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# IMPROVEMENT OF THE NUTRITIONAL QUALITY OF CASSAVA THROUGH BIOTECHNOLOGY

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## ABSTRACT

A solid state fermentation (SSF) method was developed as a cheap and low technology process converting cassava into a form rich in proteins and amino acids suitable for human and/or animal feed. The protein content of cassava was increased from 0.64% to a range of 9.71 to 10.29% exhibiting a 15.17 to 16.08 increase using *Aspergillus candidus, A. oryzae* var. *brunneus, A. tamarii, A. usamii* and *A. niger*. Tests for aflatoxins ($B_1$, $B_2$, $G_1$ and $G_2$) showed that these microorganisms are negative, indicating safety in their use. Amino acid anlaysis of the fermented cassava revealted a four-fold increase of arginine, five-fold for glutamic acid, six to eight-fold for aspragine, methionine and lysine, eight to 11-fold for alanine, his histidine, serine, tyrosine, threonine and phenylalanine, 11 to 12-fold for leucine and glycine, and 11 to 14-fold for valine. The increase in lysine and methionine both of which are limiting and essential amino acids, can have direct impact in the animal feed industry by making available a cheap source of highly digestible feed material rich in protein and amino acids.

## Introduction

Many regions of the world especially Africa, Asia and Central America are acutely deficient in protein but have abundant supply of carbohydrates. Microbial synthesis of protein from carbohydrates and inorganic nitrogen thus appears to be an economically feasible operation for improving the nutritional quality of traditional carbohydrate sources such as cassava.

In the Philippines, the area planted to cassava as of 1985 totalled 216,280 hectares representing 1.70% of the total hectarage of cultivated land. The yield amounts to 1.5 million metric tons of roots (Table 1). The bulk of this cassava production is used as animal feed considering corn and sorghum, the traditional carbohydrate-sources for feed formulations, are not easily affordable. Therefore, the utilization of cassava for feed will result in a tremendous impact on the improvement of the nutritional value of cassava.

The average food composition of fresh cassava is shown in Table 2. Analysis of specific varieties showed that the Lakan type has dry matter content of 44% and

fresh weight starch content of 32%; Datu I has 37% dry matter content and 27% fresh weight starch content; Sultan I has 34% dry matter content; and Golden Yellow has 34.42% fresh weight starch content (Sanchez, 1987).

Table 1. Production areas, quantity and value of cassava by region, 1985[1]

| *Region* | *Production areas (Has.)* | *Quantity (MT)* | *Value (₱)* |
|---|---|---|---|
| Ilocos | 2,120 | 12,930 | ₱ 27,995 |
| Cagayan Valley | 680 | 3,097 | 3,208 |
| Southern Tagalog | 7,370 | 43,183 | 47,893 |
| Central Luzon | 1,210 | 7,266 | 11,219 |
| Bicol | 30,940 | 245,687 | 239,992 |
| Western Visayas | 8,810 | 42,691 | 58,174 |
| Central Visayas | 41,120 | 88,509 | 118,940 |
| Eastern Visayas | 30,160 | 113,255 | 154,346 |
| Western Mindanao | 43,440 | 267,460 | 243,698 |
| Northern Mindanao | 12,770 | 74,439 | 82,441 |
| Southern Mindanao | 6,680 | 36,449 | 82,441 |
| Central Mindanao | 30,980 | 616,120 | 737,635 |
| Total | 216,280 | 1,551,086 | ₱1,767,175 |

[1]BAEcon Statistics, 1985.

Table 2. Composition of cassava (*Manihot esculenta* Crantz)[1]

| *Parameter* | *Value (per 100 grams)* |
|---|---|
| Edible portion (%) | 76.00 |
| Moisture (%) | 64.00 |
| Food Energy (calories) | 141.00 |
| Protein (g) | 0.70 |
| Fat (g) | 0.10 |
| Total carbohydrate (g) | 34.30 |
| Fiber (g) | 1.00 |
| Ash (g) | 0.90 |
| Calcium (mg) | 24.00 |
| Phosphorous (mg) | 37.00 |
| Iron (mg) | 1.50 |
| Sodium (mg) | 3.00 |
| Potassium (mg) | 418.00 |
| Thiamine (mg) | 0.04 |
| Riboflavin (mg) | 0.01 |
| Niacin (mg) | 0.60 |
| Ascorbic acid (mg) | 41.00 |

[1]Food composition Table:Recommended for use in the Philippines. Food and Nutrition Research Institute National Science and Technology Authority, Manila.

Protein enrichment of cassava done by the previous researches are either high input in character and involve either costly methods of pre-treatment or sophisticated methods of fermentation (Gray and Abouel. 1966; Brook, Stanton and Wallbridge, 1969; Reade and Gregory, 1975; Gregory 1977; Muindi and Hassen, 1981, Aiddo, Hendry and Wood, 1982; Mikami, *et al.*, 1982; Yasuda, 1983 Raimbault, *et al.*, 1985). The results from these researches can be applicable in commercial scale emplying high technology and expensive equipment.

