



Extraction and Quality Evaluation of Starch from Sindhoora Variety Mango Seed Kernel

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Abstract: Mango is known as the "King of Tropical Fruits" and is one of the world's most popular fruits. Mango fruit processing generates 25-40% waste consisting of peels and kernels, presenting a significant opportunity for by-product recovery. A mango seed kernel starch has been isolated using sedimentation, centrifugation, and alkali methods. The study aims to apply centrifugation, sedimentation, and alkali methods to isolate the starch from mango seed kernel and study the effects on the physiochemical, functional, and FTIR characteristics of starch. The results of this investigation revealed that the highest starch yield was observed by the alkali method (81.11%), followed by sedimentation (64.11%) and centrifugation (63.16%). The physio-chemical properties revealed that the centrifugation method had high protein (1.77 ± 0.005 g) and lipid (0.78g) content. In contrast, the highest starch yield (97.32g) and amylose (28.13g) content were observed in the alkali method. The functional properties like solubility index (23.0%) and swelling power (11.36g/g) were also increased in the alkali method, which maintained this tendency throughout time. When compared to centrifugation and alkali methods, the water-binding capacity of the sedimentation (90.23%) method was significantly increased in the mango kernel starches ($p < 0.05$). Results of FTIR analysis showed that all starches share the same structural properties, including O-H groups, O=C=O stretching, C-H bending, C=C bending, and -CH stretching. The study concludes that extraction using the alkali method had a significantly important role in the physio-chemical and functional properties of mango kernel starches, which may be used to improve the textural and sensory aspects in formulations of novel products.

Keywords: Alkali, Centrifugation, Mango Kernel, Sedimentation, and Starch.

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1. INTRODUCTION

A common tropical fruit crop, the mango (*Mangifera indica*), is a member of the *Anacardiaceae* family and the genus *Mangifera*. Over 1,000 different mango varieties are grown in India and have been for over 4000 years. More than 90 countries around the world cultivate mangoes. In the last thirty years, the mango industry has more than doubled. Asia is the world's largest mango producer, producing 77 percent of all mangoes, followed by America (13 percent) and Africa (3%)¹. Depending on the variety, mango kernels have about 77 percent of their dry weight in carbohydrates². Mango seed kernel (MSK) has a metabolizable energy (ME) value comparable to maize due to its high lipid content and significant starch content (58–80%). Dried MSK has a protein content comparable to maize (6–13%), and it has a good essential amino acid profile, particularly in terms of lysine and methionine^{3, 4}. An upward trend in mango production has been noted in India, with a quantity of 25M tonnes and a growth rate of 39% over the past five years. Mango cultivation and production have expanded steadily in India over the years. Around 1077 hectares were used for mango farming in India in 1991 and 1992. In 2013–14 and 2016–17, the area reached a maximum of 2516 hectares and 2262 hectares, respectively. Mango production has been inconsistent even though the production area is expanding. Mango production decreased in the years 2009–2010 and 2016–2017 but increased steadily after that. Mango production has been inconsistent even though the production area is expanding. Mango production decreased in the years 2009–2010 and 2016–2017 but increased steadily after that. Mango cultivation area increased by 109 percent between 1991 and 2015, while production increased by 125 percent. While Andhra Pradesh leads the states in terms of area, with 14.72 percent of the nation's total mango area, Uttar Pradesh is the state that produces the most mangoes. The other notable states with significant and commercial mango cultivation include Tamil Nadu, Maharashtra, Bihar, Maharashtra, Telangana, and West Bengal⁵. A chain of glucose molecules connected by covalent bonds makes up the polysaccharide or complex carbohydrate known as starch. Modern human diets contain a sizable amount of pure starch, a tasteless, odorless, white powder obtained from plants. Most green plants produce starch to store extra glucose produced during photosynthesis. It is one of the most significant dietary sources for people and one of the primary polysaccharides used by plants for storage. Cereals, roots, and other vegetables all contain a lot of starch. It is a -glucose polymer primarily consisting of the two substances amylose and amylopectin. Amylose is one of these and has a starch content of 15 to 20%. It is soluble in water⁶. Mango kernel starches were examined for their physicochemical, morphological, thermal, and rheological properties⁷. They discovered that these starches shared many qualities with starches from other industrial sources. Response surface methodology was used to maximize the enzymatic hydrolysis of mango kernel starch⁸. To investigate the molecular dynamics of the starches extracted from mango and Espada seeds, Tavares *et al.* (2003)⁹ used 13C solid-state NMR. In folk medicine, several portions of the *M. indica* plant are utilized for various treatments¹⁰. Several pharmacological actions of the extracts, including antioxidant^{11, 12}, anti-inflammatory¹⁰, antidiabetic¹³, and immune-modulating activities¹⁴ have been. The ameliorative effect of mango kernel-supplemented diets on the hyperglycemic conditions of the diabetic rats observed in this study could be attributed to the flavonoids and phenolic acids present in the flour, as earlier reported by Irondi *et al.* (2014)¹⁵. In addition, mango seed

