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Physicochemical and Functional Characterization of *Mucuna pruries* Depigmented Starch for Potential Industrial Applications

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Abstract

Starch is a very important biopolymer in the food industry. The velvet bean (*M. pruriens*) is an excellent potential starch source containing approximately 520 g starch per kg. The objective of this study was to evaluate the physicochemical and functional properties of velvet bean depigmented starch. The starch granules appear oval and spherical shaped. The colour registered L*, a*, b* values of 44.9, 0.324 and 0.341 respectively. The chemical composition registered values of moisture, ash, fat, protein, fibre and NFE of 110.5, 5.8, 5.7, 0.0, 34 and 954.5 g/kg respectively, as well as amylose levels of 215.3 g/kg. Gelatinization onset (T_o), peak (T_p) and final (T_f) temperatures were of 74.23˚C, 80.57˚C and 86.39˚C. The solubility (3.1% - 16.2%), swelling power (SP) (2.86% - 16.17%) and water absorption capacity (WAC) (2.67 - 15.95 g water/g starch) were directly correlated to temperature (60˚C - 90˚C). The enthalpy values (4.10 - 13.47 j/g) were directly correlated to the time (1 - 21 days). The retrogradation increased as time increased. The viscosity of *M. pruriens* depigmented starch decreased slightly during the heating stages and then increased during cooling and the refrigeration and freezing stability registered syneresis ranges from 17.65 to 23.18 mL/50mL and from 16.4 to 22.6 mL/50mL respectively, indicating that the depigmented starch was unstable in heating-cooling processes.

Keywords

Depigmented Starch, Functional Properties, *Mucuna pruriens*, Physicochemical Properties

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1. Introduction

Starch is a naturally occurring, biodegradable, cheap, renewable, and abundantly available polysaccharide molecule. The different botanical sources of starches are cereal (wheat, corn, rice, barley, oat, sorghum, millet, and rye), legume (lima bean, garbanzo bean, lentil bean, red kidney bean, navy bean, faba bean, mung bean, pinto bean, adzuki bean, field pea, cowpea, beach pea, green pea, grass pea, soybean, and groundnut), some under-utilized legume (sword bean, jack bean, and pigeon pea), root and tuber (cassava, potato, yam, cocoyam, and sweet potato), and unripe fruit (banana, plantain, mango, and pawpaw). Starch granules are mainly found in seeds, roots and tubers, as well as in stems, leaves, fruits and even pollens. The granules occur in all shapes and sizes (spheres, ellipsoids, polygon, platelets, and irregular tubules). The two main components of starch are amylose (AM) and amylopectin (AP) and they differ significantly in their properties and functionality. AM has a high tendency to retrograde and produce tough gels and strong films. In contrast, AP, when dispersed in water, is more stable and produces soft gels and weak films. It is possible for entanglements to occur between AM and AP, along with the presence of minor components (proteins, PLs, lipids), which all also have important impacts on the physicochemical properties of the starches from different botanical origin [1].

Starch is a very important biopolymer in the food industry, where it performs various functions as thickener, binder, stabilizer, texture modifier, gelling and bulking agent. Starch varies greatly in form and functionality between and within botanical species, and even from the same plant cultivar grown under different conditions. Its different properties are utilized for making diverse food products [2].

A growing demand for starches from the food industry has created the need for new sources of this polysaccharide. A potential new starch source is the legume *M. pruriens* (Velvet bean), a native of Southeast Asia. This legume has been shown to have high agricultural potential, with yields up to 1000 kg/ha, even under adverse tropical conditions. This grain has a high starch (515 g/kg) and protein (279 g/kg) content, but as with all legumes, it also contains antinutritional components. In particular, the velvet bean contains the non-protein amino acid L-3,4-dihydroxyphenylalanine (L-DOPA) in quantities of 31 g/kg, which limits its direct consumption in food and feed [3]. *M. pruriens* belongs to the family Fabaceae and is grown primarily as a soil-improving crop to control weeds. It grows even in poor soils and produces abundant seeds. The seeds are eaten by a few ethnic groups such as Igbos in Nigeria, either as a condiment or as part of a main dish. The mature seeds are also known to be eaten by Indian tribal sects, Mundari and Dravidian groups [4]. The process to obtain a protein concentrate helps to reduce or eliminate non-nutritive factors [5]. Ascorbic acid is used in the extraction due to its positive effect in terms of reduction of the dark colour [6]. Usually a starchy coloured by- product is obtained, which depending on the variety of seed may be slightly or highly coloured, and could be more or less acceptable as a food ingredient.

