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Isolation and characterization of native and modified starch from adlay (*Coix lacryma jobi*-L.)

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Abstract

Adlay (*Coix lacryma jobi*-L. var. *Tapul*) starch was isolated and subjected to heat-moisture treatment to produce a modified starch. Chemical, physical and functional properties were analysed using standard methods and compared with modified cornstarch and modified tapioca starch. Modification caused a decrease in moisture and increase in protein, dietary fibre and ash content. Starch colour showed a decrease in the lightness of the modified *Tapul* starch. Scanning electron microscopy (SEM) showed various cracks, fissures and holes on the starch surface after modification. Water absorption capacity increased while swelling and solubility decreased. Significant changes in pasting and gelatinization properties were also observed. The findings provide evidence of altered starch properties after heat-moisture treatment which may have potential application in canned food products, sauces and bread.

Introduction

In plants, starch is the main carbohydrate reserve and an important ingredient in human nutrition (Kim *et al*., 2008). It is found in different botanical sources such as cereal, legume, root, tuber and unripe fruit (Ashogbon and Akintayo, 2014). Food starches are typically used as thickeners and stabilizers in foods such as puddings, custards, soups, sauces, gravies, pie fillings and salad dressings, but still provide many other uses. However, the industrial utilization of native starch is limited due to high rate of retrogradation, insolubility in water and fluctuation in viscosity during thermal processing (Ashogbon and Akintayo, 2014). A modified food starch is produced when it undergoes one or more chemical or physical modifications, allowing it to function properly under high heat and/or shear which usually happened during processing.

Adlay (*Coix lacryma-jobi* L.) or Job's tears is an underutilized cereal crop in the Philippines which shows potential as a source of starch. There are four known adlay varieties in the Philippines which are differentiated by the colour of their seed coats. *Tapul* is a non-waxy variety of adlay which has been discovered in the Philippines in 2010 when an indigenous tribe in Zamboanga was able to survive a typhoon by substituting this grain to rice. *Tapul* grains differ in colour to the waxy adlay variety because it comes in grey orange colour but similarly spherical in shape with a groove at one end. Its grain structure is similar to the waxy adlay and resembles that of the ordinary rice, only slightly bigger. Its proximate composition based on FNRI (2011) analysis on adlay grits indicates significant amount of energy (356 kcal) and protein (12.8 grams).

Adlay has long been used in traditional Oriental and Chinese medicines, baked products, soups, tea, and distilled liquor as flour or whole grain (Arora, 1977). It has also been reported that adlay grains may have anti-tumor activity (Numata *et al*., 1994) and be effective against viral infection (Hidaka *et al*., 1992). Adlay varieties have long been used as a nourishing cereal, added in soups and broths in the form of flour and whole grain. In Thailand, a non-dairy drink from Job's tears is available in the market as an alternative health food. Animal and human clinical trials demonstrated that the consumption of de-hulled flour and seed of Job's tear can improve lipid metabolism, thereby decreasing the risk of heart disease. In addition, it could reduce liver fat accumulation and protect from tumor stimulating compounds (Chang *et al*., 2003; Yu *et al*., 2005).

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Some bioactive compounds in Job's tear, especially coixenolide, inhibited tumors, prevented cancer and provided protection against viral infection (Hung and Chang, 2003; Chun *et al*., 2004). In a recent study by Yang *et al.* (2013) adlay is also shown to prevent osteoporosis. Owing to its perceived nutritional and health value, adlay is increasingly utilized in functional foods and drinks (Li and Corke, 1999).

In the Philippines, adlay has been overlooked in the past years particularly in its suitability as a food product. Thus, despite perceived nutritional and health-promoting benefits, promoting it in a country where rice is considered as a staple food may be difficult. In order to optimize the utilization of adlay, there is a need to conduct further study on its components to produce value-added products to increase awareness and preference of the Filipinos to this underutilized crop. Transforming adlay into either flour or starch can increase its utilization since in this form it can be used as a vehicle for micronutrient fortification. However, utilization of adlay starch can be enhanced by modification.

