



Research Article

JOURNAL OF APPLIED PHARMACEUTICAL RESEARCH | JOAPR
www.japtronline.com ISSN: 2348 – 0335

ISOLATION, ALKALINE EXTRACTION, AND CHARACTERIZATION OF STARCH FROM MANGO SEEDS FOR PHARMACEUTICAL APPLICATIONS

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Article Information

Received: 8th January 2026
Revised: 8th March 2026
Accepted: 28th April 2026
Published: 15th May 2026

Keywords

Mango seeds, starch, alkaline extraction, Physicochemical characterization, Amylopectin, Agro-industrial waste, Pharmaceutical excipient.

ABSTRACT

Background: Mango (*Mangifera indica* L.) seed is an abundant agro-waste with potential as a sustainable biopolymer source. This study explores the conversion of mango seed waste into high-purity starch and evaluates its physicochemical, structural, and functional properties for pharmaceutical applications. **Methodology:** The starch extracted from mango seeds by the alkaline method was subjected to phytochemical screening, physicochemical evaluation, flow property analysis, and advanced characterization techniques, including ATR-FTIR, NMR, SEM, XRD, DSC, and mass spectrometry. **Results and discussion:** The extraction process yielded 40% (w/w) starch. Phytochemical screening confirmed the exclusive presence of carbohydrates, indicating high purity. The starch exhibited a high amylopectin content (98.47%) and a low amylose content (1.53%), indicating a highly branched structure. Moisture content was low (0.80%), while the swelling index reached 180%, demonstrating excellent water absorption. The pH was slightly acidic (6.12 ± 0.07), and flow properties were acceptable based on bulk density, tapped density, Carr's index, and Hausner's ratio. SEM showed oval and irregular granules sized 10–22 μm . ATR-FTIR and NMR confirmed polysaccharide structures, XRD revealed A-type crystallinity, DSC showed thermal degradation at 326 °C with an enthalpy of 29.63 J/g, and mass spectrometry indicated glucose polymer fragmentation. **Conclusion:** Mango seed starch demonstrates high purity with trace residual lipids/proteins detected by spectroscopic analysis, favorable functional properties, and structural stability, supporting its potential as a sustainable and effective excipient for pharmaceutical formulations.

INTRODUCTION

Starch, a vital plant polysaccharide, is one of the most abundant renewable biopolymers globally and the second largest glucose reserve after cellulose [1]. It primarily consists of two polymers:

amylose—an essentially linear α -(1→4)-linked α -D-glucose chain—and amylopectin—a highly branched polymer with α -(1→6) glycosidic bonds interspersed within α -(1→4) linked segments [2]. The amylose-to-amylopectin ratio governs its

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physicochemical properties such as gelatinization temperature, viscosity, swelling behavior, and solubility, thereby defining its suitability for diverse industrial applications [3,4]. Its biodegradability, biocompatibility, low cost, thermoplastic behavior, and renewability have positioned starch as a critical material in food, pharmaceutical, cosmetic, and biomedical sectors [5,6].

India ranks as the second-largest producer of fruits and vegetables, yet suffers approximately 18% post-harvest wastage, equating to Rs. 13,300 crore annually [7] (Indian Council of Agricultural Research [ICAR], 2019). This waste primarily consists of underutilized crops, agricultural byproducts, and discarded non-edible plant parts such as seeds, peels, and leaves, which are rich in polymeric compounds, including cellulose, hemicellulose, lignin, sugars, and starch. High moisture content in these residues promotes microbial spoilage, leading to environmental pollution and economic losses. The extraction of starch from such agro-wastes offers a promising sustainable solution to mitigate waste, add value, and meet the increasing industrial demand for starch with novel properties.

Mango (*Mangifera indica L.*), cultivated extensively in India with an annual production of about 20 million metric tons, generates significant agro-industrial waste, particularly the seeds that account for 10–25% of the fruit's weight (Food and Agriculture Organization [FAO], 2022). Mango seeds contribute around 1.5 million tons of waste each year in India alone, representing an underexploited starch source [8,9]. Preliminary studies indicate that starch extracted from mango seeds exhibits distinctive structural and functional characteristics compared with conventional starches [10], potentially broadening its industrial and pharmaceutical applications [11].

The biomedical relevance of starch is the subject of intensive research, with applications in drug delivery systems, tissue-engineering scaffolds, hydrogels, bone cements, and nanoparticulate carriers [12–14]. Its hydrophilic backbone enables hydrogen bonding and electrostatic interactions with hydrophobic drugs, facilitating enhanced stability and solubility in the amorphous state [15]. This makes starch from unconventional sources, such as mango seeds, a promising candidate for pharmaceutical formulation innovations, especially for stabilizing poorly soluble drugs & developing novel excipients [16]. This study aims to isolate, extract, and thoroughly characterize starch from mango seeds to evaluate its

physicochemical properties and potential pharmaceutical applications. Recovery and utilization of starch from mango seed waste not only contribute to eco-friendly waste management but also support the development of value-added biopolymers with unique functional advantages in pharmaceutical sciences [17].

MATERIALS AND METHODOLOGY

Materials

Mango seeds used in this study were collected from the *Mangifera Indica* species. Sodium hydroxide and all other chemicals employed were of analytical grade.

Methods

Extraction of Starch from Mango Seeds (Alkaline Method)

Starch was extracted from mango seed powder using a modified alkaline extraction method. In brief, 25 g of seed powder was dispersed in 0.1% (w/v) sodium hydroxide solution [18] and stirred continuously for 6 h at 30 °C to promote starch release. The resulting slurry was filtered through a 200 µm cloth mesh, and the residue was washed three times with distilled water. The combined filtrate was refrigerated at 4 °C overnight to induce starch precipitation [19]. The precipitated starch was collected by filtration, dried in a hot-air oven at 40 °C for 24 h, finely ground with a mortar and pestle, and the percentage yield was determined. The dried starch powder was then stored in airtight polyethylene bags at room temperature for subsequent characterization [20,21].

