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Full Length Research Paper

Mucuna sloanei, Detarium microcarpum and *Brachystegia eurycoma* seeds: A preliminary study of their starch-hydrocolloids system

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Hydrocolloids from three legumes, *Mucuna sloanei, Detarium microcarpum* and *Brachystegia eurycoma* seeds together with their starches were used in this study. Proximate analysis showed that *D. microcarpum* and *M. sloanei* are rich sources of protein (27.01 ± 0.03%; 23.92 ± 0.12%, respectively), and fat (14.45 ± 0.02%; 6.57 ± 0.02%, respectively). *B. eurycoma* had lower values for protein (9.98 ± 0.3%) and also fat (2.12 ± 0.01%) but may be regarded as a richer source of carbohydrate (68.3 ± 0.33%). The DSC thermogram for the defatted *M. sloanei, D. microcarpum* and *B. eurycoma* indicated that the gelatinization temperatures ranged from 29.52 to 98.0°C; 40 to 100°C and 40.0 to 101.3°C respectively, showing a wide gelation temperature range (Tc – To) for all the samples and consequently a wide enthalpy change was observed ranging from (229.2 to 775.8 J/g). All the samples showed a single endotherm during gelation. As a preliminary study, the findings of this study revealed the pasting and thermal characteristic properties of a starch-hydrocolloid mixture of leguminous seed flour and the possible use of these mixtures in frozen food applications are apparent.

Key words: Starch, hydrocolloids, gelatinization, legumes, seed.

INTRODUCTION

Mucuna sloanei, Detarium microcarpum, and *Brachystegia eurycoma* (Plates 1, 2 and 3, respectively) are wild plants found in some parts of the semi-arid sub-Saharan and tropical zones of Africa. The seeds are edible and are used for the thickening of soups in some parts of Nigeria. Each possesses unique characteristic behaviour in hot water displaying different degree of the viscoelastic properties.

M. sloanei seed is used in the South Eastern part of Nigeria as condiment. The seeds are toasted for easy removal of the hull or par-boiled and then ground to obtain a fine powder or paste, when wet milled. The powder may be used as recipes of some food items and in beverages (Wanjekeche et al., 2003). Consumption of *Mucuna* as food has also been reported from Mozambique and Malawi (Infante et al., 1990). Mucuna gum is a galactomannan consisting of D-galactose and D-mannose as the main sugars (Srivastava and Kapoor, 2005). The endosperm was found to constitute 67.15% of the whole seed with about 32.6% as gum. It may also be a rich source of crude protein (Nwokocha and Williams, 2009), the chemical and nutritional evaluation of the raw seed of *M. sloanei*, suggested that this could be a rich source of crude protein after cooking. The galactoxyloglucan, isolated from the cotyledon consist of Glc:Xyl:Gal in a molar ratio of 1.8:1.7:1.0 and a molar mass of 1.6×10^{6} g mol^{-1} (Teixeira-Sá et al., 2009).

D. microcarpum is also used as soup thickener and has been reported to contain a high concentration of water

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Plate 1. The seeds of *Mucuna sloanei* showing the dimensions on a centimetre (cm) scale.

Plate 2. The seeds of *Detarium microcarpum* showing the variation in sizes and the dimensions on a centimetre (cm) scale.

Plate 3. The seeds of *Brachystegia eurycoma* showing the thickness and the variation in sizes on a centimetre (cm) scale.

soluble non starch polysaccharide, which is mainly xyloglucan (Wang et al., 1996). The xyloglucan has a β-(1–4) linked D-glucan backbone that is partially substituted at the O-6 position of its glucopyranosyl residues with α-D-xylopyranose. Some of the xylose residues are further substituted at the O-2 position with β-D-galactopyranose (Nishinari et al., 2000).

