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Physicochemical, structure and functional characteristics of *Tacca leontopetaloides* starches grown in Indonesia

DIODE YONATA[1,2], PRIYANTO TRIWITONO[1], LILY ARSANTI LESTARI[3], YUDI PRANOTO[1,♥]

[1]Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada. Jl. Flora 1, Bulaksumur, Sleman 55281, Yogyakarta, Indonesia. Tel.: +62-274-5897797, ♥email: pranoto@ugm.ac.id

[2]Department of Food Technology, Faculty of Science and Agricultural Technology, Universitas Muhammadiyah Semarang. Jl. Kedungmundu Raya 18, Tembalang, Semarang 50273, Central Java, Indonesia

[3]Department of Nutrition and Health, Faculty of Medicine, Universitas Gadjah Mada. Jl. Farmako, Sekip Utara 55281, Yogyakarta, Indonesia

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Abstract. *Yonata D, Triwitono P, Lestari LA, Pranoto Y. 2023. Physicochemical, structure and functional characteristics of* Tacca leontopetaloides *starches grown in Indonesia. Biodiversitas 24: 6396-6406.* Understanding the physicochemical, structure and functional characteristics of tacca tuber (*Tacca leontopetaloides*) starch is crucial as a scientific basis for its development, especially in the food industry. Despite the abundance of starch in Indonesian tacca tubers, its potential remains largely untapped. This research aims to determine the physicochemical, structure and functional characteristics of tacca tuber starch from three different regions in Indonesia: Bangkalan, Garut and Sumenep. Wet extraction yielded tacca tuber starch with yields ranging from 21.26 to 26.42%. Significant differences ($p<0.05$) were observed in proximate composition (ash, lipid, and protein), starch purity (97.35-98.48%), amylose content (32.81-35.26%), and functional properties like swelling power (9.67-10.51 g/g at 95°C), solubility (4.93-5.87% at 95°C), water holding capacity (0.77-0.90 g/g), oil holding capacity (0.62-0.71 g/g), and relative crystallinity (24.22-27.03%). Thermal properties and pasting properties (except breakdown viscosity) exhibited significant variations. The gelatinization temperature profile of tacca tuber starch ranged from 57.92 to 76.38°C, with an ΔH value of around 3.81-4.62 J/g. Meanwhile, the temperature of tacca tuber starch paste ranged from 72.12 to 72.88°C. Tacca tuber starch granules are polygonal, elliptical, oval to slightly ellipsoidal, with an average granule diameter of 20.21-40.43 μm. Based on the X-ray diffraction pattern, tacca tuber starch shows the CA-type, containing orthorhombic and hexagonal structure crystals. Tacca tuber starch has a high lightness (92.01-93.62) and whiteness index (91.68-92.74). In conclusion, the cultivation location significantly influences the physicochemical, structural, and functional characteristics of tacca tuber starch.

Keywords: Functional, physicochemical, starch, structure, tacca tuber

INTRODUCTION

Starch, a complex polysaccharide composed primarily of amylose and amylopectin, is ubiquitous in nature, accumulating within roots, tubers, and plant seeds (Rodriguez-Garcia et al. 2021; Suastegui-Baylón et al. 2021). Starch accumulates as water-insoluble particles, available in roots, tubers and plant seeds (Bashir and Aggarwal 2019). This natural carbohydrate serves as a critical energy source for humans and a valuable biopolymer with diverse industrial applications. The global demand for starch is steadily increasing as indicated by the 2020 Global starch production that reached 97.7 million tons and is projected to reach 156.5 million tons before 2025 (Dereje 2021; Vilpoux and Junior 2022). This increase is compliant with the widespread application of starch, especially in the food industry. Starch has notable functional properties, such as its ability to form gels and pastes to serve as a thickening, gelling and film-forming agent (Ai and Jane 2015). Starch as a natural polymer has been widely applied in various sectors, such as water purification, food additives, drug delivery and food packaging (Gupta et al. 2022). Most of the world's starch is derived from cassava, corn, potatoes, wheat and sweet potatoes. However, overexploitation of these resources necessitates exploring alternative sources. Neglected and underutilized roots and tubers, often harboring abundant starch reserves, present a promising avenue for sustainable starch production (Tejavathi et al. 2020). Therefore, further research on characterizing new natural starches is imperative to serve as an alternative to reduce dependence on commercial starch sources.

Tacca (*Tacca leontopetaloides*) an often-overlooked tuberous plant native to Southeast Asia, boasts significant potential both as a food source and for industrial applications. Thriving in coastal regions at altitudes ranging from 3 to 300 meters above sea level (Lim 2016), this humble herb harbors a wealth of possibilities. Tacca tubers contain toxic compounds such as cyanide, tannin, phytic acid and saponin, which cause a bitter taste and are dangerous if consumed (Ogbonna et al. 2017). Interestingly, beyond the potential danger lies a treasure trove of starch. Fresh tacca tubers constitute a starch content of 35.82% (Binh and Dao 2020) and yield around 22.0-33.3% (Erlinawati et al. 2018). Several studies have delved into the physicochemical properties of tacca tuber starch, with researchers from Nigeria (Manek et al. 2005; Zaku et al. 2009; Nwokocha et al. 2011), Vietnam (Vu et al. 2017), and Thailand (Santibenchakul and Sudprasert 2018) contributing valuable insights. These investigations have revealed exciting characteristics, including good gel stability and high paste clarity, suggesting the potential for tacca tuber starch in a variety of food products (Nwokocha et al. 2011). Furthermore, the naturally low digestibility of tacca tuber starch adds an intriguing dimension to its potential health benefits (Vu et al. 2018).

