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Structural and physicochemical characterization of starch from water lily (*Nymphaea lotus*) for food and non-food applications

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ABSTRACT

In this study, starches were isolated from rhizomes and seeds of water lily (*Nymphaea lotus*) using cold distilled water. The structural and physicochemical properties of the isolated starches were compared with potato, rice, and maize starches. The amylose content (g/100 g) of rhizome, seed, potato, rice, and maize was 23.03, 24.5. 25.17, 21.26, and 19.83, respectively. The SEM granule size (μm) of rhizome, seed, potato, and maize starches were 11.19±3.69, 3.56±0.92, 30.63±11.09, and 7.97±1.48, respectively. The X-ray diffraction polymorph of rhizome, seed, rice, and maize demonstrated type A, whereas potato exhibited B-type. The deconvoluted ATR-FTIR indicates low level of ordered structure in the external region of rhizome starch. The RVA pasting temperature (71.9 °C) and setback viscosity (1292.5 cP) of rhizome was lower than seed (78.3 °C and 3228.5 cP, respectively). However, peak viscosity (7201 cP) of rhizome was higher than seed (4105 cP). Rhizome and seed starches can be used where high viscosifying than rice and maize starches and better shear breakdown resistance than potato starches are required. This study indicated starches of *N. lotus* have medium amylose%, small granular size, hydrophillic nature, and high peak viscosity of potential to promote for development of products in food and non-food industries.

1. Introduction

Starch, a macromolecule composed of amylose and amylopectin, has a variety of uses in the culinary, pharmaceutical, textile, paper, cosmetic, and construction sectors (Bemiller & Whistler, 2009). The main conventional starch sources are maize, cassava, wheat, rice, and potato (Waterschoot et al., 2015). Starches from these crops are used in food, pharmaceutical, textile, paper, bioplastic, and bioethanol production (Olatunji, 2020). The global production of starch has been estimated between 88.1 and 97.7 million tons in 2020 (Olatunji, 2020). Out of this, maize accounts for 75 % of this total, followed by cassava (14 %), wheat (7 %), and potato (4 %) (Vilpoux & Junior, 2023). These starchy crops are used for both human consumption and sources of industrial starches which caused shortage of cereals and tubers. As a result, exploring alternate sources of starch that can supplement the conventional starch source becomes essential. Nowadays, researchers have paid attention to exploring new sources of starch, including underutilized plants, agricultural residue and waste, and by-products of fruit and vegetables. In addition to these new sources of starch, wild edible aquatic plants are becoming good sources of starches (Wang et al., 2021).

The use of new starch sources would make more cereals and tubers available for food-based applications. The growing concern over the shrinkage of arable land and the environmental risks associated with agricultural expansion has put into question the sustainable supply of terrestrial agriculture products (Olatunji, 2020). Additionally, cultivation of aquatic plants does not require arable land and does not compete with terrestrial animals and humans for land space. Starch isolated from freshwater macrophytes possess many similarities to those of rice, potato, and maize suggesting similar industrial applicability (Syed et al., 2012). Additionally starch from aquatic plants possesses a smaller granular size, a lower to medium gelatinization temperature, and is more susceptible to enzymatic digestion as compared to starch from terrestrial plants even though these may vary depending on ecological zones such as temperate and tropical (Olatunji, 2020).

Study conducted on starches of *Nelumbo nucifera, Eleocharis dulcis, Sagittaria sagittifolia,* and *Trapa bispinosa* indicated that starches from these aquatic plants were pure and demonstrated similar properties to

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commercial starches (Syed et al., 2021). The other most important aquatic plant that provides a high starch is the *Nymphaea lotus,* also referred to as the white lily or Egyptian lotus. In Lake Ziway, Ethiopia, *Nymphaea lotus* is abundantly growing aquatic macrophyte after *Arundo donax, Echinochloa colona*, and *Potamogeton schweinfurthii.* The distribution of these freshwater macrophytes does not hold true for different water bodies. Despite being widely distributed, *A. donax, E. colona*, and *P. schweinfurthii* barely contain starches in their rhizomes and seeds. *N. lotus* grows around the shore of Lake Ziway at a water depth of 50–111 cm, light penetration of 17–21 cm depth, a water pH ranging from 8.03 to 8.92, and water temperature of 21.5–24.77 °C (unpublished results). Even though *N. lotus* has not yet been cultivated, this wild edible aquatic plant can be cultivated with a planting space of 1 m x 1 m and a fertilizer rate of N:P:K 60:40:30, which can yield 25 tons per hectare (Jana, 2019). Water lily can grow in ponds, lakes, and perennial water bodies in all types of climate. The best soil for water lily cultivation is aquatic ecosystem with soil rich in organic matter. Currently, the annual production of *N. lotus* seed and rhizome data from Ethiopia is not available. However, it was recorded that, at its full maturity, a single *N. lotus* plant on average produces 15 fruit pods, on average the fruit pods weigh 43.33 g, and contains 19.38 g seeds per pod. A plant produce, on average ten rhizomes that weigh 52.04 g (unpublished result).

Previous studies reported that the entire rhizome and seed of *N. lotus* are edible, and consumed as vegetables (Hujjatullah et al., 1967), food for impoverished people during times of famine and supplementary starch food by farmers (Ma et al., 2023). The starch percentage on dry matter basis in the rhizome of *N. lotus* was reported 28.1 % (Hujjatullah et al., 1967). Lotus starch can be used as a binding, disintegrating, and dissolution agent for tablets in pharmaceutical applications. Otherwise, there is limited information about the isolation and characterization of starch from *N. lotus* for potential future applications. As a result, the proximate composition, granular size, amylose percentage, crystallinity, and pasting properties of the starch isolated from *N. lotus* from Ethiopia is not studied. The structural and physicochemical properties *N. lotus* starch should be studied before large scale promotion in the food and non-food applications (Abelti et al., 2023). The purpose of studying the starches of *N. lotus* is to create awareness of their potential diverse applications in food and non-food industries. In light of this, starch was isolated from the rhizome and the seed of *N. lotus,* and the structural and physicochemical properties of *N. lotus* were compared with commercial starches (potato, rice, and maize) to explore its potential for future application. For comparison, maize starch was chosen because it is A-type (monoclinic crystalline structure) and is the highest globally produced starch (Vilpoux & Junior, 2023). Potato starch was chosen because it is B-type and widely used in non-food industries; and rice starch was chosen because it is widely produced worldwide. All these starches have diverse functional properties and versatile applications in the food and non-food industries (Vilpoux & Junior, 2023).