B. eurycoma belong to the family of Caesalpiniodeae. It is a legume from the Leguminosae family, and a fine tree that occurs in the forest from south of Nigeria to Cameroon (Keay, 1989). Previous study of *B. eurycoma* included the extraction and identification of the volatile oil components from the leaf (Ogunbinu et al., 2006). Some authors have reported on the chemical composition of the seed (Giami and Wachuku, 1997; Eddy and Udoh, 2005). Studies on the extraction of the hydrocolloids from the seeds and their viscoelastic properties have been reported (Uzomah and Ahiligwo, 1999; Onweluzo et al., 2004; Nwokocha and Williams, 2009). The flours from the cotyledon of these three legumes have not found much use in food systems like the tapioca starch which is used in numerous industrial and food applications, including thickening and gelling.

Most of the starch preparations need improvement to change the functions of the native starch and optimize the use. Processes such as modification, in combination with different additives and ingredients are commonly employed. The combinations of starch and hydrocolloids have a special importance and a wide variety of applications, and they are used to provide the control of moisture and water mobility that improve overall quality of the food systems (Shi and BeMiller, 2002; Funami et al., 2005). There are several reports on the pasting and thermal characteristics of different starches and hydrocolloids blends and these reports show that the different hydrocolloid affects the pasting properties of starch in different way (Rojas et al., 1999; Shi and Bemiller, 2002; Funami et al., 2005; Liu et al., 2006). Thermal studies on the native starch-hydrocolloid system such as may be found in the seed flours of *M. sloanei, D. microcarpum* and *B. eurycoma* are scanty, partly because more value is placed on the starch extract from them and because of the complexity of such systems. The thermal characteristics of the seed flour from these three legumes, which may reflect the synergetic associations of the different components in the seed flour, have not been reported.

This study reports the pasting and thermal characteristics of *M. sloanei, D. Microcarpum* and *B. eurycoma* seed - a preliminary study of their starch-hydrocolloids system, as observed in their characteristic behaviour when heated, using the rapid visco analyzer (RVA) and diffraction scanning calorimeter (DSC). These characteristic properties may be critical in determining the

Table 1. Proximate composition (in g/100 g db) of *M. sloanei, D. microcarpum and B. eurycoma.*

Parameter	CP	CF	Fat	MC	Ash	CHO
M. sloanei	23.92±0.12^{a}	3.18±0.011^{a}	6.57±0.02^{a}	9.17±0.01^{a}	1.95±0.05^{a}	55.19±0.15^{a}
D. microcarpum	27.01±0.03^{b}	2.76±0.01^{b}	14.45±0.02^{b}	5.17±0.015^{b}	1.4±0.1^{b}	49.21±0.11^{b}
B. eurycoma	9.98±0.3^{c}	3.26±0.12^{a}	2.12±0.01^{c}	14.36±0.01^{c}	1.98±0.07^{a}	68.3±0.33^{c}

CP = crude protein; CF = crude fibre; MC = moisture content; CHO = carbohydrate. Each value represents the mean of triplicate measurement. Mean value with different superscript are significantly different at p≤0.05.

functional properties they impart to food products and may be important in their modification and applications in some food systems.

MATERIALS AND METHODS

Samples of *M. slaonei, D. microcarpum and B. eurycoma,* were purchased from the Ekeonuwa market in Owerri, Nigeria. Each sample was dehulled (by scraping the seed coat with a stainless steel knife), without pre-processing such as roasting or par-boiling. However, *B. eurycoma* seeds were soaked in distilled water overnight to facilitate the easy removal of the hull. The dehulled seeds were milled using an attrition mill to generate fine powder to pass through 60 µm- mesh seize (British standard). A portion of the flour obtained was defatted using the soxhlet extraction method and air dried. Both the defatted and undefatted flour samples were kept separately in dry bottles and refrigerated at about 10°C for further analysis.

Proximate composition

The moisture content, ash, crude fat, crude protein and crude fibre were determined in accordance with the standard methods of the AOAC (1980). Crude fat was determined for each flour sample using the Soxhlet apparatus with anhydrous diethyl ether as the solvent. Crude protein determination was by the Kjeldhal nitrogen assay (N × 6.25). Crude fibre estimates were carried ou t by calculating the loss in weight on ignition of dried residue following the digestion of the fat free samples with 1.25% each of H_2SO_4 and NaOH solutions as described by AOAC method (1980). The carbohydrate content was determined by difference.