Despite research efforts in Indonesia exploring tacca tubers' morphology, cultivation techniques, and food applications (Syafi et al. 2020; Wardah and Ariani 2020), a critical gap exists in understanding their physicochemical, structural, and functional characteristics-crucial information for their development, particularly in the food industry. Currently, tacca tubers have begun to be cultivated intensively, with harvest productivity reaching 8.7 tons/ha, easily found in the Bangkalan, Garut and Sumenep areas (Erlinawati et al. 2018; Winara and Murniati 2018; Winara et al. 2019). Each cultivation location has a different growing environment. These differences will affect the biosynthesis and starch formation process, as well as the functional properties of the resulting starch (Beckles and Thitisaksakul 2014; Šimková et al. 2013; A'yuni et al. 2021). This research pioneers the exploration of tacca tuber starch from these three diverse locations (Bangkalan, Garut, and Sumenep), comprehensively characterizing its physicochemical, structural, and functional properties. By unravelling these characteristics, it aims to unlock the potential of tacca tuber starch for both food and non-food applications. This investigation bridges the critical knowledge gap and paves the way for its strategic development.

MATERIALS AND METHODS

Materials

Tacca tubers were procured from local farmers in three different regions in Indonesia: Bangkalan, Garut and Sumenep. The tubers utilized had been grown for ten months prior to their harvest in September 2022. The characteristics of the tubers' growing locations during that year are detailed in Table 1, while Table 2 outlines the growth location characteristics at the time of harvest.

Starch isolation of tacca tubers

Tacca tuber starch isolation employed a method adapted from Herawati et al. (2020). Following thorough cleaning and manual skin removal, Tacca tubers were sectioned and washed with pristine water. A blender facilitated the grinding of the tubers with distilled water at a 1:3 (w/v) ratio until a uniform slurry was attained. This slurry was filtered through a 60-mesh sieve and squeezed to yield filtrate 1. The remaining pulp underwent a second extraction with distilled water (1:3 w/v) to obtain filtrate 2. Combined filtrates were subsequently filtered again through a 200-mesh sieve for residue removal. Following a 12-hour settling period with the water being changed every 4 hours, the liquid portion was discarded. The remaining sediment was dried at a controlled temperature of ± 50°C for 24 h and finally pulverized to obtain the desired starch powder.

Determination of chemical composition

The proximate composition of tacca tuber starch, encompassing moisture, ash, lipid, and protein content, was analyzed employing the AOAC method (1995). Subsequently, the Nelson-Somogyi method (Setyaningsih et al. 2021) was utilized to determine starch purity, while amylose levels were assessed via the method outlined by Juliano (1971).

Determination of starch color

Starch color characteristics were determined using a CR-400 chromameter (Minolta, Japan), referring to the Hunter method (Hutchings 1999). The whiteness index (WI) was calculated based on Zhu et al. (2009), while ΔE was determined based on Mokrzycki and Tatol (2011). ΔE is the difference between the color of tacca tuber starch and the color of control starch, which is the color of commercial cassava starch with the values L* (lightness), a* (+a value is redness and -a value is greenness) and b* (+b value is yellowness and -b value is blueness) respectively 92.87; -1.27 and 3.33.

Determination of swelling power and solubility

The solubility (S) and swelling power (SP) of the tacca tuber starch were determined using the method of Nwokocha et al. (2011). Tacca tuber starch (Ts) of 0.1 g was prepared in a centrifuge tube, 10 mL of distilled water was added, and heated for 30 minutes at 95°C. The tube was cooled to room temperature and centrifuged for 20 min at 3000 g. The sediment obtained was weighed (Sd), while the supernatant was dried to constant weight at 105°C and weighed (Su). S and SP values are calculated based on the following formula:

S (%) = (Su/Ts) x 100%
SP (g/g) = Sd/Ts x (1-S)

Table 1. Characteristics of tacca tuber growing locations

Growing area	Parameters			
	Latitude	Longitude	Average temperature (°C) (January - September)	Average humidity (%) (January - September)
Bangkalan	6° 51' 39" - 7° 11' 39"	120° 40' 06" - 130° 08' 04"	28.43 ± 0.60	77.10 ± 4.52
Garut	6° 57' 34" - 7° 44' 57"	107° 24' 03" - 108° 24' 34"	25.42 ± 0.52	71.87 ± 2.91
Sumenep	4° 55' 00" - 7° 24' 00"	113° 32' 54" - 116° 16' 48"	28.14 ± 0.57	82.00 ± 4.43

Note: Source: BPS-Statistic Indonesia (2023)

Table 2. The growth location characteristics at the time of harvest

Growing area	Parameters		
	Type of soil (clay: sand)	Soil pH	Light intensity
Bangkalan	85-90: 10-15	5.75-5.96	Low
Garut	5-10: 90-95	7.06-7.23	High
Sumenep	75-80: 20-25	6.29-6.44	Medium

Determination of water holding capacity (WHC) and oil holding capacity (OHC)

WHC and OHC of tacca tuber starch were analyzed by the centrifugal procedure described by Chiranthika et al. (2022) with slight modifications. Water or oil (10 mL) was mixed with 1 g of tacca tuber starch in a centrifuge tube of known weight. The slurry was mixed using a vortex for 1 minute at room temperature, then centrifuged at 3000 g for 10 minutes, and the supernatant was thrown out. WHC/OHC was measured as grams of water/oil bound by one gram of dry sample.

Morphology granules observation

The morphology of starch granules was evaluated using a Scanning Electron Microscopy (SEM) instrument (JEOL JSM-6510LA, Japan) and a JEOL JEC-3000FC auto coater based on the method of Nwokocha et al. (2011) with slight modifications. Tacca tuber starch sample was placed on the specimen stub, coated with carbon tape, and with gold. The analysis was carried out in a vacuum with an accelerating voltage of 10 kV. Sample morphology was observed with 1000x and 5000x magnification.

