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Studies on some properties of starches from three *Mucuna* species

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Abstract: This study was carried out to characterize starches extracted from *Mucuna pruriens*, *Mucuna rajada* and *Mucuna veracrux* into chemical, functional and pasting properties as well as the turbidity during storage. Starches were isolated from matured seeds with the amylose content varying between 24.61 and 71.33%. The turbidity values increased progressively during refrigerated storage in all the starches isolated. The swelling power of the starches differs significantly at the same temperature of 90°C and it ranged between 5.83 – 6-68% while the solubility ranged between 7.04 – 8.13%. The water binding capacity ranged between 170 – 178% with MV having the highest water binding capacity. Pasting properties measured using RVA, also differed significantly. Pasting temperatures, peak viscosity, and final viscosity of *Mucuna* starches separated from different *Mucuna* cultivars varied between 61.73 – 62.30°C, 71.63 – 323.0 and 123.0 – 378.30cP, respectively. It was observed that there was an increase in viscosity up to the end of the cooling time and also starches from cultivars with high swelling power had higher final viscosity and pasting temperature. Increase in final viscosity might be due to the aggregation of the amylose molecules. The high setback values shown by these starches make them unsuitable for food applications where low rate of syneresis is required such as in frozen or refrigerated foods. These *Mucuna* starches can however be suitable for industries where thermo-stable paste without breaking down and with restricted swelling is required.

Keywords: ***Mucuna species,*** **Starch, chemical, functional and pasting properties**

Introduction

Legumes are the edible fruits or seeds of pod-bearing plants belonging to the family Leguminosae and are widely grown throughout the world (Singh *et al*., 2004). Legume seeds are of prime importance in human and animal nutrition due to their high protein content (20 - 50%) (Singh *et al*., 2004) and have historically been utilized mainly as the whole seeds (Saio and Monma, 1993). Recently, they are now being fractionated into their main constituents which are starch and protein. *Mucuna* bean is one of the most underutilized legumes in Africa and the percentage of protein present ranges from 33.2 – 38.4% while the starch content ranges from 36.8 - 46.0% (Adebowale *et al*., 2005). Outside Africa, the seeds are eaten by Indian tribal sects, Mundari and Dravidian groups but are less exploited in Africa. *Mucuna pruriens* was used in Native American milpa agriculture and popular as green manure in the Southern USA before it was replaced by soybean in the mid-late 20th Century. *Mucuna* is also used as a food crop in eastern Nigeria but must be well processed before consumption. They are often cracked and removed from the seed coats, soaked for a period, and then boiled in water, roasted or fermented to remove most of the toxicity, which has been implicated in poisonings. Mature seed pods are regarded as less toxic than green pods and, along with leaves, have been boiled and eaten as vegetables (Bailey 1950; Duke, 1981).

Starch, the principal carbohydrate constituent of majority of plant materials, merits a detailed investigation to understand better its biochemical and functional characteristics as well as variations (El Faki *et al*., 1981). Starches from different sources vary, particularly in their quantitative and qualitative make up as well as in some of the physicochemical properties. Starch is considered of commercial importance due to its high industrial demand as an ingredient for a variety of processed foods (Whitaker and Tannenbaum, 1977). The growing demand for starches for the modern food industry has created interest for new sources of the polysaccharides (Singh *et al*., 2004). Applications of starch in food systems are primarily governed by gelatinization, pasting, solubility, swelling and digestibility properties. Processing causes an increase in the surface area (milling, grinding), decrease in crystallinity (loss of order), swelling of starch granules and depolymerisation of starch macro molecules, viscosity

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reduction and release of amylose and amylopectin. Functional properties such as viscosity of starch are important in the use of seed flour as an ingredient in food preparations and many of these properties are related to the physicochemical characteristics of the starch. Gelatinization leads to a significant change in the viscosity and rheological properties of the paste, which are characteristic of the type of starch. Water Absorption Index (WAI) measures the amount of water that is retained within the sample after full hydration and is a common method to measure the water binding capacity. In contrast, the Water Solubility Index (WSI) expresses the extent the soluble substances are dissolved by the surrounding medium (Wursch, 1989). Relatively, few studies on the characterization of starches have been reported. These include sword beans (Ekanayake *et al*., 2006); Radix Cynanchi bungei (Song *et al*., 2006); Black gram (Singh *et al*., 2004); Peas (Ratnayake *et al*., 2002); lima beans (Betancur *et al*., 2001) and chicken pea (Singh *et al*., 2004). However, no published work seems to have been reported on the characterization of starch from *Mucuna sp* to establish its potential application in the food industries. A systematic study of mucuna starches from different cultivars would be useful, as differences in starch properties between cultivars will affect the chemical, functional, and pasting properties of the starch and thus its suitability for end use. Hence, the objective of this work is to characterize starches separated from different *Mucuna* bean species.