The objective of this study was to investigate the diverse physicochemical and functional properties of depigmented starch isolated from the legume *M. pruriens*.

2. Materials and Methods

2.1. Materials

M. pruriens seeds were obtained from the 2010 harvest in the state of Yucatán, México. Reagents were analytical grade and purchased from J.T. Baker (Phillipsburg, NJ, USA), Sigma Chemical Co. (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

2.2. *M. pruriens* Flour

Impurities and damaged seeds were removed. Sound seeds were milled in a Mykros impact mill until passing through a 20-mesh screen (0.85 mm), and then in a Cyclotec 1093 (Tecator, Sweden) mill until passing through a 60-mesh screen (0.24 mm).

2.3. *M. pruriens* Starch

A single starch extraction was done with the legume flour. This was processed using the wet fractionation method of Hoover *et al.* [7]. Briefly, whole flour was suspended in distilled water at a 1:6 (w/v) ratio, pH adjusted to 11 with 1 mol/L NaOH, and the dispersion stirred for 1 h at 400 rpm with a mechanical agitator (Caframo Rz-1,

Heidolph Schwabach, Germany). This suspension was wet-milled with a Kitchen-Aid® mill and the fibre solids separated from the starch and protein mix by straining through 80- and 150-mesh sieves followed by five washings of the residue with distilled water. The protein-starch suspension was allowed to sediment for 30 min at room temperature to recover the starch and protein fractions. The starch was oven drying at 60˚C. The pH of the separated solubilized protein was adjusted to its isoelectric point (4.6) with 1 mol/L HCl. The suspension was then centrifuged at 1317 × g for 12 min (Mistral 3000i, Curtin Matheson Scientific Inc., Morris Plains, NJ, USA), the supernatants discarded and the precipitates freeze-dried at −47˚C and 13×10^{-3} mbar.

2.4. Starch Discoloration Process

M. pruriens starch was suspended in distilled water at a 1:8 (w/v) ratio containing sodium hypochlorite at 25 g/L, stirred for 10 min and centrifuged at 2500 rpm for 12 min. This step was repeated three times. The precipitate thus obtained was then suspended in distilled water at a 1:8 (w/v) ratio containing HCl 1M to neutralize, stirred for 10 min and centrifuged at 2500 rpm for 12 min. Finally, the starch was suspended in distilled water at a 1:8 ratio (w/v), stirred and centrifuged at 2500 rpm for 12 min. The discoloured starch thus obtained was oven drying at 60˚C.

2.5. Physicochemical Characterization of *M. pruriens* Starch

Starch Shape

The microscopic appearance of the starch granules was assessed using the method of Mc Master [8] by direct microscopic observation using a Leica optical microscope. Starch suspensions at 10 g/L were prepared for this assay.

Colour

The colour of the *M. pruriens* depigmented starch was assessed with a colour analyser Chroma-meter CR-200b (Minolta Camera Co., Ltd. Japan), taking five readings and reporting the average value of the CIELAB parameters (L*, a*, b*).