Phytochemical screening

Phytochemical screening of the extracted mango seed starch was performed to qualitatively detect proteins, tannins, alkaloids, saponins, and carbohydrates [21]. The tests were conducted using standardized procedures with defined reagent concentrations and volumes to ensure reproducibility. Briefly, the Biuret test was performed by adding 1 mL of 1% (w/v) CuSO_4 and 1 mL of 10% (w/v) NaOH to 2 mL of the sample solution. The Ferric chloride test employed 2–3 drops of 5% (w/v) FeCl_3 solution. Alkaloids were assessed using 1 mL of Mayer's reagent added to 2 mL of sample extract.

The froth test for saponins was performed by vigorously shaking 5 mL of the aqueous sample with distilled water. Carbohydrates were detected using Molisch's test, in which 2 drops of 1% (w/v) alcoholic α -naphthol solution were added to 2 mL of the sample, followed by careful addition of 1 mL of concentrated H_2SO_4

along the test tube wall [21]. All tests were conducted in triplicate to verify the presence or absence of these constituents.

Determination of amylose and amylopectin content

Amylose and amylopectin contents were determined using the iodine-binding colorimetric method [3]. A starch sample (100 mg) was moistened with 1 mL of 95% ethanol and dissolved in 9 mL of 1 N NaOH. After standing overnight to ensure complete solubilization, the volume was brought to 100 mL with distilled water. A 5 mL aliquot was neutralized with 1 mL of 1 N acetic acid, followed by the addition of 2 mL iodine solution (I₂/KI). The mixture was diluted to 100 mL and left for 20 min to allow color development. Absorbance was measured at 620 nm using a UV-Vis spectrophotometer [22]. Amylose content was calculated from the amylose standard curve, and amylopectin was obtained by difference, assuming starch consists of amylose and amylopectin only [23].

Loss on Drying (LOD)

Five grams of mango seed starch were accurately weighed and transferred into a clean, dry Petri dish [24]. The sample was dried in a hot-air oven (Bionics Scientific) at 105°C for 24 hours or until a constant weight was reached. The percentage loss on drying, representing the moisture content, was calculated using the equation:

$$LOD \% = \frac{\text{Initial weight} - \text{Final weight}}{\text{Final weight}} \times 100$$

Flow property

Thirty grams of mango seed starch were transferred into a 250 mL graduated measuring cylinder to determine the density and density-related properties. The bulk density and tapped density were measured using standard protocols. From these values, Carr's index and Hausner's ratio were calculated to assess flowability. The angle of repose and flow rate of the starch powder were measured using 50 g samples.

Swelling index

The swelling capacity of starch extracted from mango seeds was evaluated using the method described by Leach et al. (1959), with slight modifications. The experiment was conducted to determine the swelling index (SI) of the isolated starch at various temperatures [25]. Approximately 1 g of starch powder was accurately weighed and transferred into a graduated test tube. Subsequently, 10 mL of distilled water was added, and the contents were mixed thoroughly to obtain a uniform suspension.

The test tubes were then placed in a thermostatically controlled water bath maintained at 85°C for 30 minutes, with intermittent shaking to ensure homogeneous heating [26].

After the heating period, the samples were cooled to room temperature and then centrifuged at 4000 rpm for 10 minutes to separate the swollen granules from the supernatant. The final volume (V₂) of the sediment was recorded, while the initial volume (V₁) was the volume of dry starch before hydration. The swelling index (SI) was calculated using the following equation:

$$SI = \frac{V_2 - V_1}{V_1} \times 100$$

Determination of the pH of the solution

The pH of a 0.5% (w/v) aqueous solution of mango seed starch was measured with a calibrated digital pH meter (Onyx Aqua Farm, Digital pH Meter Pen Type). Triplicate readings were recorded at 25 °C to ensure accuracy and reproducibility [27].

Rheological Characterization

The viscosity of mango seed starch solution (0.1-0.5 % w/v in distilled water) was measured using a Brookfield Rheometer (DV3T, USA) with spindle No.4 at 25 °C. The analysis provided essential information on the rheological behavior, stability, and processability of the polymeric solution [28].

ATR-FTIR Analysis

The ATR-FTIR spectrum of the purified starch extracted from mango seeds was recorded using a Bruker Alpha II ATR-FTIR spectrophotometer (Germany). The sample was scanned within the 400–4000 cm⁻¹ range at a resolution of 4 cm⁻¹, averaging 25 scans per sample to improve signal clarity & spectral precision [29].

Powder X-ray Diffraction

The crystallinity of purified starch extracted from mango seeds was analyzed using a Bruker instrument in Germany. Model: D2 Phaser diffractometer with Cu Kα radiation (λ = 1.5405 Å). The instrument operated at 40 kV and 30 mA, scanning over a 2θ range of 5°–90° at 23 °C with a rate of 1° min⁻¹ to obtain high-resolution diffraction patterns [30].

Differential Scanning Calorimetry

The thermal behavior of the purified starch was evaluated using a Hitachi STA 7300 DSC. The instrument was calibrated using indium, and a baseline scan was performed to verify accuracy. Approximately 5 mg of the sample was heated in a platinum pan

under a nitrogen atmosphere (50 mL/min) from ambient temperature to 500 °C at 10 °C/min to assess thermal stability [31].

Scanning Electron Microscopy

Surface morphology analysis of starch extracted from mango seeds was performed using a JEOL JSM-6360 scanning electron microscope (SEM), Japan model. The sample was mounted on an aluminum stub and coated with platinum. The stubs containing the samples were then placed in the SEM chamber for observation and surface morphology analysis [18].