Malnutrition due mostly to protein deficiency is a common occurrence in developing countries. In several of these areas, vegetable protein constitutes a much larger proportion of the diet than does animal protein. This is most common in the Orient due to animal protein shortage and the influence of religious beliefs which excludes meat in the diet.

Availability of greater amounts of animal proteins, however, involves utilization of even greater amounts of vegetable proteins. This phenomenon is explained when vegetable proteins like soybeans are fed to animals like chicken, hog and cattle. Protein losses result due to the inefficiencies inherent in animal metabolism unlike when protein is eaten directly by man.

Improved traditional agricultural methods could easily cope with the needs of vast populations through high yielding varieties of crops. The real problem, however, is providing increasing amounts of proteins to keep pace with an increasing populations. Gray (1970) cited three points regarding the solution of this problem: (1) the total quantity produced must be usch that about 65 g protein are available per person per day; (2) the protein must be of good quality, i.e. it must contain the essential amino acids in quantities approaching the proper amounts; and (3) it must be concentrated form that an individual need not attempt to ingest almost impossible quantities of bulk to obtain his daily quota of protein.

The application of biotechnology on our abundant raw materials might be one of the answers in providing nutritious foods. Microorganisms have long been known as sources of direct food and as agents in food processing. Their capability in converting raw materials into nutritions products is already a matter of public record. Fungi specifically have been and are still being used directly as food (mushroom) as well as in the processing of various food products either for the purpose of making a different, a more palatable, or a more nutritious food (cheese, tempeh and soysauce). Fungi also significantly to the world protein supply thus helping in the solution of the major problem facing mankind (Gray, 1970).

Technological breakthroughs, therefore, must seek to increase the availability of vegetable proteins because the majority of the edible plant materials have low concentration of proteins. Let us take for example cassava, a staple food in African countries. It has a high calorie content but is very low in protein with an average of 0.7%. One has to eat large amounts of cassava to obtain his daily protein requirements. In combining the carbohydrate-synthesizing capacities of fungi it will logically give the answer to the problem.

## Materials and Methods

Nine-month old cassava (*Manihot esculenta* Crantz) of the Golden Yellow root vareity was used in the studies. The cassava was peeled and passed through a chipping machine. The chipped cassava was dried in a forced convection dryer for five hours at temperatures of 60-65°C. Cassava roots are highly perishable due to microbial, physiological and mechanical damage. It should be eaten or processed into chips 24 hours after harvest.

Several strains of molds belonging to genera Aspergillus, Mucor and Rhizopus were tested for their ability to enrich cassava (Table 3). The strains were obtained

Table 3. Molds screened for high protein production

| *Strain No.* | *Mold* | *Scource*[1] |
|---|---|---|
| 1 | *Rhizopus cohnii* | NFRI |
| 2 | *R. hangchow* | NFRI |
| 3 | *R. javanicus* | IFO 5442 |
| 4 | *R. karso* | NFRI |
| 5 | *R. oligosporus* | NRRL 2710 |
| 6 | *R. oryzae* | NFRI 1029 |
| 7 | *R. peka II* | NFRI |
| 8 | *R. pseudochinensis* | NFRI 1027 |
| 9 | *R. stolonifer* | NFRI 1030 |
| 10 | *R. tamarii* | NFRI |
| 11 | *Aspergillus niger* | IFST 59 |
| 12 | *A. niger* | NFRI 1020 |
| 13 | *A. niger* | NFRI 1021 |
| 14 | *A. niger* | NFRI 1022 |
| 15 | *A. oryzae* | IAM 2640 |
| 16 | Strain No. 1 | Isolated from tane koji |
| 17 | Strain No. 2 | Isolated from tane koji |
| 18 | Strain No. 3A | Isoalted from tane koji |
| 19 | Strain No. 3B | Isoalted from tane koji |
| 20 | Strain No. 57 | Isolated from cassava |
| 21 | Strain No. 58 | Isolated from cassava |
| 22 | Strain No. 60 | Isolated from cassava |
| 23 | Strain C | Isolated from tane koji |
| 24 | *Monascus anka* | NFRI 4478 |
| 25 | *M. anka* | IFO 6540 |
| 26 | *Mucor pyriformis* | IFST 44 |
| 27 | *M. hiemalis* | IFST 68 |

[1]NFRI = National Food Research Institute
IFO = Institute of Fermentation Technology
NRRL = Northern Regional Research Laboratory
IAM = Institute of Applied Microbiology
IFST = Institute of Food Science and Technology

from the culture collections of National Food Research Institute (17 strains), Institute of Food Science and Technology (2 strains), and isolated from rotting cassava (3 strains) and from tane koji (5 strains).

Preliminary screening of high protein mold strains was done using liquid shake culture. The preparation of starter and culture medium were based on the method of Yasuda (1983). Modification was made by substituting 2% raw cassava flour as carbohydrate source. The nutrients added to the medium were 1% ammonium sulfate, 0.2% urea, 0.2% potassium phosphate (dibasic), 0.005% magnesium sulfate, 0.05% potassium chloride and 0.001% ferrous sulfate. The medium was sterilized at 121°C for 20 minutes, cooled and inoculated with 1% mold spores suspension consisting of $10^6$ spores per mL. Inoculation was done under aseptic condition. Incubation was made at 30°C for 40 hours in a shaker.