kernel could be used as a potential source for functional food ingredients, antimicrobial compounds, and cosmetics due to its high quality of fat and protein and high levels of natural antioxidants. The mango stone obtained after the mango seed's decortication can be used as an adsorbent. The therapeutic potential of mango kernel has been extensively investigated for its anti-inflammatory, analgesic, antidiabetic, immune-modulatory, anti-oxidative, and anti-carcinogenic activity. Mango kernel powder has been reported to be beneficial in treating diarrhea and reducing blood cholesterol and blood sugar levels. The mango kernel contains diverse bioactive compounds such as phytosterols, sitosterol, tocopherols, mangiferin, isomangiferin, and polyphenols. Apart from mangiferin, gallic acid derivatives and sesquiterpenoids contribute to the very high antioxidant potential of mango kernel¹⁶. The ellagic acid and its derivatives in mango kernel have been shown to prevent DNA damage and tumor formation. Compared to most nuts and grains, mango kernel has a high concentration of phytochemicals and hence a much high therapeutic value. Mango kernel is an excellent health and preventive food. This study aims to extract starch from mango seed kernels and then investigate its physio-chemical, functional, and structural qualitative properties using Fourier Transform Infrared Spectroscopy (FTIR).

2. MATERIALS & METHODS

2.1. Materials

Mangifera indica L. Sindhoora mangoes, acquired from a neighborhood market in Krishnagiri, Tamil Nadu, provided the starch used in this study. The extraction and analysis were completed at the Bio Vision Focus laboratories in Thanjavur, Tamil Nadu, India. The collected mango species were carefully identified using Mathew K.M Flora of Tamilnadu Carnatic (1983). Dr. M. Jegadeesan, Department of Environmental and Herbal Science, Tamil University, Thanjavur, Tamil Nadu, India, identified and authenticated the mango species. For future reference, a voucher specimen has been deposited at the Herbarium of Tamil University in Thanjavur, Tamil Nadu, India.

2.2. Centrifugation method

A modified version of method¹⁷ was used to extract the starch from the mango seed. First, 50g of mango seeds were steeped at 50°C for 24 hours in a 0.16 percent aqueous sodium hydrosulfide hydrate solution. Next, the liquid was decanted, and the seeds were ground in a kitchen blender. The ground slurry was carefully rinsed with distilled water, filtered through a cotton bag (roughly 200 mesh), and then reslurried for an additional hour in distilled water. The settled starch layer was resuspended in distilled water after decanting the filtrate's supernatant. The starch was then dried in a 50°C oven for 6 hours after being centrifuged for 30 minutes at 5000 rpm. A plastic bag was used to seal the starch after it had been ground in a mortar and pestle, and it was kept at room temperature until it was required (figure -1).

2.3. Sedimentation method

The Oates and Powell (1996)¹⁸ method was used to extract the starch from the mango seed with a few minor modifications. First, 50 g of mango seeds were steeped in water for an entire night before being rinsed and ground in a blender. After filtering the slurry through a cotton bag with a

200-meter-long screen, the filtrate was saved to sediment the starch. After being re-slurred in distilled water three times, the starch was sedimented. Three hours were spent settling the finished product in 0.1 M sodium chloride (NaCl) and 1/10 volume toluene. The obtained starch was then dried for 24 hours in a 50°C oven after being thoroughly rinsed with distilled water (figure-1).

2.4. Alkali method

The method described by Noor *et al.* (2014)¹⁹ was modified to extract the mango seed starch. Beginning with a 0.5 percent sodium hydroxide (NaOH) solution, less than 10 g of mango seed flour was added, and the mixture was continuously

stirred for six hours at room temperature. Next, the remaining residues were washed three times with distilled water after the slurry had been filtered through a cotton bag with an approximate mesh size of 200. After that, the filtrate was combined and overnight precipitated at 4 °C. The starch was then filtered and dried at 40 degrees Celsius for 24 hours. Finally, a plastic bag was used to seal the starch after it had been ground in a mortar and pestle, and it was kept at room temperature until it was required (figure -1).

2.5. Determining the starch yield

According to Noor *et al.* (2014)¹⁹, the equation determined the percentage yield of isolated starches (1).

$$\text{Starch yield} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Final weight}} \times 100 \quad \text{Equation (1)}$$

2.6. Physiochemical properties

2.7. Crude protein

The nitrogen concentration was approximated using the micro Kjeldahl method, and the crude protein content was assessed using the AOAC Official method (AOAC, 2016)²⁰. Both an alcohol-ether mixture and a chloroform-methanol mixture were used to extract and measure the amount of fat (AOAC, 2000)²¹. The extracted starch's amylose content was measured in triplicate using the Williams *et al.* (2002)²² method. The amylose was calculated using a standard curve with a range of amylose concentrations.

2.8. Protein and Lipid content

The protein and lipid contents of the mango seed kernel starch were determined in triplicate according to the methods of the AOAC (2000)²¹. According to Matyash *et al.* (2008)²³, total lipids were extracted utilizing an MTBE (methyl tertiary-butyl ether)-methanol-water system with certain changes as previously described²⁴. First, the extract was evaporated under a fume hood to remove the solvents. Then, a pre-weighed aluminum Petri dish containing the dried residuals was filled with it.