Chemical Composition

Standard AOAC procedures were used to determine nitrogen (method 954.01), fat (method 920.39), ash (method 925.09), crude fibre (method 962.09) and moisture (method 925.09) contents in the *M. pruriens* starch [9]. Nitrogen (N_2) content was quantified with a Kjeltec Digestion System (Tecator, Höganäs, Skåne län, Sweden) using cupric sulphate and potassium sulphate as catalysts. Protein content was calculated as nitrogen × 6.25. Fat content was obtained from a 1 h hexane extraction. Ash content was calculated from sample weight after burning at 550˚C for 2 h. Moisture content was measured based on sample weight loss after oven-drying at 110˚C for 2 h. Carbohydrate content was estimated as nitrogen-free extract (NFE) by difference from the sum of the protein, fat, ash and crude fibre content. Apparent amylose content was estimated after iodine complexation using the method of Morrison and Laignelet [10]. Amylopectin content was calculated by the difference of total starch minus amylose content.

2.6. Functional Characterization of *M. pruriens* Starch

Gelatinization. Differential Scanning Calorimetry (DSC)

Starch gelatinization was determined with a DSC-7 (Perkin-Elmer Corp., Norwalk, CT) using the technique described by Ruales and Nair [11]. The DSC was calibrated with indium and the data were analysed using the Pyris software program. Two milligrams (d.b.) of starch was placed in an aluminium pan and moisture level adjusted to 700 g/L by adding de-ionized water. The pan was then hermetically sealed and left to equilibrate for 1 h at room temperature. It was then placed in the calorimeter and heated from 30˚C to 120˚C at a rate of 10˚C/min by using an empty pan as reference. Gelatinization temperature was determined by automatically computing onset temperature (T_o), peak temperature (T_p), final temperature (T_f) and gelatinization enthalpy (ΔH) from the resulting thermogram.

Solubility, Swelling Power (SP) and Water Absorption Capacity (WAC)

Solubility, SP and WAC patterns at 60˚C, 70˚C, 80˚C and 90˚C were determined using a modified version of Sathe *et al.* [12] method. Briefly, 40 mL of a 10 g/L starch suspension was prepared in a previously tared 50 mL centrifuge tube. A magnetic agitator was placed in the tube, and it was kept at a constant temperature (60˚C, 70˚C, 80˚C or 90˚C) in a water bath for 30 min. The suspension was then centrifuged at 2120 *x* g for 15 min, the

supernatant decanted and the swollen granules weighed. From the supernatant, 10 mL was dried in an air convection oven (Imperial V) at 120˚C for 4 h in a crucible to constant weight. Percentage solubility and SP were calculated using the following formulas:

% Solubility = dry weight at 120˚C × 400 sample weight

% SP = weight of swollen granules × 100/sample weight × (100 − % solubility).

WAC was measured using the same conditions as above, but was expressed as weight of the gel formed per sample, divided by treated sample weight.

Retrogradation

Retrogradation was determined with a DSC-7 (Perkin-Elmer Corp., Norwalk, CT) using the technique described by Gudmundsson and Eliasson [13]. The DSC was calibrated with indium and the data were analysed using the Pyris software program. Two milligrams (d.b.) of starch was placed in an aluminium pan and moisture level adjusted to 700 g/L by adding de-ionized water. The pan was then hermetically sealed, left to heat at 105˚C for 15 min and stored for 1, 2, 3, 7, 14 and 21 days at 4˚C. After that, it was then placed in the calorimeter and heated from 30˚C to 120˚C at a rate of 10˚C /min, using an empty pan as reference.

Pasting Properties

These properties were evaluated in a Viscoamylograph (Brabender PT-100, Duisburg, Germany) according to Wiesenborn *et al*. [14]. Briefly, 400 ml of 60 g/L (d.b.) starch suspension was heated to 95˚C at a rate of 1.5˚C/min, held at this temperature for 15 min, then cooled to 50˚C at the same rate and held at this second temperature for another 15 min. Maximum viscosity, consistency, breakdown and setback were calculated in Brabender Units (BU) from the resulting amylograms.

Breakdown: peak viscosity [BU] – viscosity at 95˚C × 15 min [BU]

Consistency: viscosity at 50˚C [BU] – peak viscosity [BU].

Setback: viscosity at 50˚C [BU]) – viscosity at 95˚C × 15 min [BU].