Determination of particle size distribution

Starch size distribution was measured using a Laser Particle Size Analyzer instrument (LPSA - LabTron, LLPA-C10, UK) referring to the method of Joshi et al. (2013) with slight modifications. Samples were added to pure water as a dispersing medium. The slurry obtained was then put into the cuvette and run for 120 seconds. Starch particle size is expressed in µm.

X-Ray diffraction

The crystal structure of tacca tuber starch was determined using an X-ray diffractometer (XRD) (Bruker D2 Phaser, Germany) with CuKa radiation nickel filter (λ=1.542 Å) following the method of Nwokocha et al. (2011) with slight modifications. The diffraction angle scanning area (2θ) is 5-30°. Amorphous and crystalline diffraction areas were determined using OriginPro Trial Version software (OriginPro 2023, OriginLab Corporation, Northampton, MA, USA). Relative crystallinity (%) was obtained by comparing the crystal area with the total area under the curve, before it was calculated as a percentage.

Thermal properties

Thermal properties were determined by a Differential Scanning Calorimeter (DSC-60Plus, Shimadzu, Japan) and TA-60WS collection monitor software following the method of Nwokocha et al. (2011) with slight modifications. Tacca tuber starch (4.0 mg) was prepared in a standard aluminum pan, and 9 µL of distilled water was added. The container was tightly closed, and the sample was left for 2 h, before the pot was heated at 30-110°C at 10°C/min under nitrogen flow.

Pasting properties

Pasting properties of tacca tuber starch were evaluated using a Rapid Visco Analyzer (RVA-4500, Perten Instruments, Australia) equipped with Thermocline for Windows 3 (TCW3) software referring to the method of Ratnaningsih et al. (2016) with slight modifications. A total of 3.5 g of starch (14% moisture basis) was prepared into the sample container tube, then 25 g of distilled water was added. The starch slurry was heated for 1 minute at 50°C, before increased to 95°C, with a heat rate of 5.2°C/min. The starch was held at 95°C for 5 minutes, then cooled to 50°C at a rate of 5.2°C/minute, and held at 50°C for 2 minutes. The stirring speed for the first 10 seconds was 960 rpm, and then the speed was maintained at 160 rpm for the remainder of the experiment.

Data analysis

All measurements (except SEM and particle size) are presented as mean ± standard deviation. Data from each treatment was subjected to analysis of variance (ANOVA), and significant differences between means were determined using Duncan Multiple's Range Test (DMRT) analysis at a confidence level of 95% using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Yield and chemical composition

The yield and chemical composition of tacca tuber starch are presented in Table 3, while the characteristics of the growth location of the tacca tubers at harvest are shown in Table 2. Garut tacca tubers yielded the highest starch content (26.42%), significantly exceeding Sumenep (23.19%) and Bangkalan (21.26%). Garut tacca tubers grow in sandy soil with low acidity and moderate lighting intensity (Table 2). Syarif et al. (2014), observed that tacca tubers thrive in diverse soil types, with a preference for sandy soil and a near-neutral pH. Sandy soil's macro-sized pores facilitate tuber formation. On the other hand, clay's density and higher water content restrict root growth and limit tacca tuber size (Erlinawati et al. 2018). Smaller tubers generally contain less starch (Binh and Dao 2020). Apart from the structure and composition of the soil, the lack of light availability during photosynthesis also affects the process of starch formation (Šimková et al. 2013). Tacca tuber starch's moisture content (9.25-10.29%) meets the recommended safe storage level of 13% (Tejavathi et al. 2020). The very low ash (0.17-0.21%), lipid (0.20-0.63%), and protein (0.42-0.83%) content further signifies the minimal presence of impurities, classifying tacca tuber starch as high-quality (Velásquez-Barreto et al. 2021).

The starch purity of tacca tubers in this study reached 97.35 to 98.48%, higher than that of Vietnamese tacca tubers (85.70%) reported by Vu et al. (2017). This variation in purity across locations aligns with prior observations for pigeon pea (A'yuni et al. 2021) and *Curcuma karnatakensis* (Tejavathi et al. 2020) starches. However, Estrada-León et al. (2016) emphasize the isolation method's greater influence on purity, with values exceeding 95% indicative of a highly precise technique (Fuentes et al. 2019). Furthermore, significant variation exists in the amylose content of tacca tuber starch, ranging from 32.81-35.26%, with the lowest found in Bangkalan samples. Notably, this is higher than previously reported values of 27.2-31.6% (Gwer et al. 2018; Nurhayati et al. 2022; Vu et al. 2018). While some literature suggests environmental factors do not impact starch amylose content (Seila et al. 2014; Pelpolage et al. 2016; Tappiban et al. 2020), others report the opposite (Guo et al. 2019; A'yuni et al. 2021; Shi et al. 2021). However, a majority of studies conclude that amylose content is influenced by both genetic and environmental temperature factors (Du et al. 2014; Li et al. 2014; Nhan and Copeland 2014). Low temperatures favor increased amylose synthesis, while high temperatures conversely decrease it (Shi et al. 2021). Notably, the average temperature during Garut tuber growth was the lowest (Table 1), coinciding with the production of high-amylose starch (Table 3). This suggests that the amylose content of tacca tuber starch is influenced by the location of its growth. According to Santoso et al. (2021), starch can be categorized based on its amylose content: low (<20%), medium (20-25%), and high (>30%). With its high amylose content, tacca tuber starch offers potential health benefits, particularly in the production of resistant starch (Ma et al. 2020).

