Extraction, Characterization, and Nanoreduction of *Syzygium cumini* (Jamun) Seed starch

A thesis submitted in partial fulfillment of the requirement for the award of the degree of

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Submitted by

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(July 2024)

DECLARATION

I hereby declare that the work presented in the thesis **"Extraction, characterization and nanoreduction of *Syzygium cumini* (Jamun) Seed starch"** in the partial fulfillment of the requirement of the award of the degree of Master of Science in Biotechnology at Thapar Institute of Engineering and Technology (TIET), is an original and genuine work completed by me between January 2024 and July 2024. This research was carried out under the guidance and supervision of Dr. Ovais S. Qadri, an Assistant Professor in the Department of Biotechnology, TIET. The content presented in this thesis has not been previously submitted, either in its entirety or in part, to any other educational institution or university in India or abroad for the purpose of obtaining any degree. Whenever references have been made to the previous works of others, they have been clearly indicated as such and included in the Bibliography.

Kanu

Place – Patiala Kanu

Date – 31 August 2024

CERTIFICATE

This is to certify that the thesis entitled "Extraction, characterization, and nanoreduction of *Syzygium cumini* (Jamun) Seed starch", submitted by Ms. Kanu (302201004) in the partial fulfillment of the requirement of the award of the degree of Master of Science in Biotechnology at Thapar Institute of Engineering and Technology (TIET), Deemed to be University, Patiala is a record of student's own work carried out under my supervision and guidance. This work has not been submitted in part or full to any other university or institute for the award of any other degree

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Table Of Contents

List of symbols

Acronym	Definition
°C	Degree Celsius
cm	Centimetre
g	Gram
%	Percentage
min	minute
nm	Nanometre
W	Watt
sec	Seconds
µm	Micrometer

List of Tables

List of Figures

Abstract

Syzygium cumini is reported to be the richest source of non-conventional starch. This study aimed to extract and characterize starch from *S. cumini* seeds. The starch was extracted by precipitation using six steeping additives: distilled water, NaOH, acetic acid, ascorbic acid, citric acid, and lactic acid. Scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and pasting properties analyses were performed to assess the extracted starch's qualities. Depending on the extraction medium, the starch granules' average diameters ranged from 2.8 to 27.5 µm for citric acid, 7 to 22 µm for ascorbic acid, and 7 to 32 µm for lactic acid. Despite the limited availability of research publications on the starch extracted from *S. cumini* seeds, this study performed comprehensive research establishing its extraction, characterization, and nano-reduction.

Chapter – 1

Introduction

The predominant carbohydrate in seeds, fruits, and tubers is starch, a polysaccharide abundant in plants. Starch possesses key attributes such as biodegradability, biocompatibility, and non-toxicity. The physicochemical variations of starch are influenced by its molecular structure and the specific plant sources from which it is derived (de Castro et al., 2019). The industry's heightened attention to starches derived from waste, especially fruit waste, is due to the growing demand for starch. This is driven by the need to find new starch sources that do not conflict with human populations as a food source (Kringel et al., 2020). Due to their diverse qualities, non-traditional starches offer a viable alternative to conventional starches in various industries and food production. Their global production is far lower than that of conventional sources (Henning et al., 2022). These alternative starches offer technological advantages and flexibility in modifications and applications, giving them an edge over traditional starches (Chen et al., 2023; Tarahi et al., 2022).

Syzygium cumini, or Jamun, belongs to the Myrtaceae family and is a deep purple-blue fruit with a pinkish plum, slightly acidic/astringent flavor. This fruit is majorly produced in India but is also grown in the West Indies, Thailand, the Philippines, Madagascar, East and West Africa, and Israel (Kaur et al., 2024). The *S. cumini* fruit has a calorific value of 83/100 g, chiefly composed of carbohydrates (19.7%) and excessively in the seed (89.68% total carbohydrates) (Bajpai et al., 2020), also rich in phytochemicals and antioxidants (M. Kumar et al., 2022b; Tak et al., 2022). The biochemical known as "jamboline" is found in the fruit, juice, and seed. It is thought to prevent the pathological conversion of starch into sugar in cases of excessive glucose synthesis (Marufa et al., 2019; Tosif et al., 2023b). There is an increasing demand for starches that possess specific characteristics for particular applications. Unconventional starches offer a more favourable alternative to conventional starches in various industries, including food, due to the wide range of properties exhibited by diverse forms of starches (Chen et al., 2023). The industry's growing emphasis on utilizing starches obtained from waste materials, namely fruit waste, is driven by a growing need for starch. This demand is accompanied by the need to find alternative sources of starch that do not compete with human populations for food. While starch is commonly derived from traditional sources like potatoes, cassava, corn, and wheat, there are also some fruits that contain significant amounts of starch and can serve as alternative sources of starch (Kringel et al., 2020).

S. cumini seeds, Loquat seeds (Cao & Song, 2019), jackfruit seeds (Zhang et al., 2019), litchi seeds (Kaur et al., 2024), mango kernel (Nawab et al., 2018), pearl millet (Sandhu et al., 2020), faba beans (Sharma et al., 2020), peas (Zhou et al., 2021), and sweet potato (Ballesteros-Mártinez et al., 2020), were the richest source of non-conventional starch. It has techno-industrial importance as it can be used for thickening, gelling, coating, sticking, and encapsulating. The morphology of starch granules, crystalline structures, the amylose-to-amylopectin ratio, and other factors all contribute to the significant differences in the physicochemical and functional qualities, such as pasting, gelatinization, solubility, swelling, and rheological features of different starch sources (Tarahi et al., 2022)

On the other hand, starch-based nanoparticles are drawing more interest because of their enhanced suspending capability, bio-accessibility, and controlled release behavior. As a result, starch nanoparticles are used in a wide range of applications, such as paper products, hydrogel, aerogel, emulsion stabilizers, drug delivery systems, and biocomposites (Dukare et al., 2021a). The small particle size of the nano starch provides a high specific surface area, high absorption, and reactivity capacity. It is biodegradable, non-toxic, and digestible with the lowest possible diffusion constraints. Usually, a variety of approaches, including chemical, physical, enzymatic, and nanoprecipitation procedures, are used to produce nano starch (Zuo et al., 2024). It serves as a food additive, such as a stabilizer, thickener, and texture enhancer, to control homogeneity and increase the shelf life of processed foods.

It is important to explain the distinctions between starch nanoparticles (SNP) and starch nanocrystals (SNC). The semi-crystalline structure of NS granules has an amorphous area that is hydrolyzed by acid to produce crystallites, microcrystalline starch, and hydrolyzed starch, which are referred to as SNC. The precipitation of non-crystalline starch granules produces the SNP (Kumari et al., 2022).

Given the limited availability of research publications on the starch extracted from *S. cumini* seeds, there is a need for comprehensive investigations into its extraction, characterization, and nano-reduction. The bioactive properties of *S. cumini* seed starch are important, yet there is insufficient literature on its properties and characteristics. Therefore, our research emphasizes the use of different steeping additives for extraction techniques and focuses on the proximal analysis, characterization, and nanoreduction of *S. cumini* seed starch.

Chapter – 2
Review of Literature

The literature pertains to a study on the extraction, characterization, and nanoreduction of starch. The fruit *Syzygium cumini,* commonly known as Jamun, is rich in nutrients and has been used traditionally as medicine and as an edible. The *S. cumini* fruits are oval, fleshy berries that have a single, dark brown seed in the centre that contains bioactive compounds. It originated in India, Myanmar, and Sri Lanka and was then brought to the Southeast Asian islands, China, Eastern Australia, and the Himalayan region. The Indian fruit *Syzygium jambolanum*, sometimes referred to as black plum, is called "jamun" in Hindi. *S. cumini* can also be defined as "a fried sweet doused in sugar syrup."

The distinctive blend of phenolics and glycosides, specifically ellagic acid and jamboline, inhibits the enzymatic breakdown of starch into sugar and aids in preventing diabetic consequences such as neuropathy and cataracts. Therefore, the seed possesses the ability to combat diabetes. Multiple studies have shown the advantages of taking *S. cumini* seed powder while undergoing cancer therapy. These benefits can be attributed to the presence of anthocyanins (0.54%) and other phytochemicals such as jambosine, gallic acid, ellagic acid, corilagin, and related tannins, as well as quercetin. Considering an increase in lifestyle disorders caused by stress, sedentary lifestyles, and excessive intake of highly processed foods, these *S. cumini* seeds, which are rich in phytochemicals and antioxidants, can be utilized as therapeutic agents (Kumar et al., 2022).

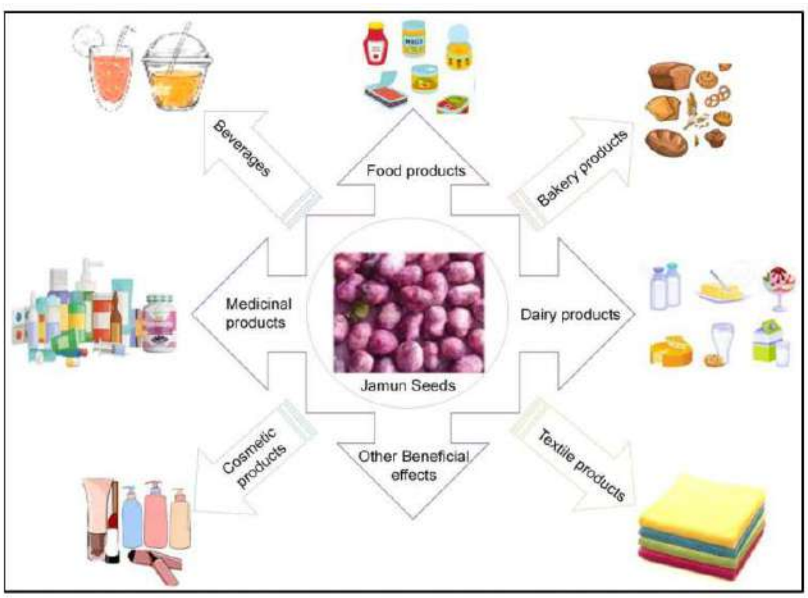


Figure 1. Multifunctional properties of *S. cumini* seeds (Das et al., 2023)

2.1 Taxonomy

Kingdom: Plantae—planta, plantes, plants, vegetal

Division: Magnoliophyta

Class: Magnoliopsida

Order: Myrtales

Family: Myrtaceae

Genus: *Syzygium*

Species: *Syzygium cumini* L.

2.2 Nutritional value of seed

The inedible byproduct of the *S. cumini* fruits is their seeds. According to proximate analysis, the moisture content of jamun fruit seeds ranged from 12.45 to 52.4 g/100 g. On a dry matter (dm) basis, the yielded contents were: crude protein 4.7-8.2 g/100 g, total lipids 0.35-1.28 g/100 g, total carbohydrates70.9-91.0 g/100 g, and ash contents 2.0-22.3 g/100 g, respectively. These seeds have been linked to several biological activities, including antidiabetic, anticancer, anti-inflammatory, antioxidant, antimicrobial, antihyperlipidemic, antihypercholesterolemic, cardioprotective, hepatoprotective, and neuroprotective effects. These bioactive compounds include hydrolyzable tannins, phenolic acids, flavonoids, other phenolics, terpenoids, and phloroglucinol derivatives. Jamun seeds also include a significant quantity of minerals, dietary fibre, ascorbic acid, and carbohydrates (Tak et al., 2022).

