Effect of Fermentation on Nutrient and Anti-nutrient Composition of Rice (*Orisa sativa*) and Mucuna (*Mucuna pruriens*) Blend Flours.

Anthony Ojokoh, Ph.D.¹; Babatunde Bello, Ph.D.²; and Fiy infloluwa Adegb enle, PGD¹

¹ Department of Microbiology, Federal University of Technology, PMB 704, Akure, Nigeria.
² Department of Chemical Sciences, Ondo State University of Science and Technology, Okitipupa, Nigeria.

E-mail: tunlapa2k3@yahoo.com

ABSTRACT

In recent years there has been a shift in the focus of biotechnological progress to find new approaches in food fermentation and develop multifunctional microorganisms to improve the nutritional and health benefit of food. In the present study, rice-mucuna blends of different formulation, (100:0, 100:0, 90:10, 80:20, 70:30, 60:40, and 50:50) respectively, were subjected to solid state fermentation for 72 hours using pure strain of *lactobacillus plantarum* and investigated the effect of fermentation on the pH, titratable acidity, and the proximate and anti-nutrient content of the blend.

We observed that the pH decreased with fermentation period while increase in the titratable acidity was noted after fermentation. The percentage protein, fat, and moisture content increased with fermentation period while carbohydrate and crude fiber decreased after fermentation. The anti-nutrient composition showed a decreased in the value with fermentation. Crude fiber recorded the lowest percentage of all the proximate content in the samples, the value ranged from 3.00±0.00 to 2.65±0.00 while carbohydrate recorded the highest value which ranged from 45.29±0.01 to 43.74±0.14. We hypothesized from our findings that prolong fermentation of rice-mucuna blends for 72 hours can increase the nutritional composition and reduce the anti-nutritional composition of the sample. However, the result obtained from this study revealed that using a pure strain of lactobacillus coupled with fermentation can enhance the nutritional benefits of rice-mucuna blends when fermented.

(Keywords: fermentation, rice, mucuna, lactobacillus, proximate, antinutrient)

INTRODUCTION

Rice is one of the world’s most important food crops. It is a grain, like wheat and corn. Rice is the seed of the grass species *Oryza sativa* (Asian rice) or *Oryza glaberrima* (African rice). As a cereal grain, it is the most widely consumed staple food for a large part of the world’s human population, especially in Asia.

Rice is the most important grain with regard to human nutrition and caloric intake, providing more than one fifth of the calories consumed worldwide by humans (Smith and Bruce, 1998). Genetic evidence has shown that rice originates from a single domestication 8,200–13,500 years ago Molina et al., 2011. Rice has been genetically modified by Ventria Bioscience to express lactoferrin, lysozyme which are proteins usually found in breast milk, and human serum albumin,

These proteins have antiviral, antibacterial, and antifungal effects. Rice containing these added proteins can be used as a component in oral rehydration solutions which are used to treat diarrheal diseases, thereby shortening their duration and reducing recurrence. Such supplements may also help reverse anemia.

*Mucuna pruriens*

*Mucuna pruriens* is a tropical legume known as velvet bean and by other common names, native to Africa and Asia and widely naturalized. The plant is notorious for the extreme itchiness it produces on contact, particularly with the young foliage and the seed pods. It has value in agricultural and horticultural use and has a range of medicinal properties. The chemical compounds responsible for the itch are a protein, mucunain and serotonin. Reddy, et al.
The seeds are shiny black or brown seeds. The dry weight of the seeds is 55 to 85 g/100 seeds.

Nutritional importance of *Mucuna* seeds as a rich source of protein supplement in food and feed has been well documented (Siddhuraju et al., 2000, Siddhuraju and Becker 2001a, Bressani 2002). *Mucuna* seeds constitute excellent raw material for indigenous Ayurvedic drugs and medicines due to the presence of 3, 4-dihydroxy-L-phenylalanine (L-DOPA), which provides symptomatic relief in Parkinson’s disease (Shaw and Bera 1993, Prakash and Tewari 1999). Standley and Steyermark (1946) have reported the use of one of *Mucuna* species as dye (*Mucunaargyrophylla*Standl.).