Heat-moisture treatment or HMT is a physical method of starch modification. It is based on the principle of heating the starch at a temperature above its gelatinization point (70-130^{o}C) for one to several hours under controlled moisture conditions (15-35%) (Haghayageh and Schoenlechner, 2011). This type of modification is simple because it only requires heating, safe because no chemicals are used and therefore relatively cheaper to produce (Jacobs and Delcour, 1998; Chung, Moon and Chun, 2000; Jobling, 2004). Morever, HMT has been shown to provide important properties for food application as this method is shown to restrict swelling, increase starch paste stability and increase gelatinization temperature which are all desirable for noodle manufacture and canned foods (Collado *et al*., 2001; Adebowale *et al*., 2005; Takahashi *et al*., 2005; Julianti *et al*., 2013).

Although adlay grains are high in starch, few studies have been done on the physicochemical properties of its starches (Li and Corke, 1999; Ramirez, 1996a,b; Thongngam and Dechkunchon, 2007; Kim *et al*., 2008; Chaisiricharoenkul *et al*., 2011) which may provide useful information for industry purpose and future product development. In addition, its suitability as a raw material for HMT had not been studied. In order to develop value-added products from adlay, understanding and knowledge of its functional properties must be considered. Hence, the objective of this study is to characterize adlay starch modified by heat-moisture treatment in terms of its chemical composition (moisture, protein, fat, ash, dietary fibre and amylose), physical (colour, starch granule structure and pH) and functional properties (water absorption, swelling, solubility, pasting and gelatinization). The results may be compared to that of commercially available modified starches to ascertain whether the former can be an alternative modified starch source. Moreover, since there is no sustainability issue, the use of this cereal crop to produce modified starch may provide added livelihood opportunities to Filipino farmers and people in the rural areas.

Materials and Methods

Materials

Adlay (*Coix lacryma jobi*-L var. *Tapul*) was obtained from Southern Tagalog Integrated Agricultural Research Center (STIARC) in Lipa City, Batangas, Philippines. Modified corn starch and modified tapioca starch were purchased from Wills International, Bicutan, Taguig City, Philippines and used as standards. All chemical reagents used were of analytical reagent grade.

Starch isolation

The *Tapul* starch was obtained following the modified alkaline steeping method of Sira and Amaiz (2004). The grains were steeped in 0.25% sodium hydroxide solution overnight at room temperature, washed in excess water until neutral and ground for 2 min at high speed in a Waring blender with enough volumes of distilled water. The homogenate obtained was passed through an 80-mesh sieve and then further filtered in a 200-mesh sieve. The filtrate were pooled and re-suspended in water, manually stirred for 5 min and allowed to settle for 2 hours. The supernatant is discarded and the starch is repeatedly re-suspended in water, stirred and allowed to settle and re-suspended in water again until the wash water has reached neutral pH. Finally, the starch is dried in a drying oven at 45^{o}C overnight until 12-14% moisture content is reached. The dried starch was ground, passed through an 80 micron sieve and stored in LDPE bags at ambient temperature till further use.

Removal of protein from starch

The starch from *Tapul* was isolated using the modified method of Sira and Amaiz (2004). However, initial chemical analysis showed the starch to be contaminated with protein (>1%), hence, the method was modified to remove the remaining adhering protein. The concentration of the alkaline steeping medium was increased from 0.25% to 0.50%. To ensure removal of any remaining adhering protein, the starch isolated was re-suspended in 500 ml of 0.25% NaOH, stirred manually for 10 minutes and allowed to soak overnight. The soaked starch was decanted, washed and re-suspended again in 0.10% NaOH overnight. The starch was allowed to settle, decanted and washed several times with tap water until the washings reached neutral pH. The starch was then dried in an oven at 45°C overnight. The dried starch was then blended for 2 minutes, sieved at 80-mesh, packed and stored at room temperature. The stored *Tapul* starches were then subjected to HMT (30% moisture, 110°C for 8 hours).