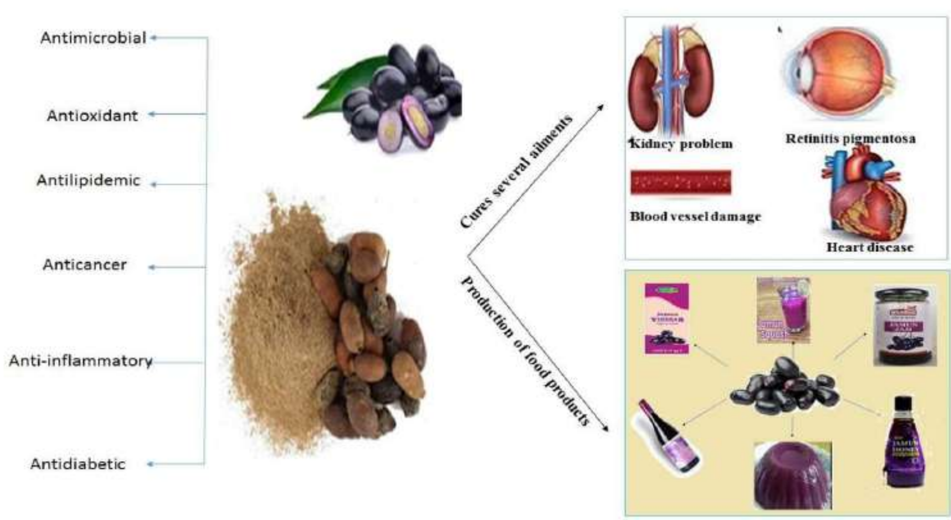


Figure 2. Biological activities of *S. cumini* seed (Kumar, n.d 2023)

2.3 Carbohydrate profiling of *S. cumini* seed flour

Approximately 20–80% of the overall fruit weight is composed of *S. cumini* seeds. The seed contains 89.68% total carbohydrates. Starch makes up most of the composition of *S. cumini* seeds. It decreases blood sugar levels through hypoglycemic effects, and it has also been called the "diabetes fighter" (Bajpai et al., 2020).

Starch is produced by plants as a reserve carbohydrate and is an important source of energy. For humans, rhizomes, roots, and tubers are the most significant sources. Various plants develop various morphologies of storage starch in their amyloplasts, which can range in size from sub-microns to more than 100 µm in diameter. These morphologies include circular, oval, ogival, or elongated to flat, lenticular, or polyhedral. The two main polysaccharides, amylose, and amylopectin, make up nearly all of the starch granules. Both are made up of chains of D-glucose residues linked by (1,4) links, which are joined by (1,6) glycosidic bonds to produce branches within the polymers. Although branched amylose molecules have fewer branches than linear amylose molecules, both types of amylose have long chains with hundreds or even thousands of glucosyl units, whereas amylopectin has relatively short chains and is highly branched (Bertoft, 2017)

Table 1. Composition of *S. cumini* seeds (Kshirsagar et al., 2019)

Nutrients	Content (per 100 g)
Moisture	53g
Fat	1.02g
Protein	3.84g
Carbohydrate	31.62g
Fiber	7.01g
Ash	1.51g

2.4 Review on study of different fruit seeds' characterization

Numerous studies have been conducted on the extraction of starch from different sources. With a focus on methods, challenges, and developments in the field, this study attempts to examine the existing literature on starch extraction from a variety of sources. This review aims to provide insights into how to optimize starch extraction procedures for industrial applications, ensuring efficiency and sustainability, by looking at the many botanical origins and advanced extraction techniques.

Loquat seed

The potential for unconventional starch sources to offer affordable substitutes for traditional starch has drawn attention in recent times. Loquat (Eriobotrya japonica) seed starch is a newly discovered non-conventional starch source that contains almost 20% starch. Because of its special composition, useful qualities, and innovative uses, it might be used as an ingredient. It's interesting to note that this starch shares many characteristics with commercial starches, such as a high amylose concentration, tiny granule size, high viscosity, and heat stability, which makes it a desirable choice for a range of culinary applications. Thus, the primary focus of this review is on the basic knowledge of how loquat seeds are valued by extracting starch using various isolation techniques that have desirable structural, morphological, and functional characteristics. It is discovered that several techniques for isolation and modification—such as wet milling, acid, neutral, and alkaline—are useful in achieving greater concentrations of starch. Also covered are insights into several analytical methods for determining the starch's molecular structure, such as X-ray diffraction, differential scanning calorimetry, and scanning electron microscopy. Furthermore, the impact of temperature and shear rate on rheological characteristics such as color, swelling power, and solubility index is disclosed. In addition, this starch has bioactive ingredients that have been demonstrated to improve the fruits' shelf life. All things considered, loquat seed starches have the potential to offer affordable, sustainable substitutes for conventional starch sources as well as new uses in the food sector. To create large-scale, high-value products with added value, further research is required to optimize processing methods. However, the structural and morphological properties of loquat seed starch are the subject of comparatively little published scientific research. Therefore, we concentrated on the various methods of isolating loquat seed starch in this review, as well as its structural and functional qualities and uses (Tosif et al., 2023).

Litchi seeds

A common ingredient in food, starch has a variety of uses in both industry and medicine due to its adaptable qualities. The behavior of starch in various reactions and cooking conditions can be understood by evaluating its physicochemical and functional characteristics, such as water binding, oil absorption, gelatinization, turbidity, swelling power, solubility, and emulsification. This will result in optimal industrial utilization and a decrease in agricultural and food waste. In this investigation, the starch from litchi (Litchi chinensis) seed flour was extracted utilizing procedures involving acid, alkali, and pure water extraction. The untreated flour and extracted starch underwent a thorough examination of their physicochemical characteristics. The following parameters were examined: bulk density (gm/cc), turbidity, syneresis, and retrogradation properties; moisture content (6.25%); ash content (2.9%); pH (water extract-5.0, alkali extract-6.85, acid extract-3.33); water absorption (water extract-81%, alkali extract-96.25%, acid extract-64.65%); water solubility swelling power; and oil absorption capacity. Notably, the most encouraging results were seen when starch was extracted using water. The sample also showed a favorable resistant starch concentration, suggesting that it might be used as a prebiotic. Based on the findings, litchi seeds have the potential for use in medicinal and industrial settings (IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES, 2012)

Avocado seeds

Environmental effects are caused by agro-industrial waste, and new materials and technologies are being used as alternatives to reduce it. The objective of this effort was to separate and analyze avocado seed starch in preparation for potential uses. After the grains were removed, the following were extracted: lipids (1.68%), proteins (1.60%), carbs (55.07%), moisture (41.35%), ash (0.33%), titratable acidity (4.64%), and starch (19.54%). The water solubility was 0.888%, the oil absorption capacity was 0.691 g/g, the water activity was 0.986 g/g, and the water absorption index was 0.333 g/g. The amount of amylose in the extracted starch is considerable (39.56%). The granule had an oval form and a smooth surface, and thermogravimetric measurement revealed stability up to 366 ◦C. Regarding the pasting qualities of the starches, RVA indicates that the beginning temperature was 88.5 ◦C, and the viscosity at the setback was 2880.5 cP. The findings imply that there may be further uses for the starch derived from avocado seeds, such as the production of edible or biodegradable films (Martins et al., 2022).

Mango seeds

The methods of distillation, alkaline, sedimentation, and centrifugation were used to extract the starch from mango (Mangifera indica) seeds. Comparative research was done on the effects of extraction techniques on the morphological, structural, and physicochemical qualities that were extracted. Under a scanning electron microscope (SEM), morphological features such as the size and shape of the starch granules show notable variations. Despite using a distinct extraction technique, the extracted mango seed starch granules had a smooth surface and an irregular or oval shape. The alkaline method (15.83 ± 3.65 μm), sedimentation method (15.97 ± 4.44 μm), centrifugation method (19.91 ± 6.82 µm), and distillation method produce the smallest starch granules (13.80 ± 2.90 µm). The isolated mango seed starch was shown by the X-ray diffraction pattern to be of the A-type, which supports the hypothesis that the size of the starch granules indicates their morphology. Mango seed is one of the possible sources of starch, as evidenced by the fact that starch separated using the alkali method had the highest starch yield of 91.72% compared to starch recovered using distillation (81.87%), sedimentation (74.22%) and centrifugation (74.56%) (Shahrim et al., 2018).

Jackfruit seeds

In Brazil, there were two primary varieties of jackfruit: soft and hard, both of which contain high amounts of starch. The purpose of this study was to assess how extraction techniques (aqueous and alkaline treatments) affected the physicochemical and structural characteristics of starches from soft and hard jackfruit seeds. The round, bell-shaped, and tiny (6.4–11.1 mm in diameter) jackfruit seed starch (JSS) granules had a smooth surface. The soft type had a greater apparent amylose content, ranging from 24 to 32%, which affected a number of characteristics. The high peak gelatinization temperatures (72–81°C) and a crystallinity index ranging from 9.3 to 36.9% were caused by the starches' A-type XRD patterns. Peak viscosities (between 2362 and 3373 cP) resembled those of cassava starch. However, when compared to the soft JSS, the hard JSS had comparatively stronger pasting qualities (peak viscosity and breakdown), swelling power, and solubility index (90°C), which may have been related to the lower amylose concentration. This was true regardless of the extraction method. There were notable variations in the lipid, protein, and amylose composition of every starch that was extracted using the NaOH solution. The JSS average particle size was reduced by approximately 36% by the alkaline solvent, most likely due to a chemical gelatinization. NaOH furthermore produced slight alterations in granule shape and swelling power, as well as a considerable decrease in the crystallinity index (Luciano et al., 2017).

Arrowroot starch

An essential biopolymer, arrowroot starch is well-known for its potential uses in a variety of sectors, most notably the manufacture of biodegradable films. Optimizing the usage of arrowroot starch in film production requires an understanding of its characteristics and how it reacts with plasticizers like glycerol. This review assesses the effects of different starch and glycerol concentrations on the characteristics of the resultant films and provides an overview of the literature on the extraction and characterization of arrowroot starch. The amylose content of arrowroot starch is significant, as it has been estimated to be approximately 35%. Amylose has a crucial role in determining the functional characteristics of starch, influencing its capacity to gelatinize, undergo retrogradation, and form films. Usually, the extraction procedure entails separating the starch from the arrowroot tubers and purifying them to get rid of any contaminants. Arrowroot starch's swelling power and solubility index are two of its key attributes. Research has revealed that arrowroot starch exhibits very low values for both criteria, suggesting restricted solubility and water absorption. In some situations, where water resistance is desired, these qualities are beneficial. A "C" type crystalline structure, which lies in between the "A" type found in cereal starches and the "B" type found in tuber starches, is revealed by X-ray diffraction examination of arrowroot starch. The mechanical and thermal properties of starch are influenced by this structural feature. The stability of starch under heat is indicated by glass transition temperatures (Tg), which are found in the range of 118 to 120°C according to thermal analysis techniques such as differential scanning calorimetry (DSC). Thermogravimetric analysis (TGA) sheds light on how arrowroot starch degrades over heat. It has been noted that substantial mass loss happens between 330 and 410°C, when the starch polymer breaks down. Plasticizers such as glycerol must be used when using arrowroot starch in filmmaking in order to increase the films' manageability and flexibility. The physical characteristics of the films are mostly determined by the concentrations of glycerol and starch. The films that were generated demonstrated the arrowroot starch in film applications since they were uniform, clear, and controllable (Nogueira et al., 2018).

