occurrence isolates from the fermented cassava peels are represented (Tables 2-4). The microorganisms isolated were: *Lactobacillus plantarum, Bacillus subtilis, B. megatarum, L. fermentum, L. bulgaricus, L. casei, L. delbrueckii* and five species of fungi *Aspergillus flavus, Mucor mucedo, Penicillium citricum, A. flavus, Rhizopus racemosus* (Table 2 and 3). The most occurring microorganisms throughout the fermentation process were *Lactobacillus plantarum, Bacillus subtilis, B. megatarum, L. fermentum, L. bulgaricus, L. casei, L. delbrueckii* (Table 4).

The percentage proximate composition of naturally and pretreated fermented cassava peels show an increase in

Table 1: Total microbial counts from fermenting blanched and unblanched cassava peels (Data are represented as mean ± standard deviation (n=3) with the same superscript down the column are not significantly different ($p<0.5$)) (NA: Nutrient Agar; MRS: de Mann Rogosa and Sharpe Agar; CZ: Czapek Dox Agar)

	Blanched			Unblanched		
Time (h)	**NA (10^6) (cfu/g)**	**MRS (10^6) (cfu/g)**	**CZ (10^3) (sfu/g)**	**NA (106) (cfu/g)**	**MRS (10^6) (cfu/g)**	**CZ (10^3) (sfu/g)**
24	7.01 ± 0.00^a	15.01 ± 0.02^a	0.00 ± 0.00^a	56.01 ± 0.01^a	35.01 ± 0.02^a	0.00 ± 0.00^a
48	12.01 ± 0.02^c	58.01 ± 0.02^c	1.01 ± 0.01^b	96.01 ± 0.01^c	98.01 ± 0.01^c	2.00 ± 0.01^b
72	18.01 ± 0.02^d	10.01 ± 0.01^b	4.01 ± 0.02^c	128.01 ± 0.02^d	135.00 ± 0.01^d	7.01 ± 0.02^d
96	8.00 ± 0.01^b	6.01 ± 0.02^a	1.01 ± 0.01^b	58.01 ± 0.01^b	67.01 ± 0.01^b	3.01 ± 0.02^c

Table 2: Colonial, morphological, biochemical characterization and presumptive identification of bacterial isolates (A: Acid production; Ag: Acid production without gas; AG: Acid production with gas; ng: no gas or color changes; Ng: partial color change without gas; a: no acid formed; Ch: chains; -: negative; +: positive; X: un-blanched; R: Rod; F: Fat; L: Long; T: Thin; CM: Creamy)

														Sugar fermentation					
Isolate code	**Colony shape**	**Cell shape**	**Colony appearance**	**Cell arrangement**	**Gram reaction**	**Motility**	**Catalase**	**Methyl red**	**Indole**	**Voges-Proskauer**	**Nitrate**	**Ornithine**	**Casein hydrolysis**	**Hygrogen sulphide**	**Glucose**	**Maltose**	**Insitol**	**Lactose**	**Mannitol**	**Possible organisms**
AX1	FL	R	CM	Ch	+	+	-	+	+	-	-	-	+	-	AG	AG	Ng	Ag	Ag	*Lactobacillus plantarum*
AX2	TL	R	CM	Ch	+	+	-	-	+	-	-	+	+	+	AG	Ag	Ng	Ag	Ag	*Bacillus megaterium*
AX3	FS	R	CM	Ch	+	+	-	+	-	-	-	+	+	+	AG	AG	Ag	AG	AG	*L. fermetum*
AX4	FL	R	CM	Ch	+	+	-	+	+	-	+	-	+	-	AG	AG	Ag	AG	AG	*L. plantarum*
BX1	FL	R	CM	Ch	+	-	+	+	+	+	-	+	-	+	AG	AG	AG	AG	AG	*L. bulgaricus*
BX2	TL	R	CM	Ch	+	+	+	+	-	-	-	+	+	+	AG	AG	AG	AG	AG	*L. fermentum*
CX1	TS	R	CM	Ch	+	+	+	-	+	-	+	+	+	+	Ag	Ag	AG	AG	AG	*B. subtilis*
CX4	TL	R	CM	Ch	+	+	+	-	-	-	+	+	+	+	Ag	Ag	AG	AG	AG	*L. plantarum*
DX1	FT	R	CM	Ch	+	+	-	+	-	-	+	-	+	+	Ag	Ag	Ng	Ag	Ag	*L. delbrueckii*
DX2	FT	R	CM	Ch	+	+	-	+	-	-	-	-	-	+	Ag	Ag	Ng	Ag	Ag	*L. casei*
DX3	FL	R	CM	Ch	+	+	-	+	-	-	+	-	+	+	AG	Ag	Ng	Ag	Ag	*L. plantarum*