The growth was filtered through vacuum with the aid of Whatman filter paper No. 1. The mycelia were washed with 0.85% sodium chloride solution then dried at 100°C for 15 hours. After colling, the weight of the mycelia was taken and analyzed for crude protein by Kjeldahl using Digital Kjedahl Analysis System KN-01 Type, Mitsubishi Kasei Company.

High protein mold was tested in cassava chips by solid state fermentation (SSF) process. The dried cassava chips were hydrated with aqueous solution of ammonium sulfate and potassium dihydrogen phosphate at a concentration of 10% and 4%, respectively. The hydrated cassava was sterilized at 121°C for ten minutes. Upon cooling, 2% urea and 1% powdered spores of high protein molds were evenly mixed with the cooked cassava. The levels of nutrient addition was based on the method of Raimbault, *et al.*, (1985).

The inoculated samples were incubated at 30°C for 72 hours then dried at 90°C for 15 hours. Crude protein was determined as described by Sanchez, 1987.

Optimization of fermentation conditions for SSF such as moisture content (35, 40, 45 and 50%), temperature of incubation (25°, 30°, 37° and 40°C) and length of fermentation period (daily for seven days) were done.

Amino acid analysis of the enriched and non-inoculated cassava used High Speed Amino Acid Analyzer Model 835. Based on the data obtained the values of each amino acid in mg/100 g cassava was calculated.

The selected high protein molds were tested for aflatoxins production using the culture filtrate spot method developed by the National Institute of Hygiene and Safety (1980). The culture filtrate were spotted directly in activated precoated TLC plates (Merck G60). Aflatoxins ($B_1$, $B_2$, $G_1$ and $G_2$) purchased from Wako Pure Chemicals Industries, Inc. were used as standards. The chromatograms were developed with solvent consisting of chloroform:acetone:hexane (85:15:20). Detection of the aflatoxins was done under UV light.

Identification of isolated high protein-producing molds was done based on the standard methods (Raper and Fennel, 1965). Cultural characteristics was studied in Czapek's Solution Agar and Malt Extract Agar. The giant colony growth of the isolated molds at 25°C and 30°C was measured and growth characteristics

such as color of aerial parts including vegetative mycelium, conidial heads and cleistothecia or sclerotia (if present), pigmentation of the basal medium and mycelium, characteristics of colony margins, texture of surface growth and zonation.

Morphological characteristics such as shape and size of cells, spores and conidial structures, arrangements of parts in the conidial structures, arrangements of parts in the conidial heads, conidiophores and other structures were taken.

## Results and Discussions

*Chemical and physical analysis of cassava*

The chemical composition of cassava variety Golden Yellow used in the study was 0.64% crude protein, 45% moisture and76% starch. The analysis of cassava as reported by Food and Nutrition Research Institute (1980) is shown in Table 2.

The physical characteristic like viscosity as compared with tapioca as determined by Prabender Amylograph yielded the following analysis as shown in Table 4: gelatinization temperature, 71.5°C; maximum viscosity, 775 BU; maximum viscosity temperature, 82.5°C; lowest viscosity, 305 BU and lowest viscosity temperature, 90°C. The differences in values obtained might be due to the variety of cassava used in the preparation of the commercial tapioca.

*Protein production in liquid culture of mold strains.*

Twenty seven (27) strains of molds screened for protein production in liquid shake culture using cassava as carbon source (Figure 1) showed that the ability of the strains varied in the amounts of growth, protein production and pH and total soluble solids (TSS) after fermentation (Table 5). The liquid culture medium has

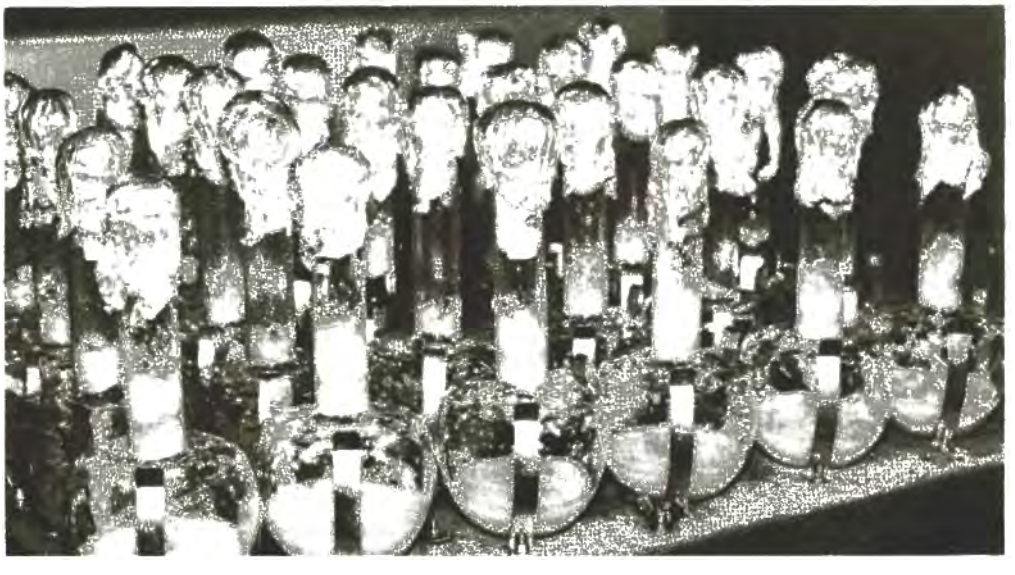

Figure 1. Screening of high protein molds by liquid shake culture.