Materials and Methods

Materials

Three species of mucuna seeds were obtained from IITA/ILRI, Ibadan, Nigeria. The species includes *Mucuna pruriens* IRZ, *Mucuna veracrux* black and *Mucuna rajada.*

Starch isolation

Procured seeds were cleaned to remove foreign materials and impurities. The seeds were dehulled and starch was isolated using the methods of Singh *et al.* (1989) and Song *et al*. (2006) with minor modifications. The seeds were steeped in distilled water 1:4 w/v overnight and washed. The steep water was drained off, soaked seeds were ground in an electrical blender at high speed for 15 mins. The ground slurry was screened through a nylon cloth (100 mesh). The material left over the nylon cloth was washed thoroughly with water. The filtrate slurry was allowed to stand for 1 hour. The supernatant was decanted and the settled layer was resuspended in water and centrifuged in wide-mouthed cups at 2800 rpm for 5 min. The upper non-white layer was scrapped off. The white layer was resuspended in water and recentrifuged for 2- 3 times. The starch was then collected and dried in an oven at 50^0C for 12 hours, then ground and sieved through a 400 mesh screen.

Determination of chemical properties

Mucuna starches extracted were estimated for their moisture, fat, ash and protein (%N × 6.25) content by employing standard methods of analysis (AOAC, 2000) in triplicates as highlighted below.

Moisture content- 5 g of samples was weighed into a pre-weighed clean petri-dish and placed in an oven maintained at 103^0C. After 3 hours, the dish was removed and transferred into a dessicator to cool. When cool, the weight was taken and returned into the oven for another hour; it was cooled and weighed again until constant weight.

Ash content- 5 g of the sample was weighed into an empty porcelain crucible, which had been previously ignited and weighed. This was transferred into the muffle furnace set at 600ºC and left for about 6 hours. About this time it had turned to white ash. The crucible and its content were cooled to about 100ºC in air, then room temperature in a dessicator and weighed.

Fat content was determined using the soxhlet extraction method. The extraction flask was dried in the oven to a constant weight. 4 g of each dried sample was weighed into fat free extraction thimble and pug lightly with cotton wool. The thimble was placed in the extractor and fitted up with reflux condenser and a 250 ml soxhlet flask which has been previously dried in the oven, cooled in the desiccator and weighed. The soxhlet flask is then filled to $^3/_4$ of its volume with petroleum ether (b.pt. 40º – 60ºC), and the soxhlet flask. Extractor plus condenser set was placed on the heater. The heater was put on for six hours with constant running water from the tap for condensation of ether vapour. The set was constantly watched for ether leaks and the heat source was adjusted appropriately for the ether to boil gently. The Ether was left to siphon over several times say over at least 10 – 12 times until it was short of siphoning. The thimble containing sample was then removed and dried on a clock glass on the bench top. The extractor, flask and condenser was replaced and the distillation continued until the flask was practically dry. The flask which now contains the fat or oil was detached, its exterior cleaned and dried to a constant weight in the oven.

Protein content by weighing 1 g of samples was weighed into the digestion flask and Kjeldahl catalyst

tablets were added. 20 ml of concentrated H_2SO_4 was added. The flask was fixed into the digester at 410°C for 6 hours until a clear solution is obtained, this is then cooled and the digest was transferred into 100 ml volumetric flask, and made up to mark with distillled water. The distillation apparatus was set up and it was rinsed for 10 min, after boiling 20 ml of boric acid was pipette into a conical flask, 5 drops of indicator and 75 ml of distilled water was added and 10 ml of the digest was pipette into the Kjedahl distillation flask. The conical flask and the distillation flask were fixed in place. 20 ml of 20% NaOH was added through the glass funnel into the digest. As distillation proceeded, the distillate was collected in the boric acid for 15 min until pink colour changes to green. The content of the flask was titrated with 0.05N HCl. %Protein was the calculated.