Malay-red apple seed

In this study, a novel naturally phosphorylated starch was isolated from the seeds of the *Syzygium malaccense* stone fruit—an unconventional and underutilized source. The morphological and chemical properties of the extracted starch were investigated using techniques including scanning electron microscopy, FTIR, -NMR, HPAEC-PAD chromatography, XRD, DSC, and RVA. A highly pure starch (95.6%) with an average granule size of 13 μm was obtained from the extraction process. Through HPAEC-PAD chromatography, the examination of the starch components indicated an amylose content of 28.1% and a majority (65%) of B-chains (B1–B3 65%) in the amylopectin. The deconvolution of the C1 signal in the 13C-CP/MAS-NMR verified that the X-ray diffractogram was consistent with B-type starch. Additionally, the X-ray diffractogram revealed that 28.5% of *S. malaccense* is crystalline. The results of the DSC study for Tc and ΔH were 82.6 ◦C and 12.41 J g 1, respectively. These values are consistent with a highly organized structure of starch granules. Peak (4678 mPa•s), trough (3055 mPa•s), and ultimate viscosity (6526 mPa•s) values were recorded, suggesting that *S. malaccense* might be utilized as a thickening in hot meals (Santos et al., 2024).

Pea starch

When isolating starch from pea flour, organic acids (5% lactic acid and 5% acetic acid, w/v) and biosolvents (5% ethyl lactate, 5% and 10% d-limonene, w/v) are employed as extraction agents. As a control, 0.4% w/v NaOH was used. The findings reveal little variations in the granular morphology of starch, apparent amylose concentration, and the majority of temperature parameters, as well as insignificant variations in the crystalline structure of starch. Nonetheless, discernible variations are seen in the protein and starch damage composition, as well as pasting viscosities, across starches extracted using various extraction methods. The starch extracted using 5% ethyl lactate has the lowest pasting viscosities, whereas the starch extracted using 5% acetic acid has the greatest protein residue (1.1%) when compared to the other methods (≤0.4%). Since pea starch has the highest pasting viscosities and the least amount of leftover protein and solvent residue, 5% d-limonene appears to be a viable biosolvent for pea starch isolation. Utilizing Fourier transform infrared spectroscopy (FTIR), residual protein bands are identified in every sample. Additionally, distinct bands at 1564, 1575, and 1720 cm^{-1} are linked to acid residue found in starches that were extracted using the two organic acids. Starches extracted by biosolvents are distinguished from those extracted by alkali and acidic agents by chemometric analysis of FTIR spectra (Lu et al., 2019).

Nanostarch reduction

Reduced to nanoscale dimensions, nanostarch has drawn a lot of attention recently because of its special qualities and its uses in a wide range of industries. In contrast to regular starch, nanostarch has more features, like more surface area, better solubility, and different mechanical and thermal properties. These characteristics make it a viable material for a variety of uses, such as food packaging, medicine delivery systems, bioplastics, and as a useful component in food items. This review provides an overview of nanostarch research, with particular attention to the methods used to reduce it, the qualities that result, and the wide range of uses it has. This study aims to provide insights into future research directions and industrial applications by combining the most recent discoveries and highlighting the challenges and advances in the field of nanostarch.

A high-speed jet (HSJ) was used to manufacture nanostarches successfully following a pretreatment of micronization. After 60 minutes of micronization treatment, one cycle of 240

MPa of HSJ (188.1 nm) was applied to obtain the nanostarches. Furthermore, the particle size might approach that of nanoscale materials (66.94 nm) following three cycles of HSJ treatment. Nanostarches physicochemical characteristics have been described.
The viscosity of nanostarches dramatically dropped when compared to native tapioca starch, and it declined slightly as the number of HSJ processing cycles increased, according to Rapid Visco-Analysis (RVA). All materials showed pseudoplastic, shear-thinning behavior, according to steady shear analysis, and the HSJ processing cycles had no effect on the nanostarch flow curves. Following HSJ treatment, the tapioca starch crystalline structure was completely destroyed, according to X-ray diffraction measurements. The analysis of molecular features revealed that amylopectin chains were broken down following HSJ and micronization treatments, as demonstrated by the drop in weight-average molar mass. The outcomes showed that the disintegration of starch molecules produced nanostarches. This research will offer valuable insights into nanostarches and their potential industrial applications (Xia et al., 2017).

Mung bean nanostarch

Using acid hydrolysis, mung bean starch nanocrystals (SNC) were created. The thermal, rheological, and morphological characteristics of the resultant nanocrystals were examined. formed by acid hydrolysis, irregular or spherical nano-scale crystals with an average hydrodynamic diameter of 179 nm. Using X-ray diffraction (XRD), the mung bean SNC showed a C-B-type crystalline structure with increased crystallinity. In comparison to its native starch, mung bean SNC has a lower negative zeta potential. The disappearance of thermal peaks for nanocrystals suggested that the SNC from mung beans was more thermally unstable. Even at high concentrations of SNC in the suspension, a shear-thinning behavior was observed. At every concentration under investigation, the elastic behavior was noted, and it was unaffected by changes in frequency (Kumari et al., 2022).

Cereal and tuber nanostarches

In this study, an enzymatic method was used to extract and analyze nanostarch from a tuber (cassava and potato) and cereal (maize) crops. The standard acid hydrolysis procedure was used to create nanostarch. Numerous methods, including differential scanning calorimetry (DSC), X-ray diffraction (XRD), dynamic light scattering (DLS), Fourier-transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM), were used to study these nanostarches. When it came to maize starch, the lowest sizes were 18 ± 3 nm when hydrolyzed with an acid and 162 ± 23 nm when hydrolyzed with an enzyme. For the starches of maize, potatoes, and cassava, the enzymatic method produced yields of nanostarch of 18, 29, and 41 wt%, respectively. After enzyme hydrolysis, a significant reduction in the crystalline area of starch was observed as determined by relative crystallinity using XRD spectra. DSC showed that the reduction of the amorphous region in nanostarch resulted in a decrease in its melting enthalpy. Because enzyme hydrolyzed nanostarch is renewable and biodegradable, it has the potential to be used as filler in biocomposites due to its reinforcing qualities (Dukare et al., 2021).

Corn and waxy corn nanostarch

In order to create nanoparticles (nano-WCS and nano-CS), corn (CS) and waxy corn starch (WCS) with varying amylose contents (as determined by UV-vis spectroscopy) were treated to acid hydrolysis, ultrasound, and a combination of both, carried out at a raised temperature for a short period. Scanning transmission electron microscopy (TEM-STEM) demonstrated the viability of the suggested technique for producing nanoparticles smaller than 5 nm.

X-ray diffraction (XRD) and FTIR spectroscopy provide information on their molecular structure. A gaseous product of thermal decomposition was also analyzed using FTIR along with thermogravimetric analysis (TGA). The degree of loss in thermal stability varied between nano-WCS and nano-CS. It has been demonstrated that waxy corn starch is less modifiable. It is anticipated that nanoparticles will work well in biomedical and functional food applications (Bajer, 2023).

Gaps in Literature

Starch is a valuable and important ingredient in different food preparations as a functional ingredient. However, the food industry is not the only industry that uses starch. There is an ever-growing market in the starch industry worldwide. The present study aims to explore another source of starch that has not yet been tapped. The importance of the present study is listed below.

- Presently, no study has been reported in the literature based on the extraction and characterization of starch from *S. cumini* seeds.
- The *S. cumini* seed starch will be a 'novel starch', and the rheological properties have to be determined in order to establish the appropriateness of this starch as an ingredient in different food applications. No such study presently exists.

Objectives

- To extract starch from *S. cumini* seeds using different solvents for maximum yield
- To characterize the structure of the extracted starch

- To determine the rheological properties of the extracted starch
- To perform nanoreduction of the extracted starch using physical and chemical methods
- To characterize the structure of nanostarch

Chapter – 3
Methodology

Chemicals and Materials: The fruit of *Syzygium cumini* was purchased from the local market of Patiala. The steeping additives citric acid, ascorbic acid, acetic acid, sodium hydroxide, and lactic acid were purchased from Loba Chemie Pvt. Ltd. Company. The lab's distillation apparatus generated the distilled water used for the extraction.

***S. cumini* seed processing:** Before analyzing the physical features of the *S. cumini* seeds, a few preparatory steps were required to be completed. The *S. cumini* fruit was washed with tap water to remove any unnecessary debris. This culinary and food processing method, known as blanching, involves boiling food and then quickly cooling it in ice water. Blanching stops enzyme activity, which is essential for maintaining food quality. Heat during the blanching process inactivates deteriorating enzymes in fruits and vegetables, resulting in gradual changes to their color, texture, flavor, and nutritional content. If these enzymes are allowed to remain active, they may continue to degrade cellular structures, producing strange tastes, vitamin deficiencies, and textural alterations. Blanching efficiently stops these enzymatic reactions by heating the food quickly and then rapidly cooling it in cold water, preserving its crispness and nutritional value. It has several benefits, including maintaining the vibrant color and crunchy texture of vegetables, preserving nutrients by deactivating enzymes, extending the shelf life of vegetables for freezing, lowering surface microorganisms, making skin peeling easier, enhancing flavors and odors, lessening the bitterness in some vegetables. This process improves various foods' overall quality, safety, and usability.

Figure 3. Extracted seeds from *S. cumini*

Drying

Prior to analysis, the seeds obtained from the *S. cumini* fruit were subjected to hot air drying at a temperature of 50 ºC. This process efficiently decreases the moisture content in the seeds, which is vital for several reasons. Excessive moisture in seeds can cause mold to grow quickly and increase microbial activity, resulting in a considerable decline in the quality and viability of the seeds. The formation of mold not only impacts the physical characteristics of seeds but also presents health hazards due to the possible generation of mycotoxins. In addition, elevated moisture levels might expedite metabolic activities in the seeds, resulting in a decline in both vigor and germination capacity. By reducing the moisture content through the process of hot air drying, the negative impacts are minimized, therefore maintaining the seeds' ability to survive and remain strong during storage. This technique guarantees the preservation of the seeds' optimal condition for future utilization, whether it be for cultivation or additional examination, by upholding their structural integrity and biological functionality.

Grinding

Following the drying process, the Jamun seeds were cooled to room temperature to ensure their stability and ease of handling. After being cooled, the seeds underwent grinding in order to transform them into a consistency similar to flour. The initial grinding process fragmented the seeds into smaller particles, hence enhancing the efficiency of the subsequent phase. Subsequently, the initial seed material was sifted to obtain an extremely fine powder, guaranteeing consistency in the size of the particles. Sieving is a process that eliminates bigger particles or impurities that could potentially impact the quality and uniformity of the end product.