2.9. Determination of amylose

100 mg of each type of starch were sampled and transferred to a 100 mL volumetric flask with 1 mL ethyl alcohol P. A and

9 mL 1 N NaOH solution. They were then heated in a 100 °C water bath for 10 minutes, with subsequent cooling for 30 minutes. Volume was completed with distilled water. A 5 Next, an aliquot was taken from each sample and transferred to a 100 mL volumetric flask, in which 1 mL 1 N acetic acid and 2 mL of 2% (w/v) iodine were added, completing the volume with distilled water. The absorbance at 610 nm was measured 30 min after adding the iodine solution. Results were compared to the standard curve obtained from 40 mg of pure amylose (Sigma), according to Zavareze *et al.* (2009)²⁵.

2.10. Determination of amylopectin content

It was obtained by the difference between total starch and amylose contents.

2.11. Solubility and swelling power (SP)

Swelling power and solubility (SP) were calculated using the method of Crosbie, (1991)²⁶, and Leach *et.al.*, (1959)²⁷, with a few minor modifications²⁸⁻³⁰. The prepared one percent starch solution was heated in a water bath to 90°C for 30 minutes while stirring continuously, then cooled. The supernatant was collected after the suspension was centrifuged for 10 minutes at 3200 rpm. The weighted precipitated portion was computed along with the SP value. After weighing and draining the supernatant into a Petri dish, the S value was calculated, and the results were then represented as g/g and percent, respectively.

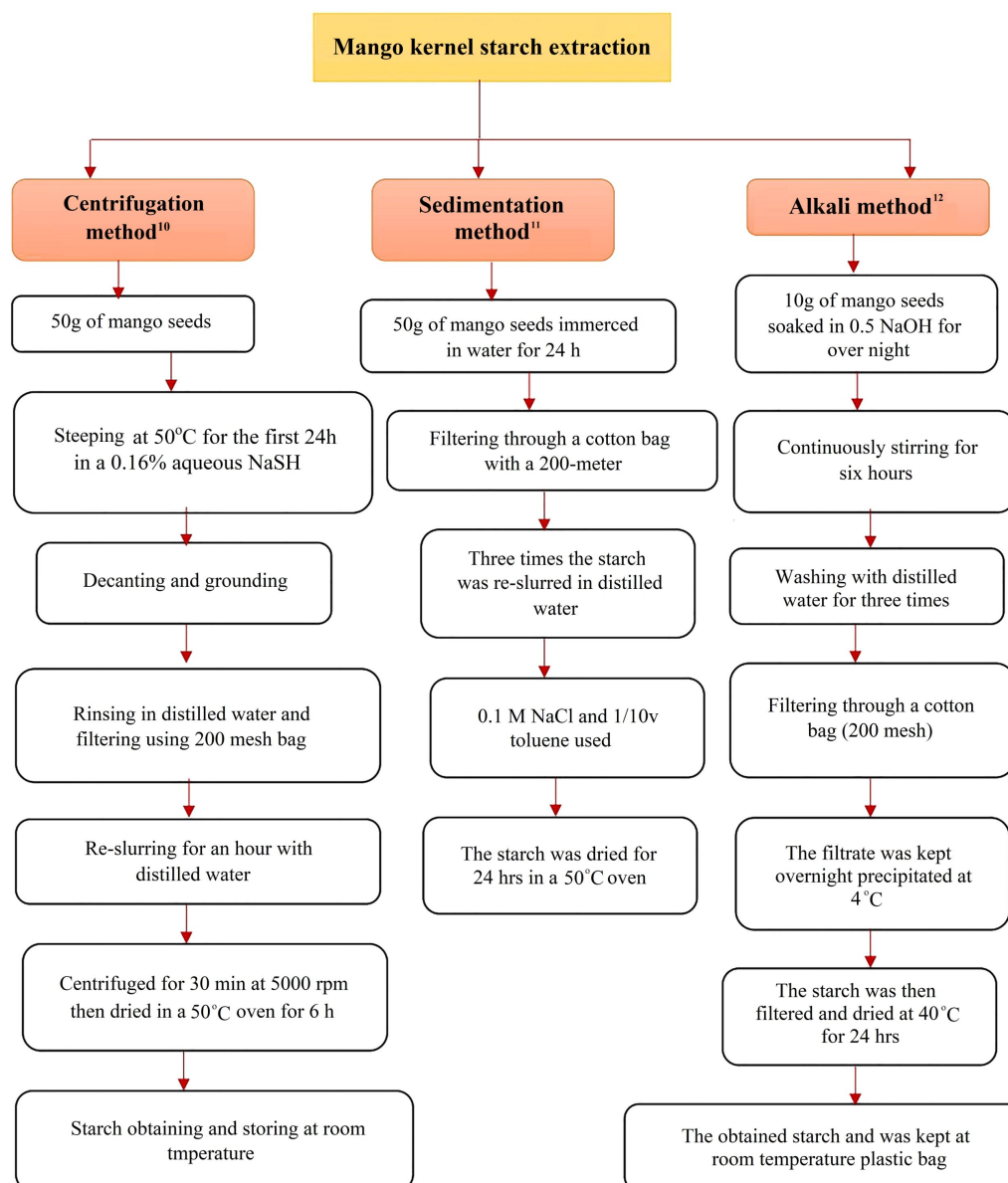


Fig 1: Extraction of starch from mango kernel

2.12. Water Binding Capacity (WBP)

The modified Medcalf method was utilized to calculate the WBC³¹. After being stirred for an hour, a suspension of 3g (dry basis) starch in 60ml distilled water was centrifuged for 10 minutes at 3200 rpm. After draining the extra water, it weighed the precipitated material and computed the WBC value.