*Mucuna* is also being extensively used as cover crop, mulch and to control weeds in agriculture. *Mucunapruniens* contains L-DOPA, a precurser to the neurotransmitter dopamine and formulations of the seed powder have been studied for the management and treatment of Parkinson’s disease (Katzenschlager, et al., 2004, Lieu et al., 2010, and Manyam, 2004).

Fermented foods are those foods which have been subjected to the action of micro-organisms or enzymes so that desirable biochemical changes cause significant modification to the food. However, to the microbiologist, the term ‘fermentation’ describes a form of energy-yielding microbial metabolism in which an organic substrate, usually a carbohydrate, is completely oxidised, and an organic carbohydrate acts as an electron acceptor (Adams, 1990).

The primary benefit of fermentation is the conversion of sugars and other carbohydrates into preservative organic acids, e.g. converting juice into wine, grains into beer, carbohydrates into carbon dioxide to leaven bread, and sugars in vegetables. Food fermentation has been said to serve six main purposes (Steinkraus, 1995).

Enrichment of the diet through development of a diversity of flavors, aromas, and textures in food substrates, Preservation of substantial amounts of food through lactic acid, alcohol, acetic acid, and alkaline fermentations, Biological enrichment of food substrates with protein, essential amino acids, and vitamins, Elimination of antinutrients, A decrease in cooking time and fuel requirement, Enhancement of digestibility of foods (FAO, 1998). Owing to the fact that fermented foods play an important role in providing food security, enhancing livelihoods, improved food preservation, increasing the range of raw materials that can be used to produce edible food products and removing anti-nutritional factors to make food safe to eat and improving the nutrition and social well-being of millions of people around the world. These facts has stimulated our strong interest to elucidating the effect of fermentation on the chemical changes (pH, Titrtatable acidity, proximate and anti-nutrient composition) of rice-mucuna beans blends using pure strains of *Lactobacillus plantarum*.

**MATERIALS AND METHODS**

**Source of Samples**

Dry rice sample was obtained from a local market in Akure metropolis Ondo State, Nigeria while dry mucuna beans was obtained from International Institute of Tropical Agriculture (IITA) Ibadan Akinyele Local Government Area of Nigeria and was transported in a polythene bag directly into the laboratory for analysis.

**Preparation of Samples**

The procedure for the treatment of the rice and the mucuna beans was as previously described by (Wakil et al., 2008). The method involved drying and dry-milling of rice and removal of extraneous matter, soaking in water, de-hulling, drying and dry-milling of mucuna beans.

**Formulation of Samples**

The rice-mucuna beans blends were formulated in ratios 100:0, 100:0, 90:10, 80:20, 70:30,60:40 and 50:50 (Malleshi et al., 1989) and were labeled and coded appropriately to avoid mixed up of samples as follows R, M, RcM1, RcM2, RcM3, RcM4, and RcM5.

**Fermentation of Samples**

Each sample was fermented in a transparent, sterile container using pure strains of *Lactobacillus plantarum* for 72 hours at room temperature and chemical analysis (pH,
titratable acidity, proximate analysis and anti-nutrient analysis) were carried out on the raw and fermented samples.

**pH and Titratable Acidity**

The pH was measured by the method of AOAC (1996). The samples were thoroughly stirred to achieve uniformity before the pH was measured. The pH electrode was dipped in each of the sample and measurements were taken using a HANNA pH Meter 209.