2. Materials and methods

2.1. Materials

The rhizome and seed of *Nymphaea lotus* were harvested at their mature stage from four different shore areas of Lake Ziway in November 2022. Lake Ziway has an average elevation of 1636 m above sea level and is situated between latitudes 7° 52′ and 8° 8′ N and 38° 40′ and 38° 56′ E. The yearly precipitation ranges from 729.8 mm to 1227.7 mm. The lake's average yearly temperature ranges from 18.2 to 21.6 °C. The rainfall precipitation on Lake Ziway is between 454 and 995 mm (Goshime et al., 2020). The plant material was identified by a plant taxonomist at the National Plant Herbarium in the Department of Plant Botany and Biodiversity Management of Addis Ababa University and was archived in the herbarium for future reference (voucher number AL001). A reference starch potato (Badshah variety), rice (Arborio variety), and maize (Ganga 111 variety) produced by SD Fine Chemical Limited, Mumbai, India, was purchased from a local market in Addis Ababa. All commercially obtained starches were used without further treatment.

2.2. Isolation of starch

Starch was isolated as described by Man et al. (2012). Freshly harvested *N. lotus* rhizomes (2 kg) were manually peeled with stainless steel knives, and the seeds (2 kg) were washed under running tap water. The rhizome pieces and softened seeds were mixed with cold distilled water, homogenized, filtered using 250 μm muslin cloths, and settled for 12 h at 4 °C (Fig. 1). The supernatant was decanted, and white starch granules were collected and dried at 40 °C for 24 h

2.3. Proximate composition

The moisture content of starch was determined using AOAC (2015) Official method number 925.09 by oven drying method (Advantage--Lab's laboratory drying oven, Model:2030, Jachthoornlaan 8, 2970 Schilde, Belgium). The protein content was determined using Kjeldahl methods involving digestion, distillation, neutralization and titration AOAC (2015) Official method number 920.87. The total lipid content of starch granules was determined by acid hydrolysis method AOAC (2015) Official method number 922.06. The ash content of starch was determined by igniting the starch for 4 h at 550 °C in furnace (Controller, B400/B4100, Nabertherm GmbH, Germany) according to AOAC (2015) Official method number 942.05. Crude fiber of starch was determined using sequential acid and alkali extraction method according to the AOAC (2015) Official method number 978.10.

2.4. Amylose percentage

The amylose percentage of starch was determined as described by Morrison and Laignelet (1983). Briefly, starch granules (70 mg) were dispersed in 10 mL of urea (6 M)-DMSO (Dimethyl Sulphoxide) solution (1:9), heated for 10 min in a boiling water bath, and further heated in an oven at 100 °C for 1 h The solution (0.5 mL) was taken into a volumetric flask containing 25 mL of distilled water plus 1 mL of I_2/KI (100 mg I_2 and 1000 mg KI in 50 mL distilled water) and made up to 50 mL with distilled water. The absorbance of the samples was measured against a blank at 635 nm using a spectrophotometer. The blue value was computed according to the method of Sukhija et al. (2016) using Eq. (1). The percentage of amylose content was calculated in duplicate according to the method of Alvani et al. (2011) using Eq. (2). Amylopectin content (%) was determined by subtracting amylose content (%) from 100 (Chemiru & Gonfa, 2023) using Eq. (3).

$$\text{Blue value} = \frac{(\text{Absorbance} \ * \ 100)}{2 * \ \text{g solution} \ * \ \text{mg starch}} \text{ X } 100 \tag{1}$$

$$\text{Amylose content } (\%) = \ (\text{Blue value} \ * \ 28.414) - 6.218 \tag{2}$$

$$\text{Amylopectin percentage } (\%) = 100 - \text{Amylose } (\%) \tag{3}$$

2.5. Morphology and granule size

Starch morphology and starch granule size were determined using a scanning electron microscope (SEM) (Versatile Benchtop Scanning Electron Microscopy, JCM-6000Plus, Jeol Ltd, Japan), as reported in Singh et al. (2009). Before observations, starch granules were suspended in ethanol to obtain a 1 % (w/v) suspension. One drop of the suspension was mounted on an aluminum stub with carbon tape and thinly coated with gold dust. Three images were obtained from different areas to observe the morphology of starch granules. Starch granules diameter were measured using SEM image scale bar. The granule size distribution of the starches were determined using ImageJ software by measuring twenty five granules of rhizome, seed, potato, and maize starches

Fig. 1. Isolation of starch from rhizome and seed of water lily (*Nymphaea lotus*).

2.6. X-ray diffraction

The crystalline pattern of starch granules was determined using X-ray diffractometer (XRD) (Maxima_X XRD-7000 X-ray Diffractometer, Shimadzu Corporation, Japan) as described by Manek et al. (2012). Firstly, starch granules were ground using a mortar and pestle, homogeneous starch granules were tapped, compressed in the indentation of sample holder, and illuminated using Cu-Kα radiation ($\lambda = 1.54056$ Å). The XRD was operated at 40 kV voltage with filament emission of 30 mA current and a 0.25 nm divergence slit. Diffractograms were obtained from 5° to 40° in a 2θ scale at increments of 0.02°, a scanning speed of 2°/min and a preset time of 0.6 s. A nickel filter was used to reduce the contribution of radiation to the X-ray signal. The crystalline peak area and amorphous area were calculated by Origin Pro 2019 version software, and the percentage crystallinity was calculated using Eq. (4).

$$\text{Relative Crystallinity } (\%) = \frac{\text{Crystalline peak area}}{\text{Crystalline peak area } + \text{ amorphous peak area}} * 100 \tag{4}$$

2.7. Fourier transformed infrared spectroscopy

The Fourier transformed infrared spectroscopy spectra present in the starches isolated from *N. lotus* rhizome, *N. lotus* seed, potato, rice, and maize were recorded using Fourier transformed infrared spectroscopy (Thermo Scientific, Model: Nicolet IS50 ABX, Germany) (Wang et al., 2020). Finely ground starch granules were placed on the integrated attenuated total reflectance (ATR Thermo Scientific, Model: Nicolet IS50 ABX, Germany) and then pressed using a built-in pressure clamp until the sample was sufficiently in contact with the attenuated total reflectance. Prior to running the samples, a background spectrum was obtained after cleaning the crystal with isopropanol. Sample was measured directly after pressing the samples on the crystal. FTIR spectra were obtained at wave numbers between 400 and 4000 cm^{-1} and a resolution of 4 cm^{-1} recorded by the OPUS software, and the data was exported as an Excel file. The original spectra were corrected by subtraction of the baseline in the region from 1200 to 800 cm^{-1} before deconvolution was applied using Origin Pro (Lin et al., 2015). The intensity ratio (IR) was calculated at 1047/1022 cm^{-1}and 1022/995 cm^{-1} to describe the ordered structure of starch external region (Yu et al., 2013).