Starch modification

Isolated native *Tapul* starch (NTS) was modified through heat-moisture treatment (HMT) following the modified method of Collado *et al*. (2001). Fifty (50) grams of starch was adjusted to 30-35% moisture content by soaking in distilled water and equilibrated at 4°C overnight. The conditioned starch samples were placed in petri dishes, covered with an aluminum foil and heated in a drying oven at 110°C for 8 hours. The starch samples were mixed occasionally using a mixer glass for even heat distribution. The starches were removed from heat and allowed to cool at ambient temperature, packed in PE bags, sealed and stored till further use.

Chemical analyses

The chemical analysis of both native and heat-moisture treated adlay starch were determined according to AOAC (2000). The conversion factor of protein was 5.85. The amylose content was determined using the Megazyme Rapid Test Kit (Megazyme International Ireland Ltd.) based on the principle of concanavalin-A binding method (Gibson *et al.*, 1996).

Physical analyses

The colour and colour difference were analyzed with a chromameter (CR-300; Minolta, Tokyo, Japan) using the Hunter system, which identifies colour using three attributes: L (white = 100, black = 0), a (red = positive, green = negative) and b (yellow = positive, blue = negative). The colour difference (ΔE), a measure of the distance in colour space between two colours, was determined by comparison to a white standard tile with colorimeter values of L = 94.5; a = -1.0 and b = 0.2, using the following relationship:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

The pH was determined using the Jenway pH meter standardized with buffer solutions pH 4 and pH 7.

Scanning electron microscopy (SEM)

Dried and finely ground native and heat-moisture treated adlay starches were placed on aluminum specimen holders with double-sided adhesive tapes. The starches were coated with thin film of gold under vacuum using JEOL JFL-1200 FINE COATER. Coated samples were examined using JSM 5310 (JEOL Ltd., Japan) scanning electron microscope operated at 20 kV. The images were viewed at 1000x magnification.

Water absorption capacity (WAC)

The water absorption capacity (WAC) of the starches was determined using the method of Sosulski *et al.* (1976). One gram of the starch sample was mixed with 10 ml distilled water and let stand at room temperature for thirty (30) minutes, then centrifuged at 3,000 rpm for 30 minutes. Water absorption was measured as percent water bound per gram starch.

Swelling power and solubility

Swelling power (SP) and solubility were determined according to Li and Yeh (2001) with modifications. The sample was weighed and distilled water was added into a centrifuge tube with a screw cap. The centrifuge tubes were heated at 55°C, 75°C and 95°C for 30 min with occasional stirring. The tubes were cooled in an iced water bath and centrifuged at 3,000 rpm for 15 min. The supernatant was carefully removed and starch sediment was weighed. The clear supernatant was removed into a pre-weighed aluminum dish and evaporated at 130°C for 2 hours, cooled and then weighed. The swollen sediment and dried supernatant were weighed to determine the swelling power and solubility as follows:

Swelling power (%) = [sediment weight / (initial sample weight x (100% - %solubility))] × 100;

Solubility (%) = [dry supernatant weight / initial sample weight] × 100

Pasting properties

The pasting properties of the starch samples were measured with a Rapid Visco-Analyzer (RVA, Newport Scientific, Warriwood, Australia), according to Li and Corke (1999). A 2.5 g of sample (dry basis) was weighed into an RVA canister and deionized water was added to obtain the total sample weight of 28 grams. The sample was then mixed for 30 seconds to obtain a lump-free sample. The time-temperature profile was: holding for 2 minutes at 50°C, heating to 95°C in 7.5 minutes, holding for 5 minutes at 95°C, cooling to 50°C in 7.5 minutes and holding for 5 minutes at 50°C. The peak viscosity, setback, breakdown and the final viscosity were recorded.

