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Enhancing the Nutritive Values of Agrowastes for Animal Feed Production Using Selected BacterialStrains

[1]Akindele, S. T and[2]Fagade, O.E

[1]*Department of Science Laboratory Technology, Abraham Adesanya Polytechnic, Ogun State Nigeria*
[2]*Department of Microbiology, University of Ibadan, Oyo state, Nigeria*

Abstract: *The peels of cassava, plantain and pawpaw are major agricultural waste after harvest. These agro wastes after harvest were used as substrate for the production soluble protein and reducing sugar. The selected bacteria strainsincludeAlcaligenes species, Alcaligeneseutrophus, Alcaligenesaquarnarius, Cellulomonas species and Cellulomonasflavigena. The various peels were fermented either singly with one isolate or in combination of wastes of varying ratios of 1:1, 2:1, 5:1 and 10:1.The protein yield of the isolates in the preliminary experiment was used to screen the isolates.The fermented cassava peels with plantain peels had the highest level of protein production at combination 5:1 (49.3%) and lowest at ratio 10:1 (4.8%). The fermented plantain peels with cassava peels had highest protein at rates 1:1 (34.6%) lowest at ratio 10:1 (12.0%). For fermented cassava peels with pawpaw peels had the highest level of protein production ratio 5:1 (47.3%).The study shows that individual strain of the bacteria employed had potentials in the production of single-cell protein of quality using the solid state fermentation technique with recommended ratio treatment for optimal production.*

Keywords:*Agro wastes, Bacteria strains, Protein, Reducing-sugar, Fermentation*.

I. Introduction

In a world of diminishing resources and increasing needs, opportunities for the re-use of wastes materials are examined since most human endeavor result in waste products (MacDonald-Dow and Griffin, 1981). Prior to the global population explosion and the industrial were low enough and of such a composition as to allow their ready stabilization (recycling) by natural means (Arthur 1992). Among the different causes of pollution, agricultural caused pollution is one of the most important parts of the natural environmental quality problem. Agricultural wastes are excesses of agricultural production that have not been utilized (Loehr, 1974).

Recent changes in agricultural production methods have caused natural interest in the agriculturally related pollution to escalates and such pollution is no longer considered minor and uncontrollable (Loehr, 1974). Many agro-industrial wastes particularly those derived from food processing are useful as animal feed. A number of these wastes have been used directly in animal's feeds (leDividich et al., 1976). The agricultural product feeds that are in Nigeria include cereals, legumes, straws, corncobs, cocoa pod husk, coffee pulps, cassava peels, pawpaw peels, yam peels, cocoyam peels etc.

Microorganisms can be isolated from almost any environmental conditions. Microbes will adapt and grow at sub-zero temperature, as well as extreme heat, desert condition, in water with an excess of oxygen and in anaerobic conditions with the presence of hazardous compounds or any waste. The main requirements are energy source and a carbon source, because of the adaptability of microbes and other biological systems many compounds or wastes that are widely disposed into the biosphere either deliberately, for example, agro-chemicals, or accidentally, for example oil- spillages are being degraded (Karevia and Stark.,1994). The presence of microbes in any environment is advantageous especially for waste that cannot be collected for treatment and so in most cases, natural microbial activity may be sufficient to degrade these dispersed wastes.

The nutritional problem encountered in the utilization of these by products are enhanced by the use of microbial degrading enzymes from the bacteria to achieve maximum yield to a single-cell protein and reducing sugar through the use of solid state fermentation. The present paper reports the changes in the nutritive value of wastes Plantain peels (PLT) Cassava peel (CP), Pawpaw peels (PWA) and the optimalcondition for production of the single-cell protein and reducing sugartaking into consideration these factors: size of the wastes, temperature,optimization of the peels.

II. Materials And Methods

Collection of Samples

The cassava peels were obtained from cassava processing mills Eleyele while the plantain pawpaw peels, pawpaw peels, and lemon peels were obtained from Bodija market. The peels were collected in a clean polythene bags and brought into the laboratory.

Pure Culture

Bacteria used in this study were isolated from the agricultural by products which were plantain peels, cassava peels and pawpaw peels. The peels were washed with water and surface sterilization was carried out. The peels were cut into small portion and placed on sterile plates. Molten sterile plate count Agar and Nutrient Agar were poured onto different plates. The agar plates were allowed to solidify and incubated for 24hrs. The bacteria isolates were further streaked on nutrient agar and incubated at 37^0C for about 24hrs to obtain pure culture.

