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Physical and Chemical Characteristics of Waxy Proso Millet's Starch

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ABSTRACT
The granule shape, amylose, pasting, retrogradation, transparency, and thermal properties of the four varieties of waxy proso millet's starches were investigated. The starches had a substantial proportion of smooth-edged, big polygonal grains, a sparse proportion of spherical shape granules, and a combined grain size of 6.04 µm. Waxy proso millet variants' starches exhibit lower amylose level (0.24%) and greater phosphorus contents (2.32 mg/100 g) than non-waxy varieties' starches, as well as greater transparency (30.23%) and lesser volume proportions of retrogradation (4.99%); greater peak viscosities (3282 cP), thickening viscosities (2403 cP) and breakdowns (882 cP), and lesser setbacks (268 cP); and lesser onset temperatures (To) (69.65°C), peak temperatures (Tp) (74.9°C), final temperatures (Tc) (80.98°C) and enthalpies (Delta H) (11.19 J/g) in their starch gelatinization.
Keywords— Proso millet, starch, Millets, Anti-ageing

1. Introduction

Among the world's oldest cereals, proso millet (PM), also serves as primary food crop in South India's dry and semi-arid regions. It grows rapidly for only 10–11 weeks [1][2]. The crop, which can grow at a variety of elevations above sea level, is grown on about 1 million hectares of land in India. Protein, carbohydrate, dietary fibres, and numerous trace elements (Ca, Fe, Mg, Zn, etc.) are all abundant in proso millet [3]. Waxy PM (299 mg/100 g) has a methionine quantity that is greater than twice as high as that of wheat, rice, and maize. Compared to wheat, rice, and maize, PM possesses a protein concentration of roughly 13.58% and higher levels of vital amino acids. Some illnesses, including diabetes and cardiovascular conditions, can be prevented using proso millet [4], [5]. Proso millet contains something. Starch makes up the majority of the seed in proso millet and ranges in concentration from 58.5% to 73.5% [6]. The nutritional and therapeutic benefits of proso millet starch and its similar items have garnered significant attention as the standard of life rises and consumer demand for health-promoting foods rises. Therefore, it is essential to conduct research on the qualitative attributes of proso millet starch in order to encourage the industrialization of proso millet, increase the added value of products derived from PM, and boost farmers' incomes. The primary source of carbohydrates in the human diet, starch is also a key raw material that is utilised extensively across a wide range of industries. In the food business, it can be utilised as thickeners, water retention agents, stabilisers, or sweeteners.

At the moment, maize [6], potato [7], and rice [8] are the principal subjects of starch research. The benefits of proso millet for human health have sparked current interest in using it to make foods that promote health. Conferring to Liu et al. [9], diverse potato varieties and growing environments result in some variations in the physicochemical properties of potato starch, however the key variables impacting functional characteristics of potato starch are its grain sizes and phosphorus and amylose levels. Small spherical grains and copious polygonal grains, among which the latter are only rarely present and have bimodal distributions, are the two fundamental starch grain forms and sizes found in PM [10]. The size distribution of its starch pellets range from 1 to 8 µm. The least pasting temperature (PT), peak viscosity (PV), setback (SB), and breakdown (BD) of PM were observed when comparing to barnyard, kodo, foxtail, and millet. PM has amylose contents that range from 2.85% to 19.3% and starch granule diameters that are between 1.9 and 10.0 µm [11].

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The gelatinization capabilities of PM and potato starches were obviously dissimilar from one another, with proso millet starch having better thermal and cold paste chemical stability than potato starch and less ability to produce gel than maize and potato starches. Statistics from earlier studies [12] demonstrated that proso millet's starch granules were polyhedral, had plane edges, and ranged in size from 5.76 to 8.64 µm. These granule sizes were bigger than those of waxy rice and were comparable to those of non-waxy PM. Waxy PM has greater starch transparency and retrogradation than waxy rice, non-waxy PM, and non-waxy PM, but its starch gelatinization is worse because of its greater enthalpy and PT of the various waxy PM starches.