¹H NMR Spectroscopy

The ¹H NMR spectrum of starch extracted from mango seeds was recorded using a SA/AD/INS/001 spectrometer operating at 400 MHz. The sample was dissolved in a deuterated solvent such as DMSO-d₆ and transferred to an NMR tube for analysis. Spectral acquisition parameters were optimized to obtain clear proton resonance signals corresponding to the starch molecular structure.

Solid-State ¹³C NMR Spectroscopy

Solid-state ¹³C cross-polarization magic-angle spinning (CP-MAS) NMR spectra of mango seed starch were recorded on a JEOL ECX-400 spectrometer operating at 400 MHz to elucidate the carbon framework and molecular structure. The acquisition parameters included a contact time of 3.5 ms, relaxation delay of 5 s, sweep width of 35 kHz, and magic-angle spinning at 10 kHz [32].

Mass Spectrometry

The mass spectrum of starch extracted from mango seeds was obtained using an electrospray ionization mass spectrometer (SA/AD/INS/015). The purified sample was dissolved in a suitable solvent and introduced into the ion source under positive ionization mode. Spectral data were recorded to determine the molecular weight and fragmentation pattern, providing insight into the structural composition of the starch.

RESULTS AND DISCUSSION

Percent yield

The alkaline extraction method yielded 10 g of purified starch from 25 g of mango seed powder, corresponding to a yield of 40% (w/w). This substantial yield indicates that mango seeds represent a promising alternative source of starch for pharmaceutical drug delivery and food applications. The

relatively high extraction efficiency can be attributed to the alkaline conditions, which effectively solubilize proteins and other non-starch components, facilitating the release of starch granules from the cellular matrix. The use of readily available reagents and mild processing conditions further supports the method's potential for scalability. However, pilot-scale studies and process optimization will be necessary to evaluate industrial feasibility, cost-effectiveness, and regulatory compliance for pharmaceutical applications.

Phytochemical screening

The qualitative phytochemical screening of the extracted mango seed starch revealed the presence of carbohydrates, while proteins, tannins, alkaloids, and saponins were absent. The positive Molisch's test confirmed the polysaccharide nature of the isolated starch. In contrast, the Biuret, Ferric chloride, Mayer's, and froth tests showed negative results, indicating the absence of proteinaceous or secondary plant metabolites. All results were consistent across triplicate analyses, confirming the reproducibility of the findings. The presence of carbohydrates and the absence of other phytochemical constituents, such as proteins, tannins, alkaloids, and saponins, indicate the high purity of the extracted starch, as determined quantitatively. Similar results have been reported for starches isolated from mango seeds and other botanical sources, where purification effectively removes associated non-starch phytoconstituents [3].

The absence of proteins and tannins is particularly desirable, as these compounds can influence starch functionality, color, and stability during processing [33]. The results thus confirm that the extraction method yielded a carbohydrate-rich, contaminant-free starch suitable for use in pharmaceutical and food formulations [34]. The purity profile is consistent with previous studies identifying mango seed starch as a potential excipient with desirable physicochemical and functional characteristics [35].

Determination of amylose and amylopectin content

The starch sample exhibited an amylose content of 1.53% and an amylopectin content of 98.47%. The low amylose proportion indicates that the starch is predominantly amylopectin-rich, suggesting a highly branched macromolecular structure. Such compositional characteristics are typical of waxy starches, which are known to possess superior swelling capacity, paste clarity, and viscosity [3]. These functional attributes enhance their suitability for applications in film formation, coating materials, and controlled-release drug delivery systems. The present

findings are consistent with previous reports on starches extracted from tropical fruit seeds, which generally exhibit high amylopectin content [34].

Loss on drying

The moisture content of the starch sample, determined by the loss-on-drying method, was 0.80%. The sample reached a constant weight after drying at 60 °C, indicating that all surface and loosely bound moisture had been effectively removed. The low moisture content (0.80%) indicates that the starch sample possessed excellent dryness and stability. Such a low level of residual moisture minimizes the risk of microbial growth and enzymatic degradation during storage [3]. Starches with moisture levels below 12% are generally considered stable and suitable for further physicochemical and formulation studies [9]. Similar observations have been reported for starches isolated from various tropical plant sources, which exhibit comparable low moisture content and high thermal stability [33].

Swelling index

The swelling index of mango seed starch was found to be 180% higher than that of mango seed starch, indicating enhanced disruption of hydrogen bonds and greater water absorption within the granules. This behavior suggested a higher amylopectin content and lower granular crystallinity, consistent with reports on starches derived from other non-conventional sources [36]. The observed swelling capacity of mango seed starch (150–300%) indicates its potential for applications in the pharmaceutical, biopolymer, and food industries, where water uptake and gel formation are desirable properties [26].

pH determination

The pH of a 0.5% (w/v) aqueous solution of mango seed starch was 6.12 ± 0.07 , indicating a slightly acidic nature. This mild acidity may result from residual organic acids or phosphate groups in the starch granules. Similar pH values have been reported for starches from other tropical sources [27]. The slightly acidic pH suggests good stability and suitability of the starch for food and pharmaceutical applications.