Gelatinization properties

The gelatinization temperature of native and modified adlay starches were studied using a differential scanning calorimeter (DSC-Q100, TA Instruments Waters, USA) equipped with a TA Universal Analysis software. Water (8 µl) was added using a microsyringe to starch (4.0 mg, db) in the DSC pans, which were then sealed and allowed to equilibrate for at least 30 minutes. The sealed pans were then heated (20-120˚C at 10˚C/min) to gelatinize the starch. An empty pan was used as a reference. The onset of gelatinization (To), peak temperature (Tp), completion temperature (Tc) and gelatinization enthalpy (ΔH, J/g) were determined.

Statistical analysis

Analysis of Variance (ANOVA) was used to analyze significant differences between the native and heat-moisture treated *Tapul* starches, and between modified *Tapul* starches and standard modified starches.

Results and Discussion

Chemical properties

Result of the chemical analyses of native and heat-moisture treated *Tapul* starch and standard modified starches are presented in Table 1. Following modification, the moisture content of heat-moisture treated *Tapul* starch decreased in comparison to the standard modified starches. Moisture content of a powdered product depends on the drying temperature, length of drying time and humidity conditions to which the powdered product was exposed. It also played a significant role in the flow properties of a food (Lawal, 2004). Re-suspension of the starch paste in alkali was able to significantly lower the protein content of the starch as illustrated in Table 1. Protein content in starch is related to starch purity and ideally should not be present in starch. However, increase in protein content after HMT of *Tapul* starch may be attributed to the residual proteins still embedded in the starch granules which were released when the starch granules were subjected to HMT. Ash content increased after HMT which may be due to the contaminating sodium from repeated alkali treatment. This is also an indication of the process used to isolate the starch from adlay. The total dietary fibre of *Tapul* starch after HMT increased. Increase in starch digestibility after HMT is attributed to changes that occur in the starch granule particularly when starches are heated at temperatures below its gelatinization point at high moisture content (>20˚C), thereby making the starch susceptible to enzymatic attack (Jacobs and Delcour, 1998). In terms of amylose content, the value did not change even after HMT and the obtained value is slightly lower compared to the study of Li and Corke (1999) who reported a range of 15.9-25.8% for normal coix starches. The amylose content of starches is important as it affects swelling, gelatinization, pasting, retrogradation and enzymatic vulnerability of starches. Regardless of the starch origin, HMT can cause a decrease in amylose leaching and formation of amylose-lipid complexes which may cause an underestimation of the amylose content.

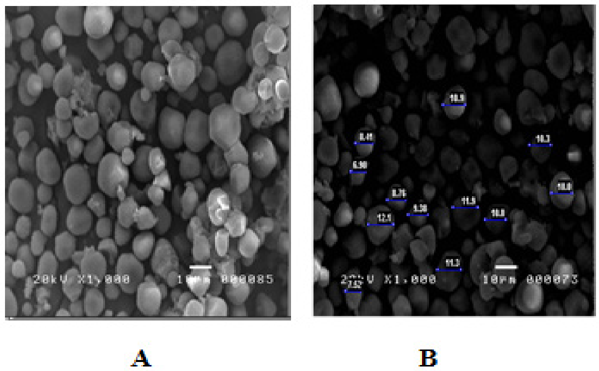

Figure 1. Scanning electron micrograph of *Tapul* starch a) Native and b) HMT starch

Physical properties

Result of the physical analyses of NTS, HMTTS and standard modified starches is presented in Table 2. The colour of the finished starch is an important characteristic with white as the ultimate objective (Wang *et al*., 2000). A starch with an L value >90 is considered to have a satisfactory lightness degree. After HMT, the L value decreased but is still within the acceptable range. In addition, low chroma value is desired for starches. The parameter a was observed to be negative (-) and reduced after heat-moisture treatment but b was observed to be positive (+) and found to increase after HMT which may affect the whiteness of the modified adlay starch. In terms of colour difference, ΔE increased after HMT which could be due to reaction of the amino group in the proteins present in the starch during modification but ΔE was small (<10) to be detected visually (Kim *et al*., 2008; Yaseen and Shouk, 2011).