2.13. Infrared spectroscopy using a Fourier transform (FTIR)

The FTIR data were collected using a Shimadzu FT-IR 8400 spectrophotometer (Shimadzu, Japan) over 64 scans at a resolution of 4 cm⁻¹. The spectra were obtained with KBr pellets containing a uniform mixture of 100 mg dry KBr and 2 mg sample (dry basis). The wavenumber range of the spectra was 400 to 4000 cm⁻¹³².

2.14. Statistical analysis

Using the SPSS 16 program, analysis of variance (ANOVA) and Tukey's test were used to compare the sample averages with a 95% confidence level ($p < 0.05$). Each analysis was carried out three times.

3. RESULTS AND DISCUSSION

3.1. Different methods of starch extraction and its starch yield

The starch yield percentages are shown in Table -I. The percentage of starch yield from mango seed kernels with the subsequent processes of sedimentation, centrifugation, and alkali was 64.12%, 63.16%, and 81.12%, respectively. The starch yield in this study was higher when compared to studies by Chowdary *et al.* (2000)³³ and Silva *et al.* (2013)³⁴, which yielded 55 percent and 59.82 percent of starch, respectively. Due to varying ecological conditions and species diversity present, these starch yields differ by region.

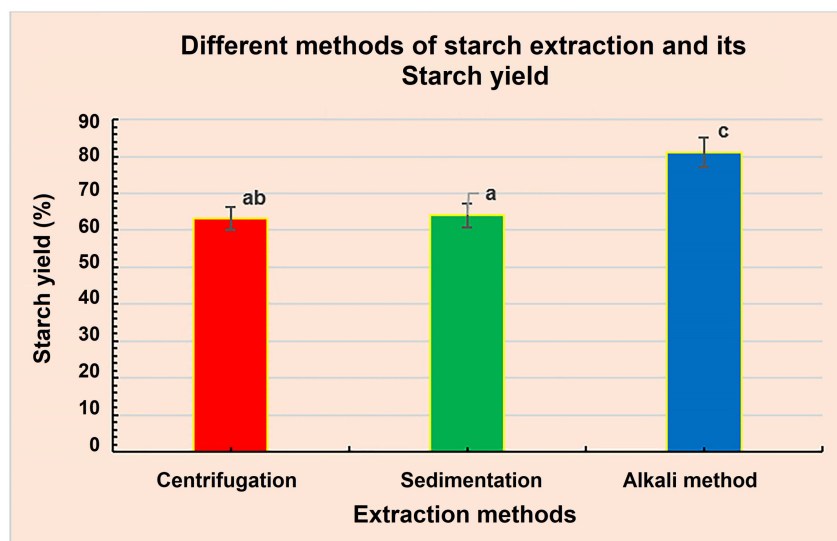


Fig 2: Different methods of starch extraction and its Starch yield from mango kernel; ^{a-c} Values denoted in the bar diagram showed statistically significant ($p < 0.05$) differences from each extraction using Duncan's Multiple Range Test

The Palmer variety of mango, widely cultivated in Ponta Grossa, Brazil, produced 58.20 percent mango starch from the kernel, according to Bet *et al.* (2017)³⁵. In contrast to earlier literature, where the starch yield was lower, our Sindhoora variety's yield was higher.

3.2. Physiochemical properties of mango kernel starch

The physicochemical properties of mango seed kernel starch are shown in Table -2. Using centrifugation, sedimentation, and

alkali methods, the protein content of the starch was determined to be 1.77g, 1.76g, and 1.05g, respectively. Centrifugation techniques produced the highest protein concentration (1.77g), which was statistically significant ($p < 0.01$) and was then followed by sedimentation and alkali techniques. In the centrifugation, sedimentation, and alkali methods, the lipid content was 0.79g, 0.53g, and 0.78g, respectively. In comparison to the sedimentation and alkali methods, the centrifugation method (0.79g) produced statistically significant amounts of lipid content ($p < 0.01$).

Table 2: Physio-chemical properties of mango kernel starch				
Physiochemical composition	Methods	Mean \pm SD	F Value	p-value
Protein (g/100g)	Centrifugation	1.77 \pm 0.005 ^b	15482.33	0.00**
	Sedimentation	1.76 \pm 0.005 ^b		
	Alkali	1.05 \pm 0.005 ^a		
Lipid (g/100g)	Centrifugation	0.79 \pm 0.01 ^b	659.111	0.00**
	Sedimentation	0.53 \pm 0.00 ^a		
	Alkali	0.78 \pm 0.01 ^b		
Starch (g/100g)	Centrifugation	92.46 \pm 0.057 ^a	8097.85	0.00**
	Sedimentation	94.26 \pm 0.057 ^b		
	Alkali	97.32 \pm 0.005 ^c		
Amylose content (%)	Centrifugation	27.06 \pm 0.05 ^a	496.16	0.00**
	Sedimentation	26.03 \pm 0.05 ^b		
	Alkali	28.13 \pm 0.11 ^c		

** Significant at the 1% level; values after triplicate analysis are shown as mean values standard deviation. According to Duncan's test ($p < 0.05$), values in a row that share a letter do statistically differ from each other.