Table 5 (Continued)

| *Strain No.*[1] | *pH*[2] | *TSS ( Brix)*[3] | *Mycelia (d.w.) mg/g cassava* | *Protein (mg/g mycelia* | *Protein (mg/g cassava* |
|---|---|---|---|---|---|
| 20 | 2.61 | 1.95 | 380 | 323 | 123 |
| 21 | 3.80 | 2.05 | 430 | 340 | 146 |
| 22 | 3.81 | 1.95 | 500 | 475 | 238 |
| 23 | 4.41 | 2.05 | 480 | 414 | 199 |
| 24 | 6.00 | 3.50 | 175 | 228 | 40 |
| 25 | 6.12 | 3.60 | 110 | 269 | 30 |
| 26 | 7.06 | 2.00 | 320 | 421 | 135 |
| 27 | 7.65 | 3 00 | 45 | 407 | 183 |

[1]Refer to Table 3.
[2]Initial pH - 5.0.
[3]Initial Total Solube Solids (TSS) = 4° Brix.

an initial pH 5.0. After fermentation for 40 hours, Mucor species and Rhizopus species (except for *R. cohnii* and *R. oryzae*) changed the pH of the medium to alkaline ranging from pH 6.39 to 7.96. The Aspergillus species on the other hand, produced acid thus lowering the pH of the medium in the ranged of pH 2.56 to 4.41. The TSS changed from the initial value of 4°Brix to a range of 1.5 to 3.35°Brix.

The highest mycelial production obtained was 500 mg/g cassava while the lowest was 45 mg/g cassava. The protein produced by the mold strains ranged from 313 to 551 mg/g mycelia and 30 to 238 mg/g cassava (Table 5). Based on these results five strains, namely Nos. 2, 3A, 60, C and A. niger NFRI 1021 were selected for the solid state fermentation of cassava.

*Effect of moisture levels on the protein production of selected molds on SSF of cassava chips*

The selected molds were grown in cassava chips with different moisture content (35, 40, 45 and 50%) for 72 hours at 30°C. Table 6 shows the percent protein and protein production in mg/g cassava obtained. Results indicated that optimum moisture for SSF of cassava depends on the strain of mold. Highest protein content was obtained at 40% moisture for *A. oryze* var. *brunneus* and *A. niger* NFRI 1021; at 45% for *A. candidus* and *A. usamii and 50% for A. tamarii.*

*Effect of incubation temperature (°C) on the protein production of selected molds on SSF of cassava chips*

The selected molds were cultured in cassava chips with optimum moisture content required by the specific mold strain. The molds were incubated at 25°C, 30°C, 37°C and 40°C for 72 hours.

The optimum temperature for protein production for *A. usamii* was 25°C, for *A. candidus*, *A. oryzae* var. *brunneus* and *A. niger* NFRI 1021 was 30°C and of *A. tamarii*, 37°C. The highest protein contents (% d.b.) produced by the mold strains at these optimum temperature were 9.86% for *A. candidus*, 9.56% for *A. oryzae* var. *brunneus*, 9.75% for *A. tamarii*, 10.29% for *A. usamii* and 9.71% for *A. niger* NFRI 1021 (Table 7).

Table 6. Effect of varying moisture levels (%) on the protein production of Aspergillus species on solid state fermentation (SSF) of cassava chips at 30°C.

| *Moisture (%)* | *A. candidus* | *A. oryzae var. brunneus* | *A. tamarii* | *A. usamii* | *A. niger NFRI 1021* |
|---|---|---|---|---|---|
| Protein (%, d.b.) | | | | | |
| 35 | 3.68 | 3.68 | 4.41 | 3.82 | 6.33 |
| 40 | 7.65 | 9.56* | 5.45 | 6.04 | 9.56* |
| 45 | 9.12* | 8.24 | 9.27 | 8.82* | 8.94 |
| 50 | 7.80 | 2.65 | 9.86* | 8.01 | 3.98 |
| Protein (mg/g cassava) | | | | | |
| 35 | 43.08 | 43.16 | 47.85 | 41.80 | 60.80 |
| 40 | 55.00 | 80.12* | 55.38 | 57.74 | 86.33* |
| 45 | 82.21* | 65.95 | 75.45 | 77.31* | 45.41 |
| 50 | 60.29 | 26.00 | 76.94* | 73.04 | 37.87 |

*Highest value.

The optimum temperature for growth was similar to that for protein production except for *A. tamarii* whose optimum temperature for growth was 30°C. This indicates that in some molds, the optimum temperature for growth is not necessarily the best temperature for protein production.

