BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, June 2024. Vol. **21**(2), p. 789-803

Effects of Extraction Methods on the Functional and Morphological Characterization of Mango Seed Kernel Starch

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http://dx.doi.org/10.13005/bbra/3265

(Received: 19 February 2024; accepted: 21 June 2024)

The mango, or Mangifera indica L., is India's national fruit. The by-products released during the processing of mangoes are peels and seeds. This study sought to understand the effects of starch extraction on the pasting, thermal, and morphological properties of mango seed kernels. Three distinct methods such as sedimentation, centrifugation and alkali methods of starch separation was used as per standard procedures. The ability of sedimentation starch had highest water binding capacity (90.2%) and alkali starch had high paste clarity (78.2%) among the isolation methods. The pasting properties of mango kernel starch indicated significant differences across each extraction method. The gelatinization and conclusion temperatures of the alkali method were high (63.4°C and 65°C, respectively) while having a low start temperature (30.1°C) and enthalpy (4.0J/g). The thermal properties of alkali process had a low onset temperature of 30.1°C and low enthalpy of 4.0 J/g, but a high gelatinization temperature of 63.4°C and conclusion temperature of 65.2°C. The starch structures could be easily observed under a light microscope and the alkali method of starch revealed a slight bulkiness in them, it was still possible to detect the shape of the starch granules. Despite using various extraction methods, the isolated mango seed starch SEM granules had smooth surfaces and oval, crooked, or cuboidal shapes. It strongly suggested that the alkali method used for starch extraction improved the starch yield from seeds and enhanced pasting, thermal, and morphological starch characteristics. These results proved the possibility of the utility of employing the alkali method of starch extraction as a functional element in food product compositions.

Keywords: Extraction; Kernel; Mango seed; Morphological; Pasting; Starch; Thermal.

In cereal crops, starch plays a major role in determining crop output and quality. Starch makes up roughly 70% of cereal grains. Near pollination, the starch synthesis is highly susceptible to unfavorable conditions[1]. Glycosidic linkages bind a vast number of glucose units together to form the carbohydrate known as starch. It is a material found in plants that serves as a food reserve and is extensively employed in the medical field. Additionally, it's employed as a food ingredient, mostly as a stabilizer and thickener. Additionally, gel and ethanol can be made with it. The separation of starch from a range of food sources, including potatoes, maize, corn, and rice, and its use in the food and non-food industries, have been the subject of much research[2].

Mango kernels have 65% carbohydrates, 2.9% reducing sugars, 5.7% protein molecules, 0.8% pectin, 9.3% lipids, and 1.1% tannins by dry weight. The remainder is made up of the remaining

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*Corresponding author E-mail: parimala1996@gmail.com moisture[3]. The mango seed kernel that remains after decortication is added to wheat flour or utilized to extract edible oil[4]. In addition to being used as animal feed, mango seed kernel flour can also be consumed. Regarding the separation of starch from mango seed kernels, very little information is available. The significant concentration of carbohydrates and oil in mango seed kernels makes them a potential seed[5].

According to the plant source of information, natural starches can have a variety of mechanical characteristics, such as variations in shape, mass, the proportion of amylose to amylopectin, patterns of crystallization, gelatinization features, and rheological or water retention[6]. These properties of starch are associated with the ratio of amylose to amylopectin and the degree of branching, which controls the gelatinization and rheological potential of the starch and, consequently, the resulting stiffness and viscosity[7]. To increase its usefulness, natural starch can also be chemically, physically, or enzymatically modified. Because of this, characterizing natural or modified starches of which gelatinization and rheological properties are the most crucial is essential to figuring out potential applications.

Starch, one of the primary macronutrients, influences food's flavor, texture, and processing qualities as well as its health impacts. However, it also serves as a new polymeric material for the industry and is used for encapsulating, thickening, and gelling among other industrial uses[8, 9]. Nowadays, starches derived from many plant sources are employed in industrial settings. After years of extensive research, potato starch is now used as a raw material for producing biodegradable plastic goods[10].

Corn starch is widely utilized in the food sector as the primary starch added as a thickening agent in processed foods[11]. Furthermore, starch-rich matrices can be utilized as substitute substrates in the manufacturing of fermented foods and beverages[12,13, 9]. Because of waste, which motivated researchers to conduct the current study. The study aimed to study the pasting, thermal, paste clarity, and morphological characteristics of starches separated from the Sindhoora variety of mango kernel.

MATERIALS AND METHODS

Materials

Good quality Mangoes (*Mangifera indica* L.) were acquired from a neighbourhood market in Krishnagiri, Tamil Nadu. The kernel of the mango seed was carefully removed, washed, and allowed to dry at room temperature. The dry kernel was finely powdered and the laboratory-based analysis was conducted at Bio Vision laboratory in Tanjore, Tamil Nadu, India. Standard procedures were followed in the starch analysis of the following list.

Starch extraction methods

Centrifugation method

The mango seed kernel starch was isolated according to the procedure described by Hassan et al. (2017)[14] with minor modifications. Initially, 50 g of mango seeds were steeped for 24 hours at 50°C in an aqueous solution containing 0.16% sodium hydrosulfide hydrate. After that, the fluid was decanted, and a household blender was used to grind the seeds. The pulverized slurry was re-slurried in distilled water for an hour after being filtered through a cotton bag with a mesh size of around 200ìm. The settled starch layer was then re-suspended in distilled water after the supernatant had been removed from the filtrate. Ultimately, the starch underwent a 30-minute centrifugation at 5000rpm and a 6-hour oven drying process at 50°C. Using a mortar and pestle, the starch was pounded, sealed in a plastic bag, and stored at room temperature until needed.

