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Effect of Fermentation with *R. oligosporus* and *R. stolonifer* on Some Physicochemical Properties of Starch Extracts from Dehulled and Undehulled Velvet Bean (*Mucuna utilis*) Seeds

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ABSTRACT

Velvet bean is an underutilized legume with high protein source but inadequate information about its nutritional qualities and it is a major factor in the poor utilization of these local crops in production of different foods. But it was known that germination can lead to the development of such functional foods that have a positive effect on the human organism and that help in maintaining the health, hence studies were carried out on the changes that occurred on selected physico-chemical properties of starch extracts from dehulled and undehulled velvet bean flour when fermented with *Rhizopus oligosporus* and *Rhizopus stolonifer* from a period of between 0 and 72 h. Some physicochemical properties such as total sugar, pH, titratable acidity, amylose and amylopectin of the starch extracts were determined using standard methods. The results showed that sugar contents reduced significantly in all the samples as fermentation progressed. Total starch (17.58-42.10 mg); amylose (15.41-28.52%); total sugar (1.74-2.64%) dehulled velvet bean was higher than undehulled velvet bean with total starch (16.5-41.35 mg); amylose (15.35-27.95%); total sugar (1.64-2.57%) but lower amylopectin (15.35-27.95%); pH (2.85-5.05) and Titratable Acidity (TTA) (4.13-5.65). The proportion of amylopectin revealed a marginal increase with fermentation as a result of the decrease in the proportion of amylose present which may be due to the *Rhizopus* spp. used for the fermentation process.

Key words: Velvet bean, *Rhizopus oligosporus, Rhizopus stolonifer,* fermentation, starch, physicochemical

INTRODUCTION

Large segments of the population in the developing countries suffer from protein malnutrition. Projections based on current trends indicate a gap between human population and protein supply. Hence research efforts are being directed to this area to identify and evaluate underexploited sources as alternative protein crops for the world of tomorrow[1]. In this regard, various studies were being carried out to assess the potential of indigenous legumes that are not widely used as food, as a dietary source of protein, as well as a genetic resource for the improvement of traditional legume crops.

Mucuna utilis (velvet bean), a tropical legume, is little known and has a low human preference for food, but has a high potential as an energy/protein source in livestock feed[2]. It was comparable to soybean in terms of amino acid and mineral profile[3,4]. The consumption of velvet bean by human as a source of protein foods has been reported by researchers to be hindered by the presence of antinutritional factors such as hydrocyanic acid, tannins, trypsin inhibitors, haemagglutinin and phytic acids[3,5]. Heat treatment was frequently used to man to improve the utilization of the nutrients in legumes. The use of fermentation to transform agricultural produce into several physical and chemical states has been used to bring about several changes through agro-processing operation. This was due to the fact that fermentation has become an appropriate technique used by developing countries and rural areas where they have limited access to this expensive equipment[3]. Fermentation process in food processing has been reported to have several inherent benefits[6], however, the operations has also been reported to have the ability to modify the functional properties of starch present in velvet bean. Also, functional properties of starches have several applications replacing other plants of microbial polymers at high cost.

Such starches are modified in a number of ways as reported by Campbell-Platt[7]. The application of starch in food processing has been reported to be affected by its swelling property which is required in baking of bread; emulsifying power and water binding capacity which is also required in salad dressing and baking power respectively[8]. Other properties of starch that is of great importance in their food applications in food industry include gelatinization, pasting, solubility, swelling, clarity or opacity and viscosity[9]. This study therefore aims to evaluate the effect of fermentation with *Rhizopus* species on some selected physicochemical characteristics of starch extracted from velvet bean seeds.

MATERIALS AND METHODS

Materials: The samples type of black coloured seed coat velvet bean (*Mucuna pruriens* var. *utilis*) was used for the study and was purchased by International Institute for Tropical Agriculture, IITA, Ibadan, Nigeria. After thoroughly drying in the sun the pods were thrashed to remove seeds. The seeds, after thorough cleaning and removal of broken seeds, foreign materials and immature seeds were stored in airtight plastic jars at room temperature (25°C). The *R. oligosporus* and *R. stolonifer* used for the study were supplied by the Indonesian Embassy, Lagos, Nigeria. All chemicals used for the experiment were of the analytical grade.