2.8. Pasting properties

The pasting properties of starches were determined by the Rapid Visco Analyzer (RVA-4500 Perten Instruments Australia Pty Ltd., Macquarie Park, NSW, Australia) (Sukhija et al., 2016). Briefly, starch granules (3 g) were added to 25 mL of distilled water to make a 12 % (w/v) starch slurry. The starch slurry was poured into an aluminum canister and stirred using plastic paddles for 30 s before insertion into the RVA machine. The paddle speed was 960 rpm in the first 20 s to disperse the slurry and then maintained at 160 rpm during measurement. The slurry was held at 50 °C for one min, and heated from 50 °C to 95 °C at a heating rate of 10 °C/min, held at 95 °C for 2.5 min, and cooled to 50 °C at 10 °C/min, and finally held at 50 °C for 2 min. Each sample was analyzed in duplicate, and from the generated pasting curve, pasting temperature, peak, breakdown, setback, and final viscosities were acquired by Thermocline for Windows software.

2.9. Swelling power and solubility

The swelling power and solubility of starches were measured as described by Guo et al. (2015). Briefly, the starch sample (0.2 g) was mixed with 10 mL of distilled water in a centrifuge tube and heated at different temperatures 45 °C, 55 °C, 65 °C, 75 °C, 85 °C, and 95 °C for 30 min in a water bath. The swelling power and solubility of starches were determined in duplicate and calculated using Eqs. (5) and (6).

$$\text{Solubility} = \frac{\text{Mass of dry supernatant}}{\text{Mass of sample}} \text{X100} \tag{5}$$

$$\text{Swelling power} = \frac{\text{Mass of sediment}}{\text{Mass of sample X } (100 - \text{Solubility})} \tag{6}$$

2.10. Syneresis

Starch syneresis was determined according to Singh et al. (2009). Briefly, starch granules (2 g) were suspended in distilled water (100 mL) and cooked at 85 °C for 30 min in a water bath. The amount of water expelled after centrifugation at 3000 rpm for 10 min was divide by the weight of the remaining starch paste and starch syneresis was determined in duplicate calculated by Eq. (7)

$$\text{Syneresis } (\%) = \frac{\text{Mass of released water}}{\text{Weight of remaining paste}} \tag{7}$$

2.11. Statistical analysis

The data generated were subjected to a one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences software version 20. The mean was separated (p<0.05) using the Tukey test when a significance difference was observed. The XRD patterns, FTIR spectra, RVA, solubility, and swelling power graph were sketched using Origin Pro 2019 software.

3. Results and discussion

3.1. Proximate composition

The proximate composition of starches isolated from *N. lotus* rhizome, *N. lotus* seed, potato, rice, and maize is presented in Table 1. The rhizome and seed of *N. lotus* yielded 16.57 % and 14.75 % starches, respectively. A comparable starch yield (12 %) was reported from *Trapa bispinosa*, which is an aquatic vegetable in China (Wang et al., 2021).

There was no significant difference ($p > 0.05$) in the moisture content of the starches of the *N. lotus* rhizomes, *N. lotus* seeds, and potato. The moisture content of maize starch was significantly higher (12.25 ± 0.25 %) as compared to other starches, whereas the moisture content of rice starch was the lowest (5.5 ± 1.5 %). The moisture content of starches isolated from *N. lotus* rhizome and seed were lower as compared with maize and potato starches. A starch with a higher moisture content

Table 1
Physicochemical properties of starches isolated from *N. lotus* rhizome and seed and a reference starches (potato, rice and maize).

Starch sources	Moisture (g/100 g)	Protein (g/100 g)	Fat (g/100 g)	Ash (g/100 g)	Amylose content (g/100 g)
N. lotus rhizome	7.25 ± 1.25^{abc}	0.028 ± 0.003^{abc}	0.00 ± 0.00^{b}	0.50 ± 0.50^{a}	23.03 ± 0.07^{a} (M)
N. lotus seed	7.50 ± 0.50^{abc}	0.034 ± 0.003^{abc}	0.92 ± 0.06^{a}	0.22 ± 0.02^{a}	24.50 ± 0.13^{a} (M)
Potato	9.25 ± 0.25^{abc}	0.041 ± 0.003^{a}	0.26 ± 0.24^{ab}	0.25 ± 0.25^{a}	25.17 ± 0.13^{a} (M)
Rice	5.50 ± 1.50^{c}	0.022 ± 0.003^{c}	0.24 ± 0.24^{ab}	0.001 ± 0.00^{a}	21.26 ± 0.34^{b} (M)
Maize	12.25 ± 0.25^{a}	0.034 ± 0.003^{abc}	0.99 ± 0.004^{a}	0.50 ± 0.00^{a}	19.83 ± 0.50^{b} (L)

Values in the same column with different letters are significantly different ($p < 0.05$), M- Medium amylose content, L- Low amylose content.

is more susceptible to microbial spoilage and deterioration as compared to a starch with a low moisture content (Moorthy, 2002). Starch granules with less than 13 % moisture content is prescribed safe for storage. As a result, starches isolated from *N. lotus* rhizomes and seeds with lower moisture content can be stored longer as compared to potato and maize starches.

