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EFFECT OF MICROBIAL FERMENTATION OF LIGNOCELLULOSE WASTES ON NUTRITIVE STATUS

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ABSTRACT

Lignocellulosic wastes (municipal wastes and cassava peels) were hydrolyzed with enzymes (cellulase and glycosidase) and fermented for ethanol using saccharamyce cerevisae. The amount of ethanol produced was found to be both substrate and concentration dependent. Higher yields of ethanol were obtained with less crystalline wastes. Higher yields of ethanol were obtained with cassava peels than with municipal wastes. In both case the best yields of ethanol were in the presence of glucose and acid-hydrolyzed starch. In both cases, the fermented wastes gave higher protein content than the unfermented wastes. The possibility of employing the fermented products food and animal feed supplement is suggested. The feasibility of employing the cassava peels and municipal wastes as sources of ethanol or alcoholic beverages is also worth consideration.

KEY WORDS. Lignocelluloses wastes, municipal wastes, cassava peels, saccharamyce cerevisae and ethanol

INTRODUCTION

The problem of municipal and agricultural wastes disposal currently encountered all over the world cannot be over emphasized. These wastes are often burnt resulting in a loss of volatile nutrient and generation of gaseous pollutants in the environments (Bussy, 1992). These wastes are mostly lignocelluloses containing lignin and cellulose which cannot be digested by human system due to absence of the enzyme, cellulase. However, these wastes can be hydrolyzed and converted into fermentable substrates using micro-organisms that can survive at unusually high temperature (Eka, 1980. Bussy, 1992, French and McGeary, 1997).

The production of ethanol by fermentation involves microbial hydrolysis of reducing sugars. These substrates are however the main sources of foods within the tropics, providing nourishment for a greater number of people. These cereals and tuber-yielding crops are dependable crops (Eka,1980; Mayo- Smith, 1997) Therefore, the use of cereals and tuber-yielding crops, which are food resources in the tropics, for the production of ethanol has been a global problem affecting food supply Thus, compression of these lignocellulose wastes would improve food supply within the tropics and temperate regions of the world (Fleming et al, 1997; Iguchi et al, 1997; Mayo-Smith, 1997; Verbanck, 1997). Several Nigerian foods are fermented at some stage in their preparation such as gari (fermented grated cassava), Kunu (Hausa), fermented sorghum pap, daddawa (Hausa), fermented seeds of locust beans, parkia filicoidea and nono (Hausa), fermented cow milk (Spickett, *et al*, 1955,Kemp,1957; Levi and Oruche, 1958; collar and Levil; Oke,1967; Akinrele,1963,Ekandem,1963; Okafor, 1966; Akinrele, 1967; Irvine, 1969. Eka and Edijala, 1972).

The fermented food products may be in the form of condiments, spices, flavoring agents, beverages or even staple foods (Mc Crachy and Langenbucher, 1996; O'Brien, CP, 1996). In the fermentation processes, microorganisms utilize the biochemical constituents of the food material, changing them from one form to the other with the aid of the microbial enzyme systems (Eka, 1984) During fermentation, changes both desirable and undesirable may occur in the food material and may result in the enrichment of the fermented products (Eka, 1976; Eka, 1979; Beidler,1991)› The present study focused on the assessment of lignocellulose wastes through fermentation to determine the possibility of using the fermented products as human food and animal feed supplements. However, it is necessary to ascertain that there are no undesirable substances (toxins) arising as a result of the yeast activity. Thus, a research into the antinutritional constituents of these fermented products may be rewarding.

MATERIALS AND METHODS

Collection and preparation of samples

Cassava peels were obtained from a gari processing mill at Ugep in Yakurr Local Government Area of Cross River State, Nigeria while municipal wastes were obtained from Calabar Urban Development Authority (CUDA). The methods of treatment of samples and analysis adopted were those recommended by the Association of Official of Agricultural Chemists (AOAC) (1965).

These lignocellulose wastes were hammer-milled, dried in a hot circulating oven at about 60°c for 36 hours and then ground into fine powder. The chemical analysis (except for the moisture content determination) was carried out using the dried forms of cassava peels, fermented cassava peels residues, municipal wastes and fermented municipal waste residues. The dried lignocelluloses wastes were ground in a mortar and sieved into fine powder using 40 mesh sieves. The pulverized sample was then stored in clean dried sample bottle containers until required for analysis.

