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Held at CIAT, Cali, Colombia, 1-7 August 1976

Edited by James Cock, Reginald MacIntyre, and Michael Graham

The International Society for Tropical Root Crops in collaboration with
Centro Internacional de Agricultura Tropical
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Protein Enrichment of Cassava by Fermentation with Microfungi and the Role of Natural Nitrogenous Supplements

G. Varghese, J. J. Thambirajah, and F. M. Wong[1]

Attempts to produce protein-enriched cassava for animal feed by solid state fermentation with selected local strains of *Rhizopus, Aspergillus,* and *Neurospora* showed that protein levels of the fermented products did not exceed 3%. Because this value is low for animal feed, the ability of natural nitrogenous supplements to increase microbial activity was tested. Supplementation with 35% chicken dung increased protein levels to 8–10.5% and with soybean, groundnut, and pineapple bran at 25%, the protein levels were 40, 10, and 7%, respectively. In combination with chicken dung (12.5 + 12.5%) the protein values varied between 8 and 18% for soybean, 8 and 10% for groundnut, and 5 and 7% for pineapple bran. The results indicated that supplementation increased fermentation efficiency and contributed to higher protein values.

A procedure for solid state fermentation of cassava with natural nitrogenous supplements has been developed as a first stage toward the design of a pilot plant for continuous production of the material.

Due to the increase in price and demand for animal feed by an expanding livestock industry, Malaysia is currently spending more on feed imports. Therefore, there is a need to produce more animal feed locally. It is in this context that cassava may have scope for large scale expansion. The ease by which the crop can be grown from cuttings on a wide range of soil types makes it a crop suitable for immediate expansion. In line with this a number of "estate type" cassava plantings along with processing plants have been recently established in various parts of the country through government-aided schemes.

Nutritionally, cassava tubers provide mainly carbohydrates and some useful amounts of calcium and vitamin C to the diet (Wood 1965). The protein levels are however low and vary according to moisture content and varieties grown. The average is usually in the region of 1.3% (Oke 1968, Sundhagul 1972). However, in Asia and Africa cassava has been traditionally enriched by microbial fermentation.

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In Nigeria, the fermented cassava "gari," which is a staple food, is produced by a two-stage fermentation with *Corynebacterium* sp. and *Geotrichum candidum* (Collard and Levi 1959). In South East Asia cassava is usually mixed with peanut cake and fermented with *Rhizopus* and *Neurospora* and sometimes boiled tubers are inoculated with *Saccharomyces* sp. to produce various forms of food for human consumption. Recently, considerable interest has been shown in using cassava as an animal feed and also in protein enrichment of cassava by microbial fermentation. The Tropical Products Institute (London) has developed a microbial method for raising the protein level of cassava to 4% with minimal additives (Woollen 1967). Solid state fermentation of cassava with *Rhizopus* spp. increases protein levels to 3.4% (Yeang 1973), and with certain selected strains up to 4% (Sprung 1974).

While all the above mentioned reports point out the feasibility of cassava enrichment by microbial fermentation, they also serve to confirm that the protein levels so far attained by this method are inadequate for animal feed. Therefore, there is a need to raise protein levels of the fermented product, possibly by the use of selected microorganisms that have a higher carbohydrate-to-protein conversion ratio and through the incorporation of necessary supplements and additives in small amounts to augment fermentation efficiency.

In the present study the authors have isolated microorganisms from cassava tubers and products and screened selected strains for their protein-enrichment ability. In addition, the role of local natural nitrogenous supplements such as chicken dung, pineapple bran, groundnut, and soybean, alone and in combination with chicken dung, was assessed. A procedure of solid state fermentation of cassava by microfungi to produce fermented cassava for animal feeding trials was developed.

Materials and Methods

Cassava tubers, chips, and other products were collected from processing centres in various parts of Malaysia. Microorganisms growing naturally on these substrates were isolated by the following techniques.