There was no significant difference ($p > 0.05$) in the crude protein contents among *N. lotus* rhizome, seed, and maize starches. The crude protein content of potato was significantly higher (0.041 ± 0.003 %) as compared to other starches, whereas the crude protein content of rice starch was significantly lower (0.022 ± 0.003 %). The crude protein content of *N. lotus* rhizome (0.028 ± 0.003 %) and seed (0.034 ± 0.003 %) was found low, as compared to other finding: in native lotus stem (0.15 ± 0.02 %) (Sukhija et al., 2016) and Indian lotus root cultivars 0.12–0.16 % (Syed et al., 2012). The crude protein content of lotus seeds was higher than that of lotus rhizomes. The presence of protein associated with starch granules has an influence such as during low-temperature storage, starch paste clarity, starches amylolytic enzymes digestion. Protein forms disulfide bond with starch and indicated to increase gel strength and restrict water migration (Wu et al., 2023).

There was a significant difference ($p < 0.05$) in the fat content of *N. lotus* rhizome and seed. Fat was not detected in the rhizome of *N. lotus.* There was no significant difference ($p > 0.05$) in the fat content of *N. lotus* seed, potato, rice, and maize starches. However, maize starch contained greater amounts of fat (0.99 ± 0.004 %), and was consistent with previous results (Dhital et al., 2011), followed by the seeds of *N. lotus* (0.92 %). Starch sample containing protein, fat, and fiber below 1 % is regarded as pure (Lawal et al., 2011). Moreover, the minimal percentage of protein and ash associated with starch granules indicate high purity (Wang et al., 2021). The functionality of starch can be limited by the presence of proteins and lipids associated with starch granules. The lipid content associated to *N. lotus* seed and maize was higher (Syed et al., 2021). In the present study, *N. lotus* seed starch had a higher fat content (0.92 ± 0.06 %) than rhizome. Starch derived from *N. lotus* seeds can form starch-lipid complexes during food processing systems due to its smaller granule size. The starch isolated from *N. lotus* seeds has the potential to replace fat in food applications, delay food staling and improve freeze-thaw stability. There was no significant difference ($p > 0.05$) in the ash content of starches. However, the amount of ash in the rhizomes of *N. lotus* (0.5 ± 0.5 %) and maize (0.5 ± 0.0 %) starch was higher as compared to the other starches. The ash content indicates the presence of trace minerals such as phosphorus, potassium, and sodium.

There was no significant difference ($p>0.05$) in the amylose content among *N. lotus* rhizome, *N. lotus* seed, and potato starches, but significant variation ($p<0.05$) from maize and rice starches were observed. The amylose content (g/100 g) of rhizome, seed, potato, rice, and maize was 23.03 ± 0.07, 24.50 ± 0.13. 25.17 ± 0.13, 21.26 ± 0.34, and 19.83 ± 0.50, respectively. The amylose content in maize starch is categorized as low whereas the amylose content of rhizome, seed, rice, and potato starch are grouped as medium. The amylopectin content (g/100 g) of rhizome, seed, potato, rice, maize was 76.97, 75.5, 74.83, 78.74, and 80.17, respectively. Yu et al. (2013) reported similar amylose content for the lotus rhizome starch (20–25 %), but higher than this result for the lotus seed starch (25–35 %). Similar to this finding, Man et al. (2012) found lotus seed starch showed higher amylose content as compared to lotus rhizome. Furthermore, Zhu et al. (2022) reported more amylose contents in the starch of lotus seeds, while the rhizomes contain more amylopectin and less amylose. This situations can be attributed to variations in the expression of eleven key related genes between lotus seeds and rhizomes. It was discovered that the expression of genes involved in the amylose synthesis was higher in lotus seeds, whereas the expression of genes involved in amylopectin synthesis was higher in rhizomes.

It was reported that the amylose content of potato, rice, and maize was 23.8 %, 21.8 %, and 27.5 %, respectively (Wang & Wang, 2001). Commercially, starch has been classified based on amylose content and recognized as low (less than 20 % amylose), medium (21–25 %), and high (more than 26 %) (Bemiller & Whistler, 2009). Starches isolated from *N. lotus* rhizome, *N. lotus* seed, potato, and rice are classified as having a medium amylose content. The amylose content in maize starch is considered as low according to this classification. The amylose content of *N. lotus* rhizomes is medium and comparable to potato starch and it can be alternatively used as similar with potato starches in foods and non-food industries such as for biodegradable packaging. Lotus starch which has a higher amylose content possesses low crystallinity and low swelling capacity. The starch of *N. lotus* seed which has a higher amylose content has the advantage to delay gelatinization which enables the cooking time to be extended similar to that of tropical cereal starches and could have features to be incorporated with cereal flours for food products processing. Generally, amylose content can affect the functional properties of starch, such as swelling power, gelatinization, solubility, rheology, and pasting properties (Singh et al., 2003).

3.2. Morphological properties

The SEM of granular size and morphological properties of starches isolated from *N. lotus* rhizome, *N. lotus* seed, potato, rice, and maize are shown in Fig. 2. Starch granules isolated from *N. lotus* rhizome were larger than *N. lotus* seed starch but smaller than potato, with a smooth surface that was oval, spherical, or elongated. It was reported that lotus rhizome starch granules were oval, and smaller in size than potato starch (Jane et al., 1994). Starch isolated from the seed of *N. lotus* was seen as having a uniform size distribution with a polyhedral, angular, and oval shapes and a remarkably small granule size. The potato starch granules were observed to be smooth surface with oval, ellipsoidal, spherical, cuboidal, or elongated shape. Similar observation was noticed by Singh et al. (2009) for the potato starch granules as smooth surface, oval and irregular or cuboidal in shape. The shape of starch isolated from maize was less smooth and polyhedral. Rice starch granules were seen to have a rough surface in the form of packed clusters with an irregular and polygonal shape. The starch granules of rice were slightly damaged to some extent, which may be because of the extraction procedures from raw materials (Singh et al., 2009). There is a difference in the shapes and sizes of starches, which shows the variation of starch morphology due to starches of different botanical origins (Lindeboom et al., 2004). In general, the granular structure of *N. lotus* rhizome starches shared a similar morphology with potato starches.