Determination of functional properties of starch

Swelling power (g/g) and Solubility (%)

Swelling power and solubility were determined using the method of Leach *et al*. (1959). A 1% aqueous suspension of starch (100ml) was heated in a water bath at 90°C for 1 hour with constant stirring. The suspension was cooled for half an hour at 30°C. Samples were then poured into preweighed centrifuge tubes, centrifuged at 30000rpm for 10 min and weight of sediments was determined. Solubility was measured by pouring into evaporating dishes and evaporated at 110°C for 12 hour and weight of dry solids was determined.

$$\text{Starch solubility (\%)} = \frac{\text{wt. of suspension (dry)} \times 100}{\text{Wt. of dry starch}}$$

$$\text{Swelling power (wt/wt)} = \frac{\text{wt. of swollen sediment}}{\text{Wt. of soluble starch}}$$

Water absorption index (WAI)

This was determined using the method described by Medcalf and Gilles (1965). A suspension of 5 g starch (dry weight) in 75 ml distilled water was agitated for 1 hour and centrifuged at 3000 rpm for 10 min and wet starch was weighed. The bound water was calculated by the formular:

$$\text{Water absorption index} = \frac{\text{bound water (g)}}{\text{Wt. of sample}}$$

Amylose content

This was determined using the method of Williams *et al.* (1970). 0.1g of starch was weighed into a 100 ml volumetric flask. 1 ml of 99.7 – 100% (v/v) ethanol and 9 ml 1N NaOH was carefully added and the mouth of the flask was covered with foil and the content was mixed very well. The samples were heated for 10 min in a boiling water bath to gelatinize the starch (timing started when boiling begins). The samples were then removed from the water bath and allowed to cool very well. It was then top to mark with distilled water. Absorbance (A) was then read using a spectrophotometer at 620nm wavelength. The blank contains 1 ml of ethanol, 9 ml of NaOH, then boiled and top up the mark with distilled water. 5 ml was then pipette into a 100 ml volumetric flask.1 ml of 1N acetic acid and 2 ml of iodine solution were added and then top up to the mark, this was used to standardize the spectrophotometer at 620nm. It was then calculated as shown below:

Calculation: Amylose content (%) = (3.06) (A) (20);

where A = absorbance value

Turbidity

This was determined using the method of Perera and Hoover (1999). A 1% aqueous suspension of starch was heated in a water bath at 90°C for 1hr with constant stirring. The suspension was cooled for 1hr at 30°C. The samples were stored for five days at 4°C in a refrigerator and turbidity was determined every 24hrs by measuring the absorbance at 640nm against a water blank with Shimadzu UV-1601 Spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The absorbance measured was interpreted as turbidity.

Pasting properties

The pasting properties of the sample were determined using a Rapid Visco Analyzer (Newport Scientific Pty Ltd, Warriewood NSW 2101, Australia). 3 g of starch was dispersed in water (25 ml) and stirred in an RVA can. A programmed heating and cooling cycle was used where the sample were held at 50°C for 1 min, heated to 95°C for 3.8min, held at 95°C for 2.5 min before being cooled to 50°C in 3.8 min and

held at 50°C for 1.4 min. Viscosities were measured in Rapid Visco Analyzer Units (RVU).

Statistical analysis

All data obtained were subjected to one way analysis of variance (ANOVA) and significant mean values at 5% were separated using Duncan's Multiple Range Test (DMRT). Pearson correlation (r) for the relationships between the investigated properties was also calculated using Statistical package for social scientists (SPSS version 16.0).

Results and Discussion

Chemical composition of starches separated from different Mucuna bean species

The chemical composition of the isolated starches is shown in Table 1. Isolation of starches from legumes is generally difficult owing to the presence of a highly hydrated fine fiber fraction, which is derived from the cell wall enclosing the starch granules (Hoover and Sosulski, 1985; Schoch and Maywald, 1968).The ash content, reflecting contamination by fine fiber, of various mucuna starches, ranged from 0.24 -0.36%. *Mucuna rajada* (MR) starch have the lowest ash content while *Mucuna veracrux* (MV) have the highest. The isolated starches of MR and MP were characterized by low fat and protein content of 0.54 and 0.15% and 0.45 and 0.36%, respectively. The low ash, fat and protein of the starches extracted from MR and MP reflect the purity of these starches but were higher than that of MV. This could be due to the granular structure of each cultivar as MV seed is black in colour, hard and it takes a very long time to take up water thus difficult to break the protein- starch matrix during the extraction process. There was no significant difference in the moisture content of the starches and it ranged between 10.84 – 11.84% with MP having the lowest and MR having the highest. The differences observed could be attributed to variety, growing conditions and physical characteristics of the seed.