Color properties

Consumer perception of color plays a vital role in product acceptance, and this principle extends to tacca tuber starch. As shown in Table 4, the Garut tacca tuber starch boasts the highest L* and WI values, indicative of its superior brightness compared to other samples. Notably, all analyzed tacca tuber starches exhibited remarkable whiteness, with average L* and WI values exceeding 90, consistent with Wang et al. (2020) definition of "bright white starch." This characteristic suggests a high-purity product with minimal impurities. Furthermore, the near-zero a* and b* values indicate negligible green and yellow hues, aligning with observations for Ceiba aesculifolia starch (Suastegui-Baylón et al. 2021) and Canna edulis rhizome starch (Deng et al. 2020). In comparison to commercial cassava starch, tacca tuber starch displays superior L* and WI values, minimal a* and b* values, and negligible color differences (ΔE*), confirming expectations. Consequently, the high L* and WI values of tacca tuber starch broaden its potential applications in food products where color plays a critical role. As Kim et al. (2018) and Akhila et al. (2022) highlight, companies prioritize starches with desirable color attributes, as they directly influence consumer preferences and market success.

Table 3. Yield and chemical composition of tacca tuber starches

Parameters	**Tacca tuber starch**		
	Bangkalan	**Garut**	**Sumenep**
Yield (%)*	21.26 ± 1.02^{a}	26.42 ± 0.89^{c}	23.19 ± 0.65^{b}
Moisture content (%)	9.25 ± 0.32^{a}	10.29 ± 0.21^{b}	10.10 ± 0.12^{b}
Ash (% db)	0.19 ± 0.03^{ab}	0.17 ± 0.03^{a}	0.21 ± 0.02^{b}
Lipid (% db)	0.63 ± 0.08^{c}	0.29 ± 0.03^{b}	0.20 ± 0.05^{a}
Protein (% db)	0.83 ± 0.03^{b}	0.42 ± 0.04^{a}	0.47 ± 0.04^{a}
Starch purity (% db)	97.35 ± 0.29^{a}	98.36 ± 0.51^{b}	98.48 ± 0.32^{b}
Amylose (% db)	32.81 ± 0.27^{a}	35.26 ± 0.19^{c}	34.35 ± 0.53^{b}

Note: Data are presented as means ± standard deviations. Values in the same row followed by the same superscript are not significantly different ($p < 0.05$). *Weight of starch/ weight of tacca tubers

Table 4. Color of tacca tuber starches

Tacca tuber starches	**Parameters**				
	L*	**a***	**b***	**WI***	**ΔE***
Bangkalan	92.01 ± 0.33^{a}	$- 0.37 \pm 0.12^{a}$	2.26 ± 0.11^{c}	$91.68 \pm 0,34^{a}$	1.68 ± 0.13^{a}
Garut	93.62 ± 0.21^{c}	$- 0.32 \pm 0.14^{a}$	1.94 ± 0.02^{b}	$93.32 \pm 0,21^{c}$	1.86 ± 0.10^{b}
Sumenep	92.94 ± 0.13^{b}	$- 0.45 \pm 0.02^{a}$	1.60 ± 0.14^{a}	$92.74 \pm 0,14^{b}$	1.92 ± 0.12^{b}

Note: Data are presented as means ± standard deviations. Values in the same column followed by the same superscript are not significantly different ($p < 0.05$)

Swelling power and solubility

The swelling capacity and solubility of tacca tuber starch in this study were measured at a temperature of 95°C. Nwokocha et al. (2011) previously reported that optimal development of tacca tuber starch can be obtained when heated at a temperature of 95°C. At lower temperatures, the starch crystal areas have not been wholly gelatinized, starch expansion will be limited and starch solubility will be disturbed (Shao et al. 2020). The swelling power of Tacca tuber starch ranged from 11.43 to 12.22 g/g (Table 3). Different growing areas show significantly different swelling power. The lowest swelling power was for Garut tacca tuber starch, and the highest was for Bangkalan tacca tuber starch. These results are lower than those reported by Nwokocha et al. (2011), who obtained the swelling power of Nigerian tacca tuber starch with a value of more than 25 g/g. Hydration and swelling of starch during heating reflect the degree of interaction between starch chains in amorphous and crystalline domains (Akarsha et al. 2022). The presence of the amylopectin component strongly influences the swelling power of starch. The stable double helix crystal region of the amylopectin component will bind water optimally during heating so that the starch granules expand more easily (Ahmed et al. 2015). Therefore, amylopectin is responsible for swelling power, while amylose is an inhibitor of granule swelling and maintains the integrity of swollen granules (Shao et al. 2020). Short-branched chain amylopectin content, high relative crystallinity and lower particle size have been reported to increase the swelling power of starch (Huang et al. 2015). Starch granules will expand as heating time and temperature increase (Akarsha et al. 2022). The ability of starch to swell is crucial, related to pasting properties and rheology (Wang et al. 2018). Starch with a swelling ability of less than 16 g/g is considered to have limited swelling behavior, which is very suitable for application in noodle products (Huang et al. 2015; Jan et al. 2017).

Solubility describes the number of starch molecules that dissolve during swelling. Tacca tuber starch exhibits a significantly higher solubility (13.96-15.33%) at 95°C compared to its counterparts. This surpasses the solubility of cassava starch (0.06-0.24% at 95°C; He et al. 2020), Chinese yam starch (7.80-11.10% at 95°C; Shao et al. 2020), and canna starch (7.89-13.54% at 90°C; Cáceres et al. 2021). Notably, its solubility is comparable to that of both white (12.80-15.30% at 95°C; Shi et al. 2021) and yellow (12.70-14.00% at 95°C; Guo et al. 2019) sweet potato starch. Starch solubility is strongly influenced by bond strength and structure, such as chain length in starch granules (He et al. 2020). The distribution of starch chain lengths can be different, and these differences can be influenced by genetic variations and the location of growth (Kim et al. 2018). Additionally, the presence of amylose has been reported to influence starch density (Deng et al. 2020). Denser starch granules cause starch solubility to decrease, generally found in starches with high amylose (A'yuni et al. 2021). When heated, high-amylose starches tend to become stiff and difficult to expand. As a result, the solubility of starch becomes low (Guo et al. 2019). The solubility and swelling power values of tacca tuber starch have a similar trend. Both parameters reflect the level of interaction between water and starch molecules (Buckman et al. 2018). There is a strong negative correlation between amylose and the swelling power and solubility of tacca tuber starch (Table 6). Due to its low solubility, tacca tuber starch can be used as a coating material for food products, especially vegetables and fruit (Guo et al. 2019).