Table 3: Cultural characterization and microscopic observation of fungal isolates (CZ: Czapek Dox)

Isolate code	Cultural characteristics	Microscopic observation	Probable isolates
CZ0	The colony are fast growing, cotton to fluffy in appearance, swarm over the plate, white base with grey or brown colour, shows brown spores varying in size	Non-septate myceliums which give rise to apical, globular sporangia with collumella, collarette at the base that hold it but lack rhizoid.	*Mucor mucedo*
CZ1	Typically black powdering, with dark spores varying in sizes	Septate mycelium with long hyphae, long conidiosphores with spherical vesicle at the apex, conidia are globose vesicle, phalliades, regularly roughened and uninucleate.	*Aspergillus niger*
CZ2	Fast growing organisms, green colouration on the surface of the plate.	Conidiosphore hyaline smooth or rough wall, conidia are globose, eelipsoidal, cylindrical or fusiform	*Penicillium citricum*
CZ3	Fast growing organisms, black to yellow colour in appearance	Septate hyphae with long filament that grows outward the medium, vesicles are circular, both the hyaline and conidia are septate, rough, granular with long conidiosphores. The conidia head were compacted radiate and unseriate.	*A. flavus*
CZ4	Fast growing organism, typically cotton-cloudy, with colour changes from white to grey and later yellowish brown	Non septate mycelium which give rise to straight sporangiosphores that contain sporangium, root like structure (rhizoid) that penetrate into the medium and collumellae.	*Rhizopus racemosus*

moisture content, protein content, ash content, fat content and decrease in crude fibre and carbohydrate content (Tables 5 and 6). The protein and fat content increased from 4.80 to 6.59 and 1.38 to 3.63. While percentage proximate composition of crude fibre and carbohydrate content showed a decrease from 13.34 to 10.21 and 9.77 to 5.67 (Table 6).

The total reduction in cyanide content to a significant level was monitored. The cyanide content of the naturally and pretreated fermented cassava peels showed a decrease in cyanide content from 10.0 to 5.59 and 0.02 to 0.01 with increase in fermentation time from 10.0 (Figures 1 and 2).

Table 4: Occurrence of microorganisms isolated from fermented cassava peels (+: Present; -: Not present/absent)

Microorganism	Time (h)				(%) occurrence
	0	24	48	72	
Bacillus subtilis	+	+	+	+	6%
Lactobacillus plantarum	+	+	+	+	25%
L. fermentum	+	+	+	+	13%
B. megatarum	+	+	+	+	6%
L. bulgaricus	+	+	+	+	6%
L. casei	+	+	+	+	6%
L. debrueckii	+	+	+	+	6%
Aspergillus niger	-	+	+	+	6%
Mucor mucedo	-	-	+	+	6%
A. flavus	-	+	+	+	6%
Penicillium citricum	-	+	+	-	6%
Rhizopus racemosus	-	+	+	-	6%

Table 5: Percentage proximate composition of naturally fermented cassava peels (Data are represented as mean ± standard deviation (n=3) with the same superscript across the row are not significantly different ($p<0.05$))

Proximate	Fermentation period (h)			
	24	48	72	96
Moisture content	49.04 ± 0.40a	50.46 ± 0.33b	58.96 ± 0.12c	60.31 ± 0.44d
Ash	5.53 ± 0.06a	5.86 ± 0.40ab	6.09 ± 0.18bc	6.37 ± 0.04d
Fat content	1.38 ± 0.09a	2.33 ± 0.09b	2.42 ± 1.20b	3.63 ± 0.25c
Crude protein	4.80 ± 0.03a	5.25 ± 0.57ab	5.46 ± 0.21b	6.59 ± 0.02c
Fibre content	16.91 ± 0.55c	14.27 ± 0.51b	13.63 ± 0.43b	11.20 ± 0.38a
Carbohydrate Content	22.33 ± 0.88c	21.78 ± 0.14c	15.43 ± 0.87b	11.91 ± 0.54a

Table 6: Percentage proximate composition on pretreated fermented cassava peels at varied inoculum concentration (Data are represented as mean ± standard deviation (n=3) with the same superscript across the row are not significantly different ($p<0.05$))