The pH is an indicator of the degree of acidity or alkalinity of a substance. It is also necessary that the pH of starch is near neutrality so it may be used in industries where changes in pH of products are

Table 1. Chemical properties of native and modified *Tapul* starch as compared to standards

Components	Sample*			
	NTS	HMTTS	MCS	MTS
Moisture (%)	14.58 ± 0.30[a]	8.97 ± 0.04[d]	11.87 ± 0.71[c]	12.80 ±0.06[b]
Protein (%)	0.83 ± 0.01[b]	0.97 ± 0.01[a]	0.23 ± 0.01[c]	n.d.
Fat (%)	0.70 ± 0.14[a]	0.58 ± 0.15[b]	n.d.	0.07 ± 0.06[c]
Ash (%)	0.22 ± 0.04[c]	0.39 ± 0.04[a]	0.35 ± 0.02[b]	0.13 ± 0.03[d]
Dietary Fibre (%)	2.25 ± 1.38[b]	7.68 ± 0.63[a]	1.27 ± 0.92[c]	1.19 ± 0.40[d]
Amylose (%)	13.20 ± 0.13[b]	13.20 ± 0.01[b]	6.70 ± 0.01[c]	30.40 ± 0.01[a]

*NTS – native *Tapul* starch; HMTTS – heat-moisture treated *Tapul* starch; MCS – modified corn starch; MTS – modified tapioca starch; n.d. = not detected

Data are reported on dry basis and values are means ± s.d.'s of duplicate determinations.

[a,b,c,d] Different letters within the same row indicate a significant difference $p<0.05$.

Table 2. Physical properties and WAC of native and modified *Tapul* starch as compared to standards

Parameter	Sample*			
	NTS	HMTTS	MCS	MTS
Colour				
L	94.89 ± 0.54[b]	93.98 ± 0.25[c]	96.58 ± 0.36[a]	96.78 ± 0.03[a]
a	-0.08 ± 0.01[a]	-0.16 ± 0.01[b]	-1.14 ± 0.03[d]	-0.95 ± 0.01[c]
b	5.22 ± 0.13[b]	6.72 ± 0.05[a]	2.41 ± 0.05[c]	1.98 ± 0.01[d]
ΔE	5.14 ± 0.13[b]	6.67 ± 0.15[a]	3.71 ± 2.06[b]	2.89 ± 0.18[c]
pH	7.40 ± 0.54[a]	5.66 ± 0.21[b]	5.26 ± 0.67[b]	5.80 ± 0.03[b]
Water absorption capacity (WAC) (%)	209.50 ± 4.48[b]	265.60 ± 2.15[a]	204.70 ± 1.99[b]	189.86 ± 14.70[b]

*NTS, HMTTS, MCS, MTS are as defined in Table 1.

Values are means ± s.d's of triplicate determinations

[a,b,c,d] Different letters within the same row indicate a significant difference $p<0.05$.

not required. After HMT, the pH value obtained was lower and not significantly different from the standard modified starches. Thus, heat-moisture treated *Tapul* starch may be used in food products where these standard modified starches are typically used.

Result of scanning electron microscopy on the shape and surface characteristics of native and treated starches are shown in Figure 1. Native *Tapul* starch granules are shown to be round and polygonal in shape that varies in size and with porous surfaces. The average granule sizes of the native starch were 5.2 and 12.7 µm respectively, hence, *Tapul* starch granules may be considered small. HMT did not alter the granule shape and size of the starch granules which was similar to the previous study on HMT of finger millet starch (Adebowale *et al*., 2005). However, when viewed under SEM, HMT adlay starch showed various cracks and holes on the surface. This may be attributed to the mode of preparation and modification used that may have affected the morphology of the starch granules.