Functional properties of starches

Mucuna starches have swelling power ranging between 5.83- 6.68g/g whereas solubility values were in the range of 7.04 -8.13% as shown in Table 2. MV has the highest solubility with MR having the lowest. Swelling power and solubility provide evidence of the strength of interaction between starch chains within the amorphous and crystalline domains (Ratnayake *et al*., 2002). The swelling power of starch has been reported to depend on water holding capacity of starch molecules by hydrogen bonding (Lee and Osman, 1991). Hydrogen bonds stabilizing the structure of the double helices in crystallites are broken during gelatinization and are replaced by the hydrogen bonds with water, and swelling is regulated by the crystallinity of the starch (Tester and Karkalas, 1996). Schoch and Maywald (1968) classified chickpea starches as of type C, characterized by restricted swelling, a limited solubilisation and a stability of swollen granules against mechanical shearing. They observed a restricted swelling power in the range of 16 - 20 for chickpea and three other legume starches. The swelling power of mucuna starches was found to be lower than these observations and the low swelling power of the investigated starches may be attributed to the presence of a large number of crystallites formed by the association between long amylopectin chains. Crystallite formation increases granular stability thereby reducing extent of granular swelling (Singh *et al*., 2004).

Water absorption index (WAI) of the starches from different varieties of *mucuna* ranged between 1.70 – 1.78%. WAI of raw legume starches has been reported to be less than 10g/g starch (Sathe and Salunkhe, 1981; Sathe *et al*., 1981; Desphande *et al*., 1982). WAI of legume starches is inversely related to solubility and directly related to swelling (Halbrook and Kurtzman, 1975). The WAI is a useful indication of whether flours or isolates can be incorporated into aqueous food formulations especially those involving dough handling where an increase in unit yield is desirable (Circle and Smith, 1972). It also indicates the gelling capacity of the starch and also very important in the texture of food systems. The MR had the lowest while MV had the highest. There was no significant difference between the water binding capacities of the starches. The engagement of hydroxyl groups to form hydrogen and covalent bonds between starch chains lowers the water binding capacity (Hoover and Sosulski, 1986). The difference in degree of availability of water binding sites among the starches may have also contributed to the variation in water binding capacity (Wotton and Bamunuarachchi, 1978). Low water binding is attributed to a close association of starch polymers within the native starch granule and legume starches are said to have lower water binding capacity than cereal starches (Halbrook and Kurtzman, 1975). *Mucuna* starch can contribute greatly to the textural properties of many foods and in industries as a thickener, gelling agent and bulking agent.

Table 1. Chemical composition of starches separated from different Mucuna bean cultivars

	MR	MP	MV
Fat (%)	0.54 ± 0.28[a]	0.15 ± 0.28[a]	0.92 ± 0.43[b]
Protein (%)	0.45 ± 0.13[a]	0.36 ± 0.37[b]	0.72 ± 0.06[c]
Ash (%)	0.24 ± 0.16[a]	0.35 ± 0.15[a]	0.36 ± 0.95[b]
Moisture (%)	11.34 ± 0.19[a]	10.84 ± 0.19[a]	10.99 ± 0.11[a]
Starch (%)	87.43 ± 0.02[a]	88.30 ± 0.11[a]	87.01 ± 0.04[a]

[a] Mean values followed by the same letter within a row do not differ significantly (P< 0.05), MR = *Mucuna rajada,* MP = *Mucuna pruriens,* MV = *Mucuna veracrux*

Table 2. Functional properties of starches separated from *Mucuna* bean cultivars

	MR	MP	MV
Swelling power (g/g)	5.83 ± 0.26[a]	6.68 ± 0.34[b]	5.87 ± 0.11[ab]
Solubility (%)	7.04 ± 1.25[a]	7.69 ± 1.24[a]	8.13 ± 2.21[a]
WAI (g/g)	1.78 ± 0.21[a]	1.70 ± 0.42[a]	1.76 ± 0.007[a]
Amylose (%)	71.33 ± 0.50[c]	55.30 ± 0.25[b]	24.61 ± 0.20[a]
Amylopectin (%)	28.68 ± 0.50[a]	44.71 ± 0.25[b]	75.39 ± 0.20[c]