Starch Clarity

Starch clarity was measured using the method of Bello-Pérez *et al*. [15], determining transmittance of a 10 g/L starch paste at 650 nm using a spectrophotometer (Beckman DU-650, Fullerton, CA). Starch suspensions (10 g/L) in tubes with threaded caps were placed in a water bath at 100˚C for 30 min, agitated using a Vortex every 5 min, and left to cool at room temperature. Percentage of transmittance (%T) was determined from these suspensions.

Refrigeration and Freezing Stability

Stability under refrigeration and freezing conditions was evaluated using a modified version of Eliasson and Kim [16] method. Pastes were prepared in a Brabender viscoamylograph. Briefly, 400 mL of 60 g/L (d.b.) starch suspension was heated to 95˚C at a rate of 1.5˚C/min, held at this temperature for 15 min, then cooled to 50˚C at the same rate and held at this second temperature for another 15 min. Portions of 50 mL were placed in centrifuge tubes, cooled to room temperature and stored at 4˚C and −10˚C, and then centrifuged at 8000 *x* g for 10 min in a J2-HS centrifuge (Beckman Instruments). The water separated from the starch gels during 1, 2, 3, 4 and 5 days was measured.

3. Results and Discussion

3.1. Physicochemical Characterization of *M. pruriens* Starch

In the micrograph, the granules of *M. pruriens* depigmented starch appear oval and spherical shaped (**Figure 1**). The granule size was heterogeneous and their shape was similar to that reported in potato, yucca, pinto bean, navy bean, field pea, as well as jack and velvet bean [17]-[20]. The images showed the presence of pores along the equatorial region of the *M. pruriens* depigmented starch granules. Similar pore arrangement has also been observed on potato starch granules, and on wheat, rye and barley granules [21]. These pores are thought to support the oxidation process that suffers the granules during the discoloration process. A similar behaviour was reported by Betancur-Ancona *et al*. [3] in granules of *M. pruriens* starch, in the micrograph the granules appear oval-shaped with a minimum diameter of 12 mm and a maximum of 45 mm as well as the presence of pores along the equatorial region that suggested the initial attack of enzymes during the germination process.

The colour analysis of *M. pruriens* depigmented starch registered L*, a*, b* values of 44.9, 0.324 and 0.341, respectively. The L*, a*, b* values registered in velvet bean depigmented starch suggested a more opaque colour than in velvet bean pigmented starch with L*, a*, b* values of 73.4, −1.73 and 3.3, respectively.

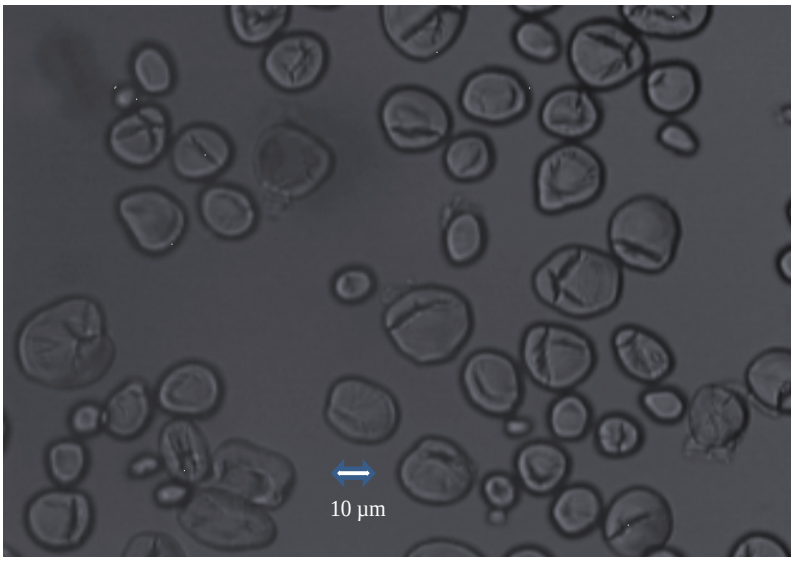

Figure 1. Photomicrograph of *M. pruriens* depigmented starch.

