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Influence of fermentation with saccharomyces cerevisiae on some biochemical and toxic components of zea mays (corn) cobs

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ABSTRACT - Zea Mays cobs were fermented with S. *cerevisiae* to determine its effect on the protein level of these starchy waste. Chemical analysis of fermented substrates showed substantial increase in crude protein (10.37%) after 7 days, and corresponding decrease in toxic components (oxalic acid, tannins, phytic acid, and hydrocyanic acid). At optimum pH 5.0, temperature of 28°C and moisture level of 130% , there were increases in level of crude protein. Supplementation of the substrates with inorganic nitrogen sources supported high protein and low toxic levels with urea giving the best results. The possibility of using fermented corn cobs in livestock feeds and thereby changing corn cobs from menace status to profitability status is highlighted.

KEY WORDS: Toxic components; fermentation; zea mays cobs; protein content

INTRODUCTION:

The need for additional protein sources for livestock production has necessitated research into the possibility of improving carbohydrate-rich substrates (Fetuga *et al* 1994). One of these approaches is in the use of micro-organisms grown on a variety of agricultural wastes of low nutritional value as a potential source of food and protein.

Corn (Zea Mays) is a widely cultivated food crop in all parts of Nigeria. It constitute a greater part of the diet of most Nigerians. However, the cobs are discarded as wastes and constitutes heaps of litter during the corn season. Sometimes it is used as source of fuel or fed to ruminants (Sundstol and Owen 1984). Unfortunately, the protein of the cob is very low and does not make a good quality feed.

The fermentative ability of micro-organisms has been and is still being used in the production and improvement of food products for both animal and human consumption (Ibrahim and Antai 1986) When selected micro organisms grow on these wastes, many biochemical changes took place and many kinds of products are formed (Fetuga *et al* 1993). These changes occur in the major food constituents especially proteins, carbohydrate and fats (Frazier and Westhaf 1978). Also the fermentation process contribute to the reduction in undesirable toxic components (oxalic acid, phytic acid, hydrocyanic acid and tannins) of the products (Antai and Obong 1992). The present study is aimed at determining the effect of fermentation on the crude protein content and on the level of toxic components.

MATERIALS AND METHODS

Source of Substrate

Corn cobs obtained from corn sellers in Watt Market, Calabar were cut into small pieces, dried in an oven at 50°C for 24 hours. The cobs were ground with an electric grinder and sieved to pass through a mesh of pore size 0.5mm. These were stored in sterile, dried plastic containers at room temperature until needed.

Source of Inoculum

The source of inoculum was baker's yeast (Saccharomyces cerevisiae) bought from Watt Market, Calabar. Five grams were weighed out into a 250ml conical flask containing 100ml of sterile distilled water maintained at 60°C in a water bath for one hour. At the end of 1 hour, the yeast was plated on Sabouraud's agar to check for viability. Seven millilitres of the inoculum was used for inoculation of each flask.

Fermentation of Substrate

The method of Antai and Mbongo (1992) was used. Thirty grams of the dried, sieved samples were weighed out in triplicates into 200ml plastic containers. Fourty millilitres of sterile mineral salts medium containing the following per litre of distilled water: KH_2PO_4, 1g; $NaHPO_4\,7H_2O$ 4g; NaCl 0.25g; $MgSO_4.7H_2O$ 0.2g; $CaCl_2.H_2O$, 0.05g; of yeast extract, 10g was added to moisten it into a mash. The mouth of the containers, were covered with calyco cloth which was fastened to the container with a rubber band. 7mls of the inoculum was

added and allowed to ferment for 7 days at room temperature after which it was dried in the oven at 70°C for 24 hours and kept for analysis.