WHC and OHC

Water holding capacity (WHC) and oil holding capacity (OHC) significantly differed between starch sources (Table 5), with Bangkalan tacca tuber starch exhibiting the highest values (WHC: 0.77-0.90 g/g, OHC: 0.62-0.71 g/g). WHC is an indicator of starch's ability to bind water content. Water binding to starch is a characteristic of the amylopectin component. Conversely, a high amylose content will reduce the ability of starch to bind water (He et al. 2020). WHC is also affected by other factors, such as variations in starch granule structure and the proportion of hydroxyl groups involved in forming covalent and hydrogen bonds (Ratnaningsih et al. 2016; Akarsha et al. 2022). OHC is also one of the functional characteristics of starch, which is very important when applied in food products, especially in taste retention, softness, and increased product palatability (Bhat and Riar 2016). Environmental factors can cause variations in OHC values during growth and the genetic makeup of each starch (Suma and Urooj 2015). While the presence of protein and lipid impurities can have some influence, their impact on OHC is generally considered minor (Rengadu et al. 2020). Notably, the WHC and OHC of tacca tuber starch are comparable to the WHC (0.89-1.02 g/g) and OHC (0.64-0.71 g/g) of potato starch (Ngobese et al. 2017), thereby highlighting its potential application as a thickening and stabilizing agent in liquid and emulsion-based food products (de Castro et al. 2019).

Table 5. Solubility, swelling power, WHC and OHC of tacca tuber starches

Tacca tuber starches	Parameters			
	Swelling power (g/g) 95°C	**Solubility (%) 95°C**	**WHC (g/g)**	**OHC (g/g)**
Bangkalan	12.22 ± 0.43^{b}	15.33 ± 0.18^{c}	0.90 ± 0.01^{c}	0.71 ± 0.04^{c}
Garut	11.43 ± 0.39^{a}	13.96 ± 0.48^{a}	0.77 ± 0.04^{a}	0.65 ± 0.01^{b}
Sumenep	11.53 ± 0.14^{a}	14.75 ± 0.14^{b}	0.80 ± 0.03^{b}	0.62 ± 0.02^{a}

Note: Data are presented as means ± standard deviations. Values in the same column followed by the same superscript are not significantly different ($p < 0.05$)

Table 6. Correlation test of amylose with functional properties of tacca tuber starch

Parameters	Pearson correlation	Sig. (2-tailed)	Correlation
Amylose with solubility	-0.777	0.000	Strong negative correlation
Amylose with Swelling power	-0.711	0.000	Strong negative correlation
Amylose with WHC	-0.832	0.000	Perfect negative correlation
Amylose with OHC	0.435	0.049	Moderate positive correlation

Note: The correlation test uses the Pearson correlation analysis method with the SPSS software

Table 7. Particle size distribution and relative crystallinity of tacca tuber starches

Parameters	Particle size of tacca tuber starch						Relative crystallinity (%)*
	d10 (μm)	d50 (μm)	d90 (μm)	Mean granule diameter (μm)	Small granule (μm)	Large granule (μm)	
Bangkalan	11.77	23.33	46.22	20.21	2.43	202.81	27.03 ± 1.16^{c}
Garut	28.47	48.94	83.99	44.71	8.23	275.18	24.22 ± 1.42^{a}
Sumenep	25.53	44.43	77.25	40.43	7.07	236.24	25.07 ± 1.36^{b}

Note: *Data are presented as means ± standard deviations. Values in the same column followed by the same superscript are not significantly different ($p < 0.05$)

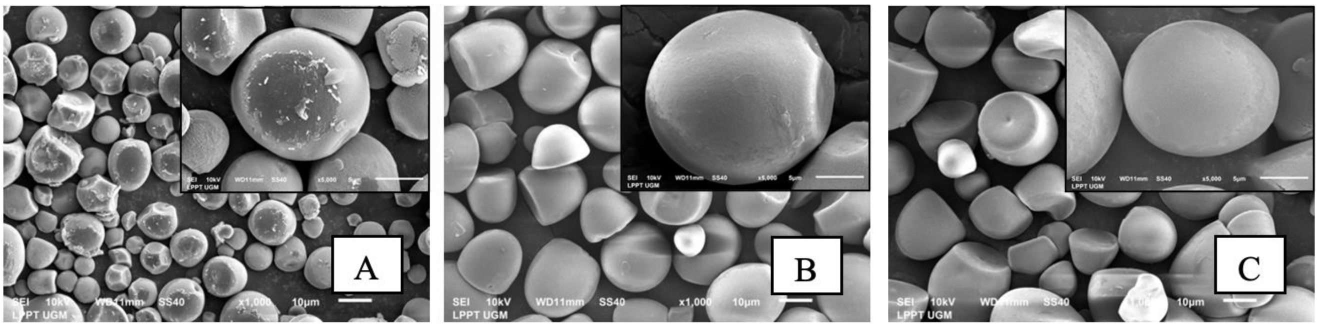

Figure 1. Scanning electron micrograph of tacca tuber starches from (A) Bangkalan; (B) Garut; and (C) Sumenep at 1000x and 5000x magnification