Flow property

The flow characteristics of the extracted mango seed starch showed consistent and correlated evidence of poor flowability. The bulk density (0.40 g/mL) and tapped density (0.56 g/mL) differed substantially, indicating high particle porosity and poor initial packing efficiency. This density gap was reflected in the

Hausner's ratio (1.40) and Carr's index (28%), both of which fall within the range interpreted as poor flow, as materials with a Hausner's ratio >1.25 or Carr's index $>25\%$ are generally cohesive and demonstrate restricted flow (Carr, 1965; USP, 2022). The angle of repose (47.98°) further supported this conclusion, as values above 40° typically indicate powders with considerable interparticle friction and low flowability [12]. Collectively, these parameters are strongly correlated and confirm that the mango seed starch exhibits poor inherent flow properties, consistent with earlier findings that native starches often possess irregular particle morphology, which contributes to limited flow. To address this limitation in pharmaceutical applications, strategies such as wet or dry granulation may be employed to improve flow and compressibility. Additionally, incorporating suitable glidants, such as colloidal silicon dioxide or talc, can enhance powder flow during tablet manufacturing. These established pharmaceutical approaches can effectively mitigate flow-related challenges and support the practical use of mango seed starch as an excipient.

Rheological Characterization

The apparent viscosity of the mango seed starch dispersion was found to be 541.7 cP at 26.1 °C. Such viscosity values indicate a moderately strong internal granular architecture, typically associated with starches possessing a balanced amylose–amylopectin ratio. Comparable viscosity profiles have been reported for starches obtained from other tropical fruit seeds, supporting the potential of mango seed starch as a functional biopolymer for pharmaceutical and food applications [7]. The moderate viscosity at ambient temperature further suggests minimal granule disruption before gelatinization, which aligns with previous findings that native seed starches exhibit restricted swelling and limited viscosity development until the thermal transition begins [1]. Collectively, these observations indicate that mango seed starch retains structural integrity and demonstrates stable flow behavior at room temperature, reinforcing its applicability as a natural thickening, stabilizing, or binding excipient in formulation systems.

IR interpretation

The FTIR spectrum (Figure 1) of starch extracted from mango seeds demonstrated characteristic absorption bands consistent with polysaccharide structures. A broad absorption band observed in the $3200\text{--}3500\text{ cm}^{-1}$ region corresponds to O–H stretching vibrations of hydroxyl groups present in starch molecules, reflecting extensive intermolecular and

intramolecular hydrogen bonding and the hydrophilic nature of starch [37]. The weak band near 3811 cm^{-1} is likely due to trace-free moisture or a minor instrumental/background effect rather than hydrogen-bonded starch hydroxyl groups. Peaks at 2914 cm^{-1} and 2849 cm^{-1} are attributed to C–H stretching vibrations of methyl and methylene groups present in the glucose units forming the starch backbone [27,38]. An absorption band observed at 1633 cm^{-1} corresponds to the bending vibrations of absorbed water molecules, reflecting the hygroscopic nature of starch granules [1]. The minor peaks detected near 1568 cm^{-1} and 1550 cm^{-1} may indicate residual protein or lipid impurities co-extracted during starch isolation, as similar bands have been reported in natural starches. Bands at 1428 cm^{-1} , 1339 cm^{-1} & 1244 cm^{-1} correspond to C–H bending & C–O–H bending vibrations, highlighting the polysaccharide framework [39].

The distinctive peaks at 1147 cm^{-1} & 1000 cm^{-1} are assigned to C–O–C glycosidic bond stretching & C–O stretching of the anhydroglucose ring, confirming the starch polymer signature [40]. Additionally, skeletal vibrations of glucose rings and C–H bending modes were apparent at 924 cm^{-1} , 854 cm^{-1} , 759 cm^{-1} , and 703 cm^{-1} . These peaks are typically associated with amylose and amylopectin components of starch [36]. This FTIR analysis confirms the successful extraction of starch from mango seeds with functional groups characteristic of typical polysaccharides. Minor alterations and additional peaks suggest the presence of trace non-carbohydrate biomolecules, which are common in natural starches and might influence their physicochemical properties. Understanding these molecular signatures is essential for potential applications of mango seed starch in food, pharmaceutical, and biodegradable material development [41].