(1) Tapioca chips were washed continuously in running water and particles were removed at 1-h intervals for 3 h and the washed particles were plated on malt agar plates incorporated with Rose Bengal, and on nutrient agar plates. Organisms originating from the plated particles were isolated and grown in pure cultures on potato dextrose agar for fungi and on nutrient agar slants for bacteria.

(2) Tapioca products that were in semi-solid state were diluted initially to 1:10 and subsequently serially into 10^3 and 10^4 dilutions. These dilutions were plated on potato dextrose agar and nutrient agar plates and organisms isolated were grown in pure cultures as in the previous case.

(3) Small pieces (2 mm^2) were removed from the tubers and chips, sterilized in 0.2% mercuric chloride solution, washed in sterile distilled water, and transferred directly on to plates containing malt agar incorporated with Rose Bengal and nutrient agar. The organisms arising from the pieces were subsequently isolated as in the previous cases.

Fermentation Trials

To screen the organisms for their relative efficiency in protein enrichment of cassava, fermentation tests were carried out in the following manner. One hundred grams of tapioca chips with 100 ml of water were sterilized for 15 min at 1.06 kg/cm^2 pressure in 500-ml jam jars. Subsequently they were inoculated with 1 ml spore suspensions of the respective test organisms, adjusted to a final count of 4×10^7 spores/ml, by using a haematocytometre. The jars were incubated in light at room temperature (28 °C ± 2 °C). After allowing for a fermentation time of 48 or 72 h, depending on the organisms, the fermented cassava in the jars was dried at 100 °C for 24 h and ground to a powder for subsequent physical and chemical analyses.

Similar fermentation tests were also carried out in the case of cassava supplemented with chicken dung, pineapple bran, groundnut, and soybean, alone and in combination.

Moisture contents of the ground product and proximate analyses for crude protein, crude fibre, crude fat, and ash were carried out according to AOAC procedure (1965).

Procedure for Solid State Fermentation of Cassava

The procedure developed for solid fermentation of cassava for feeding trials consisted of the following steps: (1) cassava chips (commercial) were steamed for 5 h in steaming boxes (2.5 kg/box) and left overnight for cool-

Table 1. Proximate analysis of cassava fermented with microfungi compared with nonfermented cassava and chicken dung.

Microorganism	Substrate	Fermentation time (h)	Moisture	Proximate analysis (%)			
				Crude protein	Crude fat	Crude fibre	Ash
Rhizopus I	Tapioca	48	8.47	3.06	0.84	2.39	1.64
Rhizopus II	Tapioca	48	9.64	3.39	1.75	3.22	1.76
Neurospora I	Tapioca	48	9.61	2.68	0.78	2.00	1.78
Aspergillus I	Tapioca	72	9.61	2.88	0.58	1.71	1.81
Aspergillus II	Tapioca	72	12.69	2.94	0.93	1.76	1.96
Aspergillus III	Tapioca	72	10.34	2.88	0.54	1.64	2.00
nil	100% tapioca	nil	11.85	2.54	0.77	1.24	1.73
nil	100% chicken dung	nil	12.28	31.68	2.47	7.18	42.20

Table 2. Proximate analysis of fermented cassava supplemented with chicken dung.

Microorganism	Substrates (%)		Fermentation time (h)	Moisture	Proximate analysis (%)			
	Tapioca chips	Chicken dung			Crude protein	Crude fat	Crude fibre	Ash
Rhizopus I	65	35	48	5.51	8.31	0.94	4.35	10.59
Rhizopus II	65	35	48	5.85	7.88	2.33	6.80	11.29
Neurospora I	65	35	48	5.36	8.75	3.91	5.50	9.24
Aspergillus I	65	35	72	5.76	10.49	1.62	5.15	8.81
Aspergillus II	65	35	72	5.68	8.75	1.89	5.82	8.71
Aspergillus III	65	35	72	5.86	8.31	2.32	5.44	8.68