Effect of pH, Temperature, Moisture, Inorganic Nitrogen level on Fermentation of Substrate

The substrate was fermented as stated above, but for the effect of pH, mineral salt medium was adjusted to different pH levels of 3.0, 4.0, 5.0, 6.0 and 6.5. for the effect of temperature, the substrate (mash) were incubated at different temperature of 8°C, 18°C, 28°C, 37°C, 40°C. The substrates were fermented for a maximum of seven (7) days after which the samples were dried and used for chemical analysis.

To determine the optimum moisture level needed for fermentation of the mash, the quantity of mineral salt medium added was adjusted to give 30, 60, 100, 120, 130, and 150% moisture (V/W).

The inorganic nitrogen sources added to the mineral salts solution were as follows: Urea (0.11g), Ammonium Sulphate (0.24g) and Sodium nitrate (0.12g). different amounts of the nitrogen sources were used in order to balance the amount of nitrogen in all cases. The corn cobs were fermented for 7 days after which they were analysed.

Chemical Analysis

The protein, ash, lipid, crude fibre, and carbohydrate contents of each flask were analysed according to the methods described by the Association of Official Analytical Chemists (AOAC 1975). The microkjeldahl method was used for determining crude protein. Lipid was estimated by extraction of sample with petroleum either (bp. 40-60°C) using soxhlet apparatus. In the case of ash, the sample was incinerated in a muffle furnance at 600°C for 24 hours.

Determination of Toxic Components in Zea Mays Cobs

The toxic components (oxalic acid, tannins, phytic acid and hydrocyanic acids) were estimated using the methods of Bassir (1969).

RESULTS

The proximate chemical composition of Zea Mays cobs and the level of toxic components are shown in Table 1. After fermentation for 3 days, there was a decrease in the lipid and carbohydrate components but a corresponding increase in the crude protein, fibre and ash components. Table 2 shows crude protein yield and toxicant level of fermented cobs. There was a general decrease in the level of toxicants as fermentation

TABLE 1: CHEMICAL ANALYSIS AND TOXIC COMPONENTS OF *Zea mays* COBS BEFORE AND AFTER FERMENTATION FOR 3 DAYS

CHEMICAL COMPONENTS	COMPOSITION (% DRY WEIGHT)	
	UNFERMENTED	FERMENTED
Crude protein	5.07 ± 0.02	8.66 ± 0.4
Crude lipid	3.11 ± 0.01	2.01 ± 0.15
Crude fibre	11.90 ± 0.09	13.4 ± 0.1
Ash	5.20 ± 0.03	9.1 ± 0.05
Carbohydrate	74.4 ± 0.05	66.83 ± 0.05
TOXIC COMPONENTS	mg / 100g	mg / 100g
Oxalic acid	3.30 ± 0.01	2.2 ± 0.02
Phytic acid	0.57 ± 0.04	0.31 ± 0.17
Tannins	0.20 ± 0.06	0.179 ± 0.02
Hydrocyanic acid	2.81 ± 0.02	1.87 ± 0.01

values are means ± SD based on triplicates.

TABLE 2: EFFECT OF FERMENTATION WITH *S. cerevisiae* ON CRUDE PROTEIN PRODUCTION AND ON LEVEL OF TOXIC COMPONENTS

Period of Fermentation (Days)	Crude protein (% dry wt)	Toxic Components (mg/100g)			
		Oxalic acid	Phytic acid	Tannins	Hydrocyanic acid
0	5.07 ± 0.02	3.30 ± 0.01	0.57 ± 0.04	0.20 ± 0.6	2.81 ± 0.02
3	8.66 ± 0.4	2.2 ± 0.02	0.31 ± 0.17	0.179 ± 0.02	1.87 ± 0.01
5	9.25 ± 0.3	2 15 ± 0.08	0.21 ± 0.03	0.160 ± 0.1	1.51 ± 0.01
7	10.37 ± 0.9	0.15 ± 0.06	0.039 ± 0.13	0.141 ± 0.02	0.09 ± 0.01