Regarding the centrifugation and sedimentation methods, the alkali method had the highest starch content (97.32g), and there was a statistically significant difference ($p < 0.01$) between the three techniques. Compared to the centrifugation and sedimentation methods, the alkali method of starch extraction has a higher concentration of amylose (28.13g). According to Maninder Kaura *et al.* (2004)³⁶ study, mango kernel starches have a lower amylose content than corn and potato starches. In native corn, potato, banana, and cassava starches, Lemos *et al.*, (2018)³⁷ reported amylose percentages of 19.7, 20.46, 16.36, and 11.19 percent, respectively, lower than the values obtained in the present study. Plant age, variety, or methodology can all affect amylose content, which could explain differences between the cited values.

3.3. Functional properties of mango kernel starch

The ability of starch granules to absorb water when heated under conditions of excess water is known as swelling power, whereas solubility refers to the number of starch components that leach into the swelling volume's supernatant³⁸. The solubility, swelling ability, and water binding of mango kernel starches are displayed in Table 3. Swelling power and solubility can be used to gauge the degree of interaction between starch chains within the amorphous and crystalline domains of the starch granule³⁹. Loss of birefringence also occurs along with starch swelling, which happens before solubilization⁴⁰.

Table 3: Functional properties of mango kernel starch				
Functional Properties	Methods	Mean \pm SD	F Value	p-value
Solubility index (%)	Centrifugation	15.13 \pm 0.05 ^a	9514.747	0.00**
	Sedimentation	17.03 \pm 0.057 ^b		
	Alkali	23.10 \pm 0.10 ^c		
Swelling power (g/g)	Centrifugation	10.06 \pm 0.05 ^a	387.000	0.00**
	Sedimentation	10.56 \pm 0.05 ^b		
	Alkali	11.36 \pm 0.05 ^c		
Water binding capacity (%)	Centrifugation	81.73 \pm 3.2 ^a	25720.333	0.00**
	Sedimentation	90.23 \pm 0.31 ^b		
	Alkali	80.36 \pm 0.51 ^c		

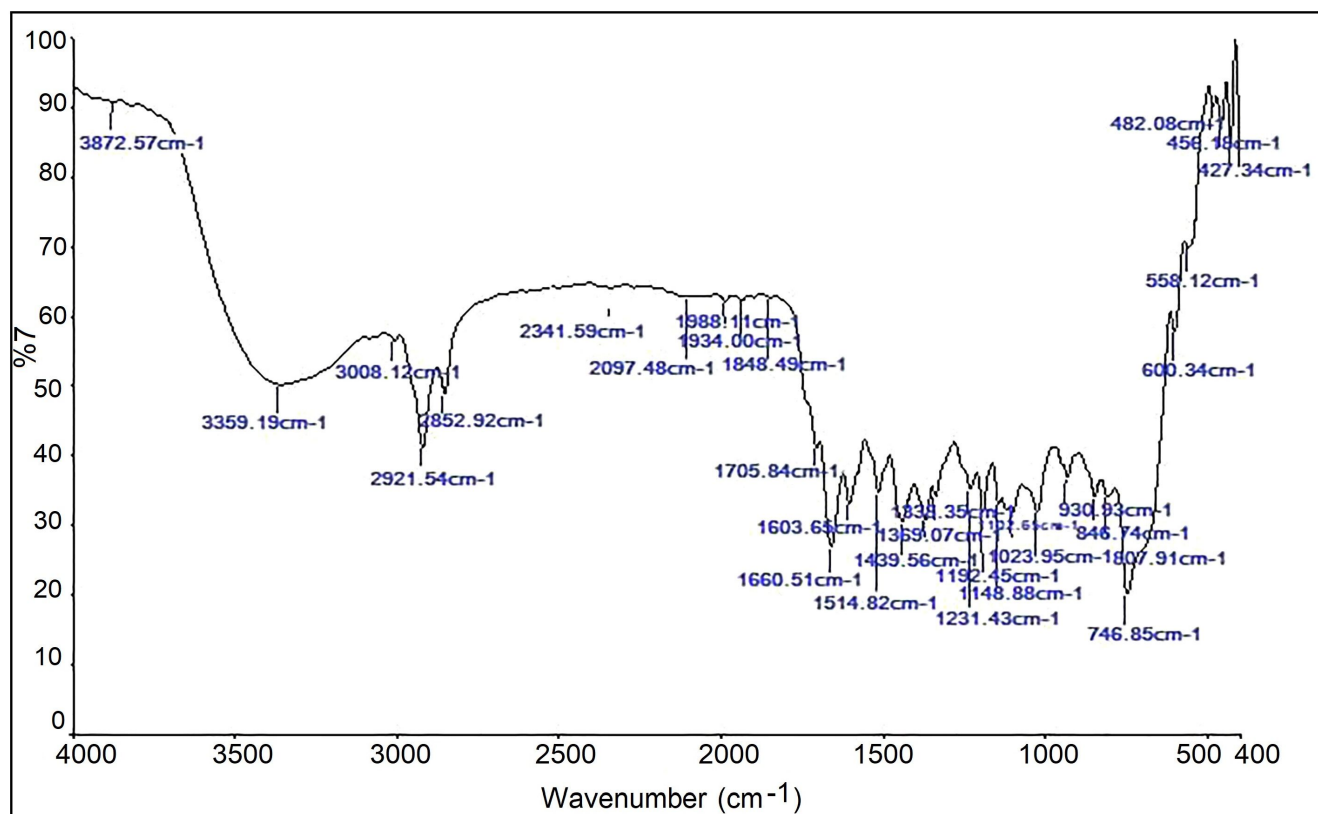
** Significant at 1% level; Values presented as mean values \pm standard deviation after analyzing in triplicate. Values followed by the same letter row-wise differ statistically by Duncan's test ($p < 0.05$).