Preparation of Inoculum

Peptone water was prepared and dispensed into bottles and sterilized at 121^0C for 15 mins. After cooling, the peptone water was inoculated with the isolates prepared from 24hrs old culture. The cultures are then incubated for 24hrs, after which the peptone water were turbidity.The turbidity shows that the isolates have grown. The isolates were read in the spectrophotometer at 600mm. Sterilized distilled water was used to standardize the inoculum to 0.50 reading at 600mm VV. Then 5ml of the 0.50 reading were inoculated into the substrates (wastes) for fermentation. Then 1ml of the inoculum was plate out into different sterilize petri-dish to show the growth count of the inoculum.

Agricultural By-product as Substrate

30g of each of the peels (Plantain peels,Pawpaw peelsandCassava peels)were weighted 250ml conical flask in four different ratios and the moisture content was adjusted to 20%. The mouth of the flask were clogged with cotton wool and then covered with aluminum foil. The substrates were autoclaved at 121^0C for 15mins. After autoclaving, the substrate were aseptically with the bacteria, another set of substrate were not inoculated, these served as the control. All the substrateswere placed in the incubator for 7 days. Samples were withdrawn after 7 days. The samples were thoroughly mixed, oven dried, grinded and stored in sterilized bottles. Samples from control flasks were treated alike.

Determination of Soluble Protein Content

Two (2) grammes of the sample was grounded into pulp and mixed with 20ml of 50% ethanol.The slurry was then filtered into macCartney bottles using a whatman No. 1 paper. 0.3ml of the filtrate is pipette into test tube. 3ml of (1ml of 0.5% $CuSO^4$) in 1% sodium potassium tartarate and 50ml of 20% sodium carbonate ($NaCO_3$) in 0.1N sodium hydroxide (NaOH) was added to it. Shaken thoroughly and was allowed to stand for 10minutes. Then 0.3 (10ml of distilled H_2O and 5ml of Folin reagent in ratio 2:1) was added to it and mixed. This was allowed to stand for 30 minutes and then it was read in spectrophotometer SP6-250 at 540nm uv optical density. Blank was prepared with 0.3 distilled water as the sample. The standard curved was determined by using Bovine Serum Albumen (BSA) (lowry et al, 1951).

Determination of Reducing Sugar

Two (2) grammes of the pulp sample were mixed with 20ml of 50% ethanol. The slurry was then filtered. 0.5ml of 5% phenol was added to it and allows it to stand for 10 mins. Thereafter 2.5ml of concentrated sulphuric acid was added. It was shaken thoroughly and allows it to cool. It was then read in the spectrophotometer at SP6-250 at 490nm uv optical destiny. Blank was prepared with 0.5m DH_2O as the sample. The amount of the sugar produced was read off from the standard curve obtained by the absorbance of increasing glucose solution(lowry et al, 1951).

Optimization of Waste

Each batch culture consisted of 30grammes of the peels. For optimizing the peels, they were combined in different ratio/treatment which were 1:1 (15g:15g), 2:1 (20g:10g), 5:1 (25:5g), 10:1 (28g:2g)

Statistical Analysis

The results obtained were analyzed with the use of student T-test to determine the significant differences in the ratio of the optimized fermented peels using the 95% confidence limit.

III. Results And Discussion

Total of sixteen bacteria isolates were obtained from the agro-industrial by products made up of plantain peels, cassava peel, pawpaw peels and lemon peels. Preliminaryscreenings of the isolates were carried out based on their protein production ability on unfermented cassava peels. The best six strains of bacteria selected were *Cellullomonasflavigena*(LM04), which produces 14.6%, *Cellulomonas specie* which gave 13.3% (LM01), *Alcaligenes specie* (PLT2) and *Alcaligenesaquarnarius* (PLT4) which gave 12.0%, and the strains from pawpaw *Cellulomonas species* (PWA4) and *Alcaligeneseutrophus* (PWA1) which produced 11.6% and 9.3% respectively as shown in table 1.