*Effect of fermentation time on the protein production of selected molds on SSF of cassava chips.*

The different mold strains were incubated from one to seven days at their optimum temperatures using cassava chips rehydrated to the optimum moisture levels required by the different strains.

Results showed that abundant growth and sporulation occurred as fermentation period is prolonged. Highest amount of protein, however, was obtained after 72 hours of incubation (Table 8). The amount of protein, mg/g cassava ranged from 79.93 to 102.61 while amount of protein (% d.b.) ranged from 9.27 to 10.00%.

*Amino acid analysis of solid state fermented cassava chips*

Cassava chips enriched by solid state fermentation process providing optimum conditions for protein production were analyzed for amino acids content. Table 9

showed a tremendous increased in all amino acids as compared with the unfermented cassava chips.

Based on the results, there was a 10-fold increase in alanine, four-fold for arginine, seven-fold for asparagic acid, five-fold for glutamic acid, 11 to 12-fold for glycine, eight to 10-fold for histicine, 10 to 11-fold for isoleucine, 11 to 12-fold for leucine, six to eight-fold for lysine, six to seven-fold for methinonine, 10 to 11-fold for phenylalanine, nine-fold for serine, nine to 11-fold for threonine, nine to 11-fold for tyrosine, 11 to 14-fold for valine and from trace amount to 358.59 mg/100 g for proline.

The impact of this study is the increase in lyzine and methionine, both essential and limiting amino acids. Large amounts of these amino acids are imported for incorporation in animal feed formulations.

Cassava enriched by mold fermentation can find applications in many processed food products. Considering that the four selected mold strains are white in color, its applicability in food presents no problem.

A study was conducted on chickens to establish the content of metabolizable energy and the content and apparent digestibility of crude protein and amino acids in cassava root meal enriched by the mold *Trichoderma harzianum* (Miundi and Hansen, 1981). Metabolizable energy content of the enrich cassava was found to be 9.1 MJ/kg dry matter, a value significantly lower than that of non-enriched cassava (12.2 MJ/kg. dry matter). The crude protein content of the enriched cassava

Table 7. Effect of varying temperature of incubation on the protein production of Aspergillus species on solid state fermentation (SSF) on cassava chips

| *Temperature (°C)* | *A. candidus* | *A. oryzae var. brunneus* | *A. tamarii* | *A. usamii* | *A. niger NFRI 1021* |
|---|---|---|---|---|---|
| Initial Moisture (%) | 45 | 40 | 50 | 45 | 40 |
| Protein (%, d.b.) | | | | | |
| 25 | 6.62 | 6.61 | 7.35 | 10.29* | 4.71 |
| 30 | 9.86* | 9.56* | 9.53 | 8.96 | 9.71* |
| 37 | 6.82 | 7.29 | 9.76* | 5.15 | 6.47 |
| 40 | 3.09 | 7.18 | 6.18 | 2.51 | 5.88 |
| Protein (mg/g cassava) | | | | | |
| 25 | 64.61 | 63.99 | 68.46 | 79.82* | 39.59 |
| 30 | 81.91* | 79.18* | 76.47 | 76.21 | 85.41* |
| 37 | 56.49 | 70.20 | 79.99* | 41.18 | 59.09 |
| 40 | 36.32 | 63.99 | 64.18 | 18.36 | 57.72 |

*Highest value.

Table 8. Effect of fermentation time (day) on the protein production of Aspergillus species on solid state fermentation (SSF) on cassava chips

| *Time (day)* | *A. candidus* | *A. oryzae var. brunneus* | *A. tamarii* | *A. usamii* | *A. niger NFRI 1021* |
|---|---|---|---|---|---|
| Initial Moisture (%) | 45 | 40 | 50 | 45 | 40 |
| Incubation Temp. (eC) | 30 | 30 | 37 | 25 | 30 |
| Protein (%, d.b.) | | | | | |
| 1 | 3.53 | 5.29 | 5.74 | 3.82 | 1.62 |
| 2 | 6.62 | 8.24 | 7.21 | 7.65 | 3.68 |
| 3 | 9.27* | 9.86* | 10.00* | 9.71* | 9.56* |
| 4 | 8.39 | 9.12 | 8.98 | 9.41 | 8.39 |
| 5 | 6.93 | 8.68 | 8.82 | 9.41 | 6.47 |
| 6 | 6.92 | 7.80 | 8.68 | 8.82 | 5.88 |
| 7 | 6.62 | 7.65 | 8.68 | 8.53 | 5.15 |
| Protein (mg/g cassava) | | | | | |
| 1 | 42.02 | 62.18 | 65.20 | 45.43 | 19.27 |
| 2 | 69.14 | 80.61 | 69.15 | 79.11 | 76.48 |
| 3 | 79.93* | 89.88* | 86.78* | 86.65* | 102.61* |
| 4 | 64.54 | 76.85 | 71.16 | 80.88 | 87.42 |
| 5 | 51.98 | 71.76 | 69.21 | 73.52 | 65.93 |
| 6 | 49.12 | 60.32 | 64.64 | 67.89 | 47.06 |
| 7 | 48.33 | 60.07 | 63.00 | 63.40 | 47.06 |

*Highest value.