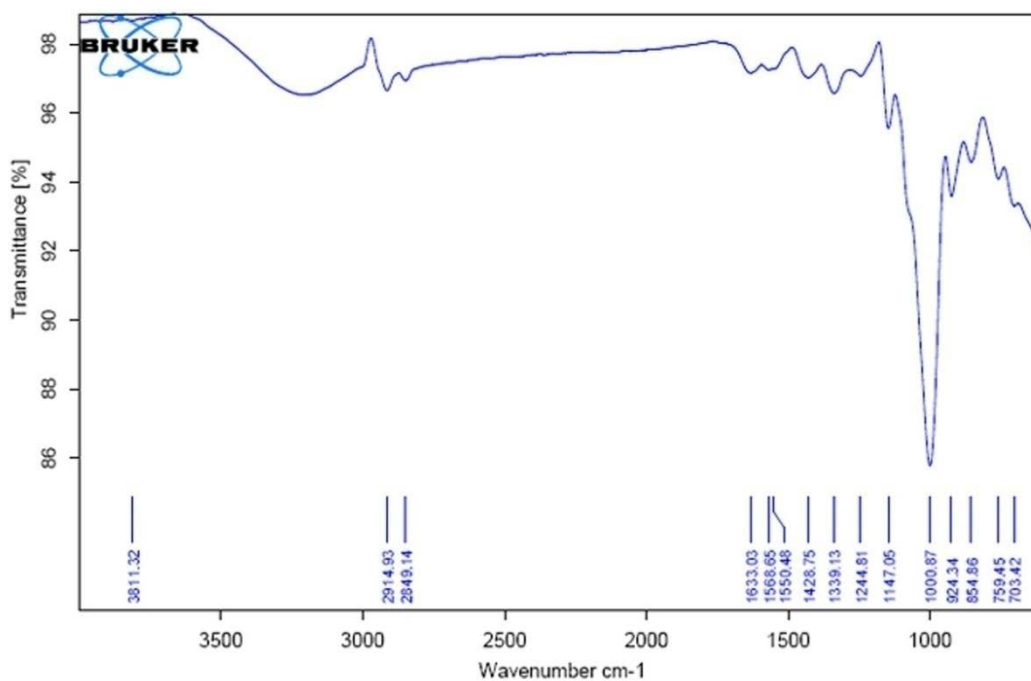


Figure 1: ATR-FTIR Spectra of the Starch extracted from the Mango seeds

¹HNMR

The spectrum exhibited (Figure 2) characteristic resonances attributable to glucopyranosyl units, confirming the polysaccharidic nature of the extracted material. The residual solvent signal of DMSO-*d*₆ was observed at δ 2.50 ppm, while a minor resonance at δ ~3.33 ppm corresponded to residual water in the solvent system. A series of broad signals were detected in the region δ 5.51–5.00 ppm, which are assignable to the anomeric proton (H-1) of α -D-glucopyranose units. The multiplicity and broad nature of these peaks are consistent with the polymeric structure of starch and the presence of intra- and

intermolecular hydrogen bonding interactions. A discernible signal around δ 4.58 ppm can be attributed to exchangeable hydroxyl (–OH) protons associated with the starch backbone, which are observable in DMSO-*d*₆ due to reduced proton exchange compared to aqueous media. The ring protons (H-2 to H-6) of the glucopyranosyl moieties appeared as overlapping multiplets in the δ 3.20–3.80 ppm region, with notable resonances at approximately δ 3.58, 3.53, 3.47, and 3.33 ppm. These signals are characteristic of the carbohydrate framework and confirm the integrity of the glucan backbone. In addition to the carbohydrate signals, weak resonances were observed in the

δ 2.67–2.32 ppm region, which may be attributed to trace aliphatic protons adjacent to carbonyl functionalities, suggesting the presence of minor residual impurities. Importantly, the spectrum did not display dominant sharp resonances in the δ 0.8–1.5 ppm range, indicating the substantial reduction of long-chain lipidic components compared to spectra typically obtained in nonpolar solvents. Overall, the spectral profile confirms successful starch extraction and effective solubilization in DMSO- d_6 , enabling clear observation of glucopyranose-derived resonances. The presence of broad but well-defined carbohydrate signals, particularly in the anomeric and ring proton regions (δ 3.0–5.5 ppm), validates the polysaccharidic structure of the material.

The minimal contribution of intense aliphatic peaks suggests improved purity and reduced lipid interference. These findings indicate that DMSO- d_6 is an appropriate solvent for structural elucidation of mango seed starch and enables more reliable characterization of its proton environment for further physicochemical investigations [42]. Although weak residual signals suggest trace non-carbohydrate components, these do not significantly affect the overall structural interpretation. Nevertheless, incorporation of additional purification steps, such as solvent washing, in future studies may further improve chemical purity and enhance the suitability of mango seed starch for high-grade pharmaceutical applications.

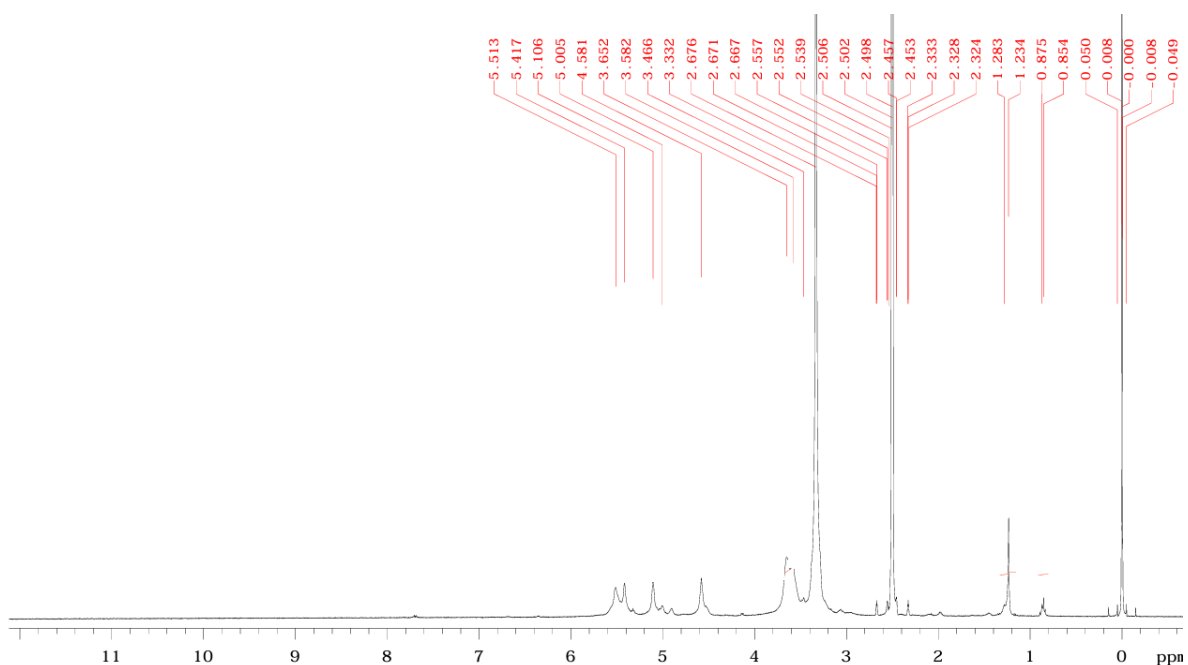


Figure 2: ^1H NMR of the Starch extracted from the Mango seeds

^{13}C NMR

The ^{13}C NMR spectrum of the starch extracted from mango seeds was recorded using solid-state cross-polarization magic angle spinning (CP/MAS) NMR. The spectrum exhibited characteristic resonances indicative of the typical polysaccharide structure of starch (Figure 3). The chemical shifts observed at approximately 101.6 ppm correspond to the anomeric carbon (C-1) of glucose units, confirming the α -glucopyranosyl structure of amylose and amylopectin components in the starch matrix. The resonance at 81.8 ppm can be assigned to the C-4 carbon, which is involved in glycosidic linkages between glucose residues. The C-2, C-3, and C-5 carbons appear as overlapping resonances around 72.5 ppm, consistent with the ring carbons in the glucopyranose unit. A

peak at about 62.2 ppm corresponds to the C-6 carbon, representing the primary alcohol group in the glucose residue. The signal near 33.6 ppm is attributed to minor impurities or possibly residual lipid components associated with the starch sample. The absence of significant peaks above 105 ppm or below 30 ppm indicates the starch is relatively pure without unusual chemical modifications [43].