The granule size distribution of *N. lotus* rhizome, *N. lotus* seed, potato, and maize is depicted in Fig. 3. The granule size (μm) of *N. lotus* rhizome, *N. lotus* seed, potato, and maize starches were 11.19 ± 3.69, 3.56 ± 0.92, 30.63 ± 11.09, and 7.97 ± 1.48, respectively. Lindeboom et al. (2004) classified starch granules as large (>25 μm), medium (10–25 μm), small (5–10 μm), and very small (<5 μm). According to

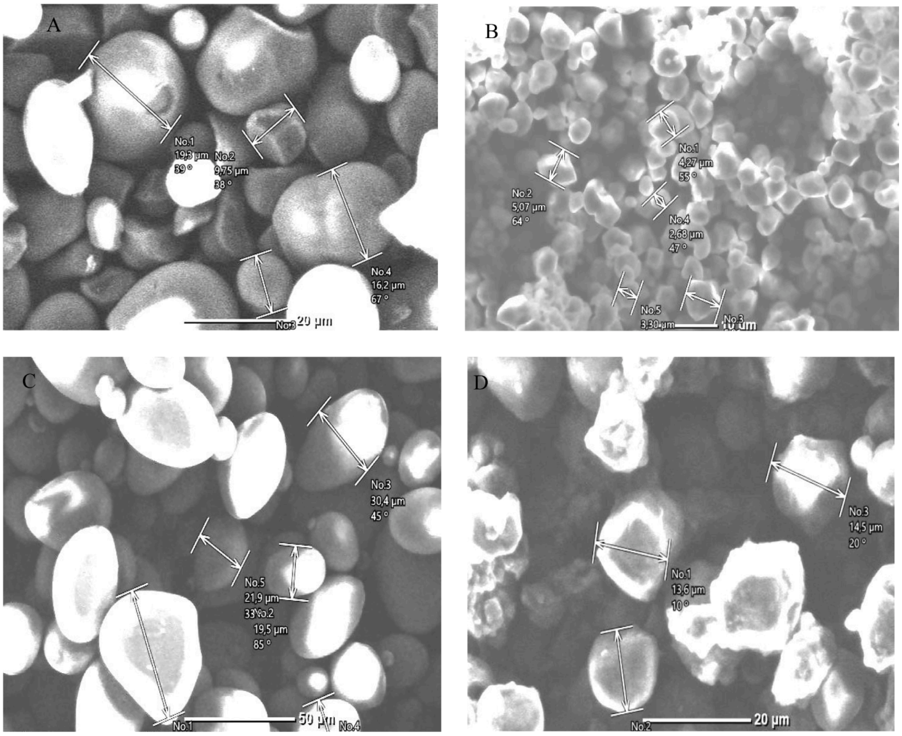

Fig. 2. Scanning electron micrograph of starch isolated from *N. lotus* rhizome (A), *N. lotus* seed (B), Potato (C), and Maize (D).

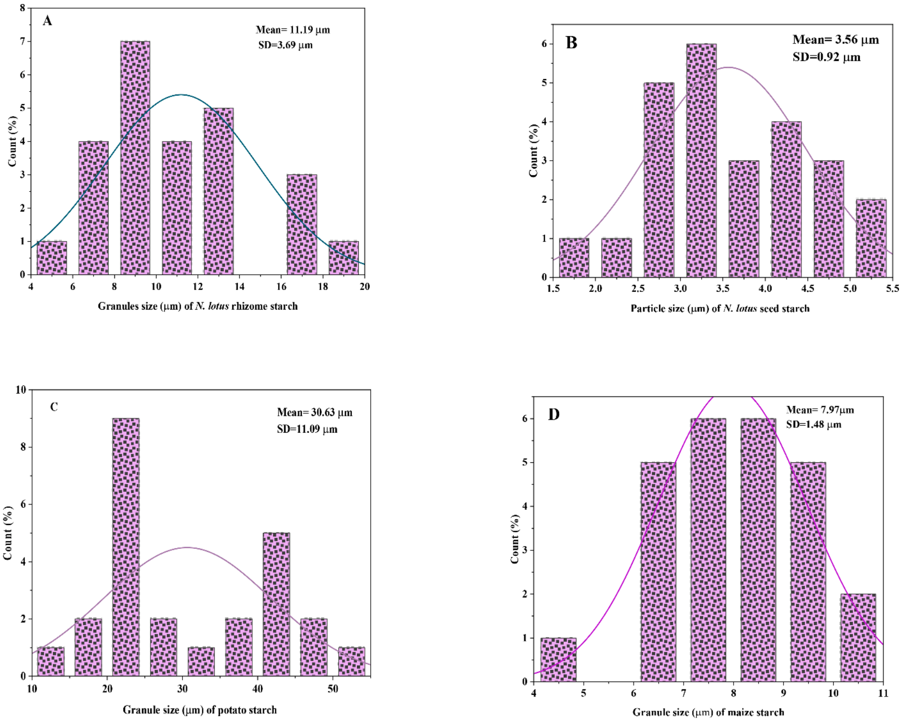

Fig. 3. Distribution of granule size of *N. lotus* rhizome (A), *N. lotus* seed (B), Potato (C), and Maize (D).

Lindeboom et al. (2004) classification, potato, *N. lotus rhizome*, maize, and *N. lotus* seed are categorized as large, medium, small, and very small, respectively. The granule length of some starches isolated from potato was reported to range from 5.13 to 87.89 μm (Abera et al., 2019), potato 12–45 μm (Singh et al., 2009), and maize 5–30 μm (Bultosa et al., 2002). Tuber starch possesses larger starch granules than cereal starch granules, and especially potato contains significant numbers of phosphate ester groups attached to the amylopectin fraction. Granules of starches isolated from *N. lotus* seed are exceptionally smaller than most of the conventional starches, viz., potato, *N. lotus* rhizome, and maize starches. Although the exact reason for the variation in starch granule size remains unclear, it is believed to be determined by the timing of the granule initiation process during the cell's starch synthesis (Vilpoux & Junior, 2023). Hence, starch isolated from *N. lotus* seed can be potentially used as a fat replacement, biodegradable films, making noodles, formulation of baby foods, carrier materials. Starch granule shape and size influences physical properties such as solubility, crystallinity, swelling, and gelatinization.

The size of starch granules matters, as it is pivotal in determining their applications in the food and non-food industries. Small granule size starch could be utilized in the food industry without further modification and for filler for biodegradable packaging films, flavor encapsulation and delivery, and fat replacers in reduced calories food products (Lindeboom et al., 2004). Additionally, small starch granules isolated from *N. lotus* seed are suitable for candy dusting, cosmetics, and filling agents for biodegradable films where small size starch granules are required. Starch with small granules size is ideal in aerosols and talcum powder preparations (Moorthy, 2002). The large granule size of lotus rhizome starches enhances their utilization as excipients and disintegrant in the pharmaceutical industry.