Though, not enough research has been done on the starch features of PM, particularly the several types of waxy PM. Therefore, the goal of the investigation was to examine the physicochemical characteristics of four varieties of waxy PM and equate them to those of non-waxy PM and viable maize starch in order to determine whether waxy PM starches could be used as a single food constituent.

2. Materials and Research Methodology

2.1 Materials

Seeds of different varieties of waxy (Neimi 6, Jichengshu1, Liaomi3, and Fu07-405) and non-waxy (Yumi3) PM have been ordered form China. From a retail store in Bangalore, India, maize starch was bought.

2.2 Starch isolation

The modified alkaline steeping technique was used to separate seed starches [16]. Separate batches of 100 g of each kind of PM were extracted, mashed with common high-speed smashing machines, and soaked in NaOH (3 g/L) at 35°C for 28 hours with a solid/liquid ratio of 1:7. Then, sieving was done on these seed samples, first using a sieve of 100 mesh (less than 150 µm) and then a sieve of 200 mesh (less than 75 µm). After the samples through sieving were centrifuged at 3000*g for 10 minutes, the supernatant and upper layer of yellowish impurities were removed, leaving only the starch samples behind. Three times were done this process. The starch specimens were submerged in distilled H2O, mixed, and centrifuged as previously mentioned. They were then neutralised with HCl (0.1 mol/L), oven dried for 24 hours at 45°C, crushed to a powder, and sieved through a 100 mesh sieve.

2.3 Scanning Electron Microscope (SEM)

By using a scanning electron microscope and a modified version of Jing's et al. [13] technique, the surface morphology of the starch grains was studied. The PM and maize starch samples were adhered to an aluminium using double-sided tape, covered in gold/palladium (60:40), and magnified by 2500.

2.4 Starch's amylose and phosphorus level

The ISO standard 6647-1-2007 was used to test the amylose content of the starches. With a spectrophotometer operating at a wavelength of 620 nm, the absorbance of a solution containing several starch kinds was determined. Based on the standard curve created using blends of amylose and amylopectin, the amylose contents were determined based on the measured absorbance (Fig. 1). Utilizing spectrophotometry, the phosphorus concentration of the starches was determined in accordance with “ISO standard 3946-1982”.

2.5 Light Transmittance

The techniques [13] were modified and used to evaluate the starch pastes' light transmittances. 1% starch pastes of various types were made in screw-cap hoses and cooked in a water bath at 100°C for 15 min while being intermittently shook. Their light transmittances (%T) at 620 nm were evaluated with a spectrophotometer utilizing water as the control after they had been cooled to a temperature of 25 °C.

2.6 Retrogradation

The samples' 1% starch pastes were made as previously mentioned, then put into 25 mL graduated assessment tubes with stoppers, 25 mL each, and kept at 25°C for 48 hours. The supernatant quantities

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of the tubes were measured every 4 hours during this storage period. As a result, a retrogradation curve of the percentages of supernatant vol. over time was created [14].

2.7 Data handling

The assessments and capacities were all three times reiterated, and SAS 9.0 was used to statistically analyse the data (SAS Institute, NC, USA). The least significant difference (LSD) at $p < 0.05$ and the analysis of variance were used to examine the implication of the variations among the means.