The NMR results confirm that the extracted starch from mango seeds possesses the characteristic crystalline and amorphous domains of native starch. The observed chemical shifts align well with previous studies on starch from various botanical sources, affirming the structural integrity of the extracted biopolymer.

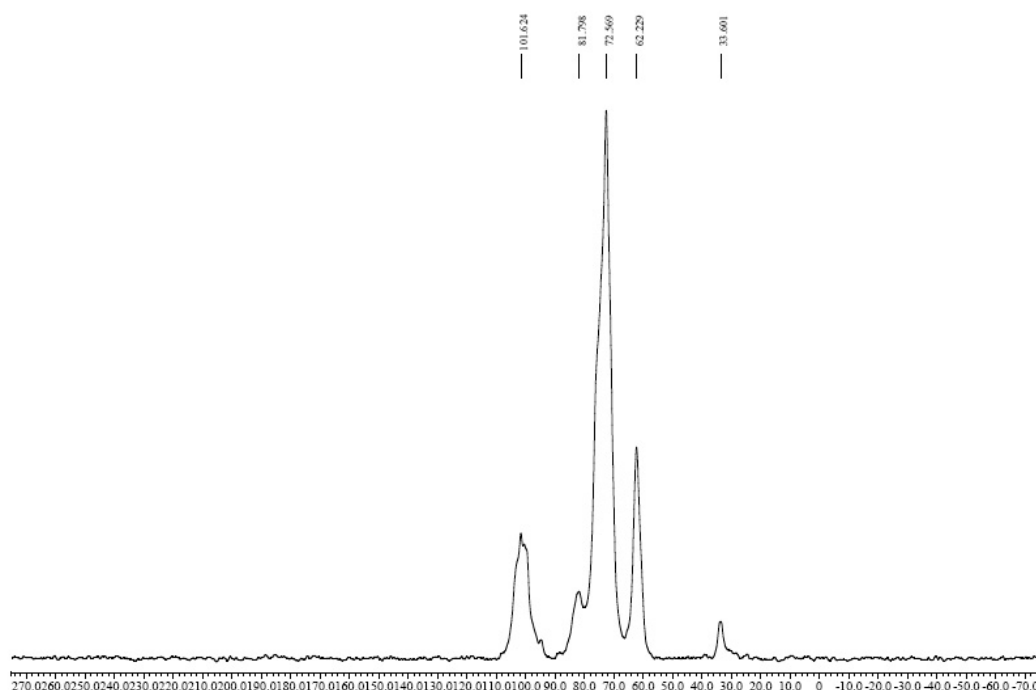


Figure 3: ^{13}C -NMR of Starch extracted from the mango seeds

SEM

The scanning electron micrographs of starch extracted from mango seeds revealed distinct morphological features (Figure 4). The granules predominantly exhibited heterogeneous shapes, including oval, elliptical, and irregular forms. Their size ranged from approximately 10 μm to 22 μm , with instances of aggregation observed in specific areas. However, this aggregation is unlikely to be due to thermal gelatinization, as the drying temperature (40°C) was well below the typical gelatinization range of starch (60–80°C). Surface analysis indicated predominantly smooth granule textures, with occasional clumping and bulking, particularly at higher magnifications, potentially influenced by extraction and sample preparation.

At lower magnifications (100x and 200x), starch granule clusters were observed, suggesting cohesive interactions possibly due to intermolecular bonding or partial gelatinization. This clustering can be attributed to hydrogen bonding and intergranular interactions that occur during alkaline extraction (0.1% NaOH), washing, and subsequent drying, rather than to complete gelatinization. Higher-magnification images (1000x and 2000x) enabled clear resolution of individual granules, revealing diverse morphological characteristics. The granule shapes, identified as oval, crooked, or cuboidal, corresponded well with documented morphologies of mango seed starch, encompassing both A-type

(disk-like, >16 μm) and B-type (spherical, 5–16 μm) granules. The impact of the extraction method on morphology was evident, with alkali extraction yielding slightly bulkier and smoother granules, consistent with rearrangements of amylopectin and amylose molecules. The observed size distribution was in agreement with previously reported data for mango seed starch, indicating the reliability and consistency of the extraction protocol [44].

These morphological characteristics are significant for the practical application of mango seed starch in the food, pharmaceutical, and biocomposite industries. The presence of smooth-surfaced yet irregular granules may confer desirable rheological and functional properties. Additionally, the detected aggregates and clumps likely reflect partial gelatinization or retrogradation, phenomena commonly associated with starch-based bioplastics or film matrices [45].

X-ray Diffraction Analysis

The X-ray diffraction (XRD) pattern of starch extracted from mango seeds exhibited characteristic peaks indicative of its semi-crystalline nature (Figure 5). Prominent diffraction peaks appeared at 2θ values near 15°, 17°, 18°, and 23°, which correspond to typical A-type crystalline patterns commonly observed in cereal starches, indicating an organized arrangement of amylopectin chains within the granules.