Least gelation concentration endpoint (LGE)

The least gelation concentration endpoints were determined following the method described by Coffman and Garcia (1977). Defatted and undefatted flour samples suspensions of 5, 10, 15, 20 and 25% were prepared in distilled water. Ten millilitre of each was placed in a test tube and heated in a boiling water bath for 1 h, followed by rapid cooling in a cold water bath. The test tubes were further cooled at 4°C for 2 h. The least gelation con centration endpoint was determined as the concentration when the sample from the inverted test tube did not slip or fall.

Pasting properties

The pasting characteristics of each sample were studied using a Rapid Visco Analyser RVA-4, (Newport scientific, NSW, Australia) following the instructions described in the manual. Three grams of each flour sample was weighed, into an RVA canister, 25 ml of distilled water was added and was inserted into the machine. A programmed heating and cooling was used where samples were held at 50°C for 1 min, heated to 95°C in 3.7 min, h eld at 95°C for 2.5 min and cooled to 50°C where it was again held f or 2 min. Parameters recorded were pasting-temperature (PT), peak viscosity (PV), hot-paste viscosity (TV), also known as viscosity at trough or minimum viscosity at 95°C, final viscosity (FV) at 50°C or cool-paste viscosity, breakdown (BD) (PV minus TV), and setback (SB).

Thermal properties

The thermal properties of the flour samples were measured using a differential scanning calorimeter (Netzsch DSC 204 F1, Phoenix). Three milligram sample was weighed into the aluminium pan with 10 µl of distilled water and hermetically sealed. The pan was kept at room temperature for 2 to 3 h. The sample was then scanned against a blank (empty pan), scanning temperature range was 20 to 250°C at a heating rate of 10°C/min. The onset (To), p eak (Tp), and final (Tc) temperatures, as well as the enthalpy (ΔH) of gelatinization of the samples were calculated by the software equipped with the instrument.

RESULTS AND DISCUSSION

Proximate composition

The results of the proximate composition of the three flour samples are presented in Table 1. The result of this study showed that *D. microcarpum* and *M. sloanei* are rich sources of protein (27.01± 0.03%; 23.92 ± 0.12%, respectively), and fat (14.45 ± 0.02%; 6.57 ± 0.02%, respectively). *B. eurycoma* had lower values for protein (9.98 ± 0.3%) and also fat (2.12 ± 0.01%) but may be regarded as a richer source of carbohydrate (68.3 ± 0.33%). The protein, fat, moisture and carbohydrate content were significantly different ($p < 0.05$).

Giami and Wachuku (1997) have earlier reported that the chemical composition with regards the crude protein of these seeds ranged from 12.2 to 23.2% while the fat varied from 4.9 to 12.0%. The protein value obtained in this study for *B. eurycoma* did not fall within this range. However, Eddy and Udoh (2005) gave a similar value for the protein composition for *B. eurycoma* (9.68%). According to Akpata and Miachi (2001), the dehulled

Table 2. The least gelation concentration endpoint (LGE) measurement of *M. sloanei, D. microcarpum and B. eurycoma.*

Concentration	*M. sloanei*		*D. microcarpum*		*B. eurycoma*	
% w/v	Undefatted	Defatted	Undefatted	Defatted	Undefatted	Defatted
5	+	-	+	+	+	-
10	++	+	++	+	++	+
15	++	+	++	++	++	++
20	++	++	++	++	++	++
25	++	++	++	++	++	++
LGE	10	20	10	15	10	15

-, viscous; +, gel; ++, firm gel.

seed flour of *D. microcarpum* contain 3.5% moisture, 3.5% ash, 2.9% crude fibre, 15% crude fat, 37.1% crude protein and 39% carbohydrate. All the samples had low crude fibre and ash content.