Data are means ± SD based on three determinations

TABLE 3: EFFECT OF pH ON CRUDE PROTEIN YIELD AND ON LEVEL OF TOXICANTS

pH	Crude protein (% dry wt)	Toxic Components (mg/100g)			
		Oxalic acid	Phytic acid	Tannins	Hydrocyanic acid
3.0	7.82 ± 0.2	2.25 ± 0.04	0.18 ± 0.01	0.172 ± 0.01	0.58 ± 0.6
4.0	8.31 ± 0.3	1.90 ± 0.02	0.17 ± 0.01	0.18 ± 0.01	0.47 ± 0.2
5.0	10.70 ± 0.1	1.80 ± 0.1	0.11 ± 0.01	0.158 ± 0.01	0.62 ± 0.2
6.0	9.70 ± 0.05	1.81 ± 0.01	0.12 ± 0.01	0.159 ± 0.01	0.72 ± 0.1
6.5	8.81 ± 0.07	1.87 ± 0.01	0.16 ± 0.01	0.161 ± 0.01	0. 75 ± 0.1

Values represents mean ± SD of three determinations

TABLE 4: EFFECT OF TEMPERATURE ON CRUDE PROTEIN YIELD AND TOXIC LEVEL OF *Zea mays* Cobs

Temperature (°C)	Crude protein (% dry wt)	Toxic Components (mg/100g)			
		Oxalic acid	Phytic acid	Tannins	Hydrocyanic acid
8°C	6.50 ± 0.6	2.5 ± 0.6	0.036 ± 0.01	0.18 ± 0.1	0.31 ± 0.01
18	7.30 ± 0.2	2.4 ± 0.5	0.035 ± 0.01	0.18 ± 0.1	0.23 ± 0.1
28	10.30 ± 0.1	2.0 ± 0.4	0.034 ± 0.01	0.178 ± 0.1	0.20 ± 0.1
37	10.00 ± 0.1	2.14 ± 0.1	0.032 ± 0.01	0.173 ± 0.1	0.21 ± 0.1
40	9.80 ± 0.3	3.1 ± 0.6	0.038 ± 0.01	0.18 ± 0.1	0.23 ± 0.2

Data are means ± SD based on three determinations

TABLE 5: EFFECT OF MOISTURE LEVELS ON CRUDE PROTEIN AND ON LEVEL OF TOXIC COMPONENTS OF *Zea mays* COBS

Moisture (%)	Crude protein (% dry wt)	Toxic Components (mg/100g)			
		Oxalic acid	Phytic acid	Tannins	Hydrocyanic acid
30	7.50 ± 0.06	2.45 ± 0.5	0.40 ± 0.1	0.172 ± 0.01	0.35 ± 0.1
60	10.91± 0.12	2.40 ± 0.3	0.38 ± 0.1	0.161 ± 0.01	0.29 ± 0.1
100	12.51 ± 0.1	2.31 ± 0.1	0.36 ± 0.1	0.140 ± 0.01	0.28 ± 0.1
120	14.97 ± 0.02	2.10 ± 0.1	0.30 ± 0.1	0.128 ± 0.01	0.26 ± 0.01
130	15.64 ± 0.03	2.0 ± 0.1	0.30 ± 0.01	0.127 ± 0.1	0.25 ± 0.1
150	15.12 ± 0.1	2.1 ± 02	0.31 ± 0.1	0.129 ± 0.1	0.26 ± 0.1

Data represent mean ± SD based on three determinations

TABLE 6: EFFECT OF SUPPLEMENTATION WITH NITROGEN SOURCES ON CRUDE PROTEIN YIELD AND ON THE LEVEL OF TOXIC COMPONENTS.