Proximate	Control	Inoculums concentration (µl/hr)			
	F/P	10^3	10^4	1.5×10^4	2.0×10^4
Moisture content	68.56 ± 0.34^a	68.43 ± 0.32^a	68.38 ± 0.23^a	68.35 ± 0.05^a	68.42 ± 0.05^a
Ash	0.65 ± 0.09^a	0.67 ± 0.06^a	1.11 ± 0.09^b	1.56 ± 0.06^c	1.82 ± 0.07^d
Fat	1.45 ± 0.10^a	1.52 ± 0.05^a	1.64 ± 0.09^{ab}	1.79 ± 0.19^b	3.85 ± 0.15^c
Crude protein	6.24 ± 0.16^a	7.57 ± 0.11^b	8.03 ± 0.33^{bc}	8.43 ± 0.43^c	10.46 ± 0.61^d
Crude fibre	13.34 ± 0.47^d	12.32 ± 0.20^c	11.68 ± 0.46^{bc}	10.87 ± 0.79^{ab}	10.21 ± 0.48^a
Carbohydrate content	9.77 ± 0.29^c	9.48 ± 0.34^{bc}	9.34 ± 0.41^{bc}	9.00 ± 0.72^b	5.67 ± 0.13^a

DISCUSSION

Apart from being a low cost inexpensive substrate, easy accessible, the use of cassava peels in food industry can be considered as a significant way to reduce cost. Also, it is noteworthy that the process will, no doubt, have a major environmental impact as it helps to resolve the problem associated with the disposal of cassava waste. This will create a safe and eco-friendly environment, especially in major cassava processing region of the world.

The microorganisms isolated from the fermented blanched and unblanched cassava peels are *Lactobacillus plantarum, Bacillus subtilis, B. megatarum, L. fermentum, L. bulgaricus, L. casei, L. delbrueckii, Aspergillus flavus, Mucor mucedo, Penicillium citricum, A. flavus, Rhizopus racemosus.* The growth and colonization of microorganisms on

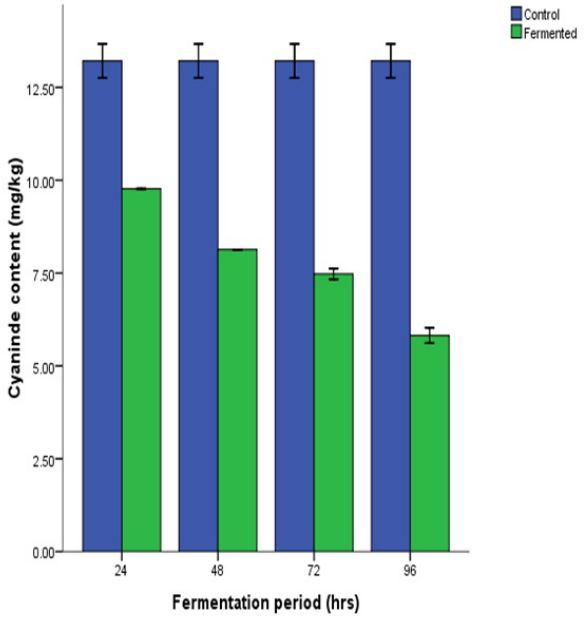

Figure 1: Effect of natural fermentation on cyanide content of cassava peels

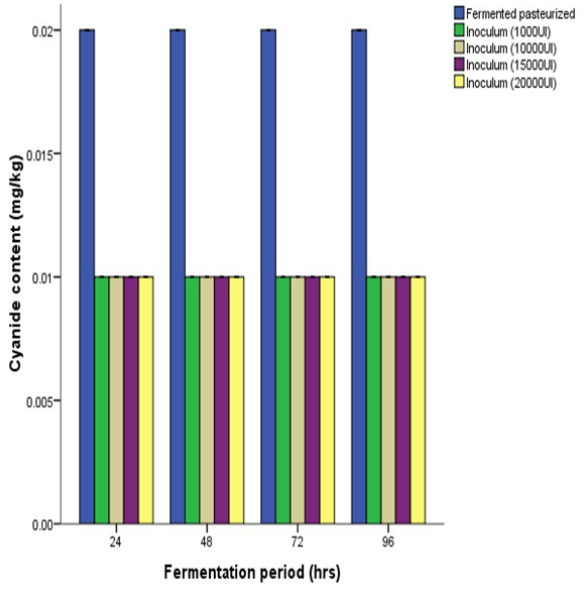

Figure 2: Effect of mono-culture on cyanide content of fermented cassava peels

fermented cassava peels might be due to its chemical compositions and certain environmental factors (pH, oxygen and temperature) (Arotupi; Olaniyi and Arotupin). The high bacterial and fungal counts may be due to lack of efficient control measures through indiscriminate discharge of this agricultural waste (cassava peels) into the environment by Arotupin. These activities tend to expose the agricultural waste to microbial contamination. The microorganisms isolated (bacteria and fungi) may probably have originated from processing site, water, processing material, human activities through handling, peeling etceteria and the fermenting substrate itself, while the variation in microbial population may be due variation in the processing techniques, depletion of available nutrients and the prevailing environmental conditions. The low bacterial counts in cassava peels may be due to the quality control measures and the processing technique (blanching) adopted in the collection and fermentation of the cassava peels.