3.3. *X-ray diffraction*

In the present study, starches isolated from *N. lotus* rhizome, *N. lotus* seed, maize, and rice showed a strong diffraction pattern at 15°, doublet peaks at 17° and 18°, 23.0° (2θ), hence, A type diffraction pattern (Fig. 4). Starch isolated from potato showed a B-type diffraction pattern, as revealed from Bragg's diffraction pattern (2θ, Å) at 5.6°, 15.26°, 17.21°, 19.75°, 22.32°, and 24.08° Although most of the bulbs, tubers, roots, and rhizomes show a B-type pattern, starch isolated from *N. lotus* rhizome, *N. lotus* seed showed A-type crystallinity pattern. It is accepted that rice and maize starches display type A-patter and potato starch displays type B pattern.

Studies on lotus seed showed a typical A-type polymorphs with peaks at angle 15.07°, doublet at 17.10°, 18.01°, and 23.09°(2θ) (Chandak et al., 2022). Some of the non-cereal starches that possess A-type X-ray diffraction pattern are *Euryale ferox* kernel, Cassava, Colocasia, and seed of lotus, rhizome of lotus, and tuber of *Cyprus esculentus*. The XRD profiles of several aquatic macrophytes such as *Colocasia esculenta, Eleocharis dulcis, Nelumbo nucifera, Sagittaria sagittifolia, Trapa bispinosa*, and *Typha angustifolia* macrophytes starches displayed in all the corms, and *N. nucifera* seed had A-type crystallinity (Syed et al., 2021). Some reports show the XRD spectra of lotus rhizome starch showed typical C-type crystallinity (Lin et al., 2015; Man et al., 2012). Generally, amylopectin side chains regular arrangement greatly contributes to the formation of crystalline structure in the starches.

3.3.1. *Degree of crystallinity*

The degree of crystallinity of starches isolated from *N. lotus* rhizome, *N. lotus* seed, potato, rice, and maize in the present study was 27.63 %, 27.03 %, 23.94 %, 34.67 %, and 36.82 %, respectively. The diffractograms revealed that all the starch granules consisted of the crystalline and amorphous regions. *Nymphaea lotus* seed starch showed a lower crystalline degree than rhizome starch. The present study is consistent with the findings of different researchers. The degree of crystallinity was reported as 38.3 % for maize (Manek et al., 2012), 20.9 % for potato (Manek et al., 2012), 27.9 to 46.6 % for lotus seed (Dhull et al., 2022). Ren (2017) reported the degree of crystallinity of lotus root, lotus seed, maize, and potato as 21 %, 20 %, 39 %, and 28 %, respectively. Even though starch with a higher amylose content shows a lower degree of crystallinity. Man et al. (2012) reported a higher degree of crystallinity of lotus seed starch (29 %) with a higher amylose content and a lower degree of crystallinity of lotus rhizome starch (23.3 %) with a lower amylose content. This is probably influenced by high degree of amylopectin phosphorylation in the lotus rhizome starches as that of potato starches that can limit high degree of crystallinity. The degree of crystallinity (%) of different starches ranged between 23.94 % (potato, A-type) and 36.82 % (maize, B-type), indicating that the starches with a lower amylose content showed higher crystallinity. It was reported that A-type starch has a higher crystalline degree than B-type or C-type starch at similar amylose content (Man et al., 2012).

The degree of crstallinity of potato starch was lower than *N. lotus* rhizome, *N. lotus* seed, maize and rice starches. The crystallinity of starch has been attributed to the well-ordered structure of the amylopectin molecules inside the granules (Moorthy, 2002; Waterschoot et al., 2015). According to Wang et al. (2021), A-type crystalline starches were more densely packed in helical structures. Hence, cereal starch like maize or rice are generally packed according to A-type packing that is denser than B-type like potato (Waterschoot et al., 2015). A-type starch has a substantially greater crystalline degree than B-type or C-type starch at similar amylose concentration. Generally, the crystalline degree and amylose level are negatively correlated (Man et al., 2012).

The degree of crystallinity of native lotus stem starch (28.12 %) indicated that the starches with lower amylose content showed higher crystallinity (Sukhija et al., 2016). The major factors affecting starch crystallinity are the interactions between the double helices, the amylose chain length distribution, the amylopectin/amylose ratio, and the arrangement of the double helices within the crystalline domain and degree of amylopectin phosphorylation.

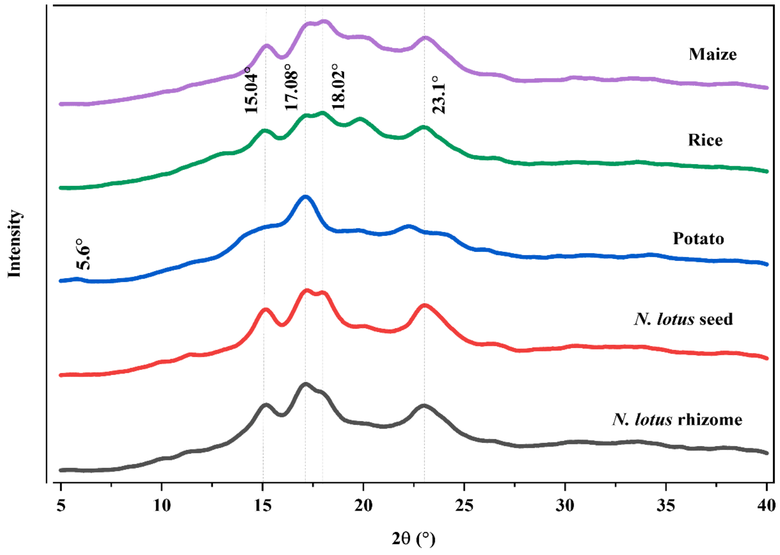

Fig. 4. X-ray diffractometry patterns of starches isolated from *N. lotus* rhizome, *N. lotus* seed, potato, rice and maize.

3.4. Fourier transformed infrared spectroscopy

The FTIR spectra of starches isolated from *N. lotus* rhizome, *N. lotus* seed, potato, rice, and maize is depicted in Fig. 5. Vibrational mode assignment of rhizome, seed, potato, rice and maize starches (Table 2). The wide and strong absorption band in the region area of 3000–3600 cm^{-1} in all starches indicate the characteristics of the stretching vibration of the -OH group, and it appears at its most intense peak at 3281 cm^{-1}. It was reported that the peak at 3281cm^{-1} in all the starch is the complex vibration stretch containing free, inter and intra-molecular hydroxyl groups (Abdullah et al., 2018).