Preparation of subculture for velvet bean fermentation: The modified method of Siddhuraju *et al.*[10] was adopted for the production of *Rhizopus oligosprus* and *Rhizopus stolonifer* subcultures for velvet bean fermentation. About 50 mL of distilled water was added to 50 g rice in a beaker and was covered with a sterile muslin cloth firmly tied with a twine and the contents was heated in an autoclave at 115 psi for 15 min and later brought to 120°C. This is followed by cooling, the cooled samples were then inoculated with each innoculum of *Rhizopus oligosporus* and *Rhizopus stolonifer* and then incubated for 4 days at 30°C; dried in a sterile oven at 40°C for 48 h, pulverized in a clean sterile dry blender to obtain a uniform granule. The pulverized fermented velvet flour was then stored in sterile sealed polythene bags until ready for analysis.

Preparation of fermented velvet bean: The production of fermented velvet bean was carried out by modified method given by Del Carmen *et al.*[11]. About 500 g of velvet bean samples were cleaned, washed with tap water and then steeped in water for 12 h. The contents were then drained after fermentation and then boiled. The boiled velvet bean was then drained, dehulled and water was then added at the ratio of 1:4 w/v and then steep for another 24 h. The steeped beans were then boiled with steep water, drained and dried at room temperature. The dehulled, cooked and steeped beans were then spread on perforated polythene bag and then inoculated with *R. oligosporus* and *R. stolonifer* and mixed thoroughly and then tightly sealed. The contents were then incubated at about 32°C for periods of time ranging between 0 and 72 h with 12 h intervals. The unfermented velvet bean sample was then used as the control sample. At regular intervals of 12 h, samples were taken out for chemical analysis.

Preparation of fermented velvet bean flour: Fermented velvet bean flour was prepared by taking samples from each fermented beans at the end of fermentation periods and blanched for 20 min and then size reduced by slicing into smaller units. The sliced samples were then drained and dried in the oven at 55°C for 24 h. The dried bean samples were then cooled and then milled in a Wiley mill (Scientific Equipment, Delhi, India) to 60-mesh size to give fermented velvet bean flour. The flour was stored and sealed in polythene bags and then kept in the freezer until required for analysis.

Extraction of starch from velvet bean flour: Starch was extracted, from fermented and unfermented samples, using a modified procedure described by Siddhuraju *et al.*[10]. The 100 g of milled flour was suspended in water and passed

through No. 70 screen to remove the hulls. The suspension was centrifuged at 1170 g for 10 min while the supernatant was discarded, precipitated and re-suspended in excess 0.2% NaOH solution and centrifuged. The starch was washed twice with water (pH 7) and then washed with 30, 50, 70% ethanol respectively. The supernatant was discarded after each washing, residue was dried in an oven at about 35°C and passed through a 100-mesh sieve. The extracted starch was then weighed and the yield determined by the modified method of Schoch and Maywald[12].

Determination of physicochemical properties of extracted starch

Determination of amylose and amylopectin contents: This was carried out as described by AOAC[13]. One hundred milligram of starch extract (ground to pass through 250 µm sieve) was weighed into 100 mL volumetric flasks and 1 mL of 95% ethanol was carefully added. Nine milliliter of 1 M NaOH was also added and this was heated for 10 min in a boiling water bath to gelatinize the starch. It was then cooled and the sample made up to volume with distilled water. A 5 mL portion of the starch solution was measured into a 100 mL volumetric flask. Also, 1 mL of 1 M acetic acid and 2 mL of iodine solution were added and made up to volume with distilled water. The absorbance was determined at 620 nm after 20 min shaking. The amylose content was calculated as:

$$\text{Amylose (\%)} = F \times \text{Absorbance} \times 20\% \quad (1)$$

$$\text{where, Factor } F = \frac{\text{mg amylose}}{\text{Unit absorbance}}$$

$$\text{Amylopectin (\%)} = 100 - \text{\% amylase} \quad (2)$$

Determination of pH: The pH of the samples was determined by the method of AOAC[13]. Twenty gram of fermented and unfermented velvet bean flour to 50 mL of distilled water while stirring for 10 min. The stirring was to prevent the starch from coating the glass electrodes and to obtain reproducible results. The pH was then measured on a pH meter (Checker HI 12 08, Hanna Instrument) using buffer 4.0 and 7.0 to standardize. The determinations were in triplicates and the mean values recorded.

