Screening of Microorganisms for Nutritive Enrichment of Non-Marketable Cassava with Organic Supplement

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Abstract

Screening of four different microorganisms which were potential to enrich the nutritive value of cassava root mash was described. Selecting which among them is best in soy hull and rice bran was identified. The effect of two organic supplements in enriching the nutritive value of cassava root was determined. *Spirulina platensis* produced high cell biomass when propagated in an alkaline (pH 10) and in saline (5-10%) freshwater medium. However, in screening microorganism that was suited for cassava root with soy hull, the yeast (*S. cerevisiae*) was more efficient in increasing the crude protein (8.93%), fat (2.29%) and fiber content (5.73%) whereas bacteria (*Lactobacillus* sp.) was more efficient in increasing the ash content (4.94%). In contrast, *Lactobacillus* sp. was more efficient in mounting the crude protein, fat, fiber and ash content (3.97%, 2.29%, 10.58% and 5.78%, respectively) of cassava root mash with rice bran except in terms of moisture where A. foetidus was consistently efficient in both organic supplements. Between the efficiency of the two organic supplements to improve the nutritive value of cassava root, both were comparable in enriching the protein and moisture content. However, crude fat, fiber and ash enrichment was better with rice bran than with soy hull.

Keywords: single cell protein, cassava, nutritive enrichment, spirulina

Introduction

Cassava (*Mahinot esculenta*) is a starchy staple food found abundant in our country. In fact, about 1.7 million metric tons were produced at 210,000 ha and bulk of the consumption is in Sulu Archipelago and Muslim population (Algerico and Bacusmo, 2002). In 2002, around 190,000 metric tons of cassava each year is utilized as feeds

Since cassava is now playing an important role in food security, equity and poverty alleviation, a lot of studies have been made to improve its nutritional value as food and feeds. Mostly the greater pie of human and animal food and feed consumption is protein, which however, is over exploited and costly. Efforts in producing feeds such as supplementation (i.e. protein, amino acid, fats, minerals vitamins etc.) have not been satisfactory because it is dictated by economics. This dilemma has led serious studies in finding viable ways and means to convert the non-marketable cassava as substrates for production of non-traditional protein enriched feeds through microbial fermentation.

However, the world of microorganisms is very diverse, and so it is worthwhile to screen which among them can efficiently carry out bioconversion of carbohydrate to protein in order to achieve the maximum yield of non-traditional protein.

Objectives

1. Compare the effect of organic supplements in terms of crude protein, fat, fiber, ash and moisture content.

2. Evaluate the performance of selected fungi, yeast, microalgae and bacteria in terms of crude protein, crude fat, crude fiber, ash and moisture content of cassava root mash.

3. Identify the most suitable microorganism for the effective enrichment of cassava with different organic supplements.

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Methodology

Preparation of the Inoculum

Pre-screening tests were performed among *Rhizopus oligosporous*, *Aspergillus niger* and *Aspergillus foetidus*. The same test was done with *Candida utilis*, *Candida tropicalis* and *Saccharomyces cerevisiae*. This method was done in order to determine which among these fungi and yeasts best yield microbial protein. *Spirulina platensis*, however, was cultivated on a freshwater and bacteria in a milk-yogurt solution. After two weeks of incubation at 29 to 30°C, spore or cell suspensions of the organisms was transferred aseptically to the cassava substrate and fermentation was allowed to take place for two weeks.

Production of Single Cell Protein (SCP) Using Cassava Substrate

In a separate vessel, a 0.5 gram spore suspension from the prepared primary inoculum of *Rhizopus oligosporous*. *Aspergillus niger*, *Aspergillus foetidus*, *Candida utilis*, *Candida tropicalis*, *Saccharomyces cerevisiae* and *Lactobacillus* sp. was aseptically transferred to the cassava root substrate and was acclimatized for fourteen days. Cell suspension of *Spirulina platensis* was mixed to the substrate after 14 days. The inoculated substrate was spread in a tray at about 2-3 cm thick and was left for 3-5 days at room temperature. The dried samples were submitted at Biotech, UPLB and PhilRice for proximate chemical analysis.

Results and Discussion

Crude Protein Content

Table 1 shows that both organic supplements can be used to enrich the protein content of cassava root. This is because rice bran having 13% of protein (*http://knowledgebank.irri.org*) and soy hull of 10% at minimum of protein (*http://www.allgreen.biz*) falls on the same range.

With respect to the ability of the microorganisms to increase the protein content of pure cassava, yeast (*S. cerevisiae*) registered the highest. This indicates that the amylolytic activity of yeast can reduce the starch content during fermentation, which only shows the better ability of *Saccharomyces cerevisiae* to carry out bioconversion of starch to protein (Padmaja and Balagopalan, 1990).