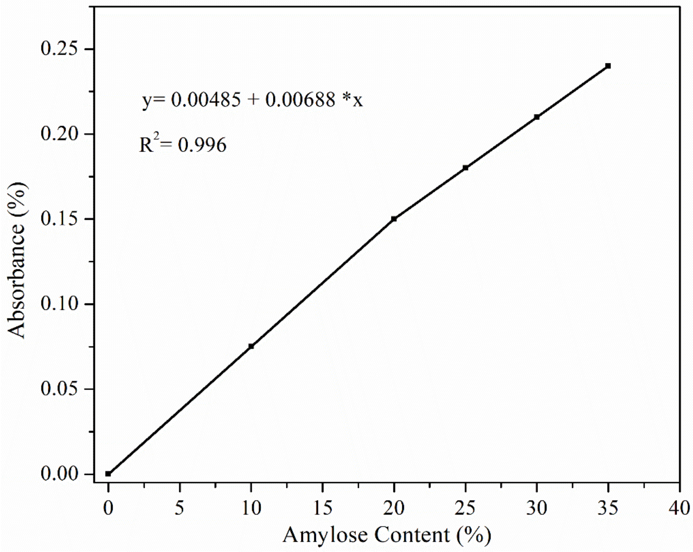

Figure 1 The mix of amylose and amylopectin's calibration curve

3. Results and Discussion

3.1 GGBS and Cement

3.1 Grain morphology

Fig. 2 displays images taken using a SEM of the starch samples where (A) Neimi6, (B) Jichengshu1, (C) Liaomi3, (D) Fu07-405, (E) Yumi3, (F) Maize. Similar to one another, the starch granules had spherical and polygonal forms with smooth edges. In addition, some proso millet starch granules had honeycomb-like patterns on their surfaces, which were probably brought on by alkali erosion during the starch extraction process [14]. The mean starch grain diameters of "Neimi6, Jichengshu1, Liaomi3, and Fu07-405" were 6.26, 6.17, 5.69, and 6.06 µm, respectively, according to statistical analysis. Waxy PM had smaller average starch grain sizes (6.04 µm) than non-waxy PM (6.32 µm) and maize (12.02 µm).

According to the findings of a prior study [15], waxy proso millet's starch granules can thus be categorised as tiny granules. Due to their higher surface area and potential for faster amylase digestion, the smaller granules may aid in boosting their digestibility. The exact reasons of some starch granule properties are unknown, but certain kinds and quantities of starch molecules are one clear component that may play a role in the development of such characteristics [16]. Other potential explanations include numerous environmental influences. The average starch granule sizes were found to be connected with some features, including amylose concentration, swelling power, pasting capabilities, rheological, and thermal characteristics [17].

3.2 Amylose and phosphorus proportions

According to Table 1, waxy PM starches had amylose levels ranging from 0.14% (Liaomi3) to 0.27% (Jichengshu1), which was much lower than the amylose levels of non-waxy PM starch (20.42%) and maize starch (24.92%). With regard to botanical sources of starch, the amylose level of the starch fluctuates and is influenced by the atmospheric and soil circumstances through the growth season as well as the timing of harvest [9]. Due to the delayed harvest, the amylose level of potato starch likely fell from 22% to 18% [18]. According to Kaur et al. [19], the amylose level of giant potato starch granules was higher than that of small and medium ones. Because it influences the functional

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characteristics of starch, amylose concentration is considered as one key indicator of the use of starch. Greater values indicate higher peak temperatures, lower PV and BD [20].