Hydrolysis of lignocelluloses

The hydrolysis of lignocellulose wastes was done in two stages using the methods of Buskel and Chuks (1992) and that of Bussy (1992). the first stage was the liquefaction of substrates using a commercially purchased enzyme solution (cellobiase), which broke down the cellulose molecules into short chains of dextrin while the second stage involved hydrolysis of the short chains of dextrin into glucose using a commercially purchased enzyme solution (glycosidase) This involved dissolving 30g each of the dried pulverized samples

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in 500ml of distilled water. The slurry was heated to 90°c with stirring and 20ml of the cellobiase solution added. The solution was again heated to 85°c with stirring, and allowed to cool to room temperature and 20ml of the glycosidase solution added, covered and allowed to stand over night.
The hydrolyzed samples were used for the microbial fermentation.

Assay of yeast activity
The yeast fermentation was carried out by seeding known weight of the hydrolyzed substrate mash with a known volume of the brewers yeast (saccharamyce cerevisae) obtained from the Nigerian Breweries Limited, Aba, Nigeria.

Preliminary investigations
Preliminary investigations were carried out to assay the yeast activity and also to find out whether lignocellulose wastes contained fermentable sugars and the extent to which these wastes could be hydrolyzed using 2% hydrochloric acid (HCl). In the experiments, three sets of flat bottom flasks were labeled in triplicate A,B and C. 20g of glucose were put into the flask A, 20g of cassava wastes powder into flask B and 20g of municipal wastes powder into a flask C.In each flask, 300ml of distilled water was added and made to mark.
Hydrochloric acid was added to flask C to give a final concentration of about 2% HCl. The flasks were sealed tightly with zinc foil and autoclaved at a pressure of 14 16/ sg.in; temperature of 121°C for about 1 hour. This allowed for the sterilization of the flasks contents and the hydrolysis of the municipal wastes by the 2% HCl. At the end of sterilization, the flasks were allowed to cool to room temperature. The content of the flask C was tested for starch using iodine. The contents of flask A and B were seeded with 10ml of the undiluted brewers yeast (saccharamyce cerevisae). The flasks were again covered with zinc foil, and left at a constant room temperature of 25°c for 7days, for fermentation to take place. The content of flask was tested for ethanol by smelling and testing with ammonium nitrate (Kemp, 1957). The ethanol was distilled off in the presence of the residue using quick fit distillation apparatus. The content of flask I was washed with 1000ml of distilled water into the distillation flask.

The ethanol content was distilled off at a temperature of 78°c into a clean 150ml flask. The percentage ethanol was determined using the specific gravity method. This involved weighing a known volume of the distillate in a specific gravity bottle at a noted temperature and calculating the specific gravity. The percentage ethanol by volume corresponding to the specific gravity at the temperature which weighing was made and was read from the standard table contained in the Association of Official Agricultural Chemist (AOAC) (1965).

Fermentation Assays
Experiments for actual fermentation of the lignocelluloses wastes powders were carried out using three sets of flasks in triplicate which were labeled 1to3. The contents of the flasks were as shown in tables 1, 2 and 3. The fermentation and determination of the percentage ethanol were carried out by using the same procedures as described for the preliminary fermentation investigations. The experiments were performed thrice and the results were based on the average of the three values obtained.

RESULTS

The results of the study are presented in tables 1,2,3,4 and 5. Table 1 show the fermentative ability of the strain of yeast used when only glucose was used as the source of carbohydrate. The amount of ethanol obtained was as high as 20.59% by volume. Table 2 shows the percentage ethanol obtained during yeast fermentation of cassava wastes powder under given conditions.
The highest percentage ethanol was obtained when both glucose and hydrolysed cassava wastes were present in the medium. When only hydrolysed cassava wastes present, the percentage ethanol was more than 50% of that of glucose. The same pattern also emerged in the case of yeast fermentation of municipal wastes (Table 3).

The results in tables 4 showed that cassava wastes contain a high percentage of moisture and carbohydrate. From the starch test before hydrolysis of the cassava wastes powder and the percentage ethanol obtained for the fermented hydrolyzed and the unhydrolysed cassava wastes powder, the results showed that most of the carbohydrate exist as polysaccharides The results for cassava wastes, hydrolyzed and fermented residues (Table 4) showed an increase in the ash content of the residual products of fermentation while there was a decreased in carbohydrate content of the residual products of Fermentation. There was an increase in protein and lipid content of the residual products of fermentation and this could be attributed to the lipid and protein content of the yeast used for fermentation.