Determination of titratable acidity: The titratable acidity was determined by the modified method of AOAC[13]. About 18 g of fermented and unfermented velvet bean flour was weighed into a 250 mL conical flask and 200 mL if carbon dioxide-free distilled water added. The flask was allowed to stand in a water bath at 40°C for 1 h so that the flask is covered to just about the level of liquid. It was swirled occasionally to ensure complete mixing and filtered. Phenolphthalein was then added after filtration before being titrated against 0.1 M NaOH solution.

$$\text{Acidity (\%)} = \frac{\text{Vol. of 0.1M NaOH used}}{\text{Weight of the sample}} \times 0.09 \times 100 \quad (3)$$

Determination of total sugar content: The total sugar content was determined by the method of AOAC[13]. About 0.025 g each of starch extract was weighed into each of the three (3) centrifuge tubes and subsequently wetted with 1.0 mL of alcohol. Distilled water (0.2 mL) and 10 mL of hot ethanol were thereafter added and sortexed. The mixture was centrifuged for 10 min at 586 x g and the supernatant decanted into test tube before making up to 20 mL extract. Also, 0.5 mL of 5% phenol was added and sortexed before adding 2.50 mL of conc. H_2SO_4 and subsequently sortexed. The mixture was then cooled and the absorbance read at 490 mm. The appropriate color was developed using an aliquot from 0.01-1 mL of extract and adding 2.50 mL of distilled water. The % sugar was thus found. The mean value of the replicates was calculated and recorded.

$$\text{Sugar (\%)} = \frac{(A - 1) \times \text{d.f} \times v \times 100}{B \times W \times 10^6} \quad (4)$$

Where:

A = Absorbance of sample
I = Intercept of sample
d.f. = Dilution factor
V = Volume of sample
B = Slope of the standard curve
W = Weight of the sample

Statistical analysis: Data are expressed as means±standard deviation (SD) from three replicate experiments. Statistical analyses were carried out using statistical program of SPSS version 11.5 for windows (SPSS Corporation, Chicago, IL). Significant differences flour samples were analyzed by using one-way (analysis of variance) ANOVA with Duncan's post hoc test. The criterion for statistical significance was set at $p<0.05$.

RESULTS AND DISCUSSION

The studied physico-chemical properties of starch extract from velvet flours during fermentation are shown in Table 1 and 2. Fermentation generally caused a reduction in pH of all starch

Table 1: Physicochemical properties of starch extract from fermented and unfermented undehulled samples of velvet bean

Sample	Starch extract (mg)	Amylose content (%)	Amylopectin content (%)	Total sugar content (%)	pH	Titratable acidity
G	41.35a	27.95a	72.05d	2.57a	5.05a	4.13d
A	16.50d	15.35d	84.65a	1.92c	3.86b	5.03c
C	20.25b	18.90b	81.06c	1.64d	3.53bc	5.13b
E	17.99c	17.03c	82.97b	2.47b	2.85c	5.63a

Means with no common letters within a column significantly differ (p<0.05). A: Undehulled sample fermented with *R. oligosporus*, C: Undehulled sample fermented with the *R. oligosporus+R. stolonifer,* E: Undehulled sample fermented with *R. stolonifer*, G: Unfermented undehulled sample (Control)

Table 2: Physicochemical properties of starch extract from fermented and unfermented dehulled samples of velvet bean