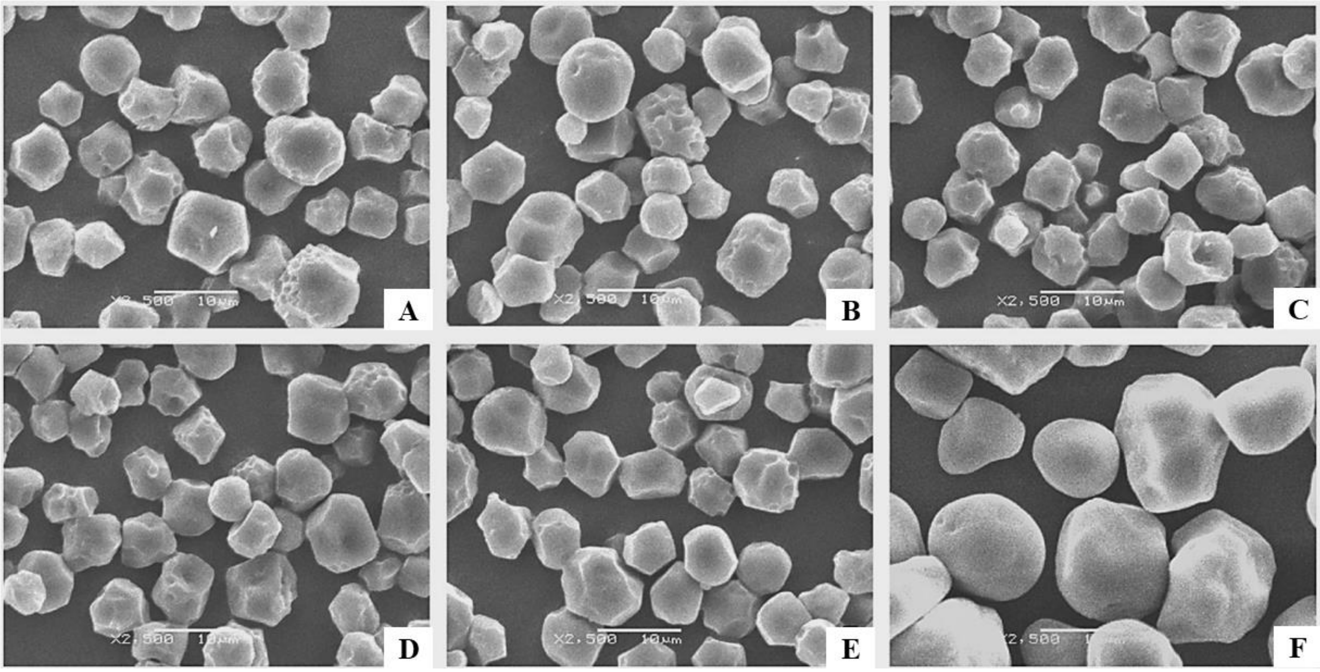

Figure 2 SEM graphs of starch grains of the samples (at 2500 magnification)

Table 1 Based on the dry starch weight, the samples' amylose and phosphorus levels

Variety	Neimi6	Jinchengshu1	Liaomi3	Fu07-405	Yumi3	Maize
Amylose content (%)	0.24 ± 0.027	0.27 ± 0.039	0.14 ± 0.005	0.22 ± 0.028	20.42 ± 0.031	24.92 ± 0.092
P content (mg/100 g)	1.61 ± 0.091	1.58 ± 0.096	2.32 ± 0.101	1.81 ± 0.091	0.59 ± 0.098	0.57 ± 0.151

Means are the averages of three replicates ± SD. Different superscripts in the same line indicate significant differences ($p < 0.05$).

The amount of phosphorus in the starch of the waxy PM under study ranged from 1.59 to 2.32 mg/100 g d.m., with Jichengshu1 and Liaomi3 having the highest and lowest levels, respectively. Its phosphorus content was higher than that of maize and non-waxy PM. The amount of phosphorus in potato starch normally rises as the plant grows [9]. The rheological and pasting characteristics of starch are also greatly influenced by phosphorus content [21]. Additionally, Alvani et al. [22] found a favourable association between the number of long-chain amylopectin fractions and the phosphorus level.

3.3 Light transmittance

Waxy PM had a starch light transmittance average of 30.23%, which was greater than non-waxy proso millet's (22.9%) and maize's (15.02%) as shown in (Fig. 3). Starch transmittance was highest in Liaomi3 at 32.05%. One of the key physicochemical characteristics of starch is transparency, which affects food taste and ultimately human meal acceptability [23]. Many variables, including the amylose content of the starch, the size of the starch granules, and the amylose/amylopectin ratio, influence the transparency of starch paste. According to this study, higher amylose content reduces the transparency of starch paste, which is in line with Tester's findings [24]. Reflecting swelling ability and starch granule dispersion in water, transparency. The uniformity of the starch solution increases with starch granule dispersion, and the transparency of the starch paste increases with starch light transmittance [13].