Figure 4. *S. cumini* seed powder

After obtaining the fine powder, it was carefully packed into aluminum packets. Aluminum packaging is selected for its exceptional barrier characteristics, preserving the powder from moisture, light, and other environmental elements that may deteriorate its quality. The compressed powder was thereafter placed in a refrigerated storage area to maintain its freshness, nutritional content, and functional characteristics until it was required for future utilization.

3.1 *S. cumini* starch extraction

The extraction of *S. cumini* seed starch was done using different steeping additives via precipitation. The different extracting agents were 0.4% w/v NaOH (control), 5% w/v lactic acid, 5% w/v acetic acid (Lu et al., 2019), 0.05 M ascorbic acid (Tosif et al., 2023), 1.0% citric acid and distilled water (Santos et al., 2024). Firstly, 20 g of jamun seed flour was added to the different concentrations above. The extraction was performed in an incubator shaker with continuous stirring of 100 rpm for 6 h at room temperature. After extraction, the slurry was filtered using a cloth bag of mesh size 200 µm. The residual was washed with distilled water three times and precipitated overnight at 4°C. The supernatant was discarded. The starch was precipitated, washed with ethanol to remove other residues, and dried at 40°C for 24h. The dried starch was ground with a mortar pestle and passed through the sieve of pore sizes 710 µ and 300 µ to convert into a uniform powder. It was further sealed in plastic bags and stored in the refrigerator for further use (Shahrim et al., 2018).

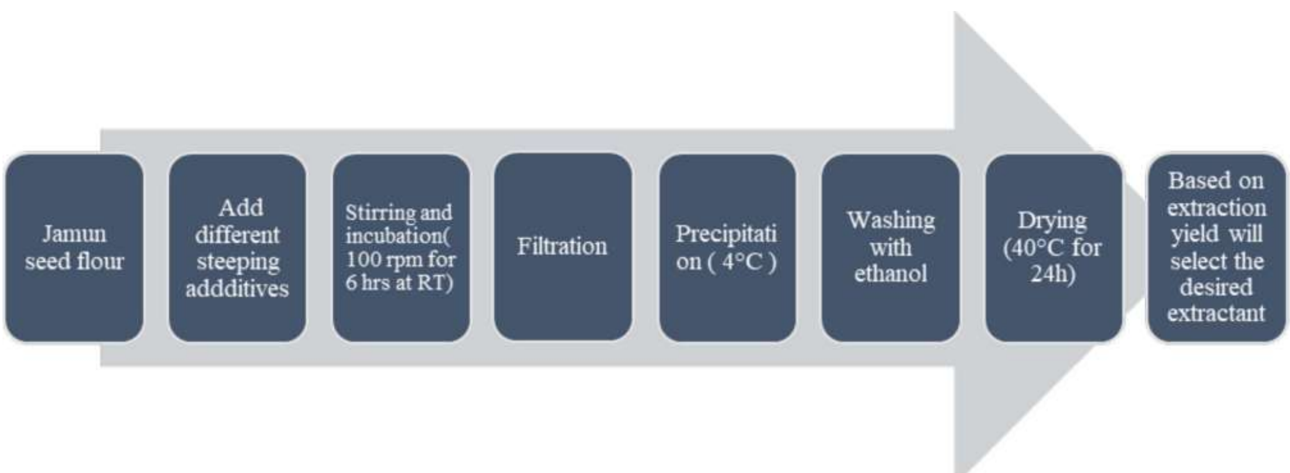


Figure 5. Preliminary extraction of starch

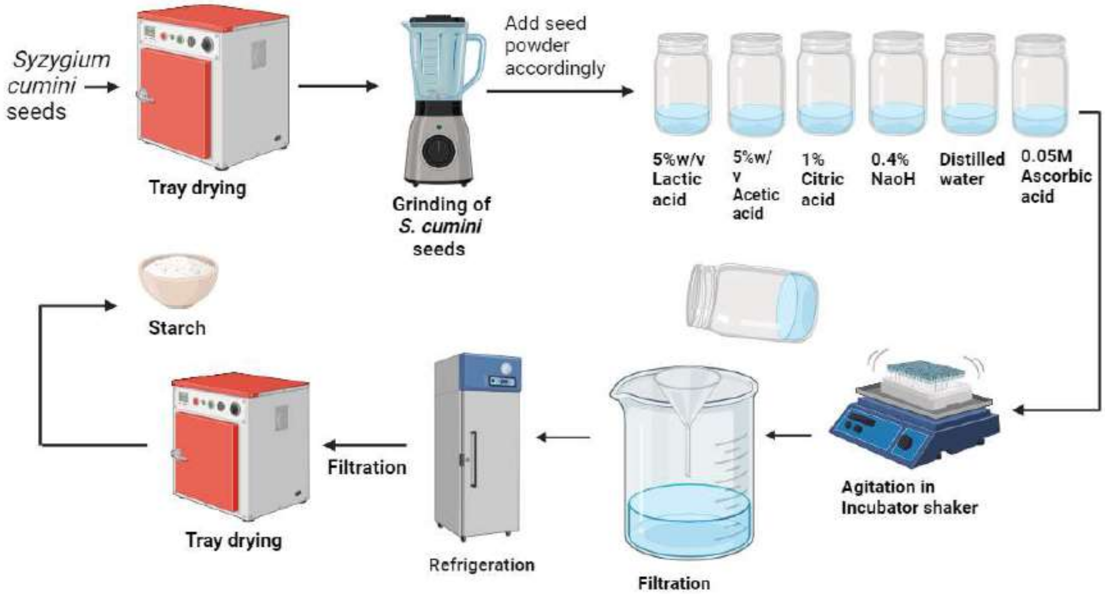


Figure 6. Extraction of starch using different steeping additives

3.2 Starch characterization

3.2.1 Starch yield

The starch yield of *S. cumini* seeds was determined by the method given by (Roy et al., 2020). Calculation of starch yield was done by using the formula :

$$Starch\ yield\ (\%) = \frac{weight\ of\ extracted\ polysaccharide}{weight\ \ of\ raw\ material} \times 100$$

3.2.2 Proximal analysis of starch

3.2.2.1 Moisture content

The *S. cumini* seeds were washed under running tap water, dried, and converted into powder form. A moisture content analyzer is a device used to measure the moisture content of the

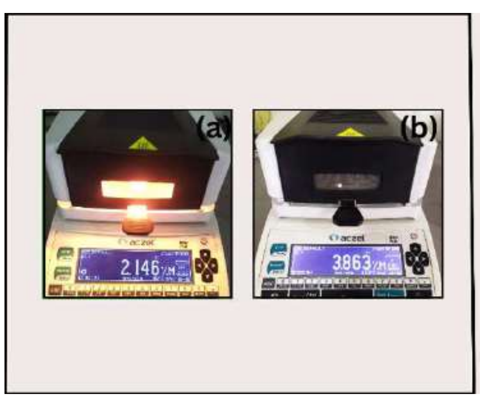


Figure 7. (a) moisture content analyzer (b) endpoint

sample. The device comprises a weighing unit and a halogen heating unit that measures moisture content based on loss using the drying method. Weighed the sample 2g of seed powder on the Petri plate contained in the weighing unit. The start button was pressed, depending on the moisture contained in the sample, and the result was displayed after some time. The moisture content analyzer Aczet Mb50 was used.

3.2.2.2 Ash content

Ash content was determined by de Castro et al. (2019) in Muffle Furnace BWF-11/07cr. Ash refers to the inorganic residue that remains after the complete burning of organic matter. Weighed 2g of sample and empty crucibles. The crucibles were placed into the muffle furnace at a temperature of 550°C till the residue was obtained of white color. Then, the sample was immediately put into the desiccator containing silica gel beads so that it would not capture moisture from the atmosphere. Then, weighed the crucibles containing the ash.

Materials are heated to extremely high temperatures in an enclosed heating device known as a muffle furnace, which keeps them safe from chemicals and pollutants outside. These furnaces are usually lined with stainless steel, which provides them with outstanding resistance to corrosion. The purpose of muffle furnaces is to remove combustion heating byproducts such as ash, soot, and gas fumes that may contaminate the material being heated. The highest temperature they can withstand is 1800°C (3270°F).

$$\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{sample taken}} \times 100$$

3.2.2.3 Fat content

According to Martins et al. (2022), fat content was determined using a fat extraction unit (Socsplus Scs04e) based on Soxhlet extraction.

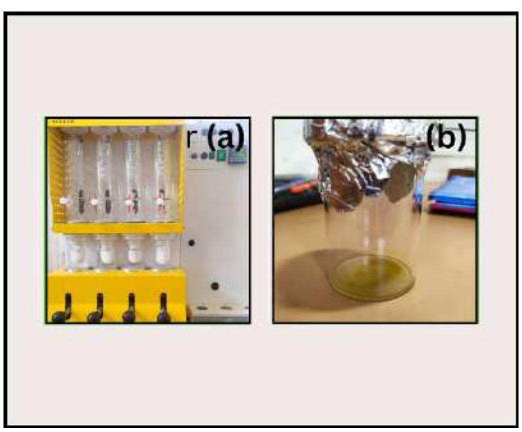


Figure 8. (a) Fat extraction apparatus (b) Extracted

Weighed 2 g of the sample and placed it into thimbles. Then, the thimbles were inserted into the solvent holder using the thimble supporting ring. 20ml of a non-polar solvent such as petroleum ether or n-hexane was poured into the thimbles, and 80ml was poured into the beaker where the thimbles were positioned. Subsequently, the beaker was positioned on a heating plate, and the solvent holder was connected to the condenser. Ensured that the water supply to the condenser was turned on before starting the apparatus. The extraction knobs were rotated to the vertical position (open) until the unit emitted a beep. Then, switched the extraction knobs to the horizontal position (closed) until the solvent was collected. After 1.5 hours of extraction, the extraction cups were removed from the Extraction Unit. The extraction cups were dried in a hot-air oven at a set temperature of 100°C for 20 minutes. Allowed the extraction cups to cool in a desiccator for 10 minutes, then weighed them to determine the crude fat content.

$$\text{Fat content (\%)} = \frac{W_1 - W_2}{\text{sample taken}} \times 10$$

Where, W_1 – weight of beaker after oil extraction; W_2 – weight of empty beaker before extraction

3.2.2.4 Protein content

The Kjeldahl method was used to determine the protein content. This method does not measure the protein content directly, and a conversion factor is required to convert the measured nitrogen concentration into protein concentration.