Sample	Starch extract (mg)	Amylose content (%)	Amylopectin content (%)	Total sugar content (%)	pH	Titratable acidity
H	42.10a	28.52a	71.48d	2.64a	4.74a	3.33c
B	16.8d	15.41d	84.59a	1.99b	3.53bc	6.43a
D	20.30b	19.10b	80.90c	1.83c	3.63b	6.03b
F	17.58c	16.90c	83.10b	1.74d	4.82a	6.13b

Means with no common letters within a column significantly differ (p<0.05). B: Dehulled sample fermented with *R. oligosporus,* D: Dehulled sample fermented with *R. oligosporus+R. stolonifer,* F: Dehulled sample fermented with *R. stolonifer,* H: Unfermented dehulled sample (Control)

extracts from the fermented flours. There were significant differences at p<0.05 in the starch extracts of the dehulled samples when compared to the control. The low pH favours the growth of microorganisms such as moulds, yeasts and lactic acid bacteria. This results into the significant increases in the values of titratable acidity as observed by Otunola *et al*.[14]. Total sugar also reduced significantly from 2.57% in the undehulled control to 1.64% in the undehulled sample fermented with both organisms. It was observed that dehulled had a secondary effect on the samples as the values were slightly higher in the undehulled samples when compared with those of the dehulled samples. The amylose contents varied from 27.95-15.53% in the undehulled fermented samples, while the values were 28.52-15.41% in the dehulled samples. The amylopectin contents varied from 84.65-72.05% in the undehulled fermented samples and from 84.59-71.48% again confirming the secondary effect of dehulling on the fermented products (Fig. 1). The organisms employed for fermentation produce enzyme, amylase, which invariably breaks down the amylase as fermentation progressed. Amylopectin however presents a contrary trend. This might be due to the fact that it can not be easily be broken down by the *Rhizopus* spp. being a branched chain polymer of glucose according to the report of Charles and Guy[8].

Amylose was observed to be significantly different in values between 0-36 and 48-72 h of fermentation (Table 1 and 2). This could be due to the action of *R. oligosporus* which produces amylase as a constituent enzyme that invariably breaks down the amylose as fermentation progressed. The low granules swelling and viscosity could be due to the low amylopectin content and possible formation of amylose-lipid complexes. Amylopectin is primarily responsible for granule swelling. The observed trend in amylose and amylopectin

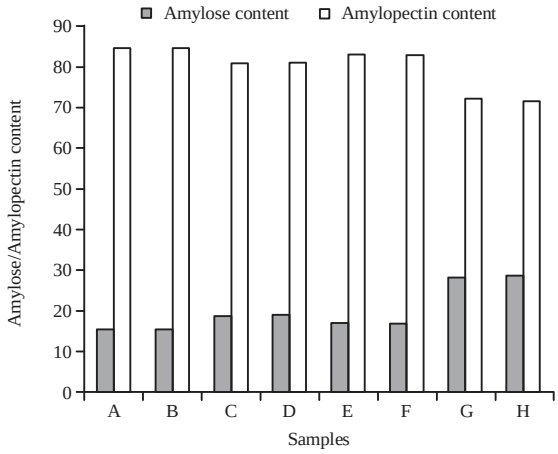

Fig. 1: Effect of fermentation on the starch contents of fermented and unfermented velvet bean flour, A: Undehulled sample fermented with *R. oligosporus,* B: Dehulled sample fermented with *R. oligosporus,* C: Undehulled sample fermented with the *R. oligosporus+R. stolonifer,* D: Dehulled sample fermented with *R. oligosporus+R. stolonifer,* E: Undehulled sample fermented with *R. stolonifer,* F: Dehulled sample fermented with *R. stolonifer,* G: Unfermented undehulled sample (Control), H: Unfermented dehulled sample (Control)

contents tend to lend to the credence assertion that granule swelling increases with increase in amylopectin probably due to prolonged fermentation of soybean to produce fermented soybean flour. However, for rice, high amylose rice varieties generally absorb more water and expand more during cooking in excess water than low amylose varieties. Higher amylose value indicate greater tendency to undergo

retrogradation in line with increased tendency of amylose to form hydrogen bonds[15]. The fermented velvet bean, depending on the period of fermentation, had variable but lower contents of amylose (2.14–26.71%) than the unfermented (27.35%).