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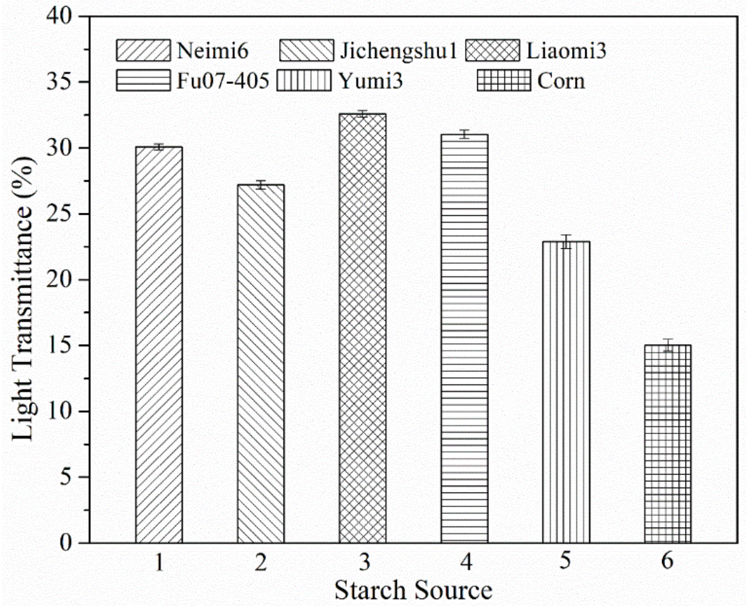

Figure 3 Light transmission through the specimens of starch

3.4 Retrogradation

Fig. 4 displays the samples' retrogradation rates for the starch paste. During the first four hours of standing, the retrogradation rates of all the starch pastes were higher. Waxy PM's starch paste retrogradations started to slow down after 8 hours of standing; after 32 hours, they tended to stabilise, with an average retrogradation percentage of 8.51%.

As a result, the waxy PM's starch paste retrogradation percentages were apparently lesser than those of the non-waxy PM (37.82%) and maize (40.84%), which most likely came from variations in their amylose concentrations [14]. The waxy PM assortments' amylose levels did not differ significantly from one another. Retrogradation of starch, a crucial factor restricting starch uses, can significantly alter the mechanical properties of starch-based meals, hence reducing their nutritive and sensory aspects. The increased transparency and lesser retrogradation of waxy PM's starches make them a useful source of raw materials for beverages.

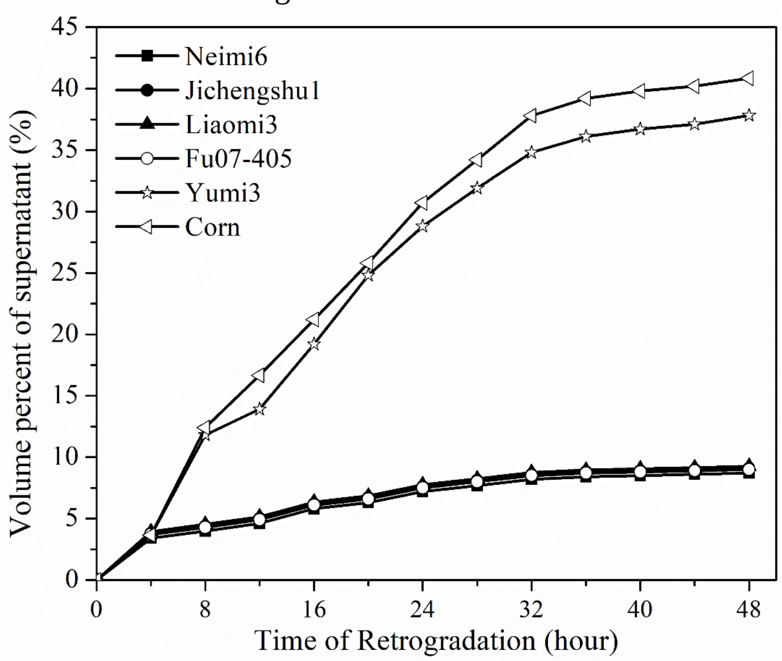

Figure 4 Starch sample retrogradation curves

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3.5 Pasting characteristics

The pasting features of the starch samples evaluated with RVA are displayed in Table 2. In comparison to non-waxy PM and maize, waxy PM's starches exhibited greater mean PV (3282 cP), TV (2403 cP), and BD (882 cP), however lower SB (268 cP) and PT (61.53°C). Among the waxy proso millet types, Liaomi3's starch had the lowest "PV, TV, FV, SB, and PT" values, which may be related to its smaller starch granule size. Starches with minor grains size likely engaged less areas and had lower viscosities [25]. Numerous parameters, such as granule size, starch molecular topographies, amylose/amylopectin ratio, and others, have an impact on the starch pasting properties [26].