ing; (2) steamed cassava was transferred into fermentation trays 60 × 60 cm and inoculated with a 100 ml spore suspension per tray of *Rhizopus* or *Aspergillus* (spore count adjusted to 4×10^7 spores/ml); (3) inoculated cassava in the fermentation trays was transferred to fermentation cabinets, maintained at room temperature and at a relative humidity at saturation point, and incubated for 48 h for *Rhizopus* and 72 h for *Aspergillus*; (4) the fermented product was dried and ground to a fine powder and stored in bins for feeding trials; and (5) the *Rhizopus*-fermented product was designated as R35 and *Aspergillus*-fermented product was designated A35, the letter denoting the fermentative agent (organism) and the number denoting the percentage supplementation with chicken dung.

Results

From the microorganisms isolated from cassava tubers and products those belonging to *Aspergillus, Neurospora,* and *Rhizopus* were subsequently used for solid state fermentation trials.

The results of the fermentation trials, with the selected species of organisms, showing proximate analysis for crude protein, crude fat, crude fibre, and ash of fermented cassava compared with those of nonfermented cassava and chicken dung are presented in Table 1. Similar results of fermentation trials of cassava supplemented with 35% chicken dung are given in Table 2. The results of fermentation with 25% supplementation of pineapple bran, groundnut, and soybean, alone and in combination with chicken dung (12.5 + 12.5%), are shown in Table 3.

Following the procedure outlined earlier we are now producing *Rhizopus*-fermented cassava with 35% supplementation of chicken dung (R35) at the rate of 300 kg/week and also *Aspergillus*-fermented product with 35% supplementation of chicken dung (A35) at the rate of 150 kg/week. These fermented products are being used for feed trials on poultry and pigs. However, based on the results of preliminary feeding trials in poultry we are now reducing chicken dung supplementation to 25%. Currently, we are also in the process of designing a pilot plant for continuous production of the fermented product.

Table 3. Proximate analysis of fermented cassava supplemented with pineapple bran, groundnut, and soybean alone and in combination with chicken dung.

Microorganisms	Substrates (%)			Fermentation time (h)	Moisture	Proximate analysis (%)			
	Tapioca chips	Chicken dung	Balance			Crude protein	Crude fat	Crude fibre	Ash
Pineapple bran									
Rhizopus I	75	—	25	48	6.21	4.09	0.20	6.35	4.32
	75	12.5	12.5	48	6.66	6.71	1.29	6.47	7.93
Rhizopus II	75	—	25	48	6.66	4.30	1.85	6.16	4.14
	75	12.5	12.5	48	5.94	7.57	2.42	6.87	7.51
Neurospora I	75	—	25	48	5.18	4.22	3.46	6.70	4.35
	75	12.5	12.5	48	6.06	7.17	2.75	6.92	6.96
Aspergillus I	75	—	25	72	5.62	3.83	1.35	5.60	4.22
	75	12.5	12.5	72	5.71	6.62	0.69	5.53	8.00
Aspergillus II	75	—	25	72	15.28	5.55	0.42	6.76	6.72
	75	12.5	12.5	72	14.38	6.18	0.84	6.13	6.66
Aspergillus III	75	—	25	72	19.00	4.77	0.37	7.36	6.50
	75	12.5	12.5	72	11.47	5.35	1.17	6.61	10.23
Groundnut									
Rhizopus I	75	—	25	48	3.49	10.42	8.90	4.03	13.20
	75	12.5	12.5	48	3.48	10.67	10.10	5.43	10.78
Rhizopus II	75	—	25	48	3.11	10.34	10.23	3.95	9.11
	75	12.5	12.5	48	3.14	9.01	8.63	4.20	10.43
Neurospora I	75	—	25	48	2.83	8.72	9.32	5.05	11.52
	75	12.5	12.5	48	2.79	9.33	8.90	5.17	11.92
Aspergillus I	75	—	25	72	2.77	9.57	9.13	5.46	10.58
	75	12.5	12.5	72	2.59	10.53	12.03	4.86	10.65
Aspergillus II	75	—	25	72	2.56	10.33	9.64	3.05	5.34
	75	12.5	12.5	72	3.99	9.13	7.51	4.65	12.39
Aspergillus III	75	—	25	72	3.21	8.65	6.53	3.89	8.82
	75	12.5	12.5	72	3.23	8.43	5.61	5.40	10.04
Soybean									
Rhizopus I	75	—	25	48	2.08	18.45	6.99	5.71	8.02
	75	12.5	12.5	48	2.28	18.03	6.62	6.06	7.79
Rhizopus II	75	—	25	48	1.40	14.07	8.72	8.07	15.00
	75	12.5	12.5	48	1.37	17.39	9.95	8.10	8.23
Neurospora I	75	—	25	48	5.07	14.45	7.52	4.90	7.66
	75	12.5	12.5	48	2.23	8.99	3.53	6.01	12.55
Aspergillus I	75	—	25	72	1.94	10.75	4.06	5.98	10.88
	75	12.5	12.5	72	1.79	11.79	4.06	5.79	12.14
Aspergillus II	75	—	25	72	1.74	14.85	15.31	6.50	5.93
	75	12.5	12.5	72	1.34	13.30	5.97	5.57	13.38
Aspergillus III	75	—	25	72	1.54	14.31	4.89	6.88	6.93
	75	12.5	12.5	72	1.62	15.38	5.91	6.31	6.43