Table 9. Amino acid (mg/100) composition of the unfermented cassava and cassava fermented by solid state fermentation (SSF) using selected molds

| *Amino acids* | *Unfermented cassava* | *Mold Strains*[1] | | | | *A. niger 1021* |
|---|---|---|---|---|---|---|
| | | *No. 2* | *No. +A* | *No. 60* | *C* | |
| Alanine | 51.72 | 552.05 | 503.25 | 556.26 | 517.97 | 514.09 |
| Arginine | 109.45 | 481.16 | 463.15 | 491.03 | 441.95 | 432.25 |
| Asparagic acid | 95.70 | 691.47 | 667.14 | 697.59 | 643.15 | 629.71 |
| Glutamic acid | 185.15 | 1087.65 | 988.68 | 1128.37 | 981.73 | 873.99 |
| Glycine | 32.43 | 393.23 | 367.36 | 394.97 | 373.81 | 357.25 |
| Histidine | 18.08 | 174.74 | 166.98 | 175.12 | 137.86 | 151.32 |
| Isoleucine | 31.68 | 359.64 | 329.46 | 355.85 | 335.10 | 307.84 |
| Leucine | 47.94 | 593.34 | 566.68 | 593.36 | 511.89 | 540.55 |
| Lysine | 52.12 | 431.22 | 464.35 | 435.83 | 321.56 | 418.04 |
| Methionine | 16.56 | 142.50 | 138.44 | 143.92 | 92.53 | 128.58 |
| Phenylalanine | 30.78 | 339.35 | 327.41 | 339.56 | 294.91 | 311.17 |

Table 11. Cultural characteristics of the selected isolated mold strains after 10 days incubation at 25°C

| *Strain No.* | *G R O W T H* | | | | | | |
|---|---|---|---|---|---|---|---|
| | *Diameter (cm)* | | *Color* | | *Texture* | | *Characteristics* |
| | *A* | *B* | *A* | *B* | *A* | *B* | |
| 2 | 5.0 | 5.8 | white | white | felt and raised | thin | Aerial mycelium and spores abundant in Czapeks than in Malt Extract Agar |
| 3A | 5.8 | 6.0 | yellowish-white | white | cottony | thin | Produced orange-brown pigment on Czapeks; thin concentric growth |
| 60 | 5.0 | 7.0 | yellowish-green to golden | green to olive green | loose | loose | Brownish to grey pigmentation in reverse in Czapecks. |
| C | 5.5 | 7.5 | white | white | cottony | cottony | Abundant aerial mycelium and spores |

A = Czapeks Solution Agar.

Table 12. Morphological characteristics of the selected isolated mold strains

| *Strain No.* | *Conidia* | | | *Conidiophore* | *Vesicle* | *Sterigmata* | *Identification* |
|---|---|---|---|---|---|---|---|
| | *Size* | *Shape* | *Color % Textuure* | | | | |
| 2 | 5.4-9.5 | ovoid spherical | hyaline smooth | hyaline; smooth 6-8 x 265 microns | globose sub-globose | biseriate and uniseriate | *Aspergillus candidus* |
| 3A | 4.5-10 | globose | hyaline to yellowish to echinulate | hyaline 0.2-2.5 mm, | globose to sub-globose | uniseriate | *A. oryzae* var. |
| 60 | 4-6.5 | globose to sub-globose | yellowish brown to smooth slightly rough | hyaline to light yellow 8-10 $\mu$ 1-2 mm | sub-globose to elongate | biseriate | *A. tamarii* |
| C | 4-10 | globose | hyaline smooth | hyaline 5.4 x 378 microns | globose | biseriate | *A. usamii* |

*didus,* Strain 3A as *A. oryzae* var. *brunneus,* Strain 3B as *A. terreus,* Strain 60 as *A. tamarii* and Strain C as *A. usamii.*

The growth of these molds are shown in Figures 2 to 6.

*Aflatoxin detection studies*

Results of the aflatoxins detection test showed that all the selected mold strains were negative for aflatoxin production. Mold strains which are known to produce aflatoxins are normally colored (green to gray). The selected strains are all producing white colored mycelia.