The presence of these peaks confirms the coexistence of crystalline and amorphous regions in the starch structure, with the crystalline domains contributing to the defined reflections. At the same time, broader humps reveal the amorphous matrix. The degree of crystallinity, calculated as the ratio of crystalline peak area to total area, indicated a moderately crystalline material, consistent with native starches extracted from botanical sources [46]. The observed crystallinity suggests that the starch granules retained their molecular order after alkaline extraction, which could positively influence their thermal and mechanical properties relevant to industrial applications. This semi-crystalline behavior aligns with previous reports for starches obtained from fruit seeds and highlights the potential of mango seed starch as a functional biopolymer.

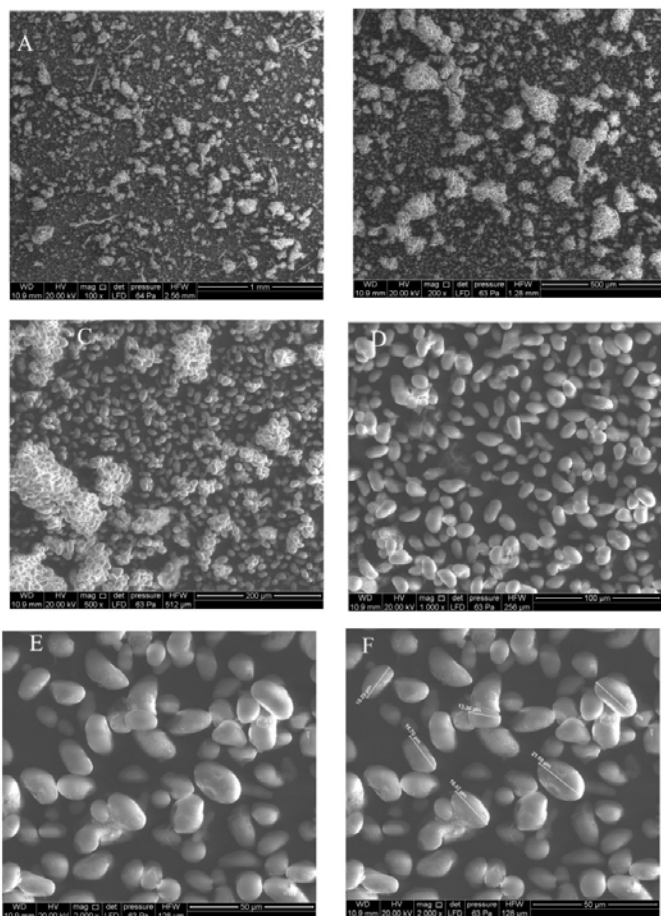


Figure 4: SEM of starch extracted from the mango seeds

DSC

The Differential Scanning Calorimetry (DSC) thermogram (Figure 6) of starch extracted from mango seeds exhibited distinct thermal transitions indicative of its physicochemical properties. An initial minor endothermic event below 120°C can be attributed to the loss of absorbed moisture. The DSC curve

showed an endothermic peak at approximately 326°C, corresponding to the thermal degradation and decomposition of the starch. This transition represents the cleavage of glycosidic linkages and the collapse of the polysaccharide backbone, indicating the material's good thermal stability at elevated temperatures [47].

The enthalpy change (ΔH) associated with this thermal event was calculated to be 29.63 J/g, indicating the energy required for thermal degradation. This value aligns with reports in the literature on botanically derived starches, indicating a typical thermal stability range for polysaccharides subjected to controlled heating under an inert atmosphere.

The weight-loss profile indicated a gradual decrease in mass starting at lower temperatures, which is typical for starch materials, with the initial loss attributed to moisture evaporation and loosely bound water molecules. Major weight loss was well correlated with the observed endothermic peak, confirming the thermal decomposition phase. The sample showed steady heat flow and decomposition kinetics. The residue remaining after thermal degradation was approximately 13.34%, indicating incomplete combustion or formation of thermally stable charred components.

Overall, the DSC analysis confirms the thermal stability and degradation pattern typical of starch extracted from natural sources such as mango seeds. The observed degradation temperature and associated enthalpy provide useful reference points for further applications in food, pharmaceutical, and material sciences where thermal processing conditions are critical.