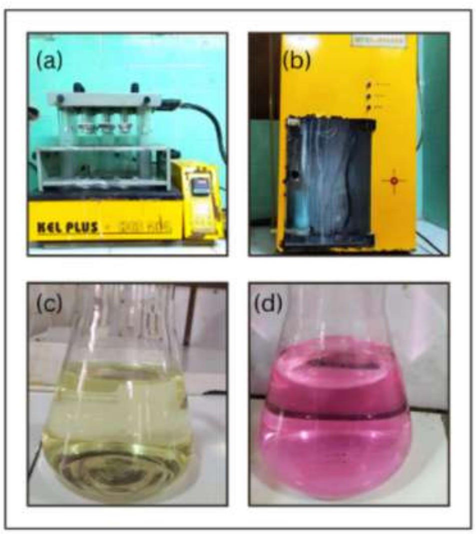


Figure 9. Kjeldahl digestion process (b) distillation process (c) after distillation showing green color (d) Pink color after titration

Preparation of reagents:

40% NaOH – Dissolve the 40g of sodium hydroxide pellets in 100 ml of distilled water.
4% Boric acid – Dissolve 4g of boric acid into 100 ml of distilled water.
0.1N HCl – To the stock of 100 ml add 1ml of HCL and 99ml of distilled water.
Mixed indicator preparation – 0.099g of bromocresol green and 0.066g of methyl red in 100 ml of ethyl alcohol.

Kjeldahl's method can be divided into three steps:

1. Digestion – 1g of food sample was added into the digestion tube, and 10g of potassium sulfate, 1g of copper sulfate, and 25 milliliters of sulfuric acid were placed within the digestion chamber and heated to 350°C for 3 hrs.

2. Distillation – The machine was turned off after 3 hrs. 50 ml distilled water along with NaOH was added until the reaction stopped. A few drops of mixed indicator were added to the 4% boric acid. Run the distillation apparatus for 15 min. Finally, the green color indicated the presence of ammonia, representing the extracted nitrogen content from the sample.

3. Titration – The solution was titrated with 0.1 N HCL, and the endpoint was characterized by a light pink color.

$$\text{Nitrogen }(\%) = \frac{\text{Sample titre} - \text{blank titre}}{\text{W} * 1000} * 14 * \text{N} * 100$$

Where N is the Normality of the standard HCl solution, W is the Weight of the sample taken.

$$\text{Protein }(\%) = \text{Nitrogen }(\%) \times 6.25$$

3.2.2.5 Carbohydrate content

The carbohydrate content in the sample can be carried out by the sum of fat, protein, moisture, and ash percentages subtracted from 100 (total percentage).

$$T_{carb}\,(\%) = 100 - (\%\text{ fat} + \%\text{ protein} + \%\text{ moisture} + \%\text{ ash})$$

3.2.3 Water Activity

The amount of water that is available for chemical and microbiological processes in a substance, like food, is measured by water activity. Pure water has a value of 1, and the scale goes from 0 to 1. The water activity of the extracted starch samples was evaluated. On a little circular plate, each starch powder was separately deposited, and the water activity was recorded by the Aqualab Pawkit water activity meter from METER group 40109.

Figure 10. Showing water activity

3.2.4 Water and oil absorption capacities

The water and oil absorption capacities were determined by the method described by de Castro et al. (2019). 10 ml of distilled water and commercial soybean oil were added to 1g of the sample in centrifuge tubes. The mixture was vortexed for 30 seconds and left to stand for 30 minutes. Further, it was centrifuged for 15 minutes at 3000 rpm, and the supernatant was discarded. Then, the dried external walls of the tubes were weighed. Then calculate the absorbed mass of water and oil by the formula:-

$$Absorbed\ mass = \frac{wet\ weight - dry\ weight}{dry\ weight} * 100$$

3.2.5 Pasting properties

The pasting properties of the samples were investigated using the method followed by Makroo et al. (2024) using a rheometer (MCR-101, Anton Paar, Austria) installed with a starch cell. Briefly, 2.5 g of sample was added with 30 mL of distilled water in a canister, and the powder/ water suspension was mixed at 960 rpm for 10 s, followed by heating to 50 °C and holding for 2 minutes. The slurry was heated from 50 to 90 °C for 4.5 minutes and held at 90 °C for 3.7 minutes. Finally, the sample was cooled to 50 °C over 5.5 minutes and held at 50 °C for 2 min. The shear viscosity was measured at 160 rpm and reported in centipoise (cp).

3.2.6 Scanning electron microscopy

The surface morphology of the starch was observed under the scanning electron microscope. The starch granules were prepared using the drop cast method. All samples were examined with a Zeiss Sigma 500 scanning electron microscope at a voltage of 5 kV.

3.2.7 FTIR

FTIR analysis of *S. cumini* seed starch was conducted before and after rapid visco-analysis using FTIR Perkin Elmer L1600300 spectrum to assess the impact of gelatinization on the functional groups of starch.

3.2.8 Nanostarch preparation

Suspensions were prepared using 5g of jamun seed starch extracted using citric acid. After that, the starch was rinsed three to four times with cold water until its pH was near a basic level. Then, 70 ml of distilled water was added. A 2 mol/L HCl solution was added dropwise to the solution until the pH was between 3 and 4 after it reached 65 °C. After that, the mixture was heated for 1 hour at 420 rpm using a magnetic stirrer. Subsequently, the suspension was sonicated for 1 hour at 60°C. Sonication was carried out at a frequency of 40 kHz with a power of 100 watts. Samples were altered chemically and physically, and a combination of the two techniques was used (sonication after HCl hydrolysis). The samples were dried at 50 °C using a tray drying method and analyzed for their microstructure using SEM.

Chapter – 4

Results and discussion

4.1 Starch yield

The quantity of extracted starch varies according to both the plant source and its specific stage of development. Thus, starch serves as the main food for storing energy during embryonic development and helps regulate seed germination and dormancy.

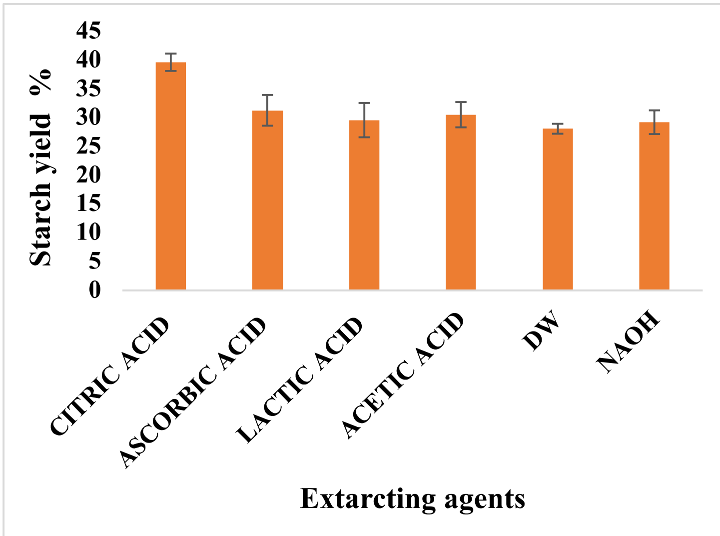


Figure 11. Extraction yield of starch

The starch was extracted using six different steeping additives: Citric acid, ascorbic acid, lactic acid, acetic acid, distilled water, and sodium hydroxide. The maximum starch yield was obtained using citric acid as the extractant, with a yield of 39.65%, while the lowest yield of 28.11% was obtained with distilled water. The starch content ranges from 28.11% to 39.65% (figure 3).

When considering stone fruits, the reported extraction yields of starch vary widely. The starch yields ranged between 24.2% and 14.0% for extracting starch with citric acid and distilled water from Malay red apple seeds (Santos et al., 2024). The other study reported a lower yield of 12% starch from litchi seeds using sodium hydroxide, 31.6%, and 24.8% using citric acid and distilled water (Jaiswal & Kumar, 2015).

Polyphenols significantly impact starch extraction yield, which increases due to their unique features. The higher starch yield from *S. cumini* seeds may be attributed to the presence of polyphenols (Santos et al., 2024). The phenolate intermediaries are formed during the catalytic conversion of phenolic compounds to polyphenols. These dark compounds bind to starch very firmly, resulting in the granules' undesirable discoloration. Additionally, the adsorption of polyphenol derivatives affects the starch precipitation rate due to the partially hydrophobic nature of some phenols. By effectively inhibiting polyphenol oxidase activity, citric acid improves the quality of extracted starch by reducing polyphenol production and its subsequent adsorption onto starch granules. As a result, citric acid extraction yields a higher starch content than other extracting agents, where polyphenol adsorption interferes with starch precipitation.

Polyphenol oxidase (PPO) activity decreases with fruit ripening. The thylakoid membranes sustain damage during ripening. This leads to the metabolism of membrane-associated materials like proteins and enzymes, and the intracellular recycling of polyphenol oxidase is probably achieved. When compared to apples, durum wheat, and mushrooms, *S. lycocarpum* PPO exhibits higher thermostability. Variations in plant species and the agronomic and climatic conditions in which they were cultivated could cause variations in the heat activation kinetics of PPO from different sources.

Batista et al. (2014) reported the inhibitory effect of citric acid on PPO activity. Both citric acid and ascorbic acid exhibit uncompetitive inhibition. Citric acid's inhibitory action is probably caused by its binding to the copper active site, which results in the formation of an inactive complex. Depending on the extracting agents used, the polyphenol content of the seeds probably affected the yield of the starch extraction process. As a result, there is an increase in starch extraction yield of citric acid compared to other acids.

4.2 Proximate analysis and water activity

The proximate analysis of the extracted starch from the different extracting agents is shown in Table 1. The starch isolated using different extracting agents showed the following properties: moisture content 1.8-10%, ash content 0.5-1%, fat content 0.5-3.5%, protein content 3.5-6.73%, and water activity 0.35-0.92.

Table 2. Proximate analysis of extracted starch from *S. cumini* seeds

Extracting agents	Moisture content (%)	Ash content (%)	Fat content (%)	Protein content (%)	Water activity
Ascorbic acid	5.0	0.5	1	3.5	0.36
Lactic acid	3.8	1	1	6.73	0.37
Acetic acid	3.6	0.5	1.5	3.67	0.58
Citric acid	7.2	0.5	3.35	3.78	0.52

NaOH	10.0	1	0.5	4.11	0.92
Distilled water	1.8	0.5	3.5	4.01	0.35

The *S. cumini* seeds exhibited a higher moisture content of 10% when treated with sodium hydroxide compared to other extractants used for starch isolation. This finding is in accordance with Jaiswal & Kumar (2015), suggesting that the presence of hydroxyl groups makes starch molecules hydrophilic, thus aiding in retaining moisture. The starch purity is indicated by the low ash values and nitrogen levels.

Lu et al. (2019) discovered that the various combinations of steeping additives (distilled water, NaOH, lactic acid, acetic acid, ascorbic acid, and citric acid) had an impact on the extracted starch products' functioning because of the variations in the residual solvent or protein content. High quantities of basic and hydrophobic amino acids are present in starch granule-associated proteins (SGAP), which bind to hydrophobic starch molecules robustly and at a high local concentration at the granule surface. The maximum protein content (6.73%) was found in starch extracted by lactic acid, whereas the lowest protein content was found in the starch extracted by the other extracting agents. The highest protein content in the lactic acid extracted sample could be attributed to the hydrophilic extracting agent repulsion with the hydrophobic protein and *S.cumini* starch.