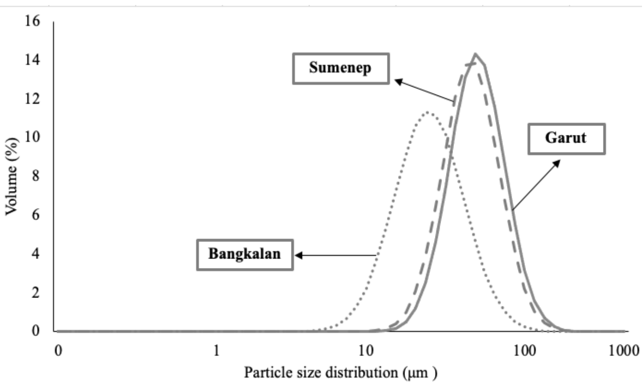

Figure 2. Particle size distribution of tacca tuber starches

Granule morphology and particle size distribution

The tacca tuber starch morphology was observed using SEM, and the findings are presented in Figure 1. Tacca tubers grown in different regions showed similar starch granular shapes due to their identical botanical origin (Guo et al. 2019). Most of the tacca tuber starch granules are polygonal, elliptical, and oval, and a few are ellipsoidal, similar to the morphology of water chestnut starch reported by Wang et al. (2018) and potato starch by Du et al. (2014). Bangkalan tacca tuber starch has a rough surface, and there are flakes around the granules, similar to the one reported by Estrada-León et al. (2016). The flakes around the starch granules are likely impurity components, such as remaining protein bodies (Palacios-Fonseca et al. 2013). According to Suastegui-Baylón et al. (2021), the fragmented debris around the starch surface is proteins and lipids. This reason is entirely rational, based on the data in Table 3. Tacca tubers from Bangkalan produce starch with higher levels of protein and lipids than those from Garut and Sumenep. Conversely, Garut and Sumenep tacca tuber starch presented a smooth surface, with some broken-out granules exhibiting a hemispherical shape. This observation aligns with Rodrigues et al. (2020) and suggests potential influence of the starch isolation process. Interestingly, Zabot et al. (2019) highlighted the superior expansion power of polygonal starch granules, while smooth-surfaced starch finds particular favor in polymer film applications.

Tacca tuber starch derived from three cultivation locations exhibited the smallest granules, ranging from 2.43 to 8.23 µm, and the largest granules, spanning from 202.81 to 275.18 µm (Table 7). Notably, a unimodal size distribution pattern was observed for all samples (Figure 2), mirroring findings for parotta starch (Estrada-León et al. 2016), potato starch, and sweet potato starch (Wang et al.

2020). The average granule diameter of tacca tuber starch ranged from 20.21-40.43 μm (Table 7), larger than that of Nigerian tacca tuber starch (12.32 μm) as reported by Nwokocha et al. (2011). In this study, the average diameter of Bangkalan tacca tuber starch was smaller than tacca tuber starch from Sumenep and Garut. Bangkalan tacca tuber starch contained less amylose and was more dominated by a crystalline structure. These results align with the report by A'yuni et al. (2021). Apart from amylose, molecular weight also influences the size of starch granules. Starch with high amylose and a larger molecular weight has a larger particle size as well. Previous findings revealed that starch granules with smaller size and lower molecular weight show high digestibility (Sukhija et al. 2016; Shao et al. 2020) and tend to have high solubility (Kumoro et al. 2021). The size of starch granules is crucial to study because it can affect the physicochemical properties of starch, such as solubility, enzyme resistance, solubility and crystallinity.

Crystalline structure

X-ray diffraction (XRD) analysis revealed a consistent crystallinity pattern among the Bangkalan, Garut, and Sumenep tacca tubers, indicative of the CA-type starch crystallinity. The main diffraction peaks are seen at 15°, 17° and 23° 2θ, further one shoulder peak at 18° 2θ (Figure 3). Based on the XRD spectrum, natural starches are usually grouped into A, B, and C types. Type A starch is characterized by strong peaks at 15° and 23° 2θ and imperfect peaks at around 17° and 18° 2θ, with orthorhombic structure. Type B starch has one strong diffraction peak at about 17° 2θ, and several smaller peaks are also seen at 15°, 22° and 24° 2θ, and a typical peak around 5.6° 2θ, with a hexagonal structure. Type C starch is a combination of types A and B, with a typical pattern having strong peaks around 17° and 23° 2θ, along with several small peaks at 5.6° and 15° 2θ, consisting of concurrently hexagonal and orthorhombic structures (He and Wei 2017; Cai and Wei 2013; Gupta et al. 2023). According to the proportion of crystallinity, type C starch is categorized into type CA (close to type A) with a shoulder peak around 18° 2θ and a strong singlet peak at 23° 2θ), type C typical, and type CB (close to type B) characterized by two the peaks of the shoulders are about 22° and 24° 2θ (He and Wei 2017; Lee and Lee 2017). Based on the XRD pattern, tacca tuber starches have CA-type starch crystallinity (Figure 3). The same crystallinity pattern was found in sweet potato starch (Zhang et al. 2018), arrowroot starch, cassava starch, corn starch, kidney bean starch (Wang et al. 2018) and pigeon pea starch (A'yuni et al. 2021). Type A starch forms and accumulates in tubers grown at high temperature environments of more than 33°C (Genkina et al. 2003) and type C starch can form in tubers grown at ambient temperatures of around 20-30°C (Shi et al. 2021). Tubers grown in a low temperature environment of around 15°C produce CC-type starch (Guo et al. 2020), while plants grown in a temperature environment of 25.94-28.69°C produce CA-type starch crystallinity (A'yuni et al. 2021). The results were the same as the crystallinity of tacca tuber starch (CA-type starch) grown in an environment with a temperature of 25.42-28.43°C (Table 1). The peaks of the XRD diffractogram can determine the phase of starch crystals. The peaks at 15°, 17°, 18° and 23° 2θ indicate that the starch contains an orthorhombic and hexagonal structure (Gupta et al. 2023; Rodriguez-Garcia et al. 2021). Even though they have the same XRD pattern, the relative crystallinity of each tacca tuber starch is different (Table 7). The crystallinity of Garut tacca tuber starch (24.22%) was the lowest, followed by Sumenep (25.05%) and Bangkalan (27.03%). Starches with low relative crystallinity generally contain more amorphous regions (high amylose) and larger particle sizes. Amylopectin has side chains that can form a crystal structure in starch, so the relative crystallinity is directly proportional to the amylopectin component (Oyeyinka et al. 2016). These results explain that environmental temperature does not affect the crystal structure of tacca tuber starch but does influence the degree of crystallinity significantly.