Nitrogen source	Crude protein (% dry wt)	Toxic Components (mg/100g)			
		Oxalic acid	Phytic acid	Tannins	Hydrocyanic acid
Urea	21.30 ± 0,8	1.78 ± 0.1	0.36 ± 0.1	0.101 ± 0.01	1.08 ± 0.1
Sodium Nitrate	18.20 ± 0.1	1.82 ± 0.2	0.38 ± 0.1	0.108 ± 0.01	1.40 ± 0.1
Ammonium sulphate	16.30 ± 0.5	1.92 ± 0.2	0.47 ± 0.1	0.112± 0.01	1.62 ± 0.2

Values represent mean ± SD based on three determinations

progressed. The percentage reduction were as follows: Oxalic acid (95.6%), phytic acid (94.4%) Tannins (49.8%) and hydrocyanic acid (99.3%) after 7 days. Crude protein increased from 5.07% to 10.37% over the same period. Table 3 shows the effect of pH on fermentation. Maximum crude protein yield occurred between pH range of 5.0 to 6.0 . There was a reduction in toxicant levels especially at the same pH range.

The effect of temperature on fermentation as shown in Table 4 illustrates that the optimum temperature for crude protein yield and reduction in level of toxic components was 28°C. Crude protein increased from 5.07% to 10.30% at 28°C. Oxalic acid reduced from 3.3 to 2.0mg/100mg, while phytic acid reduced from 0.57 to 0.034 mg/100g. Tannins reduced from 0.201 to 0.178mg/100g while hydrocyanic acid reduced from 2.81 to 0.20mg/100g.

Saccharomyces cerevisiae grew well and generated additional cell mass which led to an increase in crude protein as the moisture content of the fermenting mash was increased (Table 5). Maximum crude protein yields (15.64%) occurred at 130% moisture level. There was also a general reduction in toxicant levels. Above 130% moisture level crude protein yield dropped.

Table 6 illustrates the effect of supplementation with nitrogen sources. Urea was the best nitrogen source. The highest yield of crude protein was 21.30%. Thus addition of inorganic nitrogen greatly enhanced crude protein formation. The levels of toxic components were drastically reduced.

DISCUSSION

Many researchers have reported that fungi can grow on agricultural waste to generate additional cell mass (Labanciah *et al* 1979; Antai and Mbongo 1994). Result so obtained from the present study revealed that the protein content of corn cobs could be increased through fermentation.

Azulay (1980) reported that the yeast Candida tropicalis contributed about 40% of protein during the fermentation of cassava. Rejagopal (1977) reported on the feasibility of utilizing corn cobs waste for the growth of Aspergillus fumigatus, A. flavus, A. niger and A. tamaric. Crude protein obtained ranged from 9.28 to 14.63%. Thus fermentation helps to increase the nutritive value of starchy wastes. Abasiekong (1991) obtained higher crude protein and lower crude fibre content on the fermentation of brewers' dried grains and spent sorghum grains using cellulolytic bacteria.

The highest crude protein yeild occurred at a temperature of 28°c and pH 5.0. These environmental factors were optimum for the growth of Sacchromyces cerevisiae (Campbell and Duffus 1988). Thus the increase in crude protein was as a result of increase in cell mass of the organisms.

A moisture content of 130% supported the highest yield of crude protein. A certain amount of moisture must be needed to render the substrate soluble for assimilation by the organism. The finding is in conformity with the report of Antai and Mbongo (1994) in which 130% moisture was optimum for crude protein yield.

Supplementation of the fermenting mash with inorganic nitrogen especially urea yielded higher crude protein values. Azulay et. al (1980) also identified urea as the best inorganic nitrogen supplement for Candida tropicalis. The inorganic nitrogen supplements are easily assimilated than the nitrogen of the agricultural waste, hence more cell mass was generated.

Studies on the level of toxic components showed a general decrease as fermentation progressed. For oxalic acid, the highest reduction (95%) was obtained when the corn was fermented for 7 days. This agrees with the findings of Eka (1986) on cassava and the report of Antai and Obong (1992) on *icacinia manni*. The content of oxalic acid in food is important because it impairs the absorption of calcium and therefore renders it unavailable

Nausea, vomiting, stomach disorder and death have been known to occur due to accumulation of oxalates in the body. The lethal dose for man is 2-5mg/100g (Oke 1969).