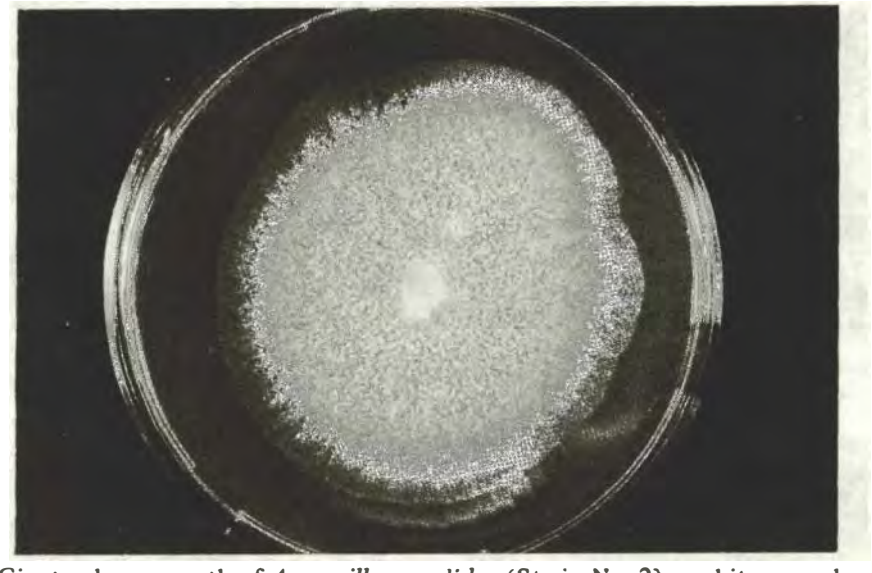

Figure 2. Giant colony growth of *Aspergillus candidus* (Strain No. 2), a white spored mutant on Malt Extract Agar.

Figure 3. Giant colony growth of *Aspergillus oryzae* var. *brunneus* (Strain 3A) a white spored mutant on Malt Extract Agar.

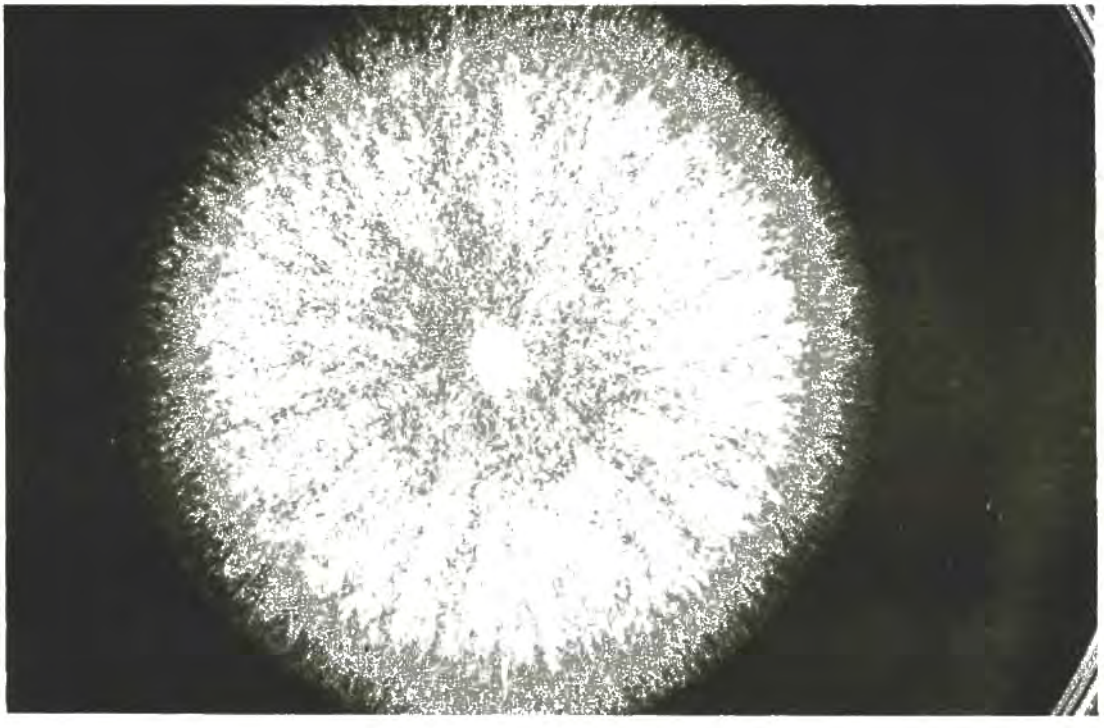

Figure 4. Giant colony growth of *Aspergillus tamarii* (Strain No. 60) on Malt Extract Agar.