Functional properties

Water absorption capacity or (WAC) is an important parameter that determines starch use in products. It affects functional properties such as viscosity, which is a very important indicator of bulking and consistency of products. Heat-moisture treatment of *Tapul* starch resulted in an increase in the WAC from 209.50% to 265.60% with the increase significantly higher than the standard (Table 2). This could be attributed to the changes in the available water binding sites when starch was modified. This finding is also in line with the studies of Balasubramanian (2011) and Iheagwara (2013) where heat-moisture treated finger millet starch and sweet potato starch obtained the highest WAC value.

The swelling power of both native and modified starches from *Tapul* increased with increasing temperature as presented in Figure 2. Beginning from the temperature of 75ºC, the swelling powers of both native and modified starches from *Tapul* increased to a maximum. However, swelling power is restricted after HMT. In general, HMT causes restricted swelling and this may be attributed to increase in starch crystallinity causing restricted entry of water within the starch matrices. This is in line with the report of Noomhorm and Hormdok (2007) on rice starches.

The solubility of both native and modified

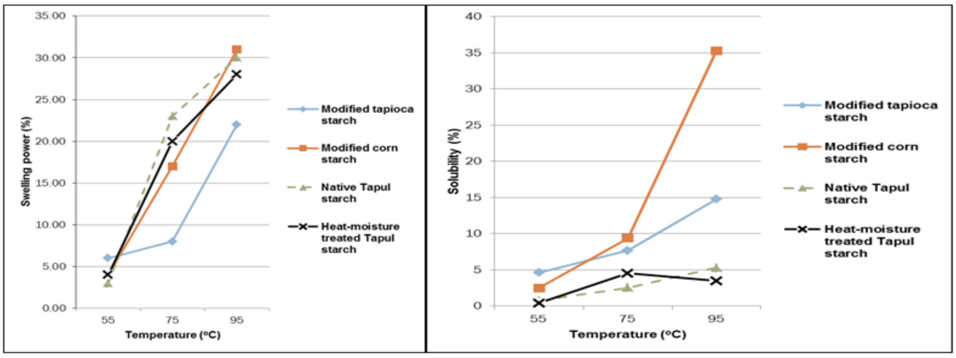

Figure 2. Effect of swelling and solubility on NTS, HMTTS, MCS and MTS

starches from *Tapul* is also shown in Figure 2. With increasing temperatures, the solubility of starches was substantially increased. This might be due to the fact that the starch contained a high amount of small size starch molecules and some big and small holes appeared on the surface of the starch granules. Upon swelling, the surface holes may allow a number of the small starch molecules to leach out into the water. However, a reduction in solubility of NTS occurred after HMT at 95°C as compared to the standard modified starches. This is in line with the results of Adebowale *et al.* (2002), who studied the effects of HMT on the solubility of Bambarra groundnut starch at 95°C where after HMT, the starch showed maximum hydrophilicity with minimum amylose leaching hence, becoming the least soluble. In addition, chemically-modified starches have higher solubility compared to physically modified starches.