The proximal composition of *M. pruriens* depigmented starch registered values of moisture, ash, fat, protein, fibre and NFE of 110.5, 5.8, 5.7, 0.0, 34 and 954.5 g/kg, respectively. Betancur-Ancona *et al.* [3] suggested that the protein content of *M. pruriens* starch (7.1 g/kg), when it is found elevated, it should be reduced by means of chemical or enzymatic treatments. The U.S. Food and Drug Administration allows a maximum level of protein in corn starch of 3.5 g/kg prevent formation of undesirable dark syrups resulting from Maillard reactions that may occur during the manufacturing process. Therefore velvet bean depigmented starch could be used in high-glucose syrup production. The apparent amylose level (**Table 1**), was lower in the velvet bean depigmented starch (215.3 g/kg) than in pigmented starch (392 g/kg) [3], native cereals and more common tubers starches such as corn (280 g/kg), sorghum (280 g/kg) and wheat (280 g/kg) [20]. This level also is lower than that of other legumes starches such as pinto bean (322 g/kg), navy bean (321 g/kg), field pea (342 g/kg) [19], mung bean (264 g/kg) [18] and jack bean (375 g/kg) [17].

3.2. Functional Characterization of *M. pruriens* Starch

Gelatinization onset (T_o), peak (T_p) and final (T_f) temperatures were 74.23˚C, 80.57˚C and 86.39˚C, respectively for the *M. pruriens* depigmented starch whereas for the pigmented starch. Betancur-Ancona *et al.* [3] reported 69.4˚C, 74.8˚C, 81.3˚C and 10.70˚C, respectively. The gelatinization temperature was higher than that of commercial corn starch (62˚C - 73˚C) and common food starches such as potato (56˚C - 67˚C), wheat (58˚C - 64˚C) and rice (68˚C - 78˚C) starches [20] [22]. It was similar to the ranges of starches from other legumes such as the 70˚C - 80˚C range of the lima bean (*Phaseolus lunatus*), 73˚C - 81˚C for soybean (*Glicine max*) [7] and 76˚C - 83˚C for jack bean (*Canavalia ensiformis*) [17]. Because of its high gelatinization temperature *M. pruriens* depigmented starch can be used in thermal food processing at temperatures higher than those in which common starches are used. This characteristic makes it extremely useful in products subjected to high temperatures, such as canned products. Gel enthalpy ΔH for depigmented starch was 10.81 J/g whereas for the pigmented starch it was 10.70, meaning that the latter requires less energy to gelatinize that the former. This coincides with Czuchajowska *et al*. [23]. Who reported that lower gelatinization enthalpy values are linked to higher amylose levels.

Solubility, swelling power and water absorption capacity in depigmented starch were directly correlated to temperature (**Figure 2**). The solubility increased as temperature increased. Gujska *et al*. [19] mention that dissolution of pinto bean, navy bean and field pea starches starts at 70˚C, probably because the swollen starch granules permit amylose liberation. The native starch (pigmented) [3], in comparison, exhibited only a moderate increase in solubility as temperature increased, reaching 16.2% at 90˚C, less than the 53.6% reached here by depigmented starch at the same temperature. This property would allow greater dispersion of *M. pruriens* depigmented starch in aqueous solutions and better water reception and retention in the preparations to which it is added.

Table 1. Chemical composition of pigmented and depigmented starch from *M. pruriens*.

Component (g/kg)	*M. pruriens* starch	
	Depigmented	Pigmented[a]
Moisture	110.5	98.2
Protein	0	7.1
Fibre	34	5.4
Fat	5.7	4
Ash	5.8	2.8
NFE	954.5	980.6
Amylose	215.3	392
Amylopectin	784.7	608

[a]Betancur-Ancona *et al.* [3].