Thermal properties

During heating of the starch-water system, the crystalline structure of starch granules undergoes irreversible disruption. It causes irreversible changes in properties such as swelling of granules, melting of crystalline areas, loss of birefringence and solubility, which is called starch gelatinization (Akarsha et al. 2022). The temperature profile used to starch gelatinize was analyzed using DSC. The gelatinization of starch is reflected by the thermal properties, as seen in Table 8. Indonesian tacca tuber starch exhibited lower gelatinization onset (To = 57.92-62.06°C), peak (Tp = 60.83-65.95°C), and conclusion (Tc = 67.25-76.38°C) temperatures compared to Nigerian tacca tuber starch (To = 74.10-77.30°C, Tp = 76.20-80.10°C, Tc = 80.10-84.20°C) (Nwokocha et al. 2011). Several factors have been identified as influencing gelatinization temperature, including granule size, amylopectin molecular structure, amylose content, relative crystallinity, and the degree of order (Guo et al. 2019; Cáceres et al. 2021). The gelatinization enthalpy (ΔH) of tacca tuber starch was quite variable (3.81-4.62 J/g), slightly higher than that reported by Manek et al. (2005) 3.49 J/g, and lower than the report by Nwokocha et al. (2011) 13.3 J/g. The ΔH value reflects the loss of molecular order and gelatinization temperature of starch; a high ΔH indicates very high crystal structure stability. Amylopectin plays a significant role in starch granule crystallinity; as a result, ΔH has a negative correlation with amylose content (Gujral et al. 2013). This relationship is also observed in tacca tuber starch. Additionally, higher ΔH values are associated with the formation of longer and more double helices by the external chains of amylopectin (Zhang et al. 2018). A wider gelatinization temperature range (Tc-To) indicates a higher level of starch crystallinity and lower amylose content, factors that hinder water penetration (Pacheco et al. 2019). Interestingly, Bangkalan tacca tuber starch demonstrated the highest To, Tp, and Tc values compared to Sumenep and Garut varieties. Ratnaningsih et al. (2016) explained that a higher gelatinization temperature profile (To, Tp and Tc) represents better crystalline stability.

Table 8. Thermal and pasting properties of tacca tuber starches

Parameters	Tacca tuber starches		
	Bangkalan	**Garut**	**Sumenep**
Thermal properties			
Onset temperature/ To (°C)	62.06 ± 0.21^{a}	57.92 ± 0.13^{c}	59.13 ± 0.09^{b}
Peak temperature/ Tp (°C)	65.95 ± 0.60^{a}	60.83 ± 0.10^{c}	61.54 ± 0.40^{b}
Conclusion temperature/ Tc (°C)	76.38 ± 0.85^{a}	67.25 ± 0.45^{c}	68.79 ± 0.81^{b}
Gelatinization temperature ranges (°C)	14.33 ± 1.05^{c}	9.33 ± 0.72^{b}	9.66 ± 0.58^{a}
Gelatinization enthalpy/ ΔH (J/g)	4.62 ± 0.25^{c}	3.81 ± 0.15^{a}	4.27 ± 0.33^{b}
Pasting properties			
Pasting temperature/ PT (°C)	72.12 ± 0.06^{a}	72.88 ± 0.03^{b}	72.66 ± 0.20^{b}
Peak viscosity/ PV (cP)	5027.00 ± 22.27^{c}	4882.33 ± 14.05^{a}	4905.67 ± 10.60^{b}
Through viscosity/ TV (cP)	1555.00 ± 38.74^{c}	1471.33 ± 20.50^{a}	1480.67 ± 72.27^{b}
Breakdown viscosity. BV (cP)	3472.00 ± 60.51^{a}	3411.00 ± 19.47^{a}	3425.00 ± 68.44^{a}
Final viscosity/ FV (cP)	2606.00 ± 12.00^{a}	2791.00 ± 10.44^{c}	2661.33 ± 25.70^{b}
Setback viscosity/ SV (cP)	1051.00 ± 44.91^{a}	1319.67 ± 23.76^{c}	1180.67 ± 89.69^{b}

Note: Data are presented as means ± standard deviations. Values in the same row followed by the same superscript are not significantly different ($p < 0.05$)