The alkali method's swelling powder and solubility content was higher than those of the centrifugation and sedimentation methods, and this tendency persisted over time. The reduction of amylose content caused by alkali hydrolysis of the granule's amorphous region (Table-3) may be the cause of this increase in swelling power by lowering the restrictive tendency of the starch granules and enabling them to swell more⁴¹. In addition, strong alkalis breaking intermolecular hydrogen bonds may cause increased solubility⁴². These results support those of Rafiq *et al.* (2016)⁴³ for horse chestnut starch that has been alkali-modified. The greatest improvement in swelling strength and solubility was seen in Chausa mango kernel starches that had been alkali-treated. In their study, Singh *et al.* (2005)³⁸ discovered that variations in swelling power and solubility among cultivars of the same botanical source could be attributed to amylose, lipid content, and granule structural organization variations. Our study does not support this argument because we could not identify variations in the techniques used to extract the same botanical source. The ability of mango kernel starches to bind to water is displayed in Table 3. Compared to centrifugation and alkali methods, the water-binding capacity of mango kernel starches that underwent sedimentation modification increased significantly ($p < 0.05$) to 90.23%. This decrease in centrifugation and alkali procedures could be brought on by sodium ions obstructing water binding sites following alkali treatment, making them inaccessible for water molecule binding. Water-binding capacity can vary depending on the type of starch, modification type, and treatment level. The crystalline and amorphous regions of the starch, the molecular

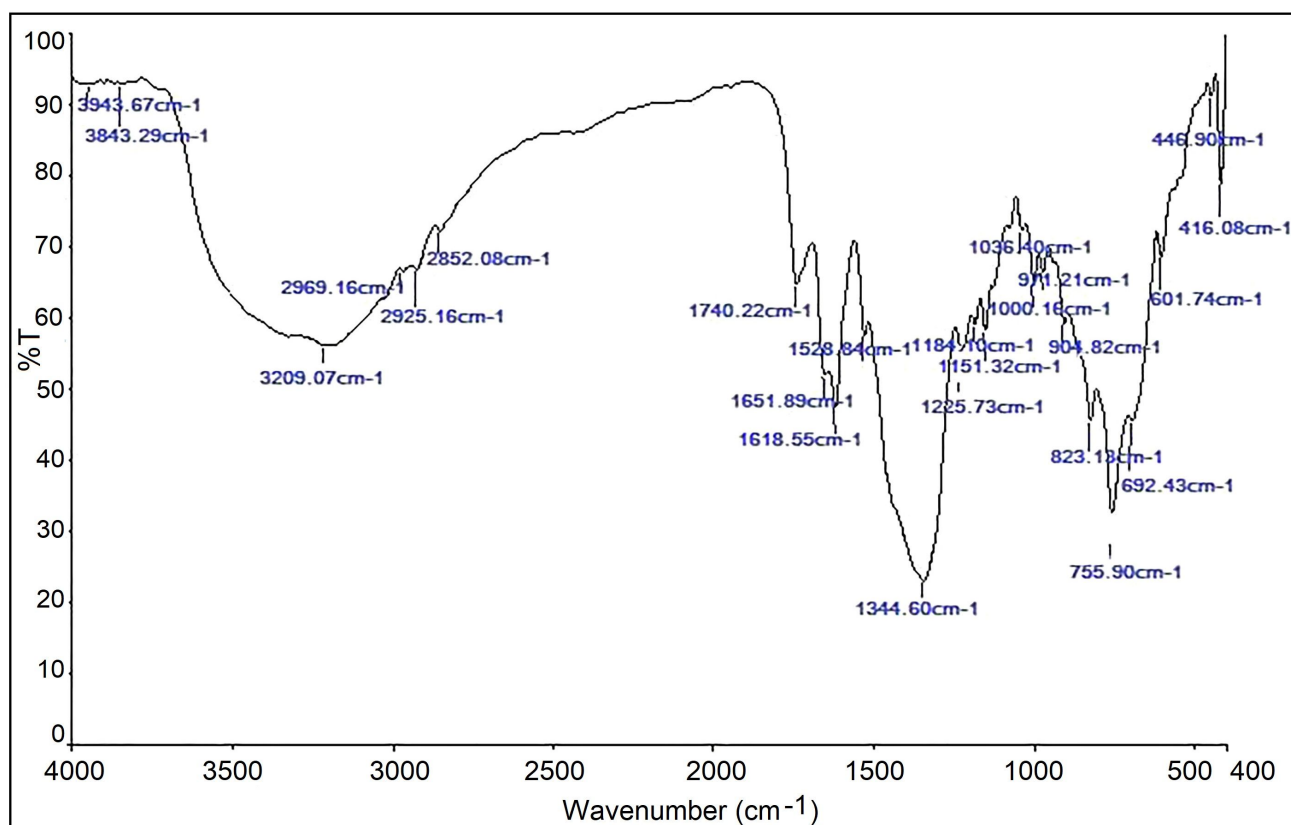
structure, and the distribution of granular sizes determine the water absorption capacity. In contrast, the physical entrapment of the water or oil in the starch structure determines the capacity to absorb it⁴⁴. The number of accessible water-binding sites may factor in the differences between the types.