Total Titratable Acidity (TTA) analysis was done using AOAC (1996) method. Approximately 10 mL of sample was pipetted into a conical flask and 2 drops of phenolphthalein indicator added. Titration was done using 0.1M NaOH to a faint pink color for at least 1 min compared against a white background. The titre volume was noted and used to calculate TTA which was expressed as percentage Lactic Acid.

\[
\text{TTA was determined and expressed as follows:} \\
\% \text{Lactic acid} = \frac{A \times 0.009}{V} \times 100 \\
\text{where } A = \text{mL of 0.1NaOH required for the titration;} \quad \text{and } V = \text{mL of sample taken for the test.}
\]

The acidity was calculated as lactic acid using the Relationship:

\[
\text{Volume of Base used} \times \text{Normality of NaOH(N)} \times 9
\]

**Volume of Sample used (average titre)**

**Proximate Analysis**: Proximate composition, such as, ash, fiber, fat, protein, moisture were determined by Nout et al., 1989 method. Carbohydrate content was determined by subtracting from 100 the sum of the percentage moisture, ash, protein, fat and fibre. The remainder value gives the carbohydrate content of the sample. \%

\[
\text{Carbohydrate} = 100 - (\% \text{Moisture} + \% \text{Ash} + \% \text{Fat} + \% \text{Protein} + \% \text{Fibre})
\]

**Antinutrient Analysis of the Sample**

**Determination of Phyrate**: The method of Young and Greaves (1940) was employed for phytin determination. 4g of finely ground samples were soaked in 100cm³ of 2%HCL for 3 hours and filtered. 25cm³ of the filtrate was placed in a 100cm³ conical flask and 5cm³ of 0.03%NH₄SCN solution was then added as indicator. 50cm³ of distilled water was then added to give it the proper acidity. This was titrated against ferric chloride solution which contained about 0.005mg of Fe³⁺ per cm³ of FeCl₃ used, the equivalent was obtained and from this, the phytate content in mg/100g was calculated.

\[
\text{Iron equivalent} = \text{titre value} \times 1.95 \times 1.19 = \text{phytin P}
\]

\[
\text{Phytic acid} = \text{titre value} \times 1.95 \times 1.19 \times 3.55 \text{mg/ phytic acid}
\]

\[
\text{Where } \alpha = \text{titre value}
\]

**Determination of Tannin**

A 0.2g of finely ground sample was weighed into a 50ml sample bottle. 10ml of 70% aqueous acetone was added and properly covered. The bottles were put in an ice bath shaker and shaken for 2 hours at 30°C. each solution was then centrifuged and the supernatant stored in ice. 0.2cm³ of each solution was pipetted into test tubes and 0.8cm³ of distilled water was added.

Standard tannic acid solutions were prepared from a 0.5mg/ml and the solution made up to 1ml with distilled water. 0.5cm³ folinciocalteau reagent was added to both sample and standard followed by 2.5ml of 20%Na₂CO₃. The solutions were then vortexed and allowed to incubate for 40 minutes at room temperature, its absorbance was read at 725nm against a reagent blank concentration of the samples from which a standard tannic acid curve was prepared (Makker and Goodchild, 1996).

**Determination of Oxalate**

A 1g of the sample was weighed into 100ml conical flask. 75cm³ of 3M H₂SO₄ was added and the solution was carefully stirred intermittently with magnetic stirrer for about 1 hour and then filtered using Whatman No. 1 filter paper. A 25cm³ of sample filtrate (extract) was collected and titrated hot (80-90°C) against 0.1NKMnO₄ solution to the point when a faint pink color appeared for at least 30 seconds.
Mg/g Oxalate = Vt x 0.9004, Where t = titre value. (Day and Underwood, 1986).

**Statistical Analysis**

Values were recorded in triplicates and statistical analysis was carried out using analysis of variance (ANOVA) and DUACAN’S Multiple Range Test for estimation of means. The “t” value was tested at 95% confidence interval.