CONCLUSION

This study reveals that the length of fermentation has a significant effect on the physico-chemical properties of velvet bean. It was also revealed that mixed (or combined) innocula, (*R. oligosporus* and *R. stolonifer*) was even more effective within the period of 0-72 h of fermentation. It was observed that fermentation for 36 h produced optimal changes, where no significant changes were observed ($p<0.05$) for the rest of fermentation period. The starch extract of the extracted starch from fermented velvet beans flour was observed to be affected by the period of fermentation with dehulled samples contributed to the significant effect of fermented when compared with the undehulled samples.

REFERENCES

1. Egbe, I.A. and I.O. Akinyele, 1990. Effect of cooking on the antinutritional factors of lima beans (*Phaseolus lunatus*). Food Chem., 35: 81-88.
2. Emenalon, O.O. and A.B.I. Udedibie, 1998. Effect of dietary raw, cooked and toasted *Mucuna pruriens* seeds (velvet bean) on the performance of finisher broiler. Niger. J. Anim. Prod., 25: 115-119.
3. Siddhuraju, P. and K. Becker, 2001. Effect of various domestic processing methods on antinutrient and *in vitro* protein and starch digestibility of two indigenous varieties of Indian tribal pulse, *Mucuna pruriens* var. *utilis*. J. Agric. Food Chem., 49: 3058-3067.
4. Iyayi, E.A. and V.O. Taiwo, 2003. The effect of diet incorporating Mucuna (*Mucuna pruriens*) seed meal on the performance of laying hens and broilers. Trop. Subtrop. Agroecosyst., 1: 239-246.
5. Udensi, E.A., O. Eke and S.N. Ukachukwu, 2001. Effect of traditional processing on the physicochemical properties of *Mucuna cochinchinensis* and *Mucuna utilis* flours. J. Agric. Food Technol. Environ., 1: 133-137.
6. FAO., 1998. A publication of food and agriculture organization of the united nations. Preventing micro-nutrient malnutrition: A guide to food-based approaches. Food Agric. Organization, Rome.
7. Campbell-Platt, G., 1980. African locust bean (Parkia Specie) and its West African fermented food product. Dawadawa Ecol. Food Nutr., 9: 123-132.
8. Charles, A. and L. Guy, 1999. Food Biochemistry. Aspen Publishers Inc., Gailtheasburg, Maryland, pp: 39-41.
9. Agboola, S.O., J.O. Akingbala and E.B. Oguntimehin, 1994. Physicochemical and functional properties of low density Cassava starch acetates and citrates, Starch/Starke, 43: 62-66.
10. Siddhuraju, P., K. Becker and H.P. Makkar, 2000. Studies on the nutritional composition and antinutritional factors of three different germplasm seed materials of an under/utilized tropical legume, *Mucuna pruriens* var. *utilis*. J. Agric. Food Chem., 48: 6048-6060.
11. Del Carmen, J., A.G. Gernat, R. Myhrman and L.B. Carew, 1999. Evaluation of raw and heated velvet beans (*Mucuna pruriens*) as feed ingredients for broilers. Poult. Sci., 78: 866-872.
12. Schoch, T.J. and E.C. Maywald, 1968. Preparation and properties of various legumes starches. Cereal Chem., 45: 564-573.
13. AOAC., 2000. Official Methods of Analysis of the Association of the Official Analytical Chemists. 17th Edn., AOAC, Artington, Virginia.
14. Otunola, E.T., O. Apoeso and I.A. Adeyemi, 1998. Development and Evaluation of Maize-Tempeh Mixes as an Instant Food Product. Biosci. Res. Commun., pp: 311-316.
15. Adeyemi, I.A., 1984. Rice production, processing and quality in Nigeria. An Extraction from Invited Lecture Delivered at the Department of Food Science and Brewing. Anambra State University of Technology Awka, Nigeria.