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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
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CONTENTS

Establishment of a Pilot Plant for the Production of Fungal Protein from Cassava

K. F. Gregory, A. G. Meiering, F. A. Azi, J. A. D. Sedgwick, J. D. Cunningham, S. J. MacLean, J. Santos-Núñez, and G. Gómez[1]

The establishment of a pilot plant for the nonaseptic, high-temperature production of protein from whole cassava mash by thermotolerant fungi was undertaken. The only nutritional supplements required by the prototype culture (*Aspergillus fumigatus* I-21A, a non-spore-forming mutant) for the conversion of 4% carbohydrate (about 15% fresh cassava) were found to be urea (0.345%), KH_2PO_4 (0.05%), and sulfuric acid (about 0.15% of 9 *N* acid to adjust the medium to pH 3.5 and supply sulfur). The plant includes devices for washing and rasping the cassava roots, a self-aspirating 300 l starter fermentor, a self-aspirating 4500 l main fermentor, a roller-press filtration device for harvesting the biomass, and a unit with a sliding roof for sun and air drying the product.

Some thermotolerant fungi have been shown to have potential for a simple, low-cost process for producing protein-rich feed from cassava (Reade and Gregory 1975, Gregory et al. 1976a). Such fungi are able to grow under the highly selective conditions imposed by high temperatures (≧ 45 °C) and acidity (ca. pH 3.5) so that sterilization of the cassava mash and maintenance of aseptic conditions do not appear to be necessary. Chemical hydrolysis of the starch derived from the cassava roots is not required prior to the fermentation process because the fungi produce amylases. Heat is generated by the growing fungi but the high fermentation temperature reduces the cost of cooling the fermentor; water at ambient temperature suffices for this purpose. Finally, the filamentous nature of the fungi enables them to be harvested by filtration, which is simpler and cheaper than the centrifugation required for harvesting yeasts or bacteria.

The initial search for suitable thermotolerant fungi resulted in the isolation of a strain of *Aspergillus fumigatus*, designated I-21 (ATCC 32722), which had a satisfactorily high protein content (over 45% crude protein under optimum conditions; ca. 38% crude protein under the selective conditions required for nonaseptic fermentation, Reade and Gregory 1975). A nonrevertible mutant of this culture, called I-21A (ATCC 32723), which is unable to produce spores, has been used for many of the studies with this strain because the inhalation of large numbers of spores of some strains of *A. fumigatus* can incite a lung infection known as aspergillosis. A subsequent, more extensive search resulted in the isolation of additional thermotolerant fungi some with excellent potential value for protein production (*Cephalosporium eichorniae* 152 and *Rhizopus chinensis* 180, Gregory et al. 1976b). A summary of a rat-feeding trial using *Aspergillus fumigatus* I-21 and I-21A and the two best new cultures is shown in Table 1. All of the isolates had similar suboptimal amounts of methionine, averaging 1.8% of the true protein. In every case the rats readily accepted the fungal mycelium as the sole source of protein in their diet. No toxic effects were noted in any of the rats. Ninety-day subchronic toxicity experiments with mycelium from *A. fumigatus* I-21 were completed in rats (G. L. Khor et al., University of Guelph, unpublished), and the extensive clinical and histological tests completed on rats fed I-21 did not indicate toxicity.

The fermentation and nutritional data obtained with these thermotolerant fungi were sufficiently encouraging to prompt the establishment of a pilot plant at CIAT for the production of fungal biomass from cassava for pig-feeding experiments. This paper describes the establishment of the minimal supplements necessary for the optimum production of protein from whole cassava mash by the prototype culture, *A. fumigatus* I-21A, the construction of simple fermentors for this system, and the assembly of a pilot plant at CIAT.

[1]Gregory, Meiering. Cunningham, Azi, and Sedgwick, University of Guelph. Guelph. Canada; and Santos-Núñez and Gómez, Centro Internacional de Agricultura Tropical, Cali, Colombia. This work was carried out with the aid of a grant from the International Development Research Centre, Canada.

Table 1. Protein content (% of dry weight) and nutritional value of selected thermotolerant fungi.

	Crude protein[a]	"True" protein[b]	PER[c]
Aspergillus fumigatus I-21	44	35	2.3*
A. fumigatus I-21A	49	37	2.3*
A. fumigatus I-21A on cassava mash[d]	37	27	2.2*
Cephalosporium eichhorniae 152	49	38	2.6*
Rhizopus chinensis 180	49	37	2.5

[a]Crude protein = total N × 6.25.

[b]"True" protein was determined by an assay for α-amino N with purified bovine serum albumin as a standard (Reade and Gregory 1975).