Table 1: percentage protein and reducing sugar produced in untreated fermented cassava peelsby bacterial strains from agro-wastes

Sample code	Reducing sugar	Protein
PLT1	24.8	6.7
PLT2	6.80	12.0
PLT3	10.4	2.6
PLT4	16.0	12.0
PWA1	15.6	9.3
PWA4	15.2	11.6
PWA5	24.0	5.3
LMO1	26.0	13.3
LMO4	26.8	14.6
CD1	24.6	6.6
CD2	24.0	8.0
Control (cassava peels)	5.10	5.67

Code: *PLT- Plantain peels, PWA- pawpaw peels, LM- Lemon peels, CD- Cassava peels*

TABLE 2: Protein and Reducing Sugar Levels of Fermented Cassava Peels with Plantain Peels at Different Treatment

	1 : 1		2 : 1		5 : 1		10 : 1	
Sample Code	Protein	Sugar	Protein	Sugar	Protein	Sugar	Protein	Sugar
PLT2	25.3	7.2	32.0	20.8	49:3	55.2	37.3	24.0
PLT4	29.3	4.8	28.0	16.0	26.6	6.8	24.6	15.2
PWA1	28.0	4.0	31.3	41.6	25.3	19.2	24.0	9.6
PWA4	32.0	4.2	34.3	39.2	32.0	23.2	30.6	17.6
LMO1	34.6	4.0	29.3	16.0	12.0	16.8	36.0	15.2
LMO4	26.6	4.2	32.3	12.0	37.3	15.0	29.3	4.8
Control	5.4	3.5	--	--	--	--	--	--

Alcaligenes specie (PLT2) had its high protein production at ratio 5:1 with 49.3% and the lowest value by *Cellulomonas specie* (LMO1) at 5:1 with 12.0%., For reducing sugar*Alcaligenes specie* (PLT2) produces highest valueat ratio 5:1 with 55.2% and lowest value was produced by *Cellulomonasflavigena* (LMO4) as shown in table 2.

Table 3: Protein and reducing sugar levels of fermented pawpaw peels with cassava peels at different treatment

	1 : 1		2 : 1		5 : 1		10 : 1	
Sample Code	Protein	Sugar	Protein	Sugar	Protein	Sugar	Protein	Sugar
PLT 2	26.3	34.2	27.6	36.6	32:6	32.0	18.3	24.0
PLT 4	24.6	31.8	28.3	36.8	34.6	28.8	16.3	25.6
PWA 1	28.0	39.2	40.0	37.0	42.6	32.4	20.0	33.6
PWA 4	30.6	47.2	46.6	47.8	50.6	47.0	27.3	46.6
Control	13.3	14.7	--	--	--	--	--	--

For fermented pawpaw with cassava peels after the period of 7 days, it was observed that *Cellulomonas specie* (PWA4) produces the highest value of protein at 5:1(50.6%) and the lowest value was produced by *Alcaligenesaquarnarius* (PLT4)at 10:1 (16.3%). for the reducing sugar the highest protein production was produced by *Cellulomonas specie* (PWA4) at 2:1 (47.8%) and lowest was produced by *Alcaligenes specie* (PLT2) 5:1 (24.0%) as shown in table 3.This shows that the bacteria had the ability to degrade the polysaccharides in the substrate to soluble sugar (Han and Anderson, 1975) produced similar results but it was carried on rye-grass straw using *Aspergillus niger*

Table 4: Protein and reducing sugar levels of fermented plantain peels with cassava peels at different treatment.

	1 : 1		2 : 1		5 : 1		10 : 1	
Sample Code	**Protein**	**Sugar**	**Protein**	**Sugar**	**Protein**	**Sugar**	**Protein**	**Sugar**
PLT 2	25.3	7.2	22.6	16.0	21:3	16.8	15.6	4.8
PLT 4	29.3	4.8	23.3	5.6	20.7	12.4	16.1	4.4
PWA 1	28.0	4.0	10.6	9.6	16.3	8.0	13.6	4.8
PWA 4	32.0	4.2	20.6	15.0	18.0	5.8	20.0	4.8
LMO 1	34.6	4.0	27.3	4.0	24.0	8.8	12.0	5.0
LMO 4	26.6	4.2	25.3	4.0	20.6	5.6	12.0	4.8

The result of fermented plantain peels with cassava peels after 7 days. All isolates had their highest production level of protein at 1:1; *Alcaligenes specie* (PLT2) 25.3%, *Alcaligenesaquarnarius* (PLT4) 29.3%, *Alcaligeneseutrophus* (PWA1) 28% *Cellulomonas specie* (PWA4) 32%, *Cellulomonas specie* (LMO1) 34.6% and*Cellulomonasflavigena* (LMO4) 26.6%. For reducing sugar, the highest value was produced by *Alcaligenes specie* (PLT2) at 5:1 (16.8%) and the lowest was at 1:1 produced by*Alcaligeneseutrophus* (PWA1) and *Cellulomonas specie* (LMO1) (4.0%.) as shown in table 4.