Sedimentation method

With a few minor adjustments, the Oates and Powell (1996)[15] method was used to separate the starch from mango seeds kernel. First, 50 g of mango seed kernel powder was pulverized in a kitchen blender after being soaking in water for the entire night. Subsequently, the slurry was passed through a cotton bag with a mesh size of approximately 200 µm, and the filtrate was set aside to separate the starch. The starch was then re-slurried three times in deionized water and sedimented. The finished product was allowed to settle for three hours in 0.1 M sodium chloride (NaCl) and 1/10 volume of toluene. After a thorough washing with distilled water, the starch was finally dried for 24 hours at 50 °C in the oven. Using a mortar and pestle, the starch was pounded, sealed in a plastic bag, and stored at room temperature until needed.

Alkali method

With minor adjustments, the method described by Noor et al. (2014)[16] was used for extracting the starch from mango seeds kernel. First, five grams of mango seed kernel powder were added to a 0.5% sodium hydroxide (NaOH) solution and stirred continuously for six hours at room temperature. Subsequently, the mixture was sieved through a cloth bag with a mesh size of approximately 200 µm, and any leftover material was repeatedly rinsed with distilled water. Next, at 4 °C, the filtrate was combined and allowed to precipitate overnight. After filtering, the resulting starch was dried for 24 hours at 40 °C in an oven. Using a mortar and pestle, the starch was pounded, sealed in a plastic bag, and stored at ambient humidity until needed.

Functional properties of starch

Water Binding Capacity (WBC)

The modified Medcalf method was utilized to compute the WBC. After an hour of agitation, a suspension of 3 g (dry basis) starch in 60 ml distilled water was centrifuged for 10 minutes at 3200 rpm. After removing the extra water, the precipitated material was weighed, and the WBC value was ascertained[17].

$$\text{WBC} = \text{Weight of wet starch (g)} / \text{Weight of starch.}$$

Paste clarity

Paste clarity was determined as the transmittance of a 1% starch paste measured at 650 nm. For thirty minutes, the starch dispersion was heated to boiling at 98°C, to fully gelatinize the granules. After allowing the pastes to cool to 20°C, the rate of transmission was determined (%). The outcomes are three repetitions' averages[18].

Pasting properties

A Rapid Visco-Analyzer (RVA) (model: RVA 4800; manufacturer: Perten Instruments, Australia) was used to examine the pasting properties. Employing starch–water suspensions (28g total matter on a dry starch basis), viscograms of starch were tracked. The same temperature and time constraints were used for testing each suspension[19].

Thermal properties

DSC-Q200 equipment (TA-Instruments, EUA) was used to carry out the DSC analysis. First, 99.99% pure indium was used to calibrate the apparatus (melting point (mp) = 156.6ºC, ÄH = 28.56 J g-1). The curves were acquired to investigate the starch's gelatinization process. The aluminium crucibles with hermetic lids, the heating rate of 10ºC min^{-1}, the airflow of 50 mL min^{-1}, and the approximately 2.5 mg samples were the conditions of the analysis. Following the sample weight measurements, 10 µL of water was added using a micropipette to achieve a 4:1 water: starch, w/w ratio. The aluminium crucibles were then sealed and left in that state for half an hour[20].

Morphological characteristics of starch

Light Microscopy

The size of the droplets and internal arrangement of the emulsions (about 50µL) were seen using a 100× magnification lens on a Primo Star optical microscope (Carl Zeiss Primo Star Microscopy GmbH, Jena, Germany). Images were taken using an optical microscope and a DCMC310 digital camera with Scope Photo software (Version 3.1.615) from Hangzhou Huaxin Digital Technology Co., Ltd., Zhejiang, China[19].

Scanning Electron Microscopy

Starch granules were photographed using a scanning electron microscope, a type of microscopy apparatus. Samples of starch were gold-coated and affixed to aluminium stubs using double-sided adhesive tape. An accelerating potential of 5 kV was used during the microscopy process[19].

Data analysis

The SPSS 25 software was utilized to compare the sample averages with a 95% confidence interval ($p<0.05$) by employing analysis of variance (ANOVA) and Tukey's test. Every analysis was carried out three times.

RESULTS AND DISCUSSION

The results on functional and morphological characteristics of starches were discussed in the below headings.

Functional characteristics of starch

Water binding capacity

Figure 1 shows the mango seed kernel starch's propensity to bind to water. Despite sedimentation methods of extraction displaying the maximum water binding capacity (90.2%) than centrifugation (81.7%) and alkali (80.4%) methods. The sedimentation process was more effective at binding water and had a reduced propensity to dissolve in it. The starch sedimentation method was able to bind water more efficiently than other ways because of its mean particle density, which is similar to that of water. The capacity to absorb water is significantly increased when the content of starch rises. This rise is also influenced by the starch's grain size. The starch's ability to absorb water rises with increasing starch content. The reason for this increase is that there are more particles in the reaction medium, which means that more water will be absorbed. There would be more hydrophilic groups that is, proteins and starch in the reaction media. This was the reason for the high-water binding capacity in sedimentation method of extraction. The settling of particles that accelerate unsteadily requires a dynamic model that incorporates the Bassett history force. For characteristics like swelling, viscosity, and gelation to be expressed, a material must have a high-water binding capacity. According to Lakshmi et al. (2016)[21], a good water absorption capacity suggests that the flour might be utilized as a liquid thickening because it can absorb water and swell. The lipophilic surroundings containing fat and protein were responsible for the high-Water Absorption Capacity (WAC). Protein's capability to absorb water is crucial for the baking of dough and cookies, among other food products[22].