Table 2 The starch samples' RVA characteristics

Variety	PV (cP)	TV (cP)	BD (cP)	FV (cP)	SB (cP)	PT (°C)
Neimi6	3527 ± 231	2501 ± 152	921 ± 49	2691 ± 231	191 ± 49	61.50 ± 0.21
Jinchengshu1	3392 ± 204	2491 ± 142	892 ± 41	2697 ± 162	231 ± 41	61.60 ± 0.11
Kiaomi3	3099 ± 219	2221 ± 298	842 ± 94	2441 ± 103	201 ± 94	61.45 ± 0.25
Fu07-405	3111 ± 231	2401 ± 281	801 ± 81	2801 ± 219	452 ± 128	61.60 ± 0.31
Yumi3	2101 ± 219	1691 ± 302	421 ± 101	2802 ± 226	1097 ± 167	61.70 ± 0.20
Maize	2100 ± 204	1598 ± 241	437 ± 28	2794 ± 267	1145 ± 103	61.70 ± 0.31

Mean values from three repetitions ± SD. Different superscripts in the same column indicate significant differences ($p < 0.05$). PT, pasting temperature; PV, peak viscosity; TV, trough viscosity; BD, breakdown (PV–TV); FV, final viscosity; SB, setback (FV–TV).

Waxy proso millet's starches showed larger BDs than other types of millet, which was likely due to their lesser amylose concentrations. The starches' decreased amylose concentrations made it easier for the granules to scatter while absorbing water and swelling, which sped up the process of shear thinning [27]. Low SBs of starches, for instance waxy and potato starches, improve starch pastes' thermostability in mechanical means and lessen their tendency to retrograde when cooling [28]. These results demonstrated that starches with lower amylose levels had greater BDs but smaller SBs, implying that their hot paste capacities were poorer than those of non-waxy PM and maize varieties. They also had improved anti-aging capabilities and better cold paste stability.

Table 3 Starch samples' DSC characteristics

Variety	T_o (C)	T_p (C)	T_c (C)	Delta H (J/g)
Neimi6	69.8 ± 0.44	74.7 ± 0.34	81.5 ± 0.18	11.63 ± 0.08
Jichengshu1	71.4 ± 0.11	76.3 ± 0.12	80.5 ± 0.19	11.11 ± 0.17
Liaomi3	68.7 ± 0.25	75.1 ± 0.19	80.7 ± 0.37	10.98 ± 0.31
Fu07-405	68.7 ± 0.69	73.5 ± 0.53	81.2 ± 0.31	11.05 ± 0.07
Yumi3	71.9 ± 0.35	75.8 ± 0.13	80.8 ± 0.39	12.98 ± 0.21
Maize	72.1 ± 0.16	78.1 ± 0.17	82.3 ± 0.10	13.32 ± 0.31

Mean values from three repetitions ± SD. Different superscripts in the same column indicate significant differences ($p < 0.05$). T_o, onset temperature of gelatinization; T_p, peak temperature of gelatinization; T_c, completion temperature of gelatinization; Delta H, gelatinization enthalpy

3.6 Thermal properties

The starch sample DSC results are displayed in Table 3. The test waxy proso millet had mean onset temperatures (To) of 69.65°C, peak temperatures (Tp) of 74.9°C, final temperatures (Tc) of 80.98°C, and enthalpy (Delta H) of 11.19 J/g, all of which were lesser than those of the non-waxy PM and maize. Liaomi3 had the lowest mean To, Tp, Tc, and Delta H of all the Although the Delta Hs in the waxy proso millet starch gelatinization were not considerably different, the lower enthalpies in this process demonstrated how much simpler it was to gelatinize waxy proso millet. According to Biliaderis et al. [36], the pace of heating, the amount of water in the starch gel, and the degree of damage to the starch granules all had an impact on the endothermic enthalpy of starch. The amylose content of starches and DH were found to be inversely associated [29]. Noda et al. [30] hypothesised that the molecular architectures of amylopectin relatively than the amylose concentrations of starches

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were what affected the DSC values. Therefore, it is necessary to continue researching the specific factor influencing the thermal characteristics of starches.