Table 5: Protein and reducing sugar levels of fermented cassava peels with pawpaw peels at different treatment.

	1 : 1		2 : 1		5 : 1		10 : 1	
Sample Code	**Protein**	**Sugar**	**Protein**	**Sugar**	**Protein**	**Sugar**	**Protein**	**Sugar**
PLT 2	26.3	34.2	30.3	36.8	32:6	32.0	20.1	24.0
PLT 4	24.6	31.8	28.0	36.8	31.3	28.0	18.2	25.6
PWA 1	28.0	39.2	34.0	37.0	42.0	36.4	24.0	33.6
PWA 4	30.6	47.2	46.6	47.8	47.3	47.0	26.6	46.8
Control	13.3	14.7	--	--	--	--	--	--

Table 5 shows the change that occurred to fermented cassava peels with pawpaw peels after 7 days. All the isolates had their highest value of production for protein at ratio 5:1 and the lowest production was at 10:1,*Alcaligenes aquarnarius* (PLT4) 18.2%. For sugar production, the highest value was 2:1*Cellulomonas specie* (PWA4) 47.8% and lowest value was at ratio 10:1 *Alcaligenes species* (PLT2) had 24%.

Table 6: **Effect of temperature on the growth of isolates**

Sample Code	**10°C**	**20°C**	**25°C**	**30°C**	**37°C**	**45°C**
PLT 2	0.36	0.40	0.58	0.46	0:31	0.05
PLT 4	0.40	0.34	0.40	0.36	0.32	0.01
PWA 1	0.26	0.25	0.34	0.33	0.38	0.01
PWA 4	0.37	0.38	0.51	0.43	0.41	0.02
LMO 1	0.26	0.27	0.42	0.38	0.28	0.02
LMO 4	0.38	0.29	0.39	0.39	0.24	0.04

Table 6 shows the effect of temperature on the isolates. Optimal growth temperature for all the isolates was 25^0Cwhile good growth was notice in*Cellulomonas specie* (PWA1)between 25^0C to 37^0C. Growth was at 45^0Cwas low for all isolates.

Alcaligenes species and *Cellulomonas species* are used as the biodegradation of the agro-waste. *Cellulomonas species* had been used in the degradation of cellulose while *Alcaligenes species* had been in the degradation of bagasse and rice straw to increase the cell mass and used in the enhancement of the protein supplement of these products (Han et al., 1979). The increase in the products which are protein and reducing sugar that occurs mostly in cassava peels against other peels could be attributed to the fact that cassava peels are less fibrous to plantain peels, pawpaw peels and lemon peels as reported by Iyayi and Lossel (2001) similar results were obtained in the fermentation of cassava products with *Trichoderma species.*

The increase in the protein level obtained could be observed as a result of the bioconversion of the sugar into protein. Solid state fermentation of biomass has been attempted as a means of elevating the protein content by many workers Rodriquez et al., (1985) similar results had been obtained in this present study.

IV. Conclusion

In conclusion, results of the present study have demonstrated the best treatment for the degradation of the peels – cassava peels, pawpaw peels and plantain peels and the best treatment or proportions that suit each peels by the isolates.,*Alcaligenes species* (PLT2), *Alcaligenesaquarnarius* (PLT4), *Alcaligeneseutrophus* (PWA1), *Cellulomonas species* (PWA4), *Cellulomonas species* (LMO1) and *Cellulomonasflavigena* (LMO4). The bacteria had the ability to degrade polysaccharide contents of the peels by converting them to simple sugar with a beneficial increase in energy and protein in these by products. As such, the method of production was simple and cheap. It was believed that the requirements for this type of production could be easily met by the technology and economy in the developing countries.Also, it can serve as a control for agro-industrial pollution, since the peels can be easily collected and used on a large small production of single cell protein and reducing sugar**.**

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