According to Okpala et al. (2013)[23] investigation of the water absorption capacity of mango seed cultivars, they had a water absorption capacity of 2.0g/g, which was higher than that of mango seed kernel (0.92g/g). The varying abundance of water binding sites across starches was the cause of the differences in WBC between various starches[24]. As per Tan et al. (2013)[25], the ability of starch to hold water is mainly influenced by the quantity of hydroxyl groups present in the molecule. When there are more exposed hydroxyl groups, the starch molecule can easily bind with water molecules through hydrogen bonding, resulting in a higher water-holding capacity. The amount of hydroxyl groups can vary depending on a number of parameters, including polarity, hydrogen bonding, chirality, resonance, electronegativity, and boiling temperature. This statement is also supported through this investigation.

Pasting clarity

Research has shown that the clarity of starch pastes is influenced by their chemical composition and amylose content, as well as the dispersion of starch granules[26]. In the context of starch extraction, two methods have been compared: centrifugation and alkali extraction. The comparative analysis revealed that the

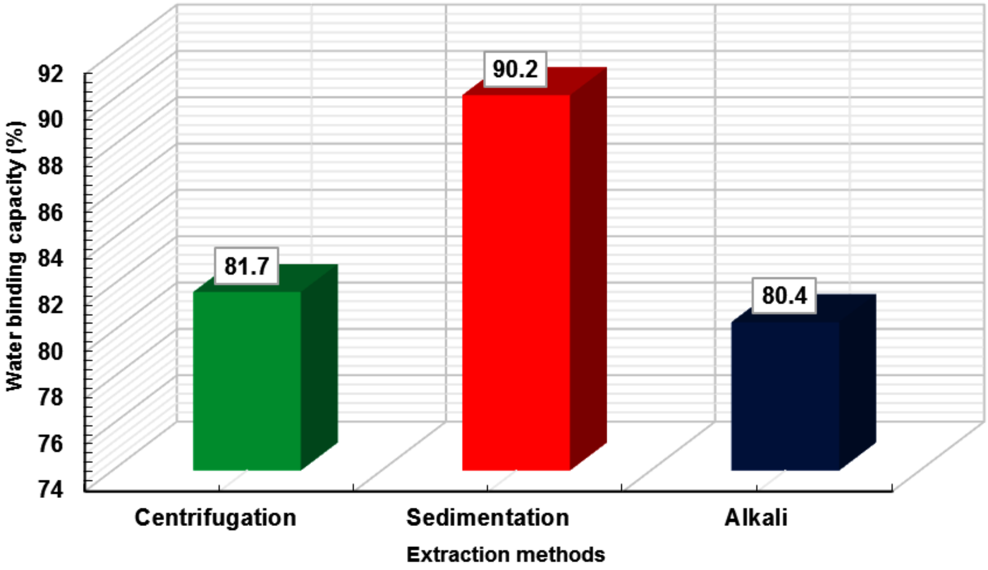

Fig. 1. Water binding capacity (%) of starch

centrifugation method produced a significantly lower light transmittance (% T) than the alkali extraction method, as illustrated in Figure 2. It is noteworthy that starch granules with high amylose levels tend to retain the integrity of granule remnants, allowing less light to pass through the paste. Therefore, this outcome can be attributed to the preservation of granule remnants in the centrifugation method, which resulted in lower light transmittance. The technique of centrifugation yielded a lower paste clarity, which is by the observations made by Hsieh et al. (2019)[27]. However, the underlying reasons for this outcome remain unclear, given the high solubility power and absence of amylose demonstrated by the centrifugation method. On the other hand, with the exception of the alkali approach, the paste clarity of starches increased with the sedimentation method as well. This finding is consistent with a study conducted by Craig et al. (2009)[28], which found that the addition of sucrose enhanced starch paste clarity by augmenting the refractive index of the solution surrounding swollen granules, thereby reducing light refraction. Moreover, sucrose was observed to inhibit the association of starch chains, resulting in lower whiteness[29]. Considering the degree of gelatinization fluctuates with different extraction techniques and is correlated with the stability of the starch, extraction techniques can have an impact on the amylose concentration of starch. The granular and crystalline structure starts to disintegrate as soon as the initial gelatinization temperature is attained, and the amylose slowly seeps out.

A study conducted by Rahul and Kawaljit (2017)[30] found that the paste clarity values of litchi

Table 1. Pasting properties of starch

Extraction methods	PV (cP)	TV (cP)	BV (cP)	FV (cP)	SV (cP)	PT (°C)
Centrifugation	2622	1600	1022	2738	1138	81.7
Sedimentation	2373	1234	1139	2281	1247	78.6
Alkali	2594	1580	1014	2854	1047	75.8

PV: peak viscosity, TV: trough viscosity, BV: breakdown viscosity, FV: final viscosity, SV: set back viscosity, PT: pasting temperature.