**RESULTS**

**Changes in pH**

We investigated the changes in pH of all the samples to detect the effect of fermentation on the pH of the fermented samples. The pH value of all the unfermented samples were basic (6.4, 4.39, 5.20, 5.01, 4.81, 4.65, 4.41) for samples R, M, RcM1, RcM2, RcM3, RcM4, and RcM5 respectively, but became acidic 2.84, 3.17, 3.16, 3.06, 2.76, 2.47, 2.20 after fermentation (Figure 1).

**Changes in Titratable Acidity**

The changes in titratable acidity of all the samples are shown in Figure 2. At 72 hours of fermentation the value of titratable acidity in (g/100 lactic acid) for the entire samples were statistically significant and higher (P≤0.05) compared to the raw samples.

**Changes in Moisture Content**

The fermented samples recorded higher moisture content (14.03±0.35, 14.89±0.00, 13.35±0.58, 14.12±0.05, 14.14±0.06, 14.09±0.09, 14.16±0.20%) compared to the raw samples with moisture content of (12.10±0.35, 12.98±0.04, 11.42±0.58, 12.19±0.07, 12.21±0.06, 12.22±0.06, 12.54±0.20% (P≤0.05) for samples R, M, RcM1, RcM2, RcM3, RcM4 and RcM5, respectively (Figure 3).

**Changes in Crude Protein Content**

To further investigate the effect of fermentation on the proximate composition of rice-mucuna beans blend, we analyze the protein content of both fermented and unfermented samples. The fermented sample with equal quantity of rice and mucuna beans (sample RcM5) recorded the highest protein content of 28.23±0.12% and is closely followed by that of sample RcM4 (which contain Rice flour (60g) + Mucuna flour (40g)) (Figure 3).

**Changes in Crude Fat Content**

We further analyzed the crude fat content of the fermented and unfermented samples. The crude fat content for all the fermented samples were higher and statistically significant (5.15±4.49, 2.88±0.17, 3.71±0.36, 5.12±0.12, 5.88±0.25, 9.74±0.31, 11.34±0.01%) compared to the raw samples which recorded 6.67±0.58, 1.66±0.17, 2.49±0.36, 3.89±0.12, 4.66±0.25, 8.52±0.31, 10.52±0.31% for sample R, M, RcM1, RcM2, RcM3, RcM4 and RcM5, respectively (Figure 3).

**Changes in Ash Content**

The ash content obtained for all the fermented samples were 4.45±0.29, 2.71±0.15, 3.97±0.03, 2.65±0.24, 2.67±0.24, 3.01±0.01 and 4.04±0.02% for sample R, M, RcM1, RcM2, RcM3, RcM4 and RcM5 respectively, while the raw unfermented samples recorded 4.43±0.03, 2.69±0.15, 3.97±0.29, 2.65±0.24, 2.65±0.24, 3.00±0.06 and 4.02±0.17%. (P≤0.05) for sample R, M, RcM1, RcM2, RcM3, RcM4 and RcM5, respectively (Figure 3).

**Changes in Crude Fiber Content**

Higher value were obtained from the raw samples (3.00±0.00, 1.00±0.00, 1.87±0.23, 1.87±0.12, 1.84±0.01, 2.10±0.75, and 3.61±0.12%) compared to the fermented samples with crude fiber content of (2.65±0.00, 0.65±0.00, 1.52±0.03, 1.52±0.02, 1.49±0.06, 1.75±0.08 and 3.26±0.01%) (Figure 3).

**Changes in Nitrogen Free Extractive (Carbohydrate)**

The carbohydrate content of the unfermented rice-mucuna blends was higher than the fermented samples the values are (45.29±1.29, 70.94±0.47, 60.11±0.53, 59.76±0.94,
56.07±0.06, 0.59±0.55, and 44.41±0.03\%)
(Figure 3).

We elucidated the effect of fermentation on the antinutritional content of rice-mucuna blend as shown in Figure 4. All the antinutritional factors analyzed; phytate, oxalate and tannin decrease significantly with fermentation.