Discussion

Rhizopus species are traditionally used in the production of "tempeh" and "ontjom," two fermented food products in Southeast Asia. Therefore, from toxicity and acceptance points of view *Rhizopus*-fermented cassava offers no serious problems. In the case of *Aspergillus* fermentation, the purity of the species must be maintained and routine tests for aflatoxin may be necessary to ensure that the fermented product is free of mycotoxins. We have adopted this procedure with regard to our *Aspergillus*-fermented materials (A35).

Direct fermentation of tapioca with *Aspergillus*, *Neurospora*, and *Rhizopus* increased protein values to about 3%. This is in conformity with the values recorded by other workers (Sprung 1974, Yeang 1973). Because the value is low for animal feed, it is necessary to raise the protein levels of fermented cassava. However the question is, how can this be

achieved? In our view, the problem may be approached in the following ways: (1) further isolation of microorganisms occurring naturally on cassava and its products and screening for their efficiency in converting starch into microbial protein; and (2) strain improvement within species and isolates by single spore isolations and possibly by hybridization and induced mutations.

At present, more attention should be given to screening work as this has not been fully explored. Another practical and immediate approach would be to use a nitrogenous supplement in small amounts to boost microbial activity and thereby increase the rate of carbohydrate-to-protein conversion. Toward this aim, we have tested naturally available nitrogenous supplements such as chicken dung, pineapple bran, groundnut, and soybean. Chicken dung is an easily available nitrogen source. However, we have observed that supplementation with chicken dung above 25% results in a high crude fibre and ash content and reduced palatability of the fermented product. Data obtained from preliminary feeding trials support this observation (Hutagalung and Tan, personal communication). The products (R35) could also be low in true protein content and may lack certain essential amino acids. Further chemical and physical analyses and feeding trials are necessary to fully evaluate the use of fermented cassava as animal feed. However, it seems clear that chicken dung supplementation above 25% is unsuitable and in our opinion this should be further reduced and substituted by a more edible natural nitrogenous supplement. Among the nitrogenous supplements we have tested, pineapple bran at 25% could give an increase in protein levels to 4–5% and when the bran was combined with chicken dung (12.5 + 12.5%) the protein level increased to about 7% (Table 3). Groundnut has the advantage that it is readily available in the region and is highly palatable. Supplementation with groundnut at 25% alone, and in combination with chicken dung increased protein levels from 8 to 11% (Table 3). Supplementation with soybean in similar amounts gave the highest protein levels. For instance, 25% supplementation with soybean raised protein level of fermented cassava to 40% and in combination with chicken dung (12.5 + 12.5%) the levels were raised to 11–18% depending on the organism used (Table 3). Soybean supplementation promoted growth and colonization of substrate by the fermentative organisms that cause a higher rate of conversion of starch to protein.