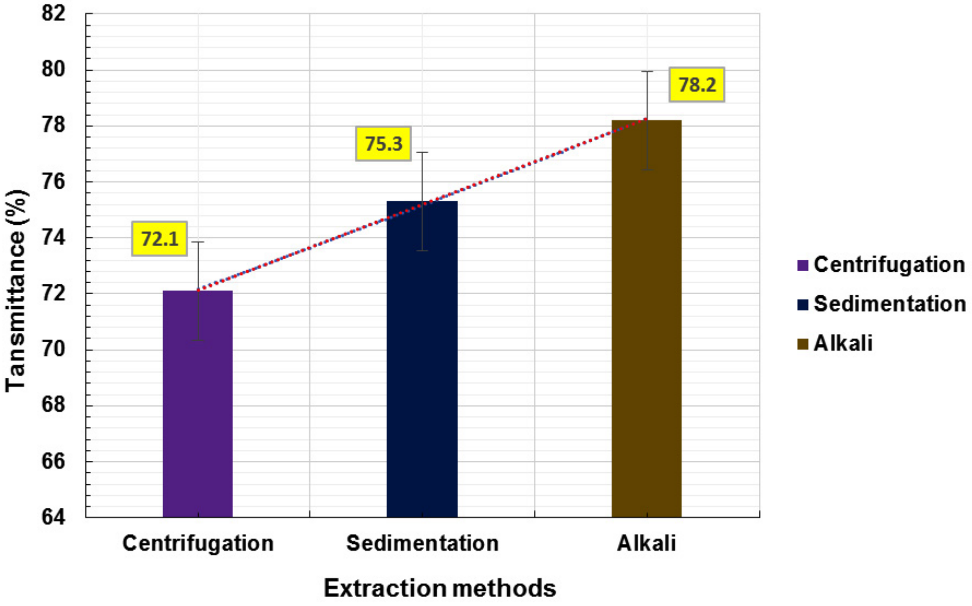

Fig. 2. Light Transmittance (%T) of starch

and mango kernel starches decreased significantly after 120 hours of storage at 4ºC. Specifically, the paste clarity of litchi starch decreased from 5.66% to 0.46%, while the paste clarity of mango kernel starch decreased from 8.96% to 1.16%. In this study, the measured paste clarity at a cooling temperature of 20ºC using sedimentation methods and found a high transmittance level of 78.2% during extraction. According to Sandhu and Singh (2007)[31], paste clarity is a representation of how starch paste behaves when light travels through it, and it is dependent on swollen and non-swollen granule remains. In contrast to water steeping (SBS) and enzyme extraction (EBS), Zhang et al. (2019)[32] reported that their investigation found that the starches recuperated from the bulbils of *Dioscoreae opposita thunb. cv. Tiegun* employing alkaline extraction (ABS) had a higher paste clarity. Because ABS has a higher amylose content and a higher swelling power value, it has superior clarity. The shorter, lower molecular weight chains of starch may also contribute to the increased light transmittance. Heating-induced alterations to the granular and molecular structure may cause the starch granules to penetrate and absorb, which will ultimately cause the starch to swell more and transmit more light. In consequence of the solubilized starch chains' molecular realignment, the paste clarity of various starches reduced as storage times increased (Ali and Hasnain, 2014)[33]. Pastes made from starch can be considerably more transparent when removed using alkaline techniques as opposed to water. Starch with distinct rheological characteristics and a greater gel strength can also be obtained by alkaline extraction techniques.

The study conducted by Alvani et al. (2011)[34] unveiled that potato starches possess a high pasting clarity and a neutral flavor. The high concentration of phosphate esters on the amylopectin chain is believed to be the underlying cause for this clarity, as indicated by Simková et

Table 2. Thermal properties of starch

Isolation method	T_o (ºC) onset	T_P (ºC) Peak	T_C (ºC) End set	R (Tc-To)	ΔH_{gel} (J/g)
Centrifugation	62.2	64.6	68.5	6.3	4.3
Sedimentation	60.2	63.2	64.1	3.9	4.2
Alkali	30.1	63.4	65.2	5.1	4.0

T_o – onset temperature, T_P – Gelatinization temperature; T_c – conclusion temperature, ΔH – enthalpy temperature

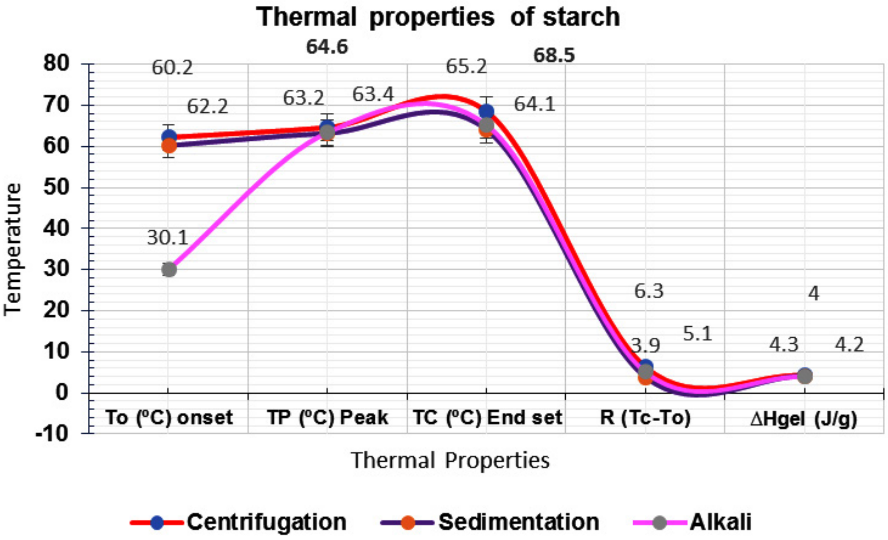

Fig. 3. Thermal properties of starch

al. (2013)[35]. According to Vasanthan et al. (1999)[36], potato starch is a remarkable and unique ingredient due to its granule dimensions, purity, amylose, and amylopectin chain measurements, the ability to transfer specific cations with corresponding effects on rheological behavior, and the capacity to generate thick viscoelastic gels on heating and subsequent cooling. This research study on mango kernel starch pasting clarity has also yielded results that are consistent with the existing literature.