Table 1: The Samples, Sample Code and Sample Ratio.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Sample Code</th>
<th>Rice (g)</th>
<th>Mucuna (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>R</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>RcM1</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>RcM2</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>RcM3</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
<td>RcM4</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>7</td>
<td>RcM5</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

Figure 1: pH Variation During Fermentation of Rice-Mucuna Blends.

Keys
R- Rice flour (100g) + Mucuna flour (0g)
M- Mucuna flour (100g) + Rice flour (0g)

RcM1- Rice flour (90g) + Mucuna flour (10g)
RcM2- Rice flour (80g) + Mucuna flour (20g)
RcM3- Rice flour (70g) + Mucuna flour (30g)
RcM4- Rice flour (60g) + Mucuna flour (40g)
RcM5- Rice flour (50) + Mucuna flour (50g)
**Figure 2:** Changes in Titratable Acidity During Fermentation of Rice-Mucuna Blends.

Keys

- **R:** Rice flour (100g) + Mucuna flour (0g)
- **M:** Mucuna flour (100g) + Rice flour (0g)
- **RcM1:** Rice flour (90g) + Mucuna flour (10g)
- **RcM2:** Rice flour (80g) + Mucuna flour (20g)
- **RcM3:** Rice flour (70g) + Mucuna flour (30g)
- **RcM4:** Rice flour (60g) + Mucuna flour (40g)
- **RcM5:** Rice flour (50) + Mucuna flour (50g)

**Figure 3:** Changes in Mean % Proximate Composition of Rice-Mucuna Blend Before and After Fermentation.

Keys

- **Rice:** Rice flour (100g) + Mucuna flour (0g)
- **Mucuna:** Mucuna flour (100g) + Rice flour (0g)
- **90:10:** Rice flour (90g) + Mucuna flour (10g)
- **80:20:** Rice flour (80g) + Mucuna flour (20g)
- **70:30:** Rice flour (70g) + Mucuna flour (30g)
- **60:40:** Rice flour (60g) + Mucuna flour (40g)
- **50:50:** Rice flour (50) + Mucuna flour (50g)
We further analyzed the phytate level of the rice-mucuna blend to know if fermentation can be used to reduce its level as phytate is known to be present naturally in many cereals and legumes and when above a certain level, phytates prevent bioavailability of essential minerals. The phytate content of fermented samples reduced significantly (P≤0.05) compared to the raw unfermented samples. The values ranged from 2.30 to 8.0 while the unfermented samples ranged from 2.5 to 10.00. Oxalate content reduced significantly within the fermentation period (P≤0.05) from 1.2 to 0.6.

The fermented samples recorded lower values of oxalate compared to the raw samples. Tannin content of fermented rice-mucuna blend showed a decreasing trend within 0-72 hour fermentation period. The values ranged from 2.8 to 12.0 for unfermented sample while the fermented samples reduced from 12.00 to 2.6 (Figure 4).

**DISCUSSION**

Fermented foods play an important role in providing food security, enhancing livelihoods and improving the nutrition and social well-being of millions of people around the world. Fermentation leads to improved food preservation and removed anti-nutritional factors to make food safe to eat. The results of this research revealed that fermentation had reduced the pH and with a corresponding increase in titratable acidity of the fermented formulated blends.
The observed increased in titratable acidity and reduced pH could be attributable to the dominance of the environment by lactic acid bacteria which degrade carbohydrates resulting in acidification. These observations are in agreement with earlier studies by Nout et al. (1989).

Since pH is the measure of acidity/alkalinity of a medium, one would think that as its value tends towards 1, the value of the titratable acidity would also decrease, practically, this is not so as the two parameters have no direct relationship connecting them. When measuring titratable acidity, sodium hydroxide (NaOH) is added to the sample, which deals with the free hydrogen, those responsible for the pH. But as one continues to add more sodium hydroxide (NaOH), one actually starts to unhook bound hydrogen and make them free. Only when sufficient sodium hydroxide (NaOH) has been added to unhook all accessible hydrogen is the measurement of titratable acidity complete.