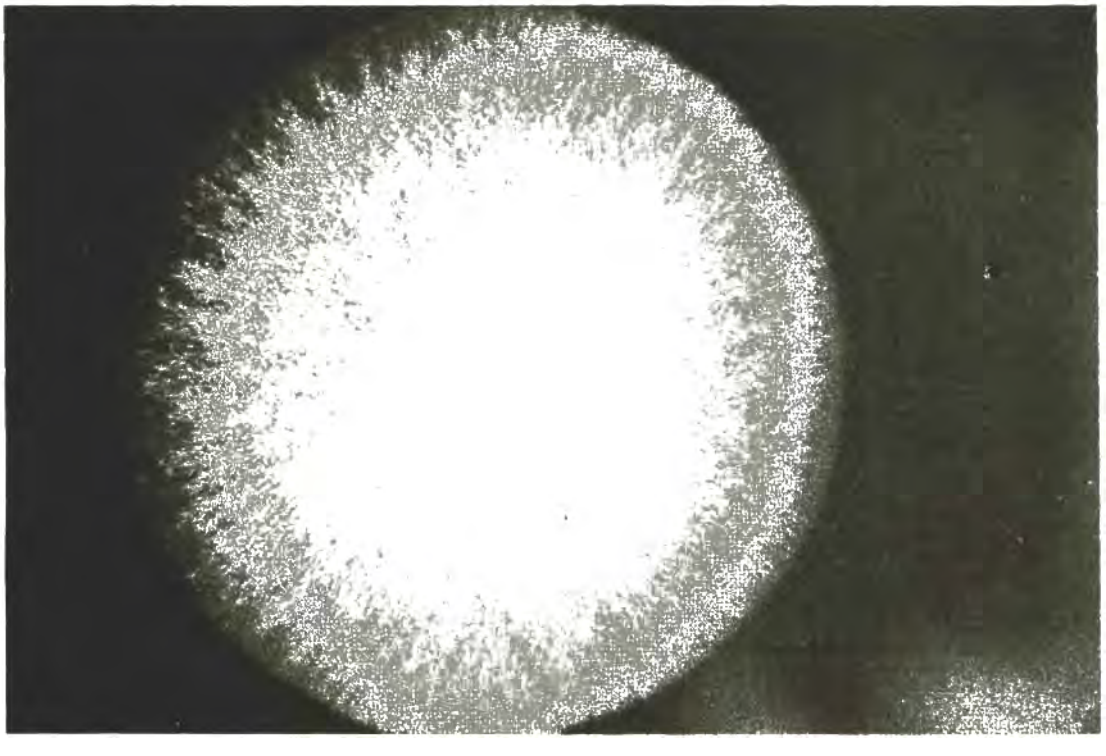

Figure 5. Giant colony growth of *Aspergillus usamii* (Strain C), a white spored mutant on Malt Extract Agar.

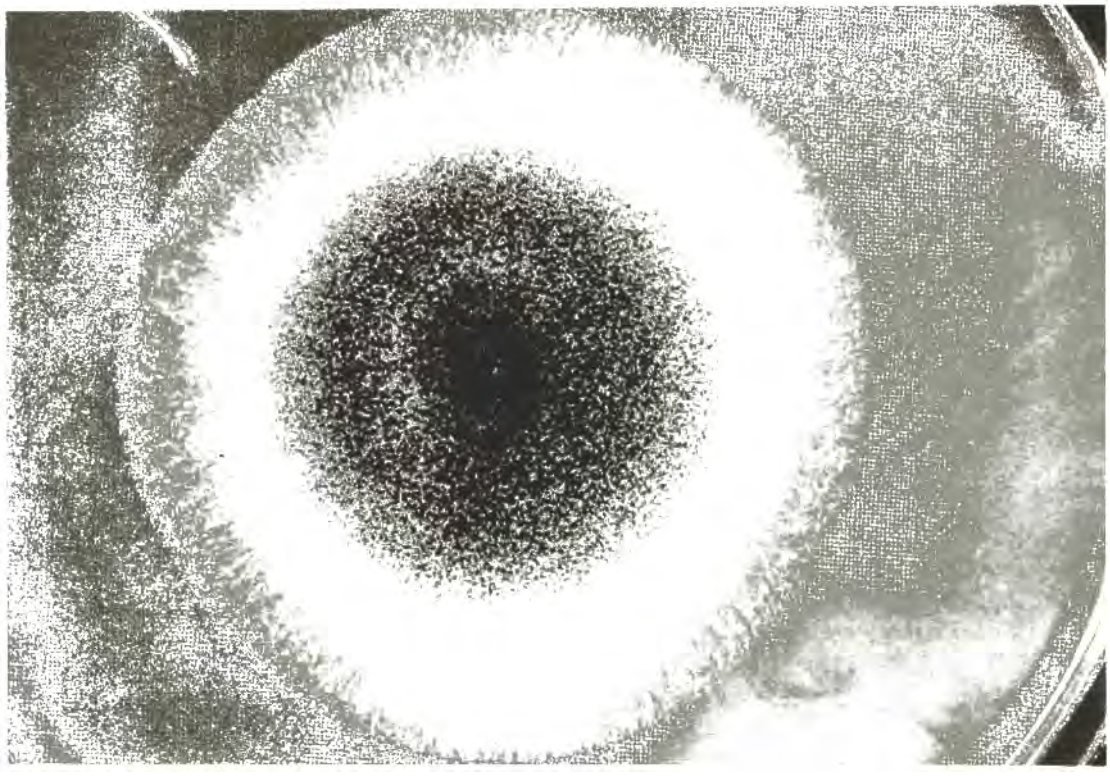

Figure 6. Giant colony growth of *Aspergillus niger* NFRI 1021 on Malt Extract Agar.

## Summary, Conclusions and Recommendations

Five strains of mold belonging to genus Aspergillus were selected from 27 strains for high protein production. Cassava was enriched with protein ranging from 9.91 to 10.29% exhibiting a 15 to 16-fold increase through solid state fermentation.

The technology developed applied the tray method. Optimization of conditions for fermentation and nutrient supplementation in this scale, however, must be established.

Utilization of the fermented cassava into different product formulations and acceptability tests on these different processed products must be undertaken. Similaly, feeding experiments both for humans and animals will prove the merit of the developed technology.

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