Pasting properties

The measurement of starch paste viscosity is a crucial aspect of studying starch pasting properties and the effects of food ingredients on starch performance. Rapid Visco Analyzers (RVA) are commonly used for this purpose[37]. The food industry often utilizes starch due to its viscosity changes during heating and cooling[38]. Table 1 demonstrates the pasting properties of mango kernel starches, with the centrifugation method showing high peak viscosity, tough viscosity, and pasting temperature compared to sedimentation and alkali methods. The centrifugation process yielded pasting values of 2622cP, 1600cP, and 81.7ºC respectively. When evaluating the properties of starches, peak temperature is an important parameter, as it represents the point at which the temperature rises above the gelatinization temperature, leading to significant absorption of water and an increase in size due to granule swelling. This results in higher viscosity[39]. The centrifugation process attained the highest peak viscosity, indicating the maximum viscosity reached during heating. Achieving optimal results in granule processing hinges on maintaining equilibrium between the rate of granule swelling and its decomposition[40, 41].

After experimenting with three distinct extraction procedures (alkali, centrifugation, and sedimentation), it was discovered that the alkali approach produced starches with greater tough viscosity and breakdown viscosity values than the other two. Mango kernel starches had tough viscosity and breakdown viscosity values of

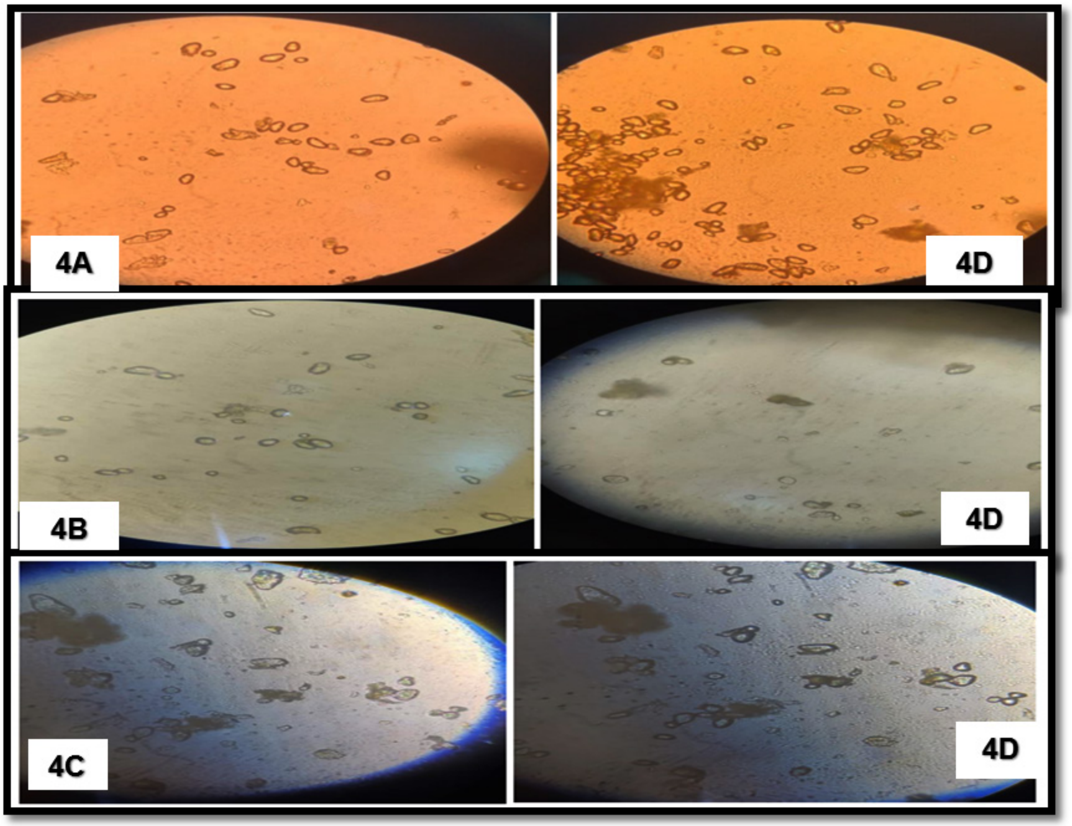

Fig. 4. 4A -Centrifugation starch, 4B- Sedimentation starch, 4C – Alkali starch & 4D – Magnification 400x

1234cP and 1139cP, respectively. The viscosity rose with increasing temperature, possibly due to the granules absorbing water from the emitted amylose as they expanded. According to research by Majzoobi et al. (2003)[41] and Sandhu et al. (2007)[42], the effectiveness of starch paste