3.4. Structural characteristics of isolated starches from mango kernel using FTIR

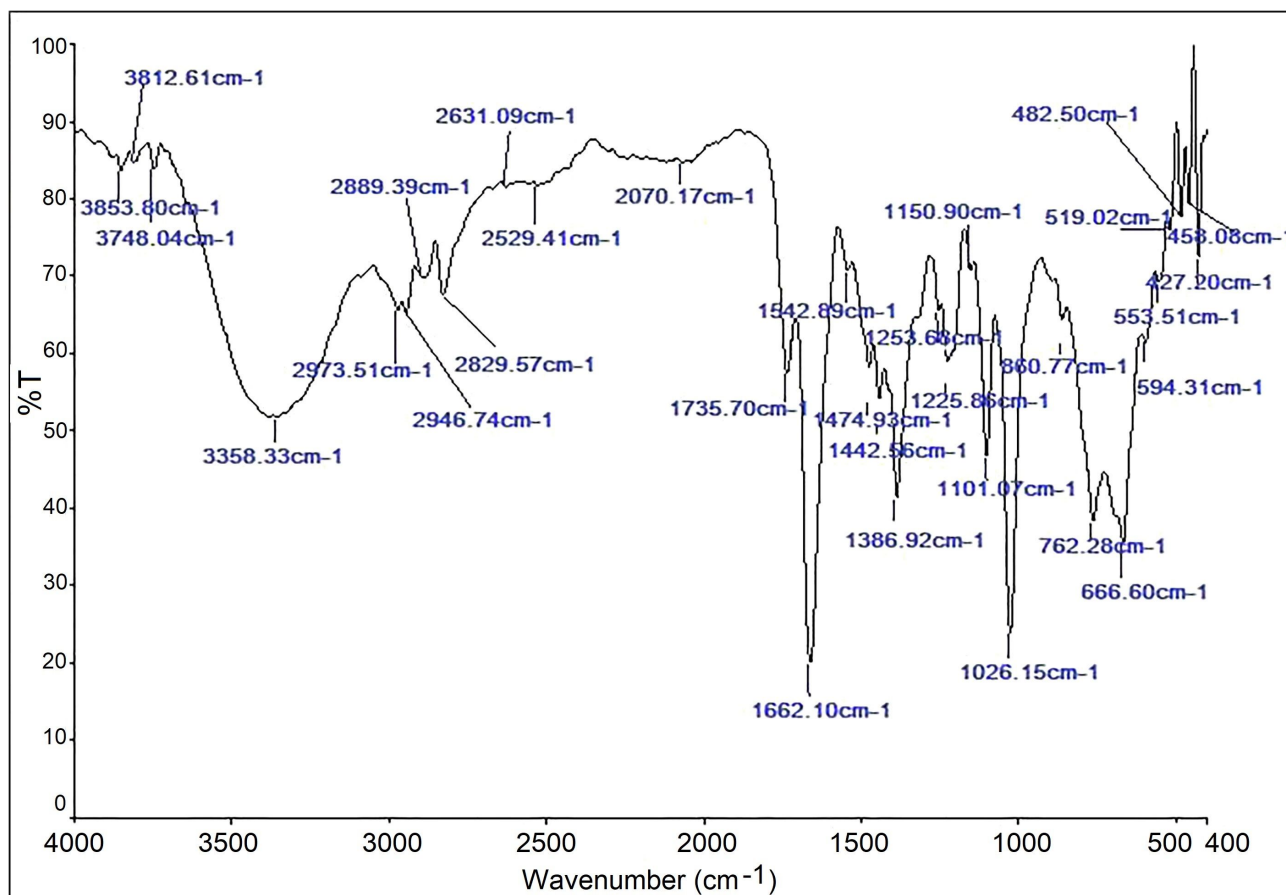
All starches had similar spectra, as evidenced by the distinct absorption peaks in Figure 1. While -CH stretching absorption is found at 2800-3000 cm⁻¹, hydrogen-bound O-H groups in starch are detected at 3000-3600 cm⁻¹. At 900-950 cm⁻¹, the skeletal mechanism of glycosidic linkage vibration was observed. The molecular structure of starch and its conformation, crystallinity, and water content may be revealed by infrared analysis⁴⁵. The figure shows the FTIR spectra of the variously processed starch samples. These had bands and properties similar to those found by Li *et al.* (2014)⁴⁶ for the phosphodiester bonds in sweet potato starch. Three spectra were found during the starch modifications that matched spectral bands from 1722 to 2867 cm⁻¹. Wavelengths from 3872.57 cm⁻¹ to 3359.19 cm⁻¹ indicate the presence of alcohol groups, 3008.12 cm⁻¹ the presence of carboxylic groups, 2852.93 cm⁻¹ to 2921.54 cm⁻¹ the presence of aromatic C-H bending groups, 1660.51 cm⁻¹ to 1848.49 cm⁻¹ the presence of C-F stretching of fluoro compound groups, and 1231.43 cm⁻¹ the presence of C-I stretching (halo compounds).