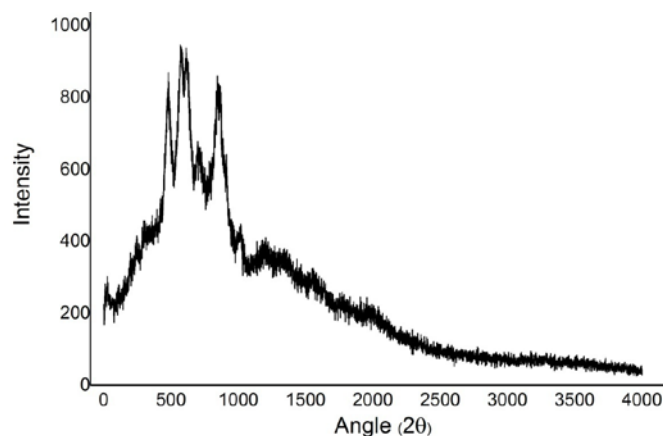


Figure 5: P-XRD study of the starch extracted from the mango seeds

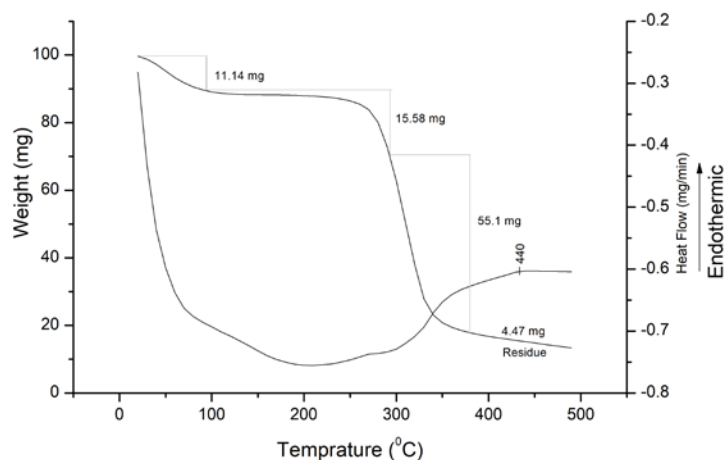


Figure 6: DSC spectra of Starch extracted from mango seeds

Mass spectra

Starch isolated from mango seeds was characterized using electrospray ionization mass spectrometry (ESI-MS) in both positive- and negative-ion modes, as shown in Figure 7. The ES+ spectrum exhibited prominent peaks at m/z 174, 194, 214, 223, 250, 279, 280, 309, 318, 345–346, 362, 382, 404, 436–446, 473–479, 512, 517, 630, 635, and 679, corresponding to oligosaccharide fragments and sodium/potassium adducts of glucose polymers. Major ion clusters indicated glucose monomers (m/z 179–223), disaccharides (m/z 279–309), trisaccharides (m/z 345–362), higher oligosaccharides (m/z 404–517), and larger glucose chains (m/z 630–679). In ES–

mode, peaks at m/z 155, 209, 254, 309, 390–407, 435, and 486 were identified as deprotonated glucose fragments and oligosaccharides, with the lower range (155–209) representing small carbohydrate fragments and the higher range (390–486) attributed to tetra- and pentasaccharide species [48]. The observed pattern, with mass increments of approximately 162 Da, confirms the presence of polysaccharide structures, specifically amylose and amylopectin. These findings collectively establish that the isolated material is starch, consisting of glucose oligomers of varying degrees of polymerization, characteristic of plant-derived polysaccharides.

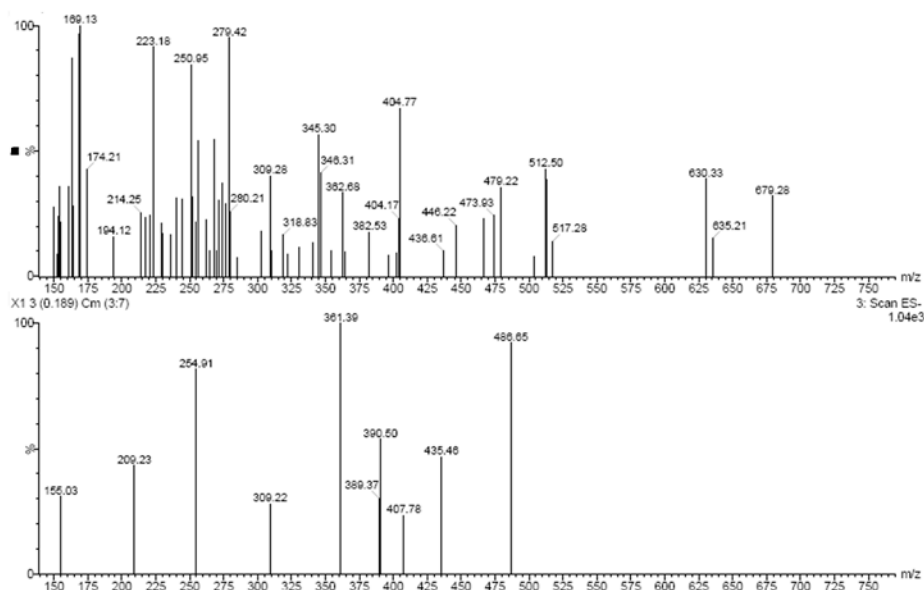


Figure 7: Mass spectra of starch extracted from mango seeds

CONCLUSION

The study successfully isolated starch from mango seeds using a modified alkaline extraction method, yielding 40% starch with

high purity as confirmed by phytochemical screening. Characterization revealed a starch composition rich in amylopectin (98.47%) with low amylose (1.53%), contributing

to superior swelling capacity and viscosity. The starch exhibited excellent dryness (0.80% moisture) and a slightly acidic pH (6.12), indicating stability suitable for pharmaceutical use. Morphological analysis showed oval to irregular granules (10–22 µm), and XRD confirmed A-type semi-crystalline patterns. Thermal analysis demonstrated good stability with degradation at 326°C (ΔH 29.63 J/g). Rheological evaluation reflected moderate viscosity (541.7 cP at 26.1°C), supporting its function as a thickening excipient. Flow property assessment indicated poor flowability, with a bulk density of 0.40 g/mL, tapped density of 0.56 g/mL, Hausner's ratio of 1.40, Carr's index of 28%, and an angle of repose of 47.98°, reflecting cohesive powder behavior typical of native starches. In conclusion, mango seed starch exhibits promising physicochemical and functional properties, indicating its potential as a sustainable biopolymer for pharmaceutical applications. Its utilization also supports the valorization of agro-waste while offering a cost-effective alternative excipient source. However, further formulation development, including tablet preparation and in vitro drug-release studies, is required to establish its performance and effectiveness as a pharmaceutical excipient conclusively.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Pooja P. Dhanawade contributed to the conceptualization, project administration, supervision, data curation, and writing, review, and editing of the manuscript. Meenakshi Bharkatiya was involved in conceptualization, supervision, data curation, writing, review, and editing. Nitin H. Salunkhe contributed to conceptualization, methodology, formal analysis, supervision, data curation, and writing-review and editing. All authors contributed to the study's methodology and formal analysis and have read and approved the final manuscript.

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