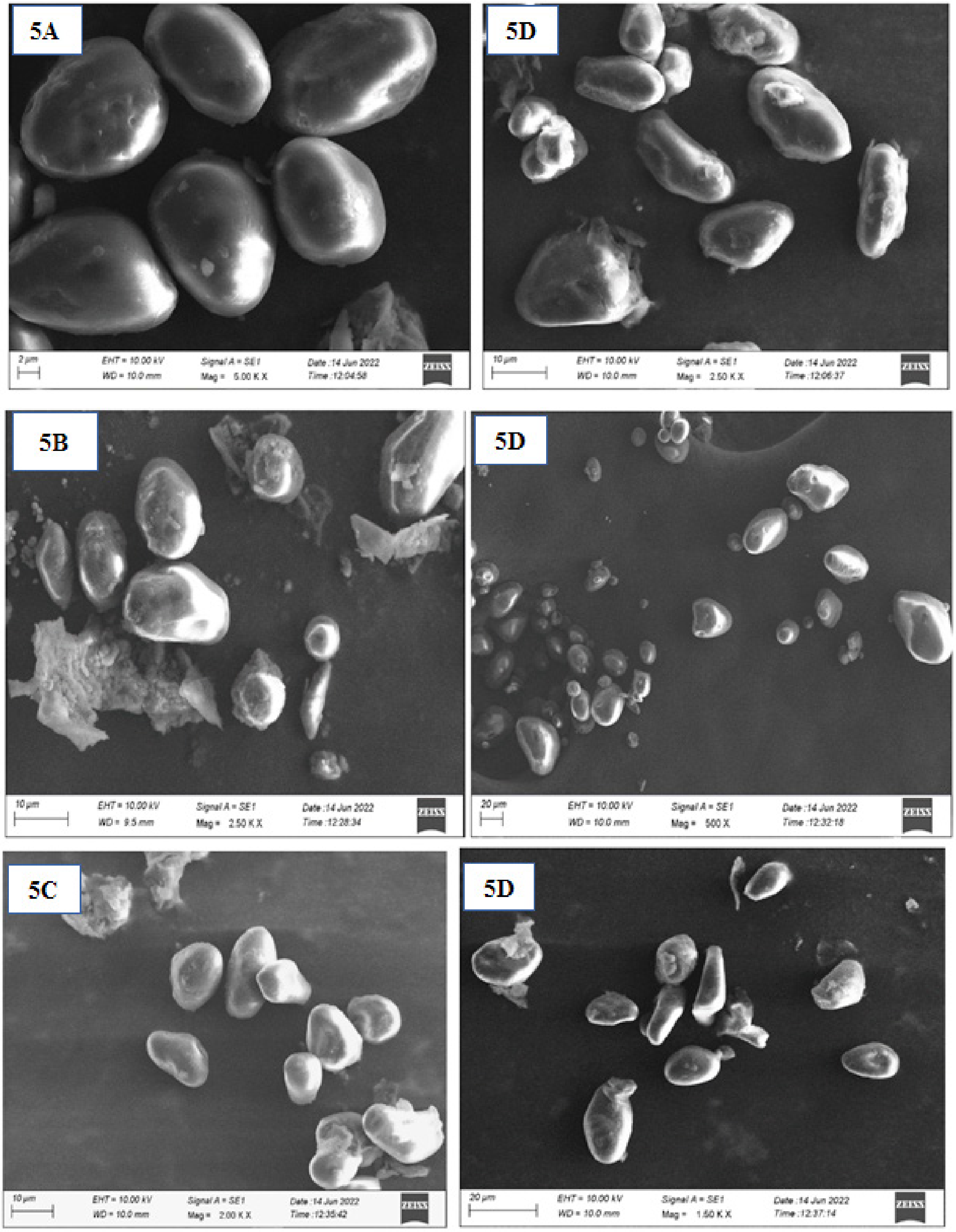

Fig. 5. Scanning Electron Microscope (SEM) of mango kernel (*Mangifera indica* L.) 5A -Centrifugation starch, 5B- Sedimentation starch, 5C – Alkali starch & 5D – Magnification figures

is affected by the concentration of amylose and the distribution of amylopectin chain lengths. Longer chains experience intra-and intermolecular entanglements which cause increment in the viscosity of the starch.

The breakdown viscosity (BV) of starch granules is an indicator of their organization, showing how quickly they disintegrate. A low breakdown viscosity value indicates that the granules are not easily agitated during heating. Bashir and Aggarwal (2017)[43] discovered that sedimentation procedures had a lower breakdown viscosity value (1247cP) than centrifugation and alkali methods. This means that sedimentation-derived starch has the weakest physical structure and is more likely to collapse after heat treatment and shearing. As a result, the alkali technique is utilized to improve starch durability under these conditions.

The alkali method showed a lower setback viscosity of 1047cP compared to the centrifugation and sedimentation methods. A low setback can affect the suitability of desserts, sauces, yogurts, emulsified products, and frozen foods, as retrogradation may cause eminence issues during storage owing to water loss[44]. The sedimentation and alkali extraction methods showed lower pasting temperature values (78.68ºC and 75.8ºC, respectively) compared to the centrifugation method (81.7ºC). The pasting temperature readings among the approaches varied only slightly. The lower pasting temperature values demonstrated a reduced level of connection in the granules' amorphous regions. Higher pasting temperature values are preferred for the preparation of soups, gravies, baked products, and canned foods[45]. Reduced retrogradation is important because it improves starch stability during storage and enables greater use in the food sector[46].

Verwimp et al. (2004)[47] investigated the effects of starch isolation conditions on the production, composition, and physicochemical characteristics and pasting of rye starch. The two techniques used by these authors to isolate rye starch were pronase-based isolation and alkaline extraction. According to the study's findings, the pronase-based process produced 81% of the rye starch yield, while the alkaline extraction approach produced only 42%. Buksa (2018)[48] observed similar results and attributed this difference to the rye starch granules' bimodal distribution.