Ezeagu et al. (2003) reported high values for protein and carbohydrate composition of 12 *Mucuna* accessions from Nigeria, giving a range of 24.50 to 29.79% for protein; 4.72 to 7.28%, fat; 59.20 to 64.88%, carbohydrate; 3.65 to 4.43%, crude fibre and starch, 39.22 to 41.17%. Their findings showed that a fairly good percentage of the carbohydrate composition is non-starch. Crude protein content of six *Mucuna* species was also found to vary from 33.2 to 38.4%. The moisture and ash contents ranged 3.65 to 5.88% and 2.74 to 3.41%, respectively, while the carbohydrate content was 43.7 to 49.7% (Adebowale et al., 2005).

Least gelation concentration endpoint (LGE)

The least gelation concentration endpoint is regarded as an index for the gelation capacity of the flour. The result showed gelation concentration (10% w/v) for the undefatted flour samples, while the defatted samples gelled at higher concentrations of 20, 15 and 15%, for *M. sloanei, D. microcarpum* and *B. eurycoma,* respectively (Table 2). Adebowale and Adebowale (2007) in their investigation of the 6 varieties of Mucuna bean flour and starch; at pH 7.0 reported an LGE of 12.5%. Similarly, Abbey and Ayuk (1991) reported an LGE range of 16 to 20% for African yam bean which was similar to that of *Vigna unguiculata* (Osuji and Uzomah, 2007).

The gelation concentration obtained for the flours may be regarded as interplay of both the starch, non-starch carbohydrate and the protein components of these flour samples. The gelation trend of the starch is the alignment of portions of both amylose and amylopectin molecules forming crystalline micelles which are woven together and united by molecular filaments. In these flour samples, however, there are possible intermolecular associations between leached molecules and the hydrocolloid molecules which favoured the ease of gel formation (Shi and BeMiller, 2002). Since gelation is important in the food system, a low value for the least gelation concentration endpoint is regarded as a better thickening agent. Thus, the flour of these seeds and particularly the undefatted flour may serve as a better thickening material than the defatted flour.

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Pasting properties

The pasting characteristics of the flours obtained from the different seeds are presented in Table 3. The defatted flour of *M. sloanei* started pasting at 83.65°C and attained a peak viscosity of 62.33 RVU in 4.33 min. On holding at 95°C, the viscosity thinned down to 36 RVU and on cooling to 50°C it retrograded to a final viscosity of 49.67 RVU (FV). *D. microcarpum* started pasting at 83.20°C, attained a peak viscosity of 82.67 RVU in 4.53 min, and on holding at 95°C, the viscosity dropped to 64.17 RVU and retrograded to a final viscosity of 95.83 RVU on cooling to 50°C . However*, B. eurycoma* started pasting at 84.70°C, attained the peak viscosity of 32.33 RVU a t the longest pasting time of 7 min which only dropped to 30.25 RVU when held at 95°C, and retrograded to 49.67 RVU on cooling to 50°C. The pasting temperature for bot h defatted and undefatted flour samples varied little, both ranging from 83.20 to 84.70°C. In contrast to the o ther two flours *B. eurycoma* showed a stable curve and the breakdown after heating at 95°C was very low (2.08 RVU). This flour also exhibited a higher pasting temperature and stability than the other two and a lower peak viscosity (32.3 RVU), indicating that there was restricted swelling of the flour granules of *B. eurycoma* and the presence of strong binding forces within the interior of the granules. Restriction to the swelling of starch granules has been associated with high amylose content (Gerald, 1999).

Ikegwu et al. (2010) investigated the pasting properties of *B. eurycoma* flour and the starch extract from the flour using the RVA. They reported a pasting temperature of

Table 3. Pasting characteristics of the defatted and undefatted seeds of *M. sloanei, D. microcarpum* and *B. eurycoma*.