The sharp peak at 2928 cm^{-1} indicates C–H stretching vibration (Abdullah et al., 2018). The feature stretching around 1641 cm^{-1} in all the starch corresponds to C—O bending associated with OH group contribution by carboxylic acids or fats contained in the starch isolate (Abdullah et al., 2018).

The major spectral differences between *N. lotus* rhizome and seed starches were observed at 1300 and 1130 indicating SO_2 stretching, at 1006 cm^{-1} the stretching of S-O-C; and 1200 and 1056 cm^{-1} is the stretching of SO_3, particularly in starch isolated from the seed of *N. lotus*. The biological activities of *Nymphaea hybrid* polysaccharide extract are related to the presence of sulfate groups (Liu et al., 2023). The stretching of SO_3 is the structural base for *N. hybrid* extracts to exert its biological activity like antioxidant, hyaluronidase inhibition, melan production, and re-epithelization (Liu et al., 2023). Generally, the FTIR spectra of the present study confirm the polysaccharide nature of the starches isolated from *N. lotus* rhizome, *N. lotus* seed, potato, rice, and maize.

The ordered structure of external region of the starch granules was determined by deconvoluting ATR-FTIR spectra in the region between 1200 and 800 cm^{-1} (Fig. 6A). The deconvoluted ATR-FTIR spectra of *N. lotus* rhizome, *N. lotus* seed, potato, rice, and maize starches are presented in (Fig. 6A). These spectral regions evaluate the degree of short-range molecular order in the external region of starch granules (Chen & Zhu, 2024).

The absorbance intensity ratio (IR) of 1047/1022 cm^{-1} and 1022/995 cm^{-1} for *N. lotus* rhizome, *N. lotus* seed, potato, rice and maize are presented in Fig. 6B. The IR ratio 1047/1022 cm^{-1} of *N. lotus* seed starch was higher than *N. lotus* rhizome starch, but lower than rice and maize starches. However, the IR ratio 1022/995 cm^{-1} of *N. lotus* rhizome starch was slightly higher than *N. lotus* seed starch and lower than rice and maize starches. The bands at 1047 and 1022 cm^{-1} are linked with order/crystalline and amorphous regions in starch, respectively (Chen & Zhu, 2024; Man et al., 2012; Yu et al., 2013). These two peak ratios 1047:1022 and 1022:995 cm^{-1} are used as predictors of ordered molecular structure in starch (Chen & Zhu, 2024). The low value for IR ratio 1047/1022 cm^{-1} of *N. lotus* rhizome as compared to *N. lotus* seed indicates the low level of ordered structure in the external region of the starch.

3.5. Pasting properties

The RVA pasting properties of *N. lotus* rhizome, *N. lotus* seed, potato, rice, and maize starches are shown in Fig. 7, and the results are summarized in Table 3. There was a significant (p<0.05) difference in the pasting temperature of starches isolated from *N. lotus* rhizome, *N. lotus* seed, potato, rice, and maize (Table 3). The pasting temperature of *N. lotus* seed starch (78.27 °C) was significantly ($p < 0.05$) higher than the pasting temperature of *N. lotus* rhizome (71.9 °C) and potato (67.02 °C) starches. The lowest pasting temperature (67.02 ± 0.02 °C) was recorded in potato starch, and the highest pasting temperature was recorded in rice (93.32 ± 0.4 °C). The pasting temperature of *N. lotus* seed starch was not significantly different from maize starch (79.47 ± 0.4 °C) indicating its potential to be incorporated into products requiring a higher temperature to cook, such as tropical cereal-based foods. The highest pasting temperature in rice starch implies more heat for structural disintegration and paste formation (Ali et al., 2020). It has been reported that protein content in rice starch is correlated with pasting temperature. In the literature, it was reported that the pasting temperature of starch isolated from potato was 61.6 °C (Gebre-Mariam & Schmidt, 1996), lotus seed 83.47 °C (Wang, Reddy, & Xu, 2018), maize 74.1 °C (Bultosa et al., 2002), and rice starch 94.7 °C (Viturawong et al., 2008).

The peak viscosity of *N. lotus* rhizome starch (7201 ± 367 cP) was higher than that of *N. lotus* seed (4105 ± 404 cP), rice (895 ± 26 cP), and maize starches (946.5 ± 25.5 cP) but lower than that of potato starch (13,180 ± 285 cP). *N. lotus* rhizome starch had the second highest peak viscosity (after potato), suggesting the high swelling capacity of lotus rhizome starch. Potato starch has extraordinarily high peak viscosity (Fig. 7)(), due to the presence of a negatively charged phosphate monoester group Waterschoot et al. (2015) which is covalently bound to amylopectin causing repulsion between adjacent amylopectin chains, and allowing rapid hydration and large swelling (Waterschoot et al., 2015). The large swelling in potato starches makes them less resistant to disintegration during shearing action (Singh et al., 2003). The high lipid content (0.99 %) in maize starch reduces swelling during heating in excess water by the formation of amylose lipid complexes (Lawal et al.,

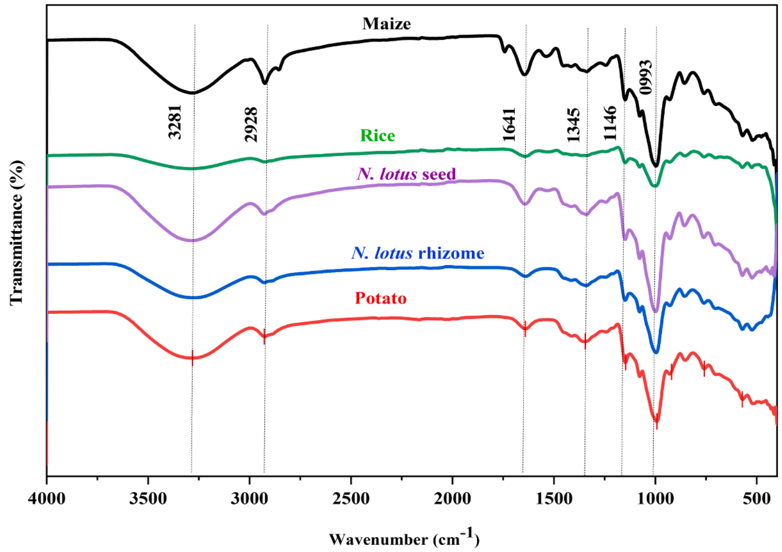

Fig. 5. FTIR spectra of starches isolated from *N. lotus* rhizome and *N. lotus* seed and reference starches (potato, rice and maize).