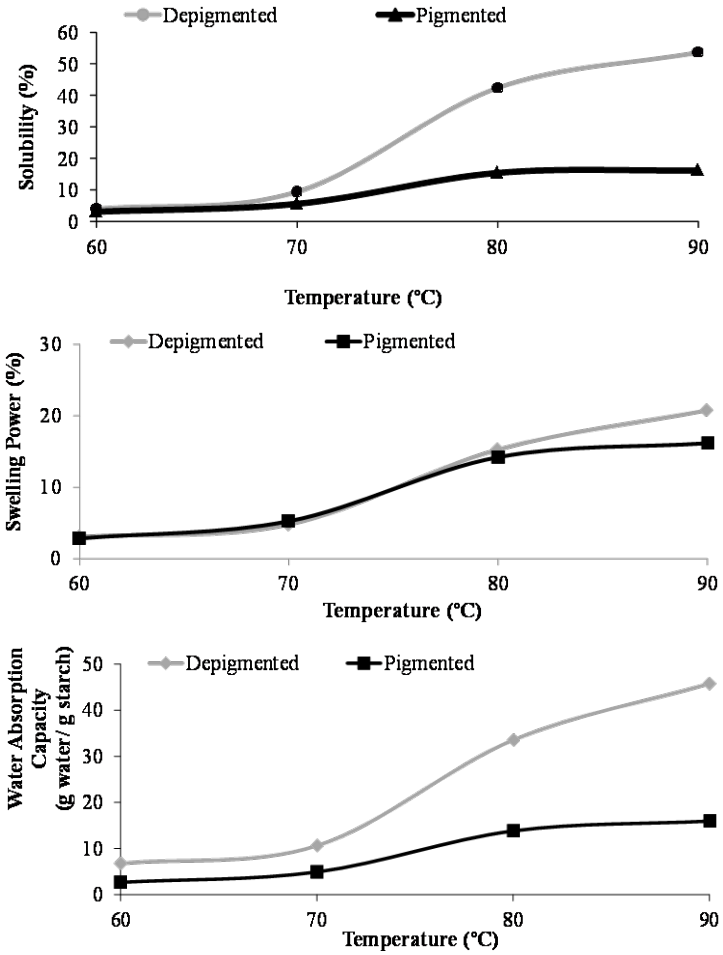

Figure 2. Solubility, swelling power (SP) and water absorption capacity (WAC) of *M. pruriens* starch. Pigmented starch values from Ref. [3].

The swelling pattern shows that at temperatures lower than 70˚C the velvet bean granules resist swelling, probably due to their high initial gelatinization temperature. From 70˚C to 90˚C, the granules gradually swell as temperature increases, a result of intermolecular hydrogen bridge rupture in the amorphous areas, which allows irreversible and progressive water absorption. In native starch (pigmented) [3], the swelling increment was gradual, beginning at 60˚C but the levels reached were lower than in depigmented starch.

The water absorption pattern showed that in the 70˚C to 90˚C range the granules swelled gradually as temperature increased due to rupture of the intermolecular hydrogen bridges in the amorphous areas, which allowed progressive water absorption. The native starch (pigmented) [3], in comparison, exhibited only a moderate increase in WAC as temperature increased, reaching 15.95% at 90˚C, less than the 45.75% reached here by depigmented starch at the same temperature.

Table 2 registers the retrogradation parameters of *M. pruriens* depigmented starch. The enthalpy values were directly correlated to time. The increased of Δ*H* means that *M. pruriens* oxided starch retrogrades in higher proportion in days 14 and 21. For the above mentioned, retrogradation increased as time increased.