Parameter	Viscosity (RVU)						
	Peak (PV)	Holding (TV)	Final (FV)	Breakdown PV - TV	Setback FV- TV	Peak Time (min)	Pasting Temp. (°C)
M. sloanei							
Defatted	62.33	36	49.67	26.333	13.67	4.33	83.65
Undefatted	64.17	36.42	50.75	27.75	14.33	4.20	83.60
D. microcarpum							
Defatted	82.67	64.17	95.83	18.5	31.66	4.53	83.20
Undefatted	85.83	67.17	100.08	18.66	32.91	4.60	83.25
B. eurycoma							
Defatted	32.33	30.25	49.67	2.08	19.42	7.00	84.70
Undefatted	33.75	31.33	51.25	2.42	19.92	7.00	84.45

88.25 and 84.10°C; pasting time 7 and 5.52 min, respectively. Their findings showed a wide variation in the pasting characteristic properties of the flour and starch. The peak viscosity (PV) was 77.58 and 275.42 RVU; TV, 23.83 and 229.25 RVU and FV, 429.50 and 462.83, respectively.

The peak viscosity which is the maximum viscosity that is attainable during the heating cycle is also an index of the water binding capacity of the flour and suggests high swelling ability as a result of more rigid polymer structures of the granules. The relatively high peak viscosity exhibited by *D. microcarpum* flour shows that of the three samples it will be most suitable for products where high gelling strength and elasticity is required. The peak viscosity of *M. sloanei* and *D. microcarpum* though not as low as that of *B. eurycoma*, decreased markedly during the shearing and heating cycle, causing the granules to rupture followed by a drop in the viscosity after their breakdown (26.3 and 18.5 RVU, respectively).

The hot paste viscosity is an index of starch granule stability to heating and it is the minimum viscosity in the constant temperature phase of the RVA profile. While *M. sloanei* and *B. eurycoma* demonstrated a weak ability to withstand breakdown during cooling (TV; 36 and 30.25 RVU, respectively), *D. microcarpum* showed greater stability (64.17 RVU). The final viscosity is used to define the particular quality of starch and indicate the stability of the cooked paste in actual use, it also indicates the ability to form viscous paste or gel after cooling and less stability of starch paste commonly accompanied with high value for breakdown. The final viscosity (cool-paste viscosity) was very high for *D. mircrocarpum* indicating that the retrogradation or the precipitation of the linear molecule is very high. The retrogradation tendency follows this order; *D. microcarpum* > *B. eurycoma* > *M. sloanei,* and this may have been enhanced by the hydrocolloid present in these flours (Shi and BeMiller, 2002). The retrogradation phenomenon has been closely related to the amylose content (Mishra and Rai, 2006) and measured by the setback viscosity. The total setback viscosity (SB) is the difference between FV and TV and it is an indication of how starch molecules behave after heating, cooking and cooling, an index of retrogradation of the linear starch molecules during cooling. Products made from *D. microcarpum* will display highest retrogradation during cooling. The low setback in *M. sloanei* and *B. eurycoma* indicates a good potential for use in frozen foods. Similar trends were observed for the undefatted samples.

The breakdown which is a measure of disintegration of the particle structures of the starch granules at the constant heating temperature illustrating the paste stability during cooking was highest for *M. sloanei* (26.33 RVU). The lowest breakdown viscosity was recorded for *B. eurycoma* (2.08, RVU) indicating very poor capacity of the paste to withstand severe processing. *M. sloanei* thus showed a more fragile polymer structure, while *B. eurycoma* may be associated with a more orderly polymer structure and the paste stability. Low breakdown viscosity may also suggest that the starch possesses cross-linking properties (Won, 1977). It is worthy of note that the values obtained for the PV, TV and FV viscosities were lower for all the defatted flour samples.