3.7 Correlation analysis

Table 4 exhibits the Pearson's association coefficients among the physicochemical characteristics of the starch samples. Amylose content had a very strong negative correlation ($p < 0.01$) with transparency, phosphorus level, PV, TV, and BD. Moreover, A significant association among the amylose level and the retrogradation rate ($p < 0.01$), SB ($p < 0.01$), and PT ($p < 0.05$). The phosphorus concentrations had a strong negative correlation with the retrogradation %, SB and PT ($p < 0.01$) and a strong positive correlation with the transparency, PV, TV, and BD ($p < 0.05$). Amylose level had a modest positive connection with PT ($p < 0.05$) even though Wiesenborn et al. [31] observed no association among amylose level and PV. Starch phosphorus level is frequently credited with subsidizing to the starch pasting characteristics of foods, and higher starch phosphorus level of potatoes was meticulously linked to its higher peak viscosity.

Although To was positively connected with Tp, Tc, and Delta H in the study, there was no clear association among the amylose content, phosphorus content, and To, Tp, Tc, and Delta H. There is only a weak relationship between the amount of phosphate bound to starch and the To or Tp of potato starch [32]. In evaluating the DSC qualities of sweet potato and buckwheat starch, the quantity of amylopectin small chains was more significant than the level of amylose [33].

Table 4 Physicochemical characteristics of the starch samples and their Pearson's correlation coefficients

	AMY	P	TRA	RET	PV	TV	BD	FV	SB	PT	To	Tp	Tc
P	-0.87[b]												
TRA	-0.89[b]	0.87[b]											
RET	1.00[b]	-0.88[b]	-0.88[b]										
PV	-0.91[b]	0.81[a]	0.83[a]	-0.95[b]									
TV	-0.91[b]	0.78[a]	0.82[a]	-0.95[b]	1.00[b]								
BD	-0.9[b]	0.84[a]	0.85[a]	-0.94[b]	0.97[b]	0.95[b]							
FV	0.27	-0.59	-0.37	0.28	-0.21	-0.14	-0.32						
SB	0.91[b]	-0.87[b]	-0.85[b]	0.95[b]	-0.96[b]	-0.94[b]	-0.98[b]	0.41					
PT	0.78[a]	-0.88[b]	-0.87[b]	0.81[a]	-0.73	-0.71	-0.76[a]	0.45	0.81[a]				
To	-0.19	-0.04	0.29	-0.13	0.21	0.21	0.23	0.14	-0.19	0.09			
Tp	-0.59	0.41	0.68	-0.53	0.55	0.57	0.61	-0.12	-0.59	-0.33	0.86[b]		
Tc	-0.65	0.49	0.76[a]	-0.59	0.59	0.59	0.64	-0.16	-0.7	-0.47	0.81[a]	0.96[b]	
del H	0.1	-0.21	0.11	0.18	-0.16	-0.12	-0.16	0.07	0.21	0.21	0.85[b]	0.8	0.64

AMY, amylose content; P, phosphorus content; TRA, transparency; RET, retrogradation.
a Significant difference at $p = 0.05$, b Significant difference at $p = 0.01$.

Conclusions

The starches from waxy PM were minute in particle sizes, with a mean size of 6.04 µm, lesser amylose levels and retrogradation proportions, greater phosphorus levels and transparencies, and were recognised by superior cold paste stability, higher viscosity, and more potent anti-aging characteristics. This suggested that waxy proso millet starches might be used as a prototype component for various industrial applications that call for low temperature during food processing. The transparency, phosphorus level, PV, TV, and BD of the starch samples were strongly inversely connected with the amylose content, while SB and PT were positively correlated with the retrogradation %age. The amount of phosphorus was adversely connected with the retrogradation rate, SB, and PT, but absolutely correlated with transparency, PV, TV, and BD. These findings suggested the potential for waxy PM to be used as a novel source of starch for a variety of specialised applications as well as the prospect of conducting an organized breeding programme to produce waxy PM with specific starch properties. Though, waxy proso millet still needs more study and development.

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