Gelatinization is a vital property for starch-based food processing. The pasting curves for mango kernel starch extractions were different (Figure 3) and the related parameters are listed in Table 1. In the presence of water (the temperature of the whole system was above the gelatinization temperature), the starch particles were swelled, all the molecular order disappeared, and amylose and amylopectin began to exude. At this stage, the particles were susceptible to mechanical stress, i.e., shearing led to particle rupture, dispersion of amylose and amylopectin, and formation of the paste[49]. Jane et al. (1999)[50] found that starches with a lower beginning gelatinization temperature had the highest proportion of short chains and the shortest average amylopectin branch chain length. The results obtained imply that the gelatinization of the more unstable crystallites may be responsible for the first peak. It's possible that the drying process damaged the short amylopectin chains that make up these crystallites. Water begins to break apart and produce mobility among the chains of amylopectin that make up the crystalline portion when starch is cooked in the presence of water. The remaining amylopectin chains begin the gelatinization process when the least stable crystallites melt and remove the barriers to water penetration. Consequently, amylopectin chains that were not impacted by the drying process may be responsible for the second peak. According to Dries et al. (2016)[51] experiments, the last transition, which has a temperature peak of 63.4°C and a beginning temperature of 30.1°C, can be linked to the melting of the V-type crystals that the amylose-lipid complex forms.

Thermal properties

The properties of starches are influenced by their molecular structures, particularly the arrangement of short amylose chains (á 1-4 linked linear (unbranched) glucose polymer). These properties include onset temperature, gelatinization temperature, conclusion temperature, and gelatinization enthalpy, as shown in Table 2. Among the different processes used, centrifugation resulted in the highest onset temperature of 62.2°C, high gelatinization temperature of 64.6°C, high conclusion temperature of 68.5°C, and high enthalpy of 4.3 J/g. Sedimentation also had a high onset temperature and enthalpy, while the alkali process had a low onset temperature of 30.1°C and low enthalpy of 4.0 J/g, but a high gelatinization temperature of 63.4°C and conclusion temperature of 65.2°C.

In a study by Chakraborty et al. (2022)[52], it was noted that a low enthalpy with peak gelatinization indicates a low amount of energy required to break down the intermolecular bonds of starch molecules in the presence of water and heat, with the peak temperature occurring at a relatively low point. The molecular structure of amylopectin and the ratio of amylopectin to amylose also play a role in determining the gelatinization properties[53].

There are three noticeable transitions were present in Table 2 and Fig 3. Furthermore, two transitions near the temperature of gelatinization were found. According to Kaur et al. (2004)[54], the cooperative melting peak is the first peak, while the "true" melting process is the second. The starch granules' absorption of water is responsible for both of their presence. The first transition has an onset temperature of 60.2°C and a peak at 63.2°C, the second has an onset temperature of 62.2°C, and a peak at 64.6°C. The onset temperature for gelatinization attributed to the first transition (sedimentation method) is considered low when compared to alkali method of extraction starch. It was found higher temperatures for the gelatinization of 5 mango cultivars from India with similar water content analysed by Kaur et al. (2004).

A recent study examined ten varieties of indigenous rice from Northeast India by utilizing DSC to analyze their gelatinization. The results of the study indicated that all ten varieties differed in their To, Tp, Tc, and ÄH values. These variations were attributed to various factors such as the architecture of the starch granules, the content of amylose-amylopectin, and crystallinity, as mentioned by Govindaraju et al. (2021)[55]. It was observed that variations in the starch's ability to gelatinize during the isolation of mango kernel starch. The investigation of thermal properties revealed that the centrifugation method had superior thermal characteristics to other extraction techniques.

Morphological characteristics of starch

Light microscopy

The optical microscope (DCMC310 digital camera with Scope Photo software (Version 3.1.615) from Hangzhou Huaxin Digital Technology Co., Ltd., Zhejiang, China*)* is a tool that employs visible light and lenses to magnify small specimens. In modern times, these images can be digitally recorded. Optical microscopes are useful for accurately determining the size and shape of specimens and for imaging live cells with minimal photodamage[56, 57]. Light microscopy (LM) has been employed in studies on the connection between the rheological characteristics of starch systems and their internal structure, as well as investigations on the microstructural changes caused by hydrothermal processing, such as granule expansion and amylose leaking. One advantage of LM is its capacity to differentiate between amylose and amylopectin using iodine staining. This allows researchers to investigate the distribution of amylose and amylopectin in starch pastes in both the scattered and continuous phases[58].

The shape and size of starch granules vary depending on their biological origin. For instance, rice starch granules are typically polygonal and less than 5ìm in diameter, although potato starch particles are elliptical and over 75ìm in diameter. This information is supported by various authors, including Sunderram and Murthy (2014)[59], Pérez and Bertoft (2010)[60], and Perez-Rea et al. (2013)[61]. In a similar vein, Saeaurng and Kuakpetoon (2018)[62] have characterized the starch granules found in mango kernels, which were about 13ìm in size. These granules may occur either individually (simple) or in clusters (compound), and are made up of growth rings that consist of both amorphous and crystalline domains. This complex structure is created by a network of amylose and amylopectin, arranged in alternating concentric shell-like structures that are 120-400 nm thick.