Pasting of starch is a process following gelatinization in the dissolution of starch. Result obtained from the RVA is presented in Table 3. It involves starch granule swelling and amylose leaching from the granule until total disruption of starch granules occurred. Starch pasting is affected by presence of amylose, lipids, protein, swelling power and chain length distribution of amylopectin (Li *et al.*, 2008). An increase in pasting temperature suggests resistance of adlay starch to gelatinization after HMT. From Table 3, it was shown that *Tapul* starches had higher peak viscosities, lower trough and final viscosities but with higher pasting temperatures compared with the standard starches. This may be attributed to increase in water absorption capacity and the interactions with traces of lipid and protein components with amylose in the starch may have affected pasting properties of starch. In terms of breakdown, HMTTS showed a reduced breakdown value compared to NTS and the standard. The breakdown value is an estimate of the paste resistance to disintegration due to heat and shear. A decrease in breakdown value after HMT is an indication that the modified *Tapul* starch is more stable during continued heating and shearing as compared to NTS and MTS which is in accordance with the study of Adebowale *et al.* (2005), Noomhorm and Hormdok (2007) and Olayinka *et al.* (2008). The final viscosity increased after modification of the *Tapul* starch. The FV value is a measure of the ability of the starch to form paste or gel during cooling. This could mean that modified non-waxy adlay starch molecules re-associate easily during cooling to produce a stable gel or starch paste. The setback value is a reflection of the degree of retrogradation of starch pastes. After HMT, the setback value was reduced. This was probably related to the low amylose content, increasing swelling power with a higher proportion of a short branch-chain of amylopectin. According to Chaisiricharoenkul *et al*. (2011), Job's tear starches are characterized by a short-chain amylopectin. A low setback value could mean that during cooling, modified *Tapul* starch paste does not retrograde easily. Overall, higher viscosities and decreased retrogradation after HMT of *Tapul* starch show potential application for food products that require thermal processing such as in canned products as well as a thickener in sauces and soups since it can extend viscosity longer.

After HMT, there was an increase in the gelatinization properties (To, Tp, Tc and Tc-To) for *Tapul* starch. Native *Tapul* starch showed a gelatinization temperature ranging from 74-77˚C corresponding to a narrow gelatinization range. After HMT, there was a widening of the gelatinization temperature ranging from 73-81ºC and is also different from the standards (61-74ºC). The enthalpy or ΔH after HMT also increased (0.50-16.98 J/g) indicative of more heat stability and may be correlated to the relative crystallinities of the adlay starch as compared to ΔH values of the standard modified starches (12-14 J/g). It may also be attributed to the characteristic of HMT starch to induce formation of new crystallites with different stabilities. A starch with higher degree of crystallinity maintains its structural stability, consequently making granule resistant toward gelatinization since water molecules

Table 3. Pasting properties of native and modified *Tapul* starch as compared to standards

Sample*	Pasting parameter (cP)						
	Peak viscosity	Trough viscosity	Breakdown	Final viscosity	Setback	Peak Time (seconds)	Pasting Temperature (°C)
NTS	4536	2007	2529	2601	594	252	74.40
HMTTS	5110	2838	2272	3321	483	282	76.80
MCS	6796	4614	2182	5949	1335	246	71.20
MTS	4994	2049	2945	2977	928	240	66.65

*NTS, HMTTS, MCS, MTS are as defined in Table 1.
Breakdown is P minus TV; Setback is FV minus PV; Peak time is time in seconds to reach the peak viscosity; Pasting temperature is the temperature at the onset of rise in viscosity

cannot easily penetrate the crystallites requiring more energy and thus exhibiting a higher transition temperature. Overall, HMT starches are shown to have different gelatinization profile from chemically modified starches and this is important to ascertain whether HMT *Tapul* starch can have functionalities similar to these types of modified starches.

Conclusion

In the development of any food products from starchy crops, the knowledge of their different physicochemical properties in particular those of the starch which is the major component, is needed to predict behaviour under given processing conditions. Consequently, the study on the chemical and functional properties of tropical starches should be carried out extensively if they are ever to become competitive with other starches already in the market. In the present study, starch from adlay was isolated and modified by HMT. Since HMT is a physical method, heat-moisture treated *Tapul* starch showed lower moisture content with significant changes in its chemical properties. Heat-moisture treated *Tapul* starch showed altered physical and functional properties in terms of colour difference, swelling, solubility, pasting and gelatinization properties. Therefore, heat-moisture treated *Tapul* starches showed potential application in foods that require high water absorption, lower swelling and solubility, higher viscosity, increase gel strength that does not retrograde easily and higher thermal stability. Moreover, pasting curves obtained showed the potential of HMT adlay starch to be an interesting alternative to be explored by food developers. This study also recommends studying other functional properties such as freeze thaw stability, paste clarity and particle size.

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