Sedimentation method



Centrifugation method



Alkali method

Fig 3: Shows FTIR spectroscopy images of the mango kernel starch processes of sedimentation, centrifugation, and alkali.

According to Kizil *et al.* (2002)⁴⁷, the spectral band between 1550 and 1750 cm^{-1} reveals the amorphous region of starch granules, which is most susceptible to acid hydrolysis, and confirms the modification of mango kernel starch in the range (1722–1752 cm^{-1}), with the appearance of a peak in the band between 1743 and 1745 cm^{-1} , as stated by Hoover (2000). The 2360–2368 cm^{-1} bands in the modified starch samples matched CN, which the samples' proteins may have produced; however, neutralization may have caused them to associate with glucose chains⁴⁸. The 2852–2854 cm^{-1} bands best captured the CH_2 deformations, with minor contributions from C–H stretch, aldehyde, COOH , and OH ⁴⁷. Recent researchers are exploring the extraction of starches from unconventional sources for application in food packaging^{49, 50, 51 & 52}.

3.5. Physio-chemical and functional parameters of extracted mango kernel starch are studied using correlation matrices.

Pearson's correlation was used to investigate the relationship between the various quality parameters used in this study (Table-4). Starch and swelling power had the highest positive correlation ($r = 0.99$) among all the quality parameters, followed by swelling power and solubility index ($r = 0.98$), starch and solubility index ($r = 0.98$), indicating a direct correlation between these parameters during the various treatment processes. The least negative correlation was found between protein and amylose content, indicating an inverse relationship between these quality parameters at a highly significant level ($p < 0.001$). The highest negative correlations were found between lipid and water binding, protein and solubility index, starch and protein, protein and swelling power, and then with amylose content and water binding.

Table 4: Correlation matrices of physio-chemical and functional parameters of extracted mango kernel starch

Correlation	Solubility index	Swelling power	Water binding	Amylose content	Lipid	Protein	Starch
Solubility index	1						
Swelling power	0.98**	1					
Water binding	-0.40	-0.25	1				
Amylose content	0.73*	0.61	-0.91**	1			
Lipid	0.26	0.11	-0.98**	0.84**	1		
Protein	-0.97**	-0.92**	0.59	-0.86**	-0.46	1	
Starch	0.98**	0.99**	-0.27	0.62	0.126	-0.93**	1

Correlation is significant at the 0.01 level (2-tailed) and at the 0.05 level (2-tailed).

The work by Przetaczek *et al.* (2018)⁵³, which stated that the functionality of starch is primarily determined by its amylopectin and amylose fractions, supports the correlation results. Additionally, the quantity of lipid, protein, and phosphorous content and the shape and surface properties of the starch granules affect how well the starch can bind water and how well it can paste. Amylose content and lipid had strong positive correlations ($r > 0.84$), and the amylose and solubility index had strong positive correlations ($r > 0.830$). It is believed that the parameters used here were inversely correlated with the physio-chemical and functional parameters for lipid, water binding, protein and solubility index, starch, and protein and amylose content. The ethnopharmacological and pharmacological efficacies of the many bioactive components of mango have recently attracted the attention of numerous studies. This research highlights the value of using mango byproducts to treat a variety of chronic illnesses, such as diabetes, cancer, asthma, hypertension, and intestinal and pulmonary bleeding, with more effectiveness and fewer adverse effects⁵⁴. According to many studies^{55 & 56}, various mango sections have anti-inflammatory, antioxidant, anticancer, anti-diabetic, antimicrobial, anti-hyperlipidemic, and immunomodulatory properties.

4. CONCLUSION

According to this study, the alkali method, centrifugation, and sedimentation were all used to extract starch from mango seed kernels. The results showed that the physio-chemical and functional properties of the alkali method were statistically

different. The FTIR study showed that all starches have equivalent structural characteristics, such as O-H groups, alkenes, aromatic compounds, and -CH stretching. The alkali method yielded more starch content than other methods. Even though numerous enzymatic and molecular processes have made it possible to create modified starch using current technology, these techniques have limitations. Therefore, physical and chemical modification are the two most common and practical ways to alter starch. This study concludes that Mango seed kernel starch is a novel starch derived from the mango fruit processing industry. As a result, all starch modification techniques can enhance the commercial use of mango seed starch. Different methods of extracting starch from mango kernel to increase its yielding can also be explored, and molecular studies of the starch granule can help to understand various properties of mango kernel starch, which can be useful in expanding its application in food processing and studying its medical consequences.

5. AUTHORS CONTRIBUTION STATEMENT

Rani. K conceptualized and gathered data for this project. Both authors Rani. K and Parimalavalli. R analyzed these data inputs to design this manuscript. To construct the final manuscript for publication, all authors discussed the methodology and results interpretation.

6. CONFLICT OF INTEREST

Conflict of interest declared none.

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