The strategy to be adopted would be to incorporate in small amounts (12–15%) a palatable nitrogen source such as groundnut or soybean. This is used not as a direct supplementation of the deficient protein in the finished product but purely as a booster for increased microbial activity. Deficiencies of a specific amino acid such as methionine, or vitamin should be directly supplemented as these would be required only in minute quantities in the animal diet.

Other workers (Gregory et al. 1974) have explored the use of *Aspergillus fumigatus* (asporogenous mutant) for submerged fermentation of cassava for the production of single-cell proteins. However, this may involve more sophisticated techniques and may not be suitable for village-level adoption. We have also initiated some screening trials with tropical edible basidiomycetous macrofungi having a higher protein content to evaluate their ability to enrich cassava.

We feel optimistic that enrichment of cassava by microbial fermentation is feasible. The use of microfungi for fermentation of cassava is not new to Africa and Asia; therefore, any new techniques developed would be accepted and could be adapted in a village-level technology. The fermented cassava may not only provide animal feed but at a later stage can be developed to provide protein enriched food for human consumption. However, a great deal more research and evaluation is necessary before a suitable product and its technology can be launched.

This study is a part of a research project on Microbiological Enrichment (Malaysia) that is carried out at the University of Malaya; the members of the research team are: R. Hutagalung; G. Varghese; B. H. Webb; and Tan Bock Thiam. Financial support from the International Development Research Centre (IDRC) is acknowledged.

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Utilization of Nutritionally Improved Cassava in Poultry and Pig Diets

R. I. Hutagalung and P. H. Tan[1]

Studies related to the improvement of cassava through nutrient supplementation and fermentation are discussed. An experiment was carried out to investigate the effect of substituting maize with increments of fermented cassava on the performance of broiler chickens. In addition, the preliminary results of iodine metabolism in pigs fed cassava diets are presented.

Substitution of maize with up to 50% fermented cassava resulted in performance that compared favourably with the control. Total substitution of the maize component of the chicken diet by fermented cassava did not appreciably depress performance. Further improvement in the protein quality of the fermented cassava and proper supplementation with other nutrients will make a significant contribution to poultry and pig diets.

One of the most important factors affecting the improvement in livestock production is the availability of cheap and good quality feedstuffs. As population increases faster than food production, expansion of existing methods of producing plant and animal protein will not meet the growing needs.

In Malaysia, one of the major problems confronted by the animal industry is the shortage of local feeds. The existing practice of heavy dependence on imported feedstuffs will continue to pose a constraint to the development of the livestock industry.

One local source that could partially remedy this shortage is cassava (*Manihot esculenta* Crantz) locally known as tapioca or "ubi kayu." Cassava has been widely used only as an energy source for poultry and swine feeds because of its low protein content.

Although the carbohydrate production of cassava exceeds other crops, our findings indicate that its extensive use in poultry and swine feeds poses some metabolic problems, including its low protein, mineral, and vitamin content, variation in HCN content resulting in cyanide toxicity, suspected goitrogenic substances causing iodine deficiency, reduction in availability of certain mineral elements resulting in zinc parakeratosis in pigs, low palatability due to dry texture, and poor performance and lack of skin and egg yolk pigmentation at higher level of supplementation. These findings indicate that the equivalent substitution of cassava with cereals is nutritionally unjustified.

Efforts to improve the nutritive value of cassava by nutrient supplementation and processing technology such as improvement in digestibility, reduction in volume and the crude fibre level by pelleting and acting as an absorbent in dehydrating palm oil mill effluent, supplementation of methionine and sodium thiosulfate, addition of palm oil and molasses, animal protein supplementation, incorporation of poultry manure and the inclusion of synthetic pigments have shown encouraging results. However, they are not sufficiently conclusive for large scale application in view of their limited ability to fulfill the protein need.

The concept of fermenting cassava for

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