In paste characterization, corn starch exhibited a classic amylogram curve, whereas the velvet bean starch (pigmented and depigmented) were different since they did not have a defined maximum viscosity peak (**Figure 3**). To obtain the paste properties of *M. pruriens* starch, the highest viscosity value developed by the paste was used. Discoloration process reduced viscosity from 256 BU in the native starch [3] to 120 BU in the *M. pruriens* depigmented starch (**Table 3**). Velvet bean pigmented starch viscosity was highly stable during a 15 min residence period at 95˚C, and remained stable even after gradual cooling. This stability makes this starch suitable for products that require cooking at high temperatures during manufacture, since it maintains adequate product consistency during processing. On the other hand, the viscosity of *M. pruriens* depigmented starch decreased slightly during the heating stages and then increased during cooling, indicating that the depigmented starch was unstable in heating-cooling processes. This must be considered when incorporating this starch into products since upon cooling its paste viscosity will increase, which will be reflected in a higher thickening capacity. The depigmented starch had higher fragility than the pigmented starch, indicating that its viscosity decreased during heating due to swelling of its starch molecules, consequently making them more fragile. The consistency and setback of the depigmented starch was lower than in corn and native *M. pruriens* starch reported by Betancur-Ancona *et al*. [3]. This indicated that the depigmented starch was more stable in heating-cooling processes, although it would have thickening capacity upon cooling in foods to which it was added.

Table 2. Retrogradation parameters of *M. pruriens* depigmented starch.

Day	T_o (˚C)	T_p (˚C)	T_f (˚C)	Δ*H* (j/g)
1	46.53	56.56	72.14	4.10
2	46.34	58.44	72.70	7.17
3	42.19	56.02	69.11	7.68
7	42.7	56.83	71.53	9.82
14	41.88	57.76	73.34	10.32
21	44.3	58.42	77.55	13.47

Table 3. Pasting properties of pigmented and depigmented starch from *M. pruriens*.

Parameter	Depigmented starch	Pigmented starch[a]
Initial gelatinization temperature (˚C)	82.5	81
Maximum viscosity (BU)	120	256
Viscosity at 95˚C (BU)	120	93
Viscosity at 95˚C for 15 min (BU)	104	276
Viscosity at 50˚C (BU)	156	350
Viscosity at 50˚C for 15 min (BU)	174	350
Breakdown	16	−20
Consistency	36	74
Setback	52	94

[a]Betancur-Ancona *et al*. [3].

The depigmented and pigmented *M. pruriens* starch transmittance (%T) values were 50.89% and 22.21%, respectively. As it was expected, the depigmented starch was more translucent than the pigmented starch because the discoloration process generated functional properties such as increased paste clarity. Segura-Campos *et al*. [24] stated that starch clarity was a result of high reflection as the characteristic arrangement of gel molecular chains reduced the intensity of the light transmitted through them. Clarity is a key parameter in starch paste quality because it provides shine and opacity to product colour. The depigmented *M. pruriens* starch's excellent clarity makes it potentially useful in products such as fruit pie fillings and candies.

Compared with the *M. pruriens* pigmented starch, the depigmented starch had higher stability in refrigeration cycles but lower stability in freezing cycles (**Figure 4**). This behavior is a disadvantage in the food industry because it means the *M. pruriens* depigmented starch behaves like a sponge, initially absorbing water and then re-

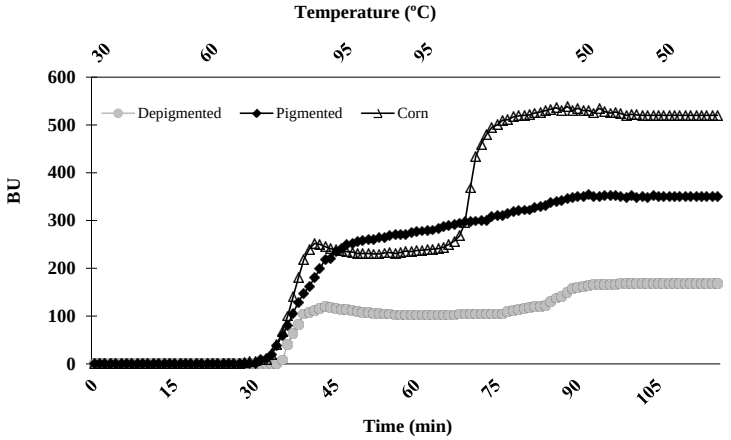

Figure 3. Viscoamylograph of *M. pruriens*.