[c]PER = Protein efficiency ratio (g weight gain/g "true" protein consumed). Values were normalized relative to casein set at 2.5. Each diet was tested with ten rats. The fungal diets were supplemented with 0.5% DL-methionine, and the casein standard diet was supplemented with 0.3% DL-methionine and 0.1% L-tryptophan. PER values that are significantly different from the casein standard at the 5% level of probability are marked with asterisks.

[d]The biomass produced on whole cassava mash contained unfermented cassava fibre as well as fungal mycelia.

Experimental Procedure

Cassava starch was used for the optimization experiments because it is less variable than whole cassava, and the mineral requirements for media containing whole cassava mash were assessed in relation to reported analytical values for cassava roots (Anonymous 1968, Ewing et al. 1969, Gamble and Snedaker 1969, Oke 1966, Pond and Maner 1974).

Growth responses in media with various salt concentrations were determined in 5-litre fermentors. Inocula for most experiments consisted of spores of *A. fumigatus* I-21 suspended in 0.02% (v/v) Tween 80 because of ease of handling; but the asporogenous mutant I-21A gave an identical response in the final optimized medium.

The mineral elements contributed by the starch were determined analytically, and the theoretical total requirement calculated by adding these analytical values to the experimentally determined supplements required. It is apparent that the average cassava variety supplies adequate amounts of all the mineral elements required except sulfur and possibly

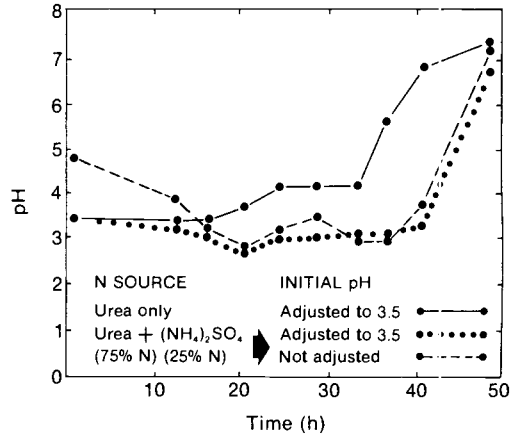

Fig. 1. The effect of nitrogen source on the pH of cassava starch medium (2% carbohydrate) inoculated with A. fumigatus I-21.

zinc. In experiments with whole cassava mash, however, the addition of zinc has not been found necessary. The amount of phosphorus supplied by the average root is very nearly equal to the amount required by the culture, but some sources of cassava would supply only about 50% of the phosphorus required. The required sulfur supplement can be supplied most easily and cheaply by means of sulfuric acid because the addition of acid is required, in any case, to adjust the initial pH of the medium to 3.5.

The only additional supplement required for the cassava mash medium is a nitrogen source. Urea and ammonium sulfate were both found to be suitable for the growth of *A. fumigatus* I-21, but ammonium sulfate alone resulted in too low a pH during the fermentation (Fig. 1). When a large mycelial starter inoculum was used, the ammonia released from the urea was utilized fast enough so that a marked rise in pH did not occur until the end of the fermentation. Accordingly, urea was selected as the nitrogen source for the fermentation.

As predicted from experimental data, when whole cassava mash was used, the cassava supplied most of the required mineral elements in adequate concentrations so that the supplements required were reduced to only three: sulfuric acid, which adjusted the pH to 3.5 and supplied sulfur; urea, as the nitrogen source; and monopotassium phosphate (Table 2). The addition of monopotassium phosphate was found to be essential for maximum protein yield with a mash prepared from the cassava

Table 2. Supplements (g/l) for *A. fumigatus* I-21 to produce maximum growth and protein yield in 2% (w/v) carbohydrate, supplied as cassava starch or whole cassava mash.[a]

	Originally proposed concentration	Optimum for cassava starch medium	Optimum for whole cassava mash medium
KH_2PO_4	1.0	0.375	0.25
$MgSO_4 \cdot 7H_2O$	0.1	0.025	0
KCl	0.05	0	0
$FeSO_4 \cdot 7H_2O$	0.01	≤0.01	0
$CaCl_2 \cdot 2H_2O$	0.01	0	0
$ZnSO_4 \cdot 7H_2O$	0.01	≤0.01	0
Urea	1.72	1.72	1.72

[a]Sulfuric acid, used to adjust the Initial pH to 3.5, provided an excess of S in all cases.