The results for the municipal wastes (table3) showed that municipal wastes contained low moisture content, carbohydrate content, protein content and lipid content as well as the ash content. The results for the unfermented municipal wastes, and the fermented municipal wastes residues showed that there was a decrease in the carbohydrate content of the residual products of fermentation and an increase in the protein, lipid and ash content.

Table 1: Ethanol Production from Glucose

Medium	specific gravity of distillate	% Ethanol by volume at 25°c
20g glucose + 0.20g $K_2H\ PO_4$ + 300ml distilled water + 10ml yeast	0.9736	20.94± 0.07

Table 2: Effect of Yeast fermentation on Cassava wastes

Medium	specific gravity of distillate	% Ethanol by volume at 25°c
(i) 20g Cassava waste powder + 0.20g $K_2H\ PO_4$ + 300ml distilled water + 10ml yeast	0.9772	17 46 ± 0.08

(ii) 20g Cassava waste powder + 20g glucose + 0.20g K_2H PO_4 + 2% distilled water + 10ml yeast	0.9867	9.56 ± 0.02

Table 3: Effect of Yeast fermentation on Municipal wastes

Medium	**specific gravity of distillate**	**% Ethanol by volume at 25°c**
(i) 20g Municipal wastes powder + 0.20g K_2H Po_4 + 300ml distilled water + 10ml yeast	0.9878	9.31 ± 0.03
(ii) 20g Municipal waste powder + 20g glucose + 0.20g K_2H PO_4 + 2% HCL + 10ml yeast	0.9817	14 27 ± 0 07
(iii) 20g Municipal wastes powder + 0.20g K_2 HPO_4 + 2%HCL + 300ml distilled water + 10ml yeast	0.9948	4 05 ± 0.03

Table 4: Effect of Yeast Fermentation on Nutritive Status of Cassava Wastes and Fermented Residues.

	Unfermented cassava wastes (%)	**Hydrolysed and fermented residues (%)**
Moisture	58.18 ± 0.41	-
Ash	1.38 ± 0.02	4.73 ± 0.05
Organic Matter	94.21 ± 0.67	87.46 ± 0.72
Lipid	6.47 ± 0.06	9.84 ± 0.09
Protein	5.69 ± 0.04	8.12 ± 0.03
Carbohydrate	86.21 ± 0.59	77.31 ± 0.56

Table 5: Effect of Yeast Fermentation on Nutritive Status of Cassava Wastes and Fermented Residues.

	Unfermented cassava wastes (%)	**Hydrolysed and fermented residues (%)**
Moisture	8.14 ± 0.03	-
Ash	1.21 ± 0.01	2.05 ± 0.02
Organic Matter	94.62 ± 0.92	96.13 ± 1.07
Lipid	0.37 ± 0.04	3.24 ± 0.03
Protein	2.17 ± 0.03	5.28 ± 0.09
Carbohydrate	96.15 ± 1.32	89.43 ± 1.40

DISCUSSION

Milling of the lignocelluloses wastes (substrates) reduced the amount of the crystalline cellulose, and increased the surface area available for attack by the hydrolyzing agent. Milling also increased the porosity of the cellulose (Bussy, 1992). Heating these lignocellulose wastes wetted and made these materials accessible to the hydrolyzing agents, hydrolysis degraded these substrates to glucose and other products. The results showed that these lignocellulose wastes can serve as good sources of ethanol industrially without affecting food supply and that the residues can serve as food supplement for both man and animals (Tables 4 and 5) On the whole, the yield of ethanol was better with cassava wastes powder than with municipal waste powder

The relative decrease in the carbohydrate content and the increase in the ash of the residual products of fermentation of cassava and municipal wastes in comparison to that of the unfermented samples gave an insight into the extent of fermentation of the samples. The relative increase in protein content in the residual products can be attributed to yeast protein and products of fermentation. In order to assess the value of the resultant product of fermentation as food supplements, attention has to be paid to the food value in terms of the essential amino acids index and the biological score. The increase in protein content of the fermented cassava and municipal wastes over that of the unfermented samples calls for a thorough investigation of the possible use of the fermented products as human food and animal feed supplements. However, it will be necessary in this case to ascertain that there are no undesirable substances arising as a result of the yeast activity.

Conclusively, the study has shown that fermentation of lignocellulose wastes may be the solution to the problem of agricultural and municipal wastes disposal currently encountered all over the world. The use of grains which are good sources of food in the tropics, for the production of ethanol would be avoided thereby improving food supply. Also these non-edible lignocellulose wastes can become palatable digestible and nutritive after a period of fermentation.

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