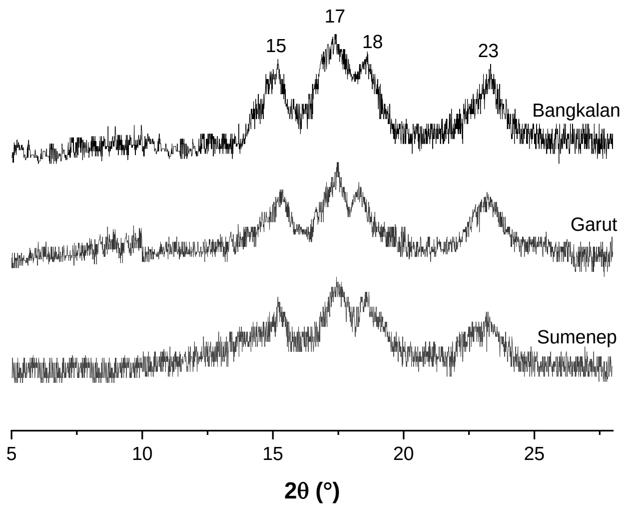

Figure 3. X-ray diffraction pattern of tacca tuber starches

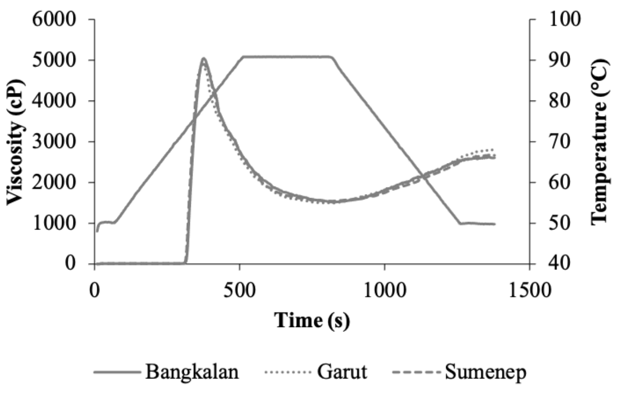

Figure 4. Pasting behavior of tacca tuber starch

Therefore, starches with high amylose content all showed lower gelatinization temperatures. This observation is consistent with the general trend of lower gelatinization temperatures in starches with higher amylose content. It is noteworthy that environmental temperature during growth may influence the thermal profile of starch, with higher temperatures tending to produce starches with higher thermal profiles (Shi et al. 2021). This suggests a potential link between environmental conditions and the thermal properties of tacca tuber starch.

Pasting properties

Tacca tuber starches from different growth areas had the same pasting profile (Figure 4) but had significantly different characteristics except for breakdown viscosity (Table 8). The pasting temperature is the minimum temperature level required to cook starch suspension (Sudhees et al. 2019). Pasting temperature of tacca tuber starch ranged from 72.12 to 72.88°C, lower than Nigerian tacca tuber starch (77.89°C) reported by Ameen et al. (2018). The high pasting temperature indicates that the tacca tuber starch is more resistant to swelling and breaking. Garut tacca tuber starch showed the highest pasting temperature, consistent with the lowest starch swelling power (Table 5). Amylose plays an essential role in the pasting temperature of starch, generally having a positive correlation (Huang et al. 2015). Peak viscosity is the maximum viscosity of starch when heated with water during gelatinization. This is related to the water retention ability of starch granules (Sudhees et al. 2019). The peak viscosity of tacca tuber starch in Bangkalan, Sumenep and Garut were 5027.00, 4905.67 and 4882.33 cP, respectively. The peak viscosity of Bangkalan tacca tuber starch was the highest. Low amylose content and higher molecular weight contribute to the high peak viscosity. In addition, the ultralong chains in amylopectin enhanced particle integrity, promoting the potential to produce more ordered and stable double helical crystallites (Shao et al. 2020). The stable double helix crystals of the amylopectin component will optimally bind water during heating; the swollen starch granules will be held before breaking when the heat is increased (Shi et al. 2021).

Breakdown viscosity serves as a crucial indicator of the resistance of the paste to heat. A lower breakdown viscosity indicates a higher ability to withstand heating (Zhang et al. 2018). The breakdown viscosity of tacca tuber starch was exhibited a narrow range, with no significant difference, oscillating between 3411.00 and 3472.00 cP. The breakdown viscosity of tacca tuber starch was lower than arrowroot starch (4104 cP), cassava starch (4901 cP), and potato starch (8360 cP) as reported by Wang et al. (2018). The low breakdown value indicates that the starch granules have cohesive strength and excellent thermal and shear stress stability (Sudhees et al. 2019). The final viscosity of tacca tuber starch was 2606.00 to 2791.00 cP. The final viscosity shows the amylose molecules reassembling to form a gel (Pineda-Gomez et al. 2021). The final viscosity is the viscosity of starch after cooling and indicates the stability of the cooled starch paste or gel (Guo et al. 2019). Starch with a high final viscosity behaves like a custard, making it suitable for infantile formulations (Gutiérrez-Cortez et al. 2021).

The setback viscosity, a tendency for starch paste to decline due to amylose rearrangement during cooling (Suastegui-Baylón et al. 2021), was significantly higher in Garut tuber starch (1319.67 cP) compared to other starches. This high setback viscosity indicates a propensity for greater retrogradation and hard gel formation (Zhang et al. 2018). This retrogradation is highly desirable for the production of resistant starch type 3 (RS3), highlighting Garut tacca tuber starch's potential in this application. Notably, observation data reveals a significant influence of cultivation location on the RVA profile of tacca tuber starch, echoing similar findings regarding sweet potato root tuber starch from different locations (Shi et al. 2021). In conclusion, a notable diversity in physicochemical, structural, and functional characteristics is observed amongst tacca tuber starches sourced from three Indonesian locations. This disparity manifests prominently in proximate composition, purity, amylose content, granule size, and relative crystallinity. These variations further influence the color profile, thermal properties, paste properties (except breakdown viscosity) and functional properties (swelling power, solubility, WHC and OHC) of starch. Notably, both granule shape and X-ray diffraction patterns remain consistent across all samples. Further investigations into modifying tacca tuber starch, through physical, chemical, enzymatic, or combined approaches, are crucial to unlocking its full potential for food industry applications.

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