With soy hull as an organic supplement, *S. cerevisiae* registered the highest protein production. The ability of yeast to convert carbohydrate into protein is due to its characteristic to multiply at a faster rate with a doubling time in terms of crude protein at 2-6 hours (Suamalainen and Cura, 1981). In addition, yeast is a good source of protein or amino acids because approximately 40% of the weight of dried yeast consists of protein (*www.worldoffungi.com*). A study carried out by Lyutskanov et al. (1989) revealed the percentage of essential amino acids lysine and isoleucine of *S. cerevisiae* were higher than that of soya bean and tryptophan increase the protein content of a corn meal (Ingram, 2002).

On the other hand, with rice bran as an organic supplement, it was bacteria (*Lactobacillus* sp.) that marked the highest protein. This is because bacterial -amylase is generally preferred for starch liquefaction due to its high temperature stability (*www.fungi.com*). Furthermore, bacteria, like yeast, proliferate rapidly. However, *Lactobacillus* sp. can produce lactic acid that catalyze the hydrolysis of starch easily converting it to glucose, which will serve as its immediate source of metabolizable energy for its rapid growth therefore, increase its bioconversion to protein.

Crude Fat Content

Comparing the two organic supplements, results in Table 2 showed higher fat content was obtained when rice bran was used. This is because according to TropRice of IRRI, rice bran contains 10-23% fat that is 100% digestible and rich in high soluble fat. Soy hull however, only contains 3% minimum of crude fat (*http://www.allgreen.biz*).

In cassava-soy hull substrate, yeast (*S. cerevisiae*) registered the highest fat content while *Lactobacillus* sp. in cassava-rice bran substrate. This confirms an earlier finding on the ability of *S. cerevisiae* to efficiently increase the crude fat of cassava using soy hull because of the ergosterol present at 6% by weight of the protoplasmic

Table 1. Percent crude protein of cassava root mash as affected by organic supplements and microorganisms

MICROORGANISMS	ORGANIC SUPPLEMENTS (%)		MEAN (%)
	Soy Hull	Rice Bran	
None	2.30 e	2.30 d	2.30 c
Fungi (*A. foetidus*)	4.47 b	3.57 b	4.02 b
Yeast (*S. cerevisiae*)	8.93 a	3.19 c	6.06 a
Microalgae (*S. platensis*)	3.53 c	3.25 c	3.39 b
Bacteria (*Lactobacillus* sp.)	2.64 d	3.97 a	3.30 b
Mean	4.55 a	3.44 a	

Legend: Means followed by a common letter are not significantly different at 1% level of DMRT

membrane and mitochondria. These sterols reach their maximum at the first two days of fermentation and decrease as days went on. Addition of fat which is high in rice bran can activate fermentation activities thereby decreasing the fat content of the substrate (*www.doctorfungus.org/thefungi/saccharomyces.htm*). This explains why yeast can best increase the fat content of cassava-soy hull substrate due to low fat content of soy hull.

Crude Fiber Content

The data shown in Table 3 discloses that after fermentation, cassava root mash with soy hull as supplement had significantly lower crude fiber content than cassava root mash with rice bran. According to IRRI, rice bran has more fiber content which consists of both water-soluble and water-insoluble fiber. On the other hand, according to the study of Garleb in 1987, soy hulls are highly digestible in the rumen due to low lignin content (*http://www.allgreeen.biz*) but not as high as rice bran.

Fungi, bacteria, microalgae and yeast have the same efficiency in increasing the crude fiber content of cassava root mash. This is because most of the microorganisms possess amylolytic enzyme and bacteria, a fibrolytic enzyme that can digest both cellulose and hemicellulose present in cassava and organic supplements because of their ability to degrade fiber content of the substrate, the increase in microbial mass enriches the substrate with fiber because the external covering of the microbes are fiber related materials.

In cassava-soy hull substrate, yeast (*S. cerevisiae*) registered the highest crude fiber content because the yeast cell undergoes growth and cell multiplication at a faster rate due to the low lignin and fiber content of soy hull (*http://www.allgreeen.biz*). High amount of fiber interferes with the gluten structure and reduces gas and tolerance of yeast which in this case affect the growth and cell multiplication of *S. cerevisiae* during its fermentative process.

In terms of cassava-rice bran substrate, bacteria (*Lactobacillus* sp.) is the most efficient in enriching pure cassava of fiber because insoluble fiber is highest in hulls to which rice bran is derived. Only fibrolytic bacteria can digest these fibers. A study of Limin Kung in the role of fiber in ruminant ration formulation indicates that where fiber is high, bacterial or probiotic population is also high therefore fermentative activity is increased (*http://ag.udel.edu*).