4.3 Water and oil absorption capacity

The amount of water and oil that a starch granule can absorb is referred to as its water and oil absorption capacity. The composition of the starch, the granule surface microstructure, long-range ordered structures, short-range ordered structures, and hydrophilic groups all impact the WAC of starch. In order to enhance the qualities of food goods, it is also reasonable to employ physical, chemical, biomodification, or compound approaches to raise or lower the WAC of starch directionally.

Water molecules enter the starch granules through their surface and enter into the interior of starch granules. Amylose and amylopectin make up the majority of the components of starch granules. Amylose is primarily found in the amorphous region of starch. As water molecules continue to penetrate, they attain the starch granule's amorphous area and hydrate. Water molecules are made more likely to penetrate by the amylose. There seems to be a small expansion and increase in volume in the starch granules. Selecting the appropriate technique is essential to determine the WAC of starch. Thus, preparing starch with a high WAC might be achieved by increasing the amount of amylose in the starch (J. Zhang et al., 2024a).

The observed water and oil absorption capacities of *S. cumini* seed starch were 160% and 124%, respectively. Kodo millet starch was found to have absorption capacities of 143% for water and 101% for oil, and for rice, porous starch was found to be 130% for water and 109% for oil, respectively (J. Zhang et al., 2024b). Native corn starch was found to have an absorption capacity of 73.25% for water and 141.75% for oil (Davoudi et al., 2022).

Depolymerization may provide an explanation for the rise in WAC. When amylose and amylopectin depolymerize, simple sugars are produced. These sugars have the ability to absorb water at their surface, which raises the WAC. Another explanation for why starch treated with plasma has a higher WAC value than untreated starch could be plasma etching. The starch's functional properties may be improved as a result of the etched surface's facilitation of heat and water absorption into the granules. Higher energy electrons striking the starch surface might cause the granules to disintegrate, which explains the increase in OAC. As disintegration increases surface area, more new connection formation becomes possible (Sonkar et al., 2023)

Citric acid is an inexpensive, nutritionally non-hazardous food ingredient frequently used in food applications. It has been discovered that the water absorption rate of banana starch has increased by 2% and 36%, respectively, by modifications with citric and succinic acids. This was linked to modifications in the starch's surface structure and acid hydrolysis of the starch layer's side chains. The higher WAC of starch prevents the formation of crystals and water precipitation brought on by unfavorable environmental conditions (J. Zhang et al., 2024c). As a result, *S. cumini* starch has a higher water-holding capacity than millet and rice starches.

4.4 Scanning electron microscopy

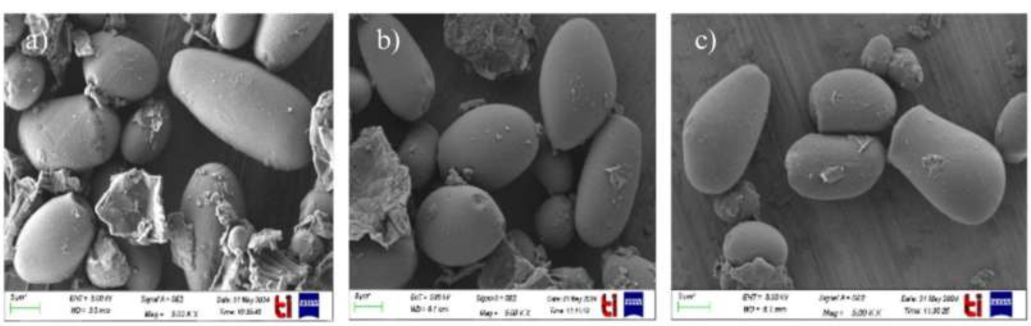


Figure 12. Surface morphology of *S.cumini* seed starch (a) Citric acid (b) Ascorbic acid (c) Lactic acid

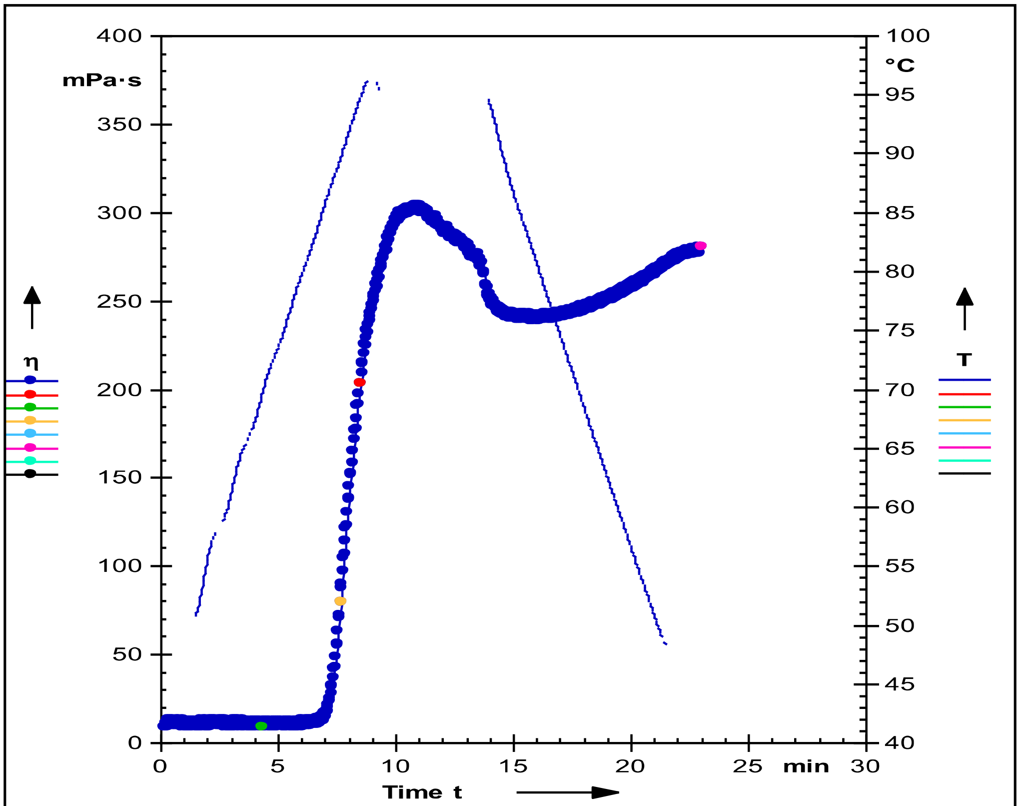
The starch granules have a round to oval shape and relatively smooth surfaces, are free from impurities, and contribute to the good extraction yield of starch from *S. cumini* seeds, as shown by scanning electron micrographs. The starch granules' average diameters recorded ranged between 2.8 to 27.5 µm in citric acid extraction, 7 to 22 µm in ascorbic acid, and 7 to 32 µm in lactic acid. Compared to Wu et al. (2023), native corn starch displays a spherical or polygonal shape with a relatively smooth surface.

Santos et al. (2024) reported the *S. malaccense* starch granule size dispersion. This starch is classified as a tiny granule; the granules' mean size is 13 µm, and the highest frequency of granule size was 8 µm. Small granule starches often have a stronger water affinity and a lower sedimentation coefficient than large granules. Specifically, *S. malaccense* starch is more appealing for applications requiring chemical modification because small granule starches have a higher surface-area-to-volume ratio than large granules. The granule size distribution (2–22 µm) shows a common feature of apple seed starches. Starches treated with lactic acid, ascorbic acid, and citric acid have comparable size and morphology.

The findings imply that no significant variations were observed in the morphology and size of the starch extracted with various extracting agents (Lu et al., 2019).

4.5 Pasting properties

The rapid visco analysis (RVA) of *S. cumini* seed starch demonstrated distinct pasting characteristics critical for its functional applications. The peak viscosity reached 303.9 mPa.s at 82 °C, indicating the maximum viscosity attained during the heating phase. The holding strength, reflecting the sample's ability to maintain viscosity under prolonged heating and mechanical stress, was measured at 240 mPa.s. The breakdown viscosity, defined as the difference between the peak viscosity and the holding strength, was 63.9 mPa.s, suggesting moderate stability against shear forces. Upon cooling, the final viscosity was recorded at 280.7 mPa.s, demonstrating the sample's capacity to regain viscosity after gelatinization. The setback viscosity, calculated as the difference between the final viscosity and the holding strength, was 23.2 mPa.s, indicating a limited tendency for retrogradation. These findings provide a comprehensive understanding of the pasting properties of *S. cumini* seed starch, which are essential for predicting its behavior in various industrial and culinary applications.



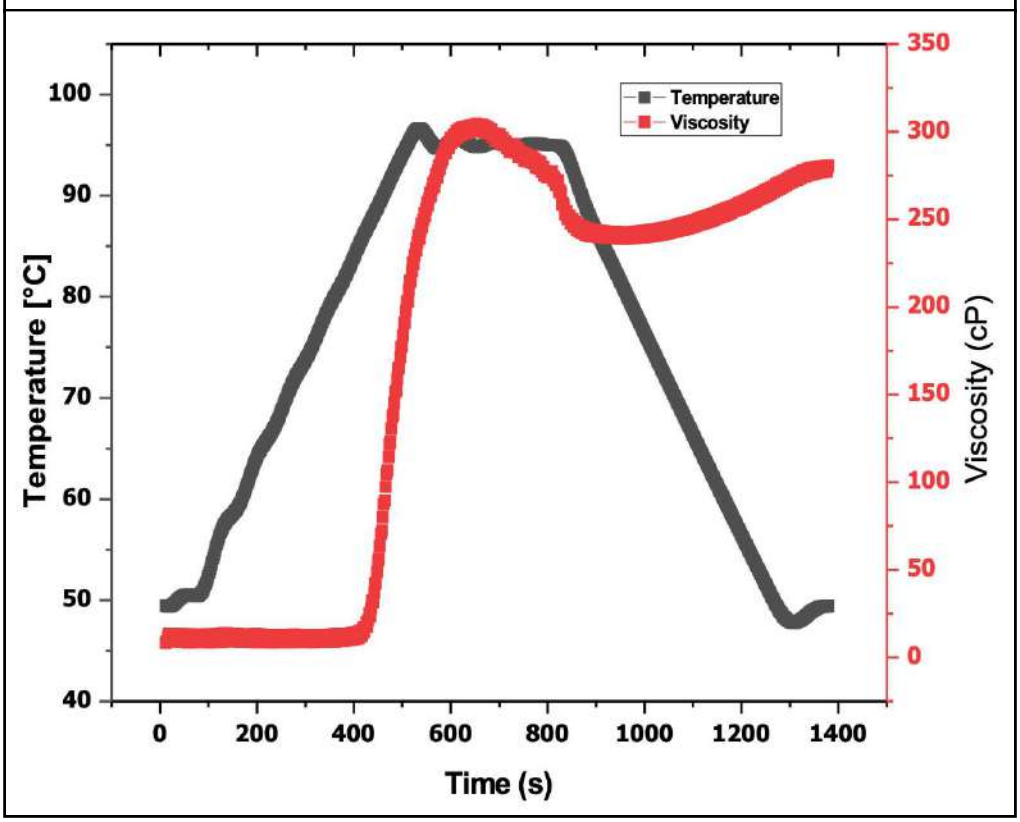


Figure 13. Rheological properties of *S. cumini* seed starch

The 82 °C is the initial paste temperature, greater than that of avocado seed starch. The maximum viscosity of the avocado starch is 5385,5 cP, its maximum viscosity upon cooling is 3085,5 cP, and its setback is 2880,5 cP, which was higher than *S. cumini* seeds starch. This shows that the starch is being converted back into a more useful form, which is good for making films, among other things (Martins et al., 2022).