While the alkali method of starch isolation revealed a slight bulkiness in them, it was still possible to detect the shape of the starch granules in centrifugation and sedimentation procedures (Figure 4). The alkali method of starch extraction swells when heated in the presence of water, unlike centrifugation and sedimentation procedures. This procedure, which is frequently regarded as the last step in the gelatinization process, necessitates the prior loss of at least some of the organized structures inside the native granule. Researchers, including Tan et al. (2004)[63], Syahariza et al.

(2010)[64], and Nadiha et al. (2010)[65], have found that the use of alkali is an effective method of starch extraction. This process involves steeping, physical shearing, and a slightly high pH value generated by sodium hydroxide. These factors can affect the multi-scale structure of the starch and lead to improved extraction results. These discoveries have been integrated into the procedures for extracting starch.

Scanning Electron Microscopy

The size and shape of starch granules in mango kernels vary significantly when viewed under a scanning electron microscope (SEM). Figure 5 demonstrates that the starch granules in mango kernels range in size from small to large and in shape from oval to elliptical. Extraction techniques significantly impact the structure of the starch. Centrifugation, sedimentation, and alkali techniques produced starch granules that were 10µm and 20µm in size, respectively. Regardless of the extraction method used, the isolated mango seed starch granules had smooth surfaces and were oval or irregularly shaped. The size and form of natural starch granules differ between plant species and with maturation level, with the granule surface being smooth with a few grooves, as seen in Fig. 5A, B, & C. The grooves were likely produced during the extraction process. The same properties from the same botanical source were discovered by Silva et al. (2013)[66]. When examining cassava starch, Schmidt et al. (2019)[67] discovered smoother surfaces and more rounded forms.

According to the research conducted by Bechtel et al. (1990)[68], Bechtel et al. (1993)[69], and Wilson et al. (2006)[70], wheat starch granules were classified into three categories based on their size - A type (larger than 16 µm), B type (between 5-16 µm), and C type (less than 5 µm) through SEM analysis. However, the size of starch granules isolated from mango kernels varied depending on the procedure used. The centrifugation, sedimentation and alkali methods showed polygonal, angular, and irregular shapes of granules with no differences in their morphological structure. According to Singh et al. (2018)[71], the alkali method's sodium hydroxide let water enter the starch, which caused granule enlargement and the degradation of amylose and amylopectin on the granules' surface. As a result, the structure was tiny and fragmented. The findings showed the presence of A- and B-type starch granules in comparison to the previously mentioned research. Zhang and his colleagues also observed that A-type starch granules have a disk-like shape, while B-type starch granules are spherical. Additionally, A-type granules are larger than B-type granules[72]. These observations are consistent with those made for other mango cultivars[73].

In a study conducted by Shahrim et al. (2004)[74], the researchers investigated the process of extracting and characterizing starch from mango seeds. It was found that the average diameter of the starch granules ranged from 13.80µm to 19.91µm. The different methods used for starch extraction, such as distillation, alkaline, sedimentation, and centrifugation, produced granules with varying sizes. However, the extraction techniques did not significantly affect the size and shape of the mango seed starch granules. The investigation supported these findings even though the granule sizes were substantially greater than those of mango seed kernel starches.

The diameter sizes of mango kernel starch granules were found to be similar to those observed in a previous study by Kaur et al. (2004)[75], with a range of 15 to 21µm. Starch granules possess a complex structure that differs in terms of the shape of their parts and chemical composition. This includes glucans, moisture, lipids, proteins, and phosphorylated residues[76]. A-type granules have a higher amylose content[77], lower crystallinity %, and lower lipid content compared to B-type starch granules[78].

CONCLUSION

The primary objective of this study was to explore the impact of the extraction of starch from mango seed kernels on the physical and chemical characteristics of pasting, thermal properties, and morphology. The study was designed to investigate the relationship between the starch extraction process and its effects on the properties of the extracted material. The research findings will provide valuable insights into the utilization of mango seed kernels as a source of starch and might offer remarkable solutions in the food and chemical industries. Three different starch isolation techniques were used. Centrifugation starch had the highest water binding capacity (90.2%) throughout the isolation techniques. Mango kernel starch's pasting qualities revealed notable variations between each extraction technique. Alkali starches showed lower pasting temperatures (75.8°C) higher viscosities (2854°cP) and more thixotropy than other starches among the extraction techniques. The system was packed tightly as a result of its improved swelling capacities. The centrifugation method had a high thermal property with high onset temperature (62.2°C) and enthalpy (4.3J/g), and also had high gelatinization and conclusion temperatures (64.6°C and 68.5°C, respectively) than other extraction methods. The starch structures could be readily observed under a light microscope, and the starch granules isolated by centrifugation and sedimentation were distinguished from one another by their distinct shapes, while the starch isolated by the alkali approach had a minor bulkiness in size. The extracted mango seed starch granules had smooth surfaces and oval, irregular, or cuboidal forms despite varying extraction techniques. According to the results of this study, centrifugation method had good pasting and thermal properties than sedimentation and alkali methods. Even though centrifugation method showed best isolation method of starch, the both sedimentation and alkali methods also exhibited the extraction characteristics in better way. So, these kinds of extracted starch can be utilized in the various food industries.

ACKNOWLEDGEMENT

None.

Authors Contribution Statement

RK designed the concept and gathered the data for the purpose of this research. The authors of this paper, RK and PR, were able to produce it after thoroughly studying the data inputs. All authors addressed the methodology and findings interpretations prior to submitting their work for publication.

Conflict of Interest

In writing this work, the author has no conflicts of interest.

Founding Source

There is no funding for this article

Data availability statement

Not applicable.

Ethical approval statement

Not applicable.

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