The proximate composition of the naturally fermented and pretreated fermented cassava peels with different inoculum concentration show an increase in ash, fat, crude protein and moisture content while the crude fibre and carbohydrate content decreased respectively when compared with control. The protein content of naturally fermented and pretreated

fermented cassava peels increased from 4.80 to 6.59 and 6.24 to 10.46 with increase in fermentation time. The increase in crude protein contents of the fermented cassava peels might be attributed to the high number of microorganisms with the ability in secreting certain extracellular enzymes such as amylases, linamarases and cellulases into the wastes in during their breakdown in the fermentation medium [11-13], ability of the microorganisms to synthesize amino acids [14], addition of crude proteins produced by bacterial isolate and combination of factors such as carbon (iv) oxide, temperature and water [1,15]. The difference in the protein content could be attributed to the kind of cassava used, agro-ecological conditions and microorganisms involved in the fermentation process.

The crude fibre content of the fermented and pretreated fermented cassava peel with bacterial inoculum showed a general decrease with increase in fermentation time. The decreased could be attributed to the ability of the fermenting microorganisms to degrade the crude fibre of fermenting cassava peel, secrete hydrolyzing and oxidizing enzymes involving in conversion of recalcitrant compounds in the waste into utilizable compounds [7] and abundant production of organic acids resulting from fermentative dissimulation of carbohydrate [16].

The ash content is always a rough measure of inorganic mineral elements in a sample [17]. The proximate analysis from this study showed an increase in the ash content of pretreated fermented substrate when compared with naturally fermented sample. The increase in ash content could be as a result of the growth and multiplication of the microorganisms in the fermentation medium [18].

The cell membrane of microorganisms contains lipid, organic, inorganic compounds and some elements such as lipid component consisting of triglyceride. The fat content of the naturally and pretreated fermented cassava peels increased with increase in fermentation time. The increase in the fat content might due to the increase in the microbial mass, activities of lipolytic microorganisms to secrete extracellular enzyme (lipase), secretion of microbial oil into the fermenting medium and other products from the metabolism [19].

The moisture content of the naturally fermented cassava peels increased with fermentation time while the pretreated fermented cassava peels showed higher increase in moisture content. The increase in moisture content might due to the availability of free bond water in the fermentation medium, the high rate of diffusion of the solute into the fermenting medium, the autolysis action of the microorganisms and presence of large volume of water in the medium. The increase in the moisture content could also account for the decrease in the carbohydrate content.

The carbohydrates content of pretreated and naturally fermented cassava peels decreased with increase in fermentation time. The decrease in carbohydrate content could be attributed to the conversion of oligosaccharides to simple sugars or the utilization of the carbohydrate nutrient as source of energy by the fermenting microorganisms for growth and metabolism by [20].

The cyanide contents of naturally fermented cassava peels were significantly higher (p<0.05) when compared with the pretreated fermented cassava peels with different inoculum concentrations that showed reduction in the cyanide content. The reduction in the cyanide content of the fermented cassava peels might due to the synergistic effect of loss of cyanogenic glycosides on hydrolysis by linamarase [21], evaporation of hydrogen cyanide during drying, changes in textural formation of the plant tissues, diffusion of vacuole-bound cyanogenic glycosides when come in contact with the membrane-bound linamarase, cleavage or maceration of the plant tissues which expose the cynanogenic glycoside to linamarase on contact with water by pressing out during cassava processing [22,23], metabolic activities of inherent microorganisms [11,24], ability of the microorganisms to secrete extracellular enzymes (amylase, xylanase and linamarase), increase in cell mass and formation of a hydrolytic complex bind force to the cyanide compound.

CONCLUSION

The results obtained from this have revealed the improvement in the nutritional component and reduction in cyanide content of cassava peels. This secretion of certain endogenous enzymes by the microorganisms might enhance the degradation of recalcitrant substances in nature. Therefore, fermentation of cassava could facilitate the decontamination of waste disposed into the environment for a desirable products formation in food processing industry under normal conditions. However, the reduction in cyanide content is an indication that the fermenting substrate could be a medium supporting growth for microorganisms involving in detoxification process.

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