Table 3. Rheological properties of *S. cumini* seed starch by RVA

Paste temperature	82°C
Peak viscosity	303.9 mPa.s
Breakdown	63.9 mPa.s
Final viscosity	280.7 mPa.s
Retrogradation	23.2 mPa.s

The firmness of crystalline zones is directly linked to the hardness of the gel. Thus, reliable and stable gels are produced when these zones are numerous, large, and strongly linked; weak and unstable gels are produced when these zones are few and small or when the forces holding the molecules together are insufficient. This parameter must be determined for the starch application industry and for creating biodegradable packaging.

For pitomba starch, the observed temperature was 77.5°C, which was lower than that of *S. cumini* seed starch. The other values, including peak viscosity, breakdown, final viscosity, and retrogradation, were 2531cP, 1475cP, 1607cP, and 551cP, respectively. These values were higher than those of *S. cumini* seed starch (de Castro et al., 2019).

4.6 Fourier Transform Infrared Spectroscopy

Starch primarily comprises two types of glucose polymers: amylose and amylopectin. The functional groups present in starch are Hydroxyl (-OH) Groups, Carbonyl (C=O) Groups, and α-Glucosidic Linkages. The Hydroxyl (-OH) Groups are present in both amylose and amylopectin; these functional groups contribute to the hydrogen bonding network within starch molecules. A broad absorption band was observed only after gelatinization at around 3300-3500 cm^{-1} due to O-H stretching vibrations. Carbonyl (C=O) Groups are found in the glucose monomers of both amylose and amylopectin. Similarly, a peak at 1640-1650 cm^{-1} due to C=O stretching vibrations was found only in the sample after gelatinization. α-Glucosidic Linkages connect glucose units in both amylose and amylopectin. Peaks at around 1000-1100 cm^{-1} due to C-O-C stretching vibrations were observed in samples before and after gelatinization.

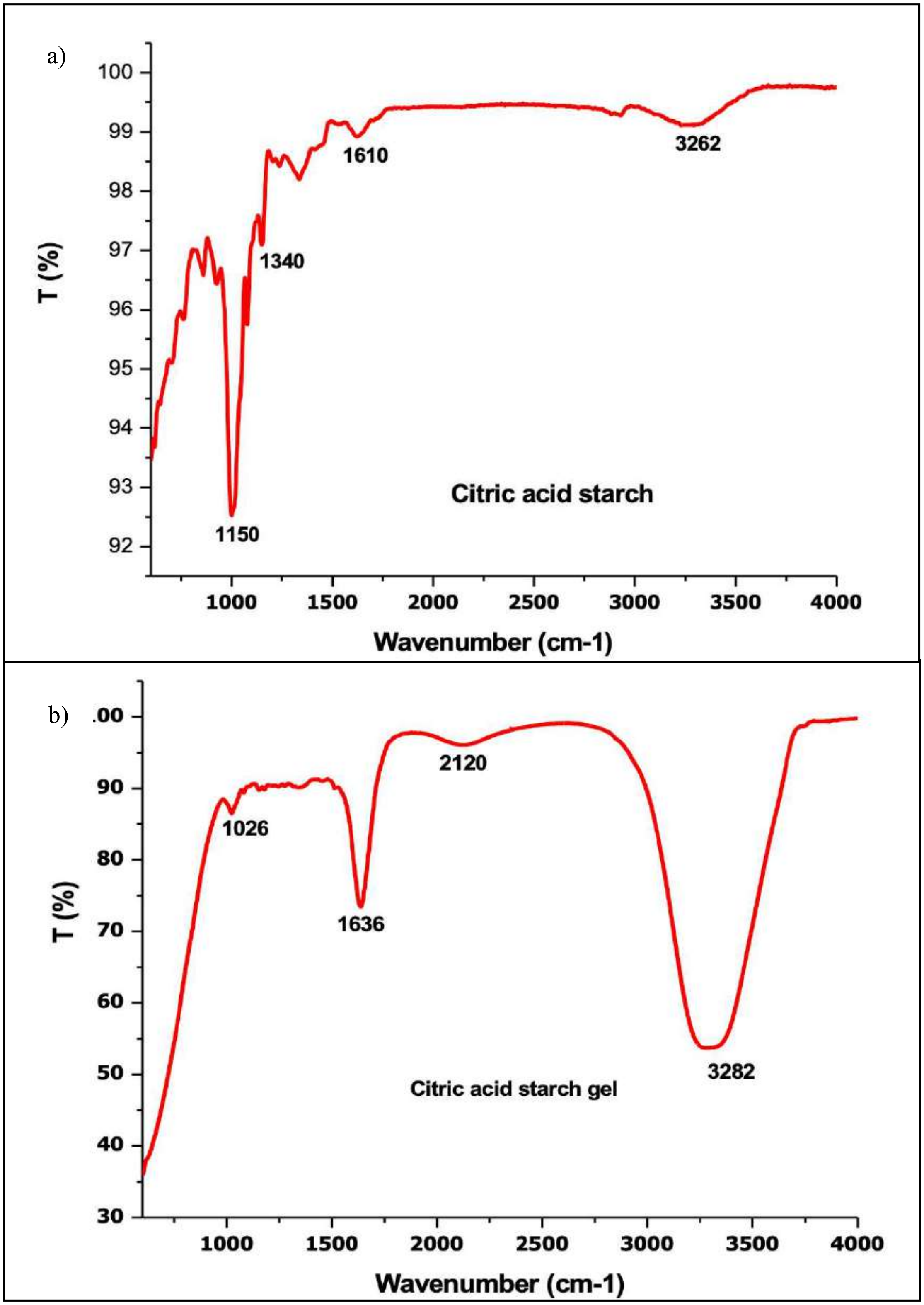


Figure 14. FTIR spectra of *S. cumini* seed starch extracted using a) citric acid before gelatinization and b) citric acid after gelatinization

The transmittance of peaks decreased after gelatinization. This may be due to the disruption of the crystalline structure during gelatinization and/or association of amorphous regions or newly formed structures, such as those in the gel phase, which may increase in intensity. Broadening of peaks was also observed due to gelatinization, indicating a decrease in molecular orderliness. This broadening occurs as the starch molecules lose their organized structure and become more dispersed in the gel matrix. New peaks in the sample after gelatinization at 3300-3500 cm^{-1} and 1640-1650 cm^{-1} appeared due to the formation of new molecular arrangements or interactions in the gel phase.

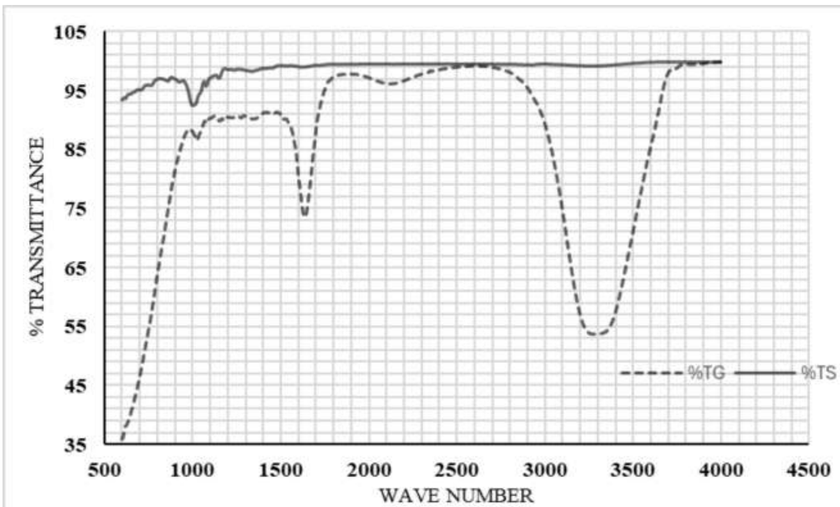


Figure 15. FTIR spectra of *S.cumini* seed starch extracted by steeping in Citric acid

Similar to the starch of *S. cumini* seeds, the broad intensity of bands 3500 and 3000 cm^{-1} in potato starch was observed (Kumar et al., 2020) in O-H bond stretching. On the other hand, C-H stretching represents a weak peak near 2900 cm^{-1}. C-O, C-C, and C-O-H stretching and C-O-H bending represent the peak ranges between 1200-1000 cm^{-1}. Pascoal et al. (2013) reported that starch from *S. lycocarpum* fruit represents stretching vibration of the O-H bond at 3420 cm^{-1}. The stretching vibration of the C-H bond was responsible for the minor peak located at about 2930 cm^{-1}. The stretching vibration of hydroxyl group O-H showed a broad stretching at a peak range of 3200–3600 cm^{-1}. The presence of C-H stretching in methyl (CH_3), methylene (CH_2), and bending vibrations showed a weak band at 2925.52 cm^{-1} (Arab et al., 2021).

The crystalline structure of the native pea starch is responsible for the bands in its FTIR spectrum at 1047 cm^{-1} and 995 cm^{-1}. The amorphous forms in starch are linked to the band at 1022 cm^{-}. It suggests that there may be overlapping bands between 1800 and 1500 cm^{-1}. As observed in the spectra of native pea starches, the bands at 1564, 1575, and 1720 cm^{-1} on the samples extracted by organic acids continued to emerge in a similar pattern (Lu et al., 2019).

4.7 Nanostarch preparation and characterization

The SEM images of acid hydrolysis, ultrasonication, and a combination of both nanostarches are given in Figure 7. Based on crystalline characteristics, starch nanoparticles (SNPs) and starch nanocrystals (SNCs) are the two main categories for nanostarch. It is classified into different shapes like nanopolyhedrons, starch nanonetworks, starch nano micelles, starch nanoflakes, starch nanosphere, starch nanoworms, and starch nanovesicles. Different preparation techniques, such as acid hydrolysis, enzymatic hydrolysis, microemulsion, high-pressure homogenization, and extrusion, can be used to obtain different structural characteristics, such as size, surface morphology, internal chains, and functional properties, such as dispersion ability, crystalline degree, pH-sensibility, etc., for nanostarch-based carriers. Moreover, a combination of one or more techniques is used to manufacture nanostarch simultaneously, increasing its huge specific surface area and structural affinity for loading bioactive compounds.

There are two approaches for making polymorphic nanostarch: "top-down" and "bottom-up" processes. Starch granules are split into tiny particles with fine structures in "top-down" processes. The techniques include high-pressure homogenization, ultrasonic assault, mechanical grinding, and acid hydrolysis. While "bottom-up" methods, which construct three-dimensional structures at the nanoscale through processes like nanoprecipitation, self-assembly, electrostatic spraying, etc., primarily rely on the anisotropy of crystal growth and the energy-driven entanglement based on distinct starch chains.