Ash Content

The ash content of cassava-rice bran substrate after fermentation was significantly greater than fermented cassava- soy hull substrate. This is because as reported by IRRI, bran contains tiny fractions of rice hull, which increases the ash content of the bran. Therefore it shows that cassava-rice bran substrate have high mineral contents fitted for animal consumption

Table 2. Percent crude fat of cassava root mash as affected by organic supplements and microorganisms

MICROORGANISMS	ORGANIC SUPPLEMENTS (%)		MEAN (%)
	Soy Hull	Rice Bran	
None	1.01 c	1.01 d	1.01 c
Fungi (*A. foetidus*)	0.78 d	2.31 ab	1.54 bc
Yeast (*S. cerevisiae*)	2.29 a	2.24 b	2.26 a
Microalgae (*S. platensis*)	0.94 c	1.83 c	1.40 bc
Bacteria (*Lactobacillus* sp.)	1.35 b	2.39 a	1.87ab
Mean	1.28 b	1.96 a	

Legend: Means followed by a common letter are not significantly different at 1% level of DMRT

Table 3. Percent Crude Fiber of Cassava Root Mash as Affected by Organic Supplements and Microorganisms

MICROORGANISMS	ORGANIC SUPPLEMENTS (%)		MEAN (%)
	Soy Hull	Rice Bran	
None	1.72 e	1.72 e	1.72 b
Fungi (*A. foetidus*)	3.60 b	8.62 c	6.11 a
Yeast (*S. cerevisiae*)	5.73 a	4.10 d	4.87 a
Microalgae (*S. platensis*)	2.47 d	9.51 b	5.99 a
Bacteria (*Lactobacillus* sp.)	2.54 c	10.58 a	6.41 a
Mean	3.21 a	6.83 b	

Legend: Means followed by a common letter are not significantly different at 1% level of DMRT (*http://www.knowledgebank.irri.org*).

The ash content of cassava root mash was highest after fermentation by *Lactobacillus* sp. The same observation was noted when soy hull and rice bran were used as supplements to cassava. This is because according to Belay (1997), *Lactobacilli* are fermentative and their growth and cell multiplication is very fast.

Moisture Content

Table 5 shows that the moisture content of substrate was not affected by the organic supplements. The moisture content of rice bran is 10-15% (*http://www.knowledgebank.irri.org*) whereas the soy hull is close to 13% maximum (*http://www.allgreeen.biz*).

In using both soy hull and rice bran as supplements to cassava root mash, fungi (*A. foetidus*) registered the highest percent of moisture. This demonstrates that among the selected microorganisms, *Aspergillus foetidus* (fungi) is the best in increasing the moisture content of cassava. A study conducted by Hutagalong (*www.unu.edu.com*) revealed that fungi became the dominant microorganisms in a moist solid fermentation because they thrive in a medium where moisture is high. Further, he added that moist is a favorable condition for the microorganisms to grow rapidly.

Conclusions

The microalgae, *Spirulina platensis* yields a high amount of single cell protein when cultivated in an alkaline freshwater medium at pH 10 and in saline medium at 5-10% salt (NaCl) concentration. Rice bran is significantly better than soy hull as an organic supplement in mounting the crude fat, crude fiber and ash content of cassava root while both supplements can be used in enriching the crude protein and moisture content of cassava root mash. *Saccharomyces cerevisiae* is the most efficient in enriching the crude protein, fat, and fiber content of cassava root mash while *Lactobacillus* sp. in ash content although it is also equally best in fat content with yeast. *A. foetidus* was the best in increasing the moisture content of cassava. Among the selected microorganisms used, *Saccharomyces cerevisiae* (yeast) performs best in increasing the crude protein, crude fat and crude fiber content of cassava -soy hull substrate while *Lactobacillus* sp. (bacteria) in ash content and *Aspergillus foetidus* (fungi) in the moisture content enrichment. In contrary, *Lactobacillus* sp. (bacteria) performs best in mounting the crude protein, crude fat, crude fiber and ash content of cassava-rice bran substrate while *A. foetidus* (fungi) in the moisture content.

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Table 4. Percent ash content of cassava root mash as affected by organic supplements and microorganisms

MICROORGANISMS	ORGANIC SUPPLEMENTS (%)		MEAN (%)
	Soy Hull	**Rice Bran**	
None	2.31 b	2.31 d	2.31 b
Fungi (*A. foetidus*)	1.61 d	5.25 b	3.43 b
Yeast (*S. cerevisiae*)	2.23 b	4.93 c	3.58 b
Microalgae (*S. platensis*)	1.90 c	5.04 bc	3.47 b
Bacteria (*Lactobacillus* sp.)	4.94 a	5.78 a	5.36 a
Mean	2.60 b	4.66 a	

Legend: Means followed by a common letter are not significantly different at 1% level of DMRT

Table 5. Percent moisture content of cassava root mash as affected by organic supplements and microorganisms.

MICROORGANISMS	ORGANIC SUPPLEMENTS (%)		MEAN (%)
	Soy Hull	**Rice Bran**	
None	3.73 d	3.73 e	3.73 c
Fungi (*A. foetidus*)	8.28 a	10.31 a	9.30 a
Yeast (*S. cerevisiae*)	4.65 c	6.05 c	5.35 b
Microalgae (*S. platensis*)	3.54 e	7.87 b	5.70 b
Bacteria (*Lactobacillus* sp.)	6.01 b	5.07 d	5.54 b
Mean	**5.24 a**	**6.61 a**	

Legend: Means followed by a common letter are not significantly different at 1% level of DMRT

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