Phytic acid inhibits the body's absorption of calcium and iron from foodstuffs by combining with them to form insoluble complexes. There was a substantial reduction in phytic acid during fermentation. This was in conformity with the findings of Antai and Obong (1992). The tannis and hydrocyanic acid contents of the corn cobs reduced with fermentation. An analysis carried out by Ofuya and Obilor (1992) on toxic components of cassava peels showed that fermentation for 96 hours caused a 95% reduction in the hydrocyanide levels and42% reduction in the soluble tannin content.

CONCLUSION

Despite the presence of toxic components in corn cobs, it has been shown that fermentation with *S.cerevisiae* reduced them to levels that can be accepted by livestock. The study indicate the potentials of fermented corn cobs as livestock feed ingredients.

However, further studies are being carried out to evaluate their acceptance by laboratory animals.

REFERENCES

Abasiekong, S. F. (1991). Effects of fermentation on crude protein content of brewers spent grains and spent sorghum grains. *Bioresource Technol.* 35:99-102.

Antai S. P.and Mbongo P.M.(1994). Utilization of cassava peels as substrate for crude protein formation. *Plant foods for Human Nutrition* 46:345-351.

Antai, S., and Obong, U.S. (1992). The effect of fermentation on the nutrient status and on some toxic components of *Icacinia manni. Plants foods for Huamn Nutrition* 42: 19-224.

AO.AC (Association of Official Analytical Chemists(1975). Official methods of Analysis. (Ed) William Horwitz, AOAC, Washington D.C.

Azulay, E., Jouanneau, F., Bertrand, J., .Janssens, J. and Lebeault J.M.(1980): Fermentation methods for protein enrichment of cassava and corn with *Candida tropicalis. Applied Environmental Microbiology 39:41-47.*

Bassir, O. (1969): Toxic substances in Nigerian foods. *West African Journal of Biological and Applied Chemistry 5: 3-6.*

Campbell, I. and Duffus, J., (1988). *Yeasts: a practical approach.* Washington, D. C. IRL Press 1-8.

Eka, O. U. (1986). Some enzymes and microorganisms in the fermentation liquor and the effect of fermentation on nutrient status of cassava products. *W. A. J. Biol. Appl. Chem* 31: 3-9.

Fetuga, B. L., Essien A. I., Babatunde, G. M. and Oyenuga, V. A. (1993). The energy value for and nutritive value of para-Rubber seed for Rats. *Arch, Anim Nutr.* 45:71-78.

Frazier, W. C. and Westhaff, D. C. (1978). *Food Microbiology* (3rd Ed.) Tata McCraw Hill, New Delhi , 538.

Ibrahim, M. H. and Antai, S. P. (1986). Chemical changes during the fermentation of African locust-bean (Parkia Filicoidea Welwl) seeds for production of "Daddawa" *Plant Food Hum. Nutr.* 36: 179-184.

Labanciah, M. E. O., Aabou-Donia S. A., Mohamad M. S. and El-Zalak, E. M. (1979). Utilization of citrus wastes for the production of fungal protein. *J. Food Technol* 14: 95-99.

Ofuga, C. O. and Obilor, S. N. (1992). The effects of solid-state fermentation on the toxic components of cassava peel. *Process Biochemistry* 29:25-28.

Oke, O. L. (1969). Oxalic acid in plants and in nutrition. In: S. Karger (ed.), *World Review of Nutrition and Dietetics*, Basel.

Rajagopal M. V. (1977). Utilization of corn cobs waste as source of carbohydrate for the growth of aspergillus fumigatus. *Journal of Food Technology* 12: 633-637.

Sundstol, F. and Owen, E. (1984): *Straw and other fibrous by-products as feed.* Elsevier. Amsterdam, 424-425.