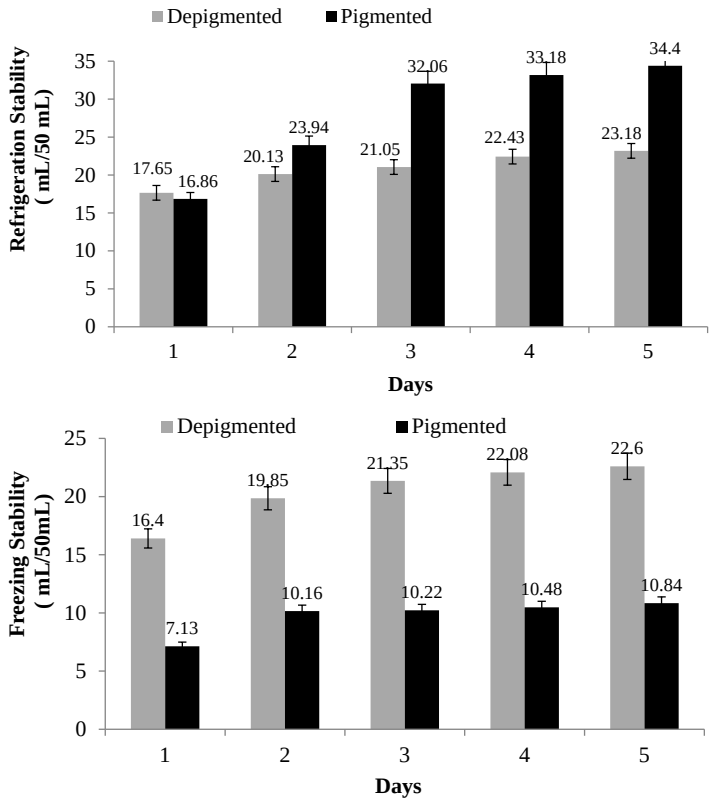

Figure 4. Refrigeration and Freezing Stability of *M. pruriens* starch.

leasing it when centrifuged. Its high syneresis can probably be attributed to the organization of the molecules (amylose and amylopectin) that leads to the gel releasing water. The depigmented starch behavior could be caused by the decrease of the starch capacity of the forming hydrogen bond, as well as by the longitude of the amylose chains, as short chains facilitate its lixiviation toward the aqueous means. Successive freezing and thawing of starches can also affect their structure since formation and melting of ice crystals can lead to starch paste redistribution and dilution [25]. This is probably what occurred in the *M. pruriens* depigmented starch. The retained water would have been released from inter- and intramolecular associations, resulting in two separate phases: one polymer-rich (gel); and another polymer-poor (liquid). Under refrigeration processes the *M. pruriens* depigmented starch was more stable than its pigmented, but under freezing processes it was unstable. This is vital to consider when using the studied depigmented starch as a food additive since it requires storage at low temperatures.

4. Conclusion

M. pruriens depigmented starch has a wide variety of possible applications as a functional ingredient in food systems and other industrial applications. An example of the above mentioned is the use of starch in the syrups production. On the other hand, the high transmittance makes depigmented starch potentially useful as an additive in jellies and candies to provide brightness. Its high gelatinization temperature, together with its solubility and high WAC, is advantageous for use in sausages, baked products, canned products, sauces, seasonings, jellies, compressed candies, gummy products, etc. However, its low syneresis (*i.e.*, low stability) under refrigeration and freezing conditions makes it inadequate for use as a thickener, stabilizer or gelling agent in refrigerated or frozen foods. The discoloration process of *M. pruriens* starch produced a derivative with more versatility as a food additive providing some new and improved functional properties. However, the election of depigmented starch as additive in food should take into account its functional properties and characteristics of other ingredients used in the preparation as well as the processing conditions. In sum, the velvet bean is an excellent starch source with many potential applications and is also a promising alternative to corn starch.

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