[a] Mean values followed by the same letter within a row do not differ significantly (P< 0.05)
MR = *Mucuna rajada*, MP = *Mucuna pruriens*, MV = *Mucuna veracrux.*

Table 3. Pasting properties of starches separated from different *Mucuna* bean

	MR	MP	MV
Peak1	323 ± 13.14[c]	278 ± 3.30[b]	71.63 ± 3.36[a]
Trough1	205.30 ± 9.02[b]	197.79 ± 2.89[b]	68.96 ± 3.48[a]
Breakdown	118 ± 4.12[b]	79.96 ± 0.41[b]	2.67 ± 0.12[a]
Final visc.	374.54 ± 17.62[b]	378.30 ± 9.72[b]	123.0 ± 3.78[a]
Setback	169.25 ± 8.60[b]	180.50 ± 6.83[b]	54.04 ± 0.30[a]
Peak time	4.57 ± 0.05[a]	4.67 ± 0.00[a]	6.97 ± 0.05[b]
Pasting temp.	61.73 ± 0.04[a]	62.30 ± 0.04[a]	61.78 ± 0.64[a]

[a] Mean values followed by the same letter within a row do not differ significantly (P< 0.05); MR = *Mucuna rajada*, MP = *Mucuna pruriens*, MV = *Mucuna veracrux*

Table 4. Pearson correlation coefficient between different functional and pasting properties.

	Peak1	**Trough1**	**Finalvisc**	**Breakdown**	**Setback**	**Peaktime**	**Pastingtemp**	**Swellingpower**	**Amylose**
Peak1	1	0.993^{++}	0.983^{++}	0.987^{++}	0.968^{++}	-0.991^{++}	0.186	0.265	0.983^{++}
Trough1	0.993^{++}	1	0.998^{++}	0.961^{++}	0.991^{++}	-0.999^{++}	0.262	0.365	0.955^{++}
Final visc	0.983^{++}	0.998^{++}	1	0.942^{++}	0.998^{++}	0.997^{++}	0.291	0.421	0.935^{++}
Breakdown	0.987^{++}	0.961^{++}	0.942^{++}	1	0.917^{+}	-0.958^{++}	0.082	0.128	0.999^{++}
Setback	0.968^{++}	0.991^{++}	0.998^{++}	0.917^{+}	1	-0.991^{++}	0.321	0.479	0.908^{+}
Peak time	-0.991^{++}	-0.999^{++}	-0.997^{++}	-0.958^{++}	-0.991^{++}	1	-0.281	-0.379	-0.953^{++}
Pasting temp	0.186	0.262	0.291	0.082	0.321	-0.281	1	0.401	0.067
Swelling power	0.265	0.365	0.421	0.128	0.479	-0.379	0.401	1	0.121

$^{++}$. Correlation is significant at the 0.01 level (2-tailed); $^{+}$. Correlation is significant at the 0.05 level (2-tailed)

Mucuna starches have amylose content ranging between 24.61 – 71.33%. The amylose content was highest for MV starch and lowest for MR starch. Legume starches have been characterized by a high amylose content of 24 – 65% (Hoover and Sosulski, 1991). Non-mutant legume starches have been reported to be characterized by a higher amylose content than cereal starches, often in the range of 30–40%, for example 30% in chickpea, 31–32% in faba bean, 34% in pea and 38% in lentil (Hoover and Sosulski, 1986). Sandhya Rani and Bhattacharaya (1989) indicated that starch granules with low amylose content being less rigid, swell freely when heated. The starch granules with higher amylose content, on the other hand, being better reinforced and thus more rigid, probably swells less freely. The starches extracted from *Mucuna* beans behaved in this manner. Starch paste behavior in aqueous system depend on the physical and chemical characteristics of the starch granules, such as mean granule size, granule size distribution, amylose/amylopectin ratio and mineral content (Maden and Christensen, 1996). The amylose content of the starch varies with the botanical source of the starch and is affected by the climatic conditions and soil type during growth (Asaoka *et al.,* 1985). The activities of the enzymes involved in starch biosynthesis may be responsible for the variation in amylose content among the various starches (Kross-mann and Loyd, 2000) isolated in this work.