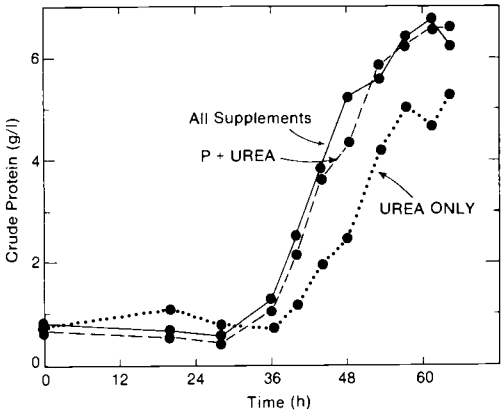

Fig. 2. Minimal supplements required for optimum protein production by A. fumigatus I-21A in medium containing whole cassava mash (4% carbohydrate).

used in these experiments (Fig. 2). In the absence of any added KH_2PO_4, total product yield was undiminished, but its protein content was lower.

Concentrations of cassava giving carbohydrate levels as high as 4% (about 15% fresh cassava) appear to be suitable for a 24-h production schedule (Reade and Gregory 1975) but require double the minimum concentration of supplements shown in Table 2.

Since the operation of a pilot plant for microbial protein production results in a large volume of waste effluent liquid, experiments were undertaken to determine the feasibility of recycling part of the effluent into subsequent fermentations. In experiments in which 50% of the effluent was recycled in sequential fermentations, the effluent became markedly toxic for the organism. The effect of recycling 25% of the effluent in sequential fermentations has not been determined, and it is questionable if recycling this low a percentage would be worthwhile.

Pilot Plant Operation

At the pilot plant constructed at CIAT, both 300 l and 4500 l batch-type fermentors of novel design are being used to process test quantities of enriched cassava (Azi et al. 1975).

The main feature of these fermentors is a self-aspirating agitator unit, by means of which air is drawn by suction down through a hollow shaft and out through the ends of the blades as the hollow impellor blades turn; thus no air compressor is required to force air into the fermentor. The process produces a high-protein product from cassava roots by means of an aerobic submerged fermentation in which nonprotein nitrogen is used for microbial protein synthesis at the expense of energy from the cassava carbohydrates.

The build-up of inoculum is made in the laboratory where the culture is first increased in multiple bottles of an agar medium. This growth is then transferred to the 14 l laboratory scale fermentor. Growth in this fermentor requires about 2 days before the culture is placed in the 300 l fermentor. About 24 h later, this 300 l volume is ready for transferring to the 4500 l fermentor.

The process at the pilot plant scale starts with fresh cassava roots, which are washed to remove soil. Next, the whole root (including the peel) is ground to break open the cell walls to release the starch granules. This is done by passing the cassava through a rasper similar to those used in small cassava starch factories. At this point the rasped cassava is lifted and transferred into the large fermentor, which is half filled with water, previously heated to about 70 °C by the passage of steam through a heat exchanger. This temperature needs to be maintained for about 10 minutes to gelatinize the starch and to prevent the development of fungistatic activity in the mash (Reade and Gregory 1975; Gregory et al. 1976a) Urea, a small amount of monopotassium phosphate and more water are added to bring the fermentor almost to its full operating volume. The addi-

tional water should lower the temperature to about 46–47 °C. Sulfuric acid is used to bring the initial pH to 3.5. The large fermentor is now ready for inoculation with the starter culture produced in the same medium in the small fermentor (300 l). This inoculum is about 6.7% of the volume of the culture in the large fermentor. The fermentation is run for about 20 h, during which time the temperature is maintained by means of a temperature controller that actuates a solenoid controlled water valve to regulate the flow of cooling water at ambient temperature.

Once the culture completes its growth, part of the biomass can be saved as a starter culture for a second batch. The remainder of the biomass is harvested by means of a roller-press device designed for this process.

The biomass produced by this process will be mixed with more cassava or any other appropriate ingredient to lower the protein content to the desired level, for feeding as a moist ration to growing pigs. Because the material would spoil if stored wet, it is preferable to obtain a stable dry product for experimental purposes. The material becomes dark and hard when oven dried, but it can be dried by exposure to the sun and air.

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Lipase Activity and the Conversion of Fat to Carbohydrate in Cassava

Frederick Nartey[1]

The activities of lipase, isocitrate lyase, and malate synthetase were investigated in cassava. The enzymes were present in mature dry seeds. Their activities increased gradually during the initial phase of germination. In the postgermination period of growth in the dark, however, the activities of these enzymes increased rapidly, and reached their peaks at the period of maximum carbohydrate synthesis and storage, which nearly coincided with the period of maximum lipid degradation. This indicated that the fat-carbohydrate mechanism in cassava involves the key enzymes of the glyoxylate cycle.

Lipids and proteins form the major constituents of the storage reserves in mature cassava seed kernels. Free fatty acids are completely absent, and carbohydrates occur to a minor extent (Nartey et al. 1973). The germination of cassava seeds is accompanied by a gradual degradation of lipids and a rapid breakdown of proteins. However, during the

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