In general, lipids (polyunsaturated fatty acids, phenolipids, fatty acid esters) and aroma chemicals readily form tight V-typed complexes with nanostarch. In the helical cavity of starch chains, particularly pure amylose, the aliphatic chain of lipid molecules is found, while the carboxyl group is likely to be found at the helical inlet.

Only the nano-clusters could be observed because the measurement particle sizes were larger and were beyond the limits of the SEM instrument. Because of partial chain breaking and crystallinity alterations, modified starches lose some of their heat resistance. When it comes to encapsulating active substances and improving their stability for use in functional food and medical applications, nanoparticles are expected to be more effective than native starch (Bajer, 2023).

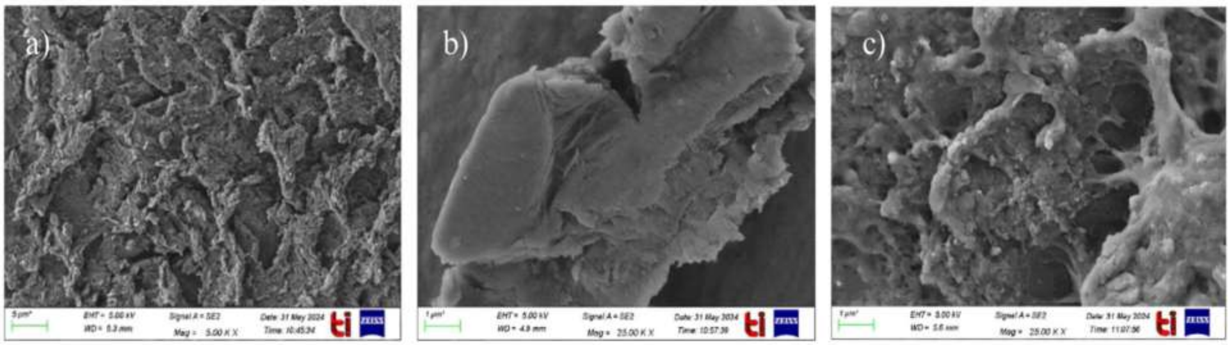


Figure 16. Citric acid starch after a) Acid hydrolysis (HCl), b) physical treatment (ultrasonication), c) combination of acid hydrolysis and ultrasonication

Acid hydrolysis and ultrasonication nano starch size from *S. cumini* seed seem to be 3µm, and the surface displays minor cracks at the granule surface and becomes loose, uneven, and rough (Xia et al., 2017), a polyhedron (Yao et al., 2024), and agglomerated form. (Roy et al., 2020b). The disruption of intramolecular and intermolecular hydrogen bonds caused by citric acid improves the flexibility of nanostarches, depending on the surface shape. Furthermore, it leads to the aggregation of nanostarches (Kaiyrbekov et al., 2021). The morphology appears to be a starch nanopolyhedron (Yao et al., 2024). The average starch size measured by the **ultrasonication** method was 8.55µm. The surface is present in an aggregated form with nonspherical morphology.

Yao et al. (2024) observed the morphology of nanoflakes in potato starch using the same acid hydrolysis method that was applied to *S. cumini* seed starch. Three stages of fast, slow, and extremely slow degradation occur during the acid hydrolysis of starch, resulting in an extensive reaction period (several days) and an inadequate recovery rate (about 5–15 weight percent). Additionally, starch that has been attacked by acid exhibits random multisite degradation in the amorphous regions, which poses a significant challenge for the structural design of nanostarch production. The crystalline distribution and structure of nanostarch cannot be directionally manipulated, and the reaction rate is unregulated. A variety of pretreatments, including extrusion, plasma, and ultrasound, can be utilized to crack up the intact starch granule structure for vigorous acid action. Nanoflake morphology of the starch can be observed when it undergoes **acid hydrolysis**. (Yao et al., 2024).

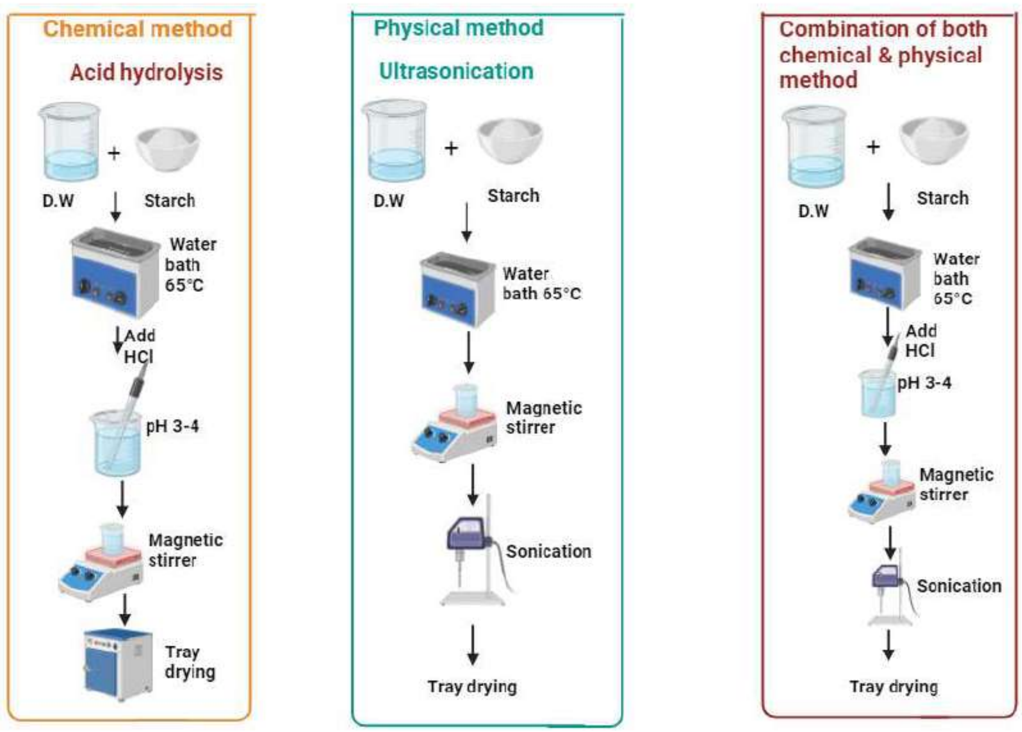


Figure 17. Preparation of starch nanoreduction using acid hydrolysis, ultrasonication, and combination of both methods.

After physical treatment (ultrasonication), the native starch of *S. cumini* seeds exhibits the same non-spherical morphology of starch as observed (Sardjono et al., 2024). When starch granules undergo conventional ultrasonic treatment, they may gradually develop physical surface defects as a result of erosion and breakage. As a result, the formation of pores, channels, or fissures may encourage the opening of starch granules, enable the acid to penetrate the polysaccharide matrix more deeply and disrupt hydrogen bonds. Secondly, the use of cavitation in acidic or aqueous conditions may encourage the production of radicals. At higher ultrasonic frequencies, it was shown that the formation of radicals was more widespread and effective. The glycosidic connections between amylose and amylopectin can then be broken by these moieties, resulting in mono- or di-saccharides, molecules with a reduced molecular weight. Compared to acid hydrolysis, the sonication of starch with reasonable amylose to amylopectin ratio appears to be less inspected. According to certain research, sonication affects the starch structure forms, such as spherical or square. Additionally, it was determined that prolonged ultrasonication action breaks down the crystalline structure, exposing a larger, amorphous region in the specimens of nanoparticles (Bajer, 2023).

Starch nanopolyhedron morphology can be observed when the starch undergoes a combination of acid hydrolysis and ultrasonication (Dukare et al., 2021b). Moreover, the appearance looks rough, loose, and irregular, with cracks on the surface (Xia et al., 2017). The amount of amylose in the starch granules was decreased following hydrolysis, sonication, and the combination of these two techniques. Greater exposure of the unbranched macro-chains (found mostly in amylose) to HCl and ultrasonic treatment was indicated by the higher amylose losses for maize starch. A modification in the starch chains' conformation is linked to this impact (Bajer, 2023).

Chapter – 5

Conclusion

There might not be much knowledge about the possible uses of *S. cumini* seed starch because it is still a relatively new and developing field of research. As previously stated, food and other industries scarcely use any *S. cumini* seed starch at all. Therefore, more investigation is needed before using this kind of material. The process of extracting, characterizing, and reducing starch at the nanoscale was conducted. A comparative analysis was conducted to evaluate the starch extraction process by employing various steeping additives. The utilization of citric acid as the extracting agent resulted in the most considerable yield of 39.65%, whereas the utilization of distilled water provided the lowest percentage of 28.11%. The starch that underwent sodium hydroxide treatment exhibited a greater moisture content of 10% in comparison to other substances utilized for starch extraction. The starch extracted with lactic acid had the highest protein content (6.73%), while the starch extracted by other agents had the lowest protein level. The starch demonstrated water and oil absorption capabilities of 160% and 124%, respectively. The rigidity of crystalline regions is closely correlated with the hardness of the gel. The starch granules exhibit a round to oval shape with relatively smooth surfaces. The mean diameters of the starch granules varied from 2.8 to 27.5 µm in the citric acid extraction, 7 to 22 µm in the ascorbic acid extraction, and 7 to 32 µm in the lactic acid extraction. During FTIR examination, the intensity of the peaks dropped following the gelatinization process. For the nanoreduction process, acid hydrolysis and ultrasonication were reduced, and a combination of these was carried out. The surface of the nano starch displayed minor cracks and became loose, uneven, rough, polyhedral, and agglomerated. Hence, additional investigation is required for appropriate industrial applications. In order to optimize the utilization and comprehension of non-conventional starch, particularly *S.cumini* seed starch, it is imperative to investigate the chemical properties and diversity of the fruit.

Chapter – 6

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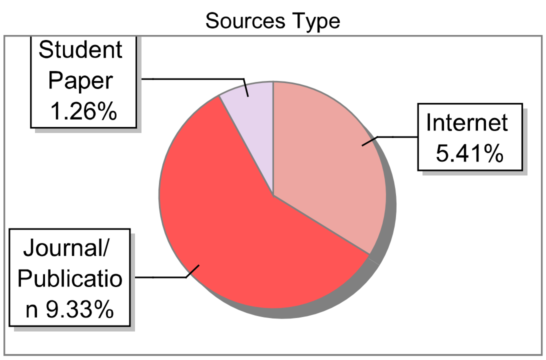

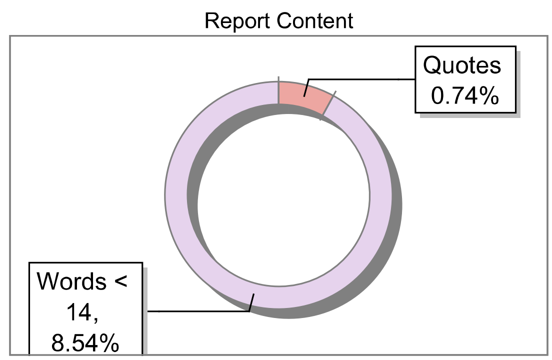

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