Our finding reveals increase in protein content after fermentation. The increase could also be due to the activities of microorganisms during fermentation, which results in extensive breakdown of protein molecules to amino acid and other simple peptides. The increase could also be attributable to the enzymatic breakdown of some protein inhibitors during fermentation. Structural proteins are known to be an integral part of the microbial cell Tortora et al., (2002).

The increase is in line with the findings of Michodjehoun et al. (2005) who reported increase in protein content of millet during fermentation of millet and the findings of Bello and Ojokoh, (2013) in their work on millet and soyabean blend. The increase in fat content observed after fermentation could be as a result of extensive break down of fat molecule to fatty acid and glycerol due to the activity of the lipolytic enzymes released by microorganisms involved in fermentation. This is in agreement with the findings of Igbabul et al., 2014.

Our findings reveal increase in ash content after fermentation. Ash content is a measure of the total amount of minerals present within a food sample, an increase in its level during microbial fermentation could be as a result of incomplete utilization of minerals by fermenting organisms during their metabolism. The decrease in crude fiber observed in this research may be attributable to the breaking down of carbohydrates by fermenting microorganisms which are used as carbon source and converted to microbial biomass, resulting in the reduction in the fiber content of the sample Rainbault (2001). The finding is also in agreement with the work of Bello and Ojoko, 2013 who reported decrease in crude fiber of fermented water yam.

We analyzed the carbohydrate content (given as nitrogen free extract) levels of all the fermented rice-mucuna blends. Our findings revealed decrease in carbohydrate after fermentation. This decrease may be attributable to utilization of carbohydrate as energy source by fermenting microorganism, the carbohydrate are usually breakdown to glucose and then use as source of carbon and energy for microbial growth. Oboh and Akindahunsi (2003) also reported decrease in carbohydrate content during fermentation of starchy substances.

Anti-nutrients such as phytate, oxalate, and tannin are compounds which affect the nutritive value of food products, before fermentation the composition of these anti-nutrients content were higher in all the samples but after fermentation, the anti-nutrients content were greatly reduced. The reduction in the antinutrient content in the fermented samples compared to unfermented samples could be as a result of microbial activities.

Phytate is naturally present in many foods especially cereals and legumes. When above a certain level, phytates reduce the availability of minerals and solubility, functionality and digestibility of proteins (Akubo and Badifu, 2004). We detected decrease in phytate level after fermentation which may be due to the activity of the endogenous phytase enzyme within the sample and inherent microorganisms which are capable of hydrolyzing the phytic acid in the fermented food sample into inositol and orthophosphate (Reddy and Peirson, 1994; Sandberg and Andlid. 2002). Decrease in phytate content of cocoyam tubers has also been reported by Marfo and Oke (1988), Marfo et al. (1990).

The reduction in tannin contents observed in our findings might be attributable to the activities of microorganism involved in fermentation. Our results corroborated the findings of Murwan et al., 2011 who reported decrease in tannin content in two sorghum cultivars (Dabar and Tabar) after fermentation with the findings of Igbabul et al., (2011).
fermentation. Our findings revealed decrease in oxalate level after fermentation. It is known that oxalate forms insoluble complex with some essential mineral preventing their bioavailability, and it is often anticipated that oxalate containing foods when consumed may interfere with calcium metabolism. Similar result which revealed decrease in oxalate contents of fermented Bambara Nut were reported in the findings of Otunola and Oyelade, 2015.

CONCLUSION

Considerable challenges remain despite the significant innovations that have been made to improve the nutritional composition of food. The present study revealed that the nutritional benefit of rice-mucuna blends can be enhanced when fermented. Fermentation as shown from this research work has been able to improve the nutrient contents with corresponding decrease in anti-nutrient content, therefore, fermentation holds promise as a food processing method that can be used to enhance the nutritional benefit of food.

REFERENCES


**SUGGESTED CITATION**