The turbidity values of the starch paste increased progressively during storage as shown in figure 1 with MP starch paste having the highest and MR having the lowest. The increase in turbidity during storage can be attributed to the interaction between leached amylose and amylopectin chains that led to development of function zones, which reflect or scatter a significant amount of light (Perera and Hoover, 1999). Turbidity development in starch pastes during storage have been reported to be affected by factors such as granule swelling, granule remnants, leached amylose and amylopectin, amylose and amylopectin chain lengths, intra- or intermolecular bonding, lipid and cross-linking substitution (Jacobson *et al.,* 1997). Legume starch contains varying amount of phosphate monoester derivatives which result in increased paste clarity and viscosity which may also have contributed to variation in paste turbidity (Jane *et al*., 1996) among different *Mucuna* starches.

The results of Rapid Visco Analysis of *Mucuna* starches of different cultivars are summarized in Table 3. Compared with cereal and tuber starches, information on the rheological characteristics of legume starches are limited (Singh *et al*., 2004). Most legume starches have been reported to exhibit type C (restricted swelling) viscosity pattern (Schoch and Maywald, 1968). The 'A', 'B', and 'C' pattern are thus, the different polymeric forms of the starch that differ in the packing of the amylopectin double helices (Singh *et al*., 2004). Pasting temperature of starches from different *Mucuna* bean ranged between 61.73 – 62.30°C. This high pasting temperature for *Mucuna* starches indicated their higher resistance towards swelling. Peak viscosity ranged from 71.63 – 323, with the highest value for MR and lowest value for MV starch. The increase in viscosity with temperature can be attributed to the removal of water from the exuded amylose by the granules as they swell (Ghiasi *et al.,* 1982). Final viscosity and setback values ranged from 123 – 378.30 and 54.04 – 169.25, respectively. Setback viscosity is a measure of syneresis of starch upon cooling of cooked starch pastes (Singh *et al.*, 2004). The high setback values shown by these starches make them unsuitable for food applications where low syneresis rate is required such as in frozen or refrigerated foods.

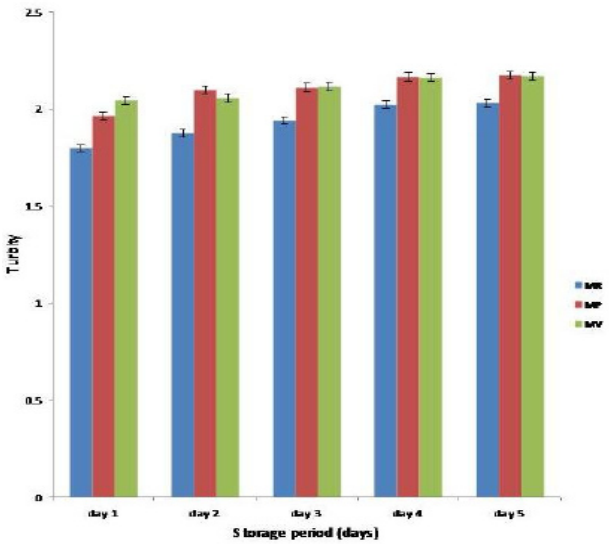

Figure 1. Effect of storage period on the turbity of starches from different Mucuna species

All the samples showed gradual increase in viscosity up to the end of cooling time. Miles *et al*. (1985) reported that increase in viscosity might be due to the aggregation of the amylose molecules. Granule swelling is accompanied by leaching of granular constituents, predominantly amylose into the external matrix resulting in a dispersion of swollen granules in a continuous matrix. MR and MP showed higher final viscosity and higher setback viscosity, indicating a higher tendency to retrograde than MV. As shown in Table 4, a positive correlation between peak 1 (r=0.983), trough 1 (r=0.955), final viscousity (r=0.935), breakdown viscousity (r=0.999)

and set back value (r=0.908). However, a negative correlation was observed between amylose content and peak time (r=-0.953).

Conclusion

Starches separated from three M*ucuna* cultivars showed significant differences in chemical, functional and pasting properties. MV starch differed significantly from other mucuna cultivars starches with respect to fat, protein, ash, amylose and swelling power. MR starch had the highest amylose content, peak and trough viscosity but had the lowest swelling power, peak time and pasting temperature. *Mucuna* starch possesses granules that are resistant to swelling, a behavior similar to other legume starches. The high setback values shown by these starches make them unsuitable for food applications where low rate of syneresis is required such as in frozen or refrigerated foods. The study showed that *Mucuna* starch is suitable for industries where thermo-stable paste without breaking down and with restricted swelling is required.

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