Thermal properties

The DSC thermogram for the defatted *M. sloanei, D. microcarpum* and *B. eurycoma* are summarised in Table 4. The gelatinization temperature ranged from 29.52 to

Table 4. The gelatinization temperature and enthalpy of *M. Sloanei, D. microcarpum and B. eurycoma.*

Parameter	Gelation temperature (°C)				ΔH (J/g)
	To	Tp	Tc	Tc - To	
M. sloanei	29.52	45.45	98	68.5	229.2
D. microcarpum	40	62.5	100	60	425.4
B. eurycoma	40	60	101.3	61.3	775.8

98.0°C; 40 to 100°C and 40.0 to 101.3°C, respective ly, showing a wide gelation temperature range (Tc – To) for all the samples and consequently a wide enthalpy change was observed ranging from (229.2 to 775.8 J/g). All the samples showed a single endotherm during gelation. While *D. microcarpum* and *B. eurycoma* gave higher and similar gelation temperature (To, 40°C) and enthalpy (425.4 and 775.8 J/g) than *M. sloanei.* This result suggests that *D. microcarpum* and *B. eurycoma* flours have a more thermo-stable granular structure and a more highly ordered granular starch structure than that of *M. sloanei*, thus their resistance to gelatinization (Donovan, 1979). This result is however a reflection of the interaction between the starch and the other constituents of the flour. During the DSC measurement, starch is converted from a semi-crystalline form to an amorphous form. The process involves the initial hydration of the amorphous regions which facilitates molecular mobility of the amorphous regions followed by the reversible swelling. The reversible swelling then leads to dissociation of the double helices within the crystalline regions and consequent expansion of the granules as the polymer hydrates. The DSC reflects each of these stages and at the conclusion temperature, the amylopectin double helices dissociated and with the increased shear, more of the swollen granules are disrupted giving rise to an amorphous gel and increased viscosity (Donovan, 1979). One factor that may be responsible for the result obtained in this study is that the flour sample is a starch-hydrocolloid-protein system, each component exhibiting different thermal characteristics.

Studies on the pasting and thermal behaviour of starch-hydrocolloid blends showed that gelation process differs with the type of starch. While the peak gelation temperatures (Tp) for *D. microcarpum* and *B. eurycoma* were 62.5 and 60°C, respectively, that of *M. sloanie* was much less (45.45°C). However, wide temperature rang e was observed between To and Tp and between Tp and Tc. The highest value between Tp and Tc (52.55°C) w as obtained for *M. sloanei*. This effect which may be attributed to the presence of the hydrocolloids within the system which also gave rise to the high values obtained for ΔH. This result suggests that the hydrocolloid composition in *M. sloanei* has the most effect and may probably consist of the highest concentration of the hydrocolloid in relation to the starch content.

Previous reports showed that hydrocolloid may cause a decrease in gelation temperature (Gudmundsson, 1991), and may also be responsible for an increase in the gelation temperature (Kim and Wang, 1999; Temsiripong et al., 2005). Both effects were attributed to the chemical composition and concentration of the hydrocolloids, and the amylose and amylopectin ratio of the starch. Similarly, Ferrero et al. (1996) reported an increase in enthalpy with increasing concentration of alginate, guar gum and xanthan gum. On the contrary, Rojas et al. (1999) reported that alginate, kappa carrageenan, guar gum and xanthan gum have the tendency to decrease the enthalpy of gelation in wheat flour. Tester and Sommerville (2003) related the increased Tc to the effect of hydration of the hydrocolloids this reduces the fraction of water available for the dissociation of the starch crystallite.

Apart from the effect of hydrocolloids, the protein constituents may also exert their thermal properties. The net energy change thus calculated under the DSC transition peak represents the total thermal energy change, which may be regarded as the sum of the energy uptake of the starch-hydrocolloid-protein system (Myers, 1990).

Conclusion

As a preliminary study, the findings of this study revealed the pasting and thermal characteristic properties of a starch-hydrocolloid system of leguminous seed flour. In order to understand the synergistic role of the specific hydrocolloid as it affects the thermal properties of the flours of these leguminous seeds, a detailed investigation involving the use of the two techniques employed in this study coupled with and dynamic rheological analysis may be necessary.

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