Table 2
Vibrational mode assignment of rhizome, seed, potato, rice and maize starches.

No.	Functional groups	Wavenumber (cm^{-1})	Rhizome	Seed	Potato	Rice	Maize
1	O-H stretching	3600–3000	3280	3276	3281	3284	3284
2	C-H stretching	2928	2923	2925	2928	2929	2931
3	C═O bending associated with OH group	1637	1641	1641	1640	1637	1639
4	C-O-C asymmetric stretching	1149	1151	1147	1146	1149	1149
5	C═O stretching	1200–800	995	1000	993	1000	1000

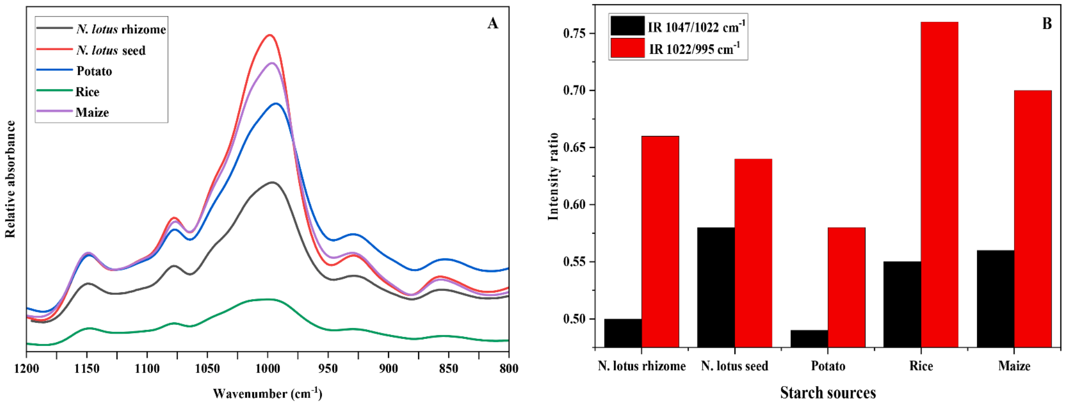

Fig. 6. Deconvoluted ATR-FTIR (A) and Intensity ratio (B) of different starches.

2011). Furthermore, maize starch possesses a low amylose content, and its tendency to gelation and retrogradation is slow as compared to rhizome, seed, and potato starches. The peak viscosity of *N. lotus* seed starch appeared to be higher than that of common cereal starches like rice and maize starches. According to Dhull et al. (2022), starches with high peak viscosity have high swelling power of fast retrogradation. Starch isolated from rhizome of *N. lotus* which has high peak viscosity as compared to seed of *N. lotus* starch can be used as thickeners, binders, and fillers in highly viscous products.

The trough viscosity of *N. lotus* rhizome and seed starches was similar ($p > 0.05$) to potato starch but different ($p < 0.05$) from maize and rice starches. The minimum trough viscosity was found in maize (637.5 ± 57.5 cP) and the maximum was in potato (2747 ± 494 cP). The trough viscosity indicates how the starch paste is shear tolerant. Starch isolated from potato was low shear tolerant and thus disintegrated easily, while rice and maize starches were shown to be relatively shear tolerant. The trough viscosity of different starches from different botanical sources was reported as 2412.7 cP for potato (Tessema & Admassu, 2021) and 1677 cP for lotus seed (Wang et al., 2018),

There was a significant difference ($p < 0.05$) in the breakdown viscosity of *N. lotus* rhizome, *N. lotus* seed, potato, rice, and maize starches. The highest breakdown viscosity (10,433 ± 779 cP) was found in potato starch, followed by *N. lotus* rhizome (4737 ± 246 cP), and the least was for rice (193 ± 62 cP) starch. The breakdown viscosity of *N. lotus* rhizome starch was higher than ($p < 0.05$) *N. lotus* seed starch. Breakdown viscosity, which is the difference between peak viscosity and trough viscosity, was highest in potato starch. Generally, larger granule starches have high peak viscosity and breakdown viscosity. The large granule absorbs water and swells more during gelatinization, but being fragile, it subsequently breaks to a greater extent, resulting in a high breakdown viscosity of starch (Dhull et al., 2022). The low breakdown viscosity in rice starch implies that the granules breakdown is more difficult. It was reported that starches with a higher swelling power are more sensitive to granule breakdown than those with a lower swelling power at a high temperature (Waterschoot et al., 2015). The degree to which the paste can break down while cooking is measured by its breakdown viscosity. In starches, the capacity to resist shear-thinning or breakdown is very important from an industrial standpoint such as for shear tolerant being not mushy and for high shear breakdown to be mushy. The breakdown viscosity of *N. lotus* seed is comparatively lower, suggesting a greater resistance to mechanical fragmentation during shear-thinning, hence increasing paste stability during heating and shear stress.

There was a difference ($p < 0.05$) in the setback viscosity of N. *lotus* rhizome, *N. lotus* seed, potato, rice, and maize (Table 3). Even though the setback viscosity of *N. lotus* rhizome and seed were not significantly different, the setback viscosity of *N. lotus* seed was higher than that of *N. lotus* rhizome starch. Setback viscosity is the difference between final viscosity and trough viscosity, which indicates the stability and aging trend of starch-cool paste. It was reported that the setback viscosity of potato was 1149 cP (Singh et al., 2009), maize 1149 cP (Singh et al., 2009), and lotus seed 743 cP (Wang et al., 2018). The setback viscosity is an indicator of the starch rapid gelation of leached amylose in starch paste (Guo et al., 2015). Starch isolated from lotus seed with a high amylose content and setback viscosity might be suitable for use as a raw material in noodle preparation (Dhull et al., 2022). Setback viscosity is a measure of the tendency of starch's gel ability to retrograde and re-associate. *N. lotus* seed starch has higher setback viscosity as compared to *N. lotus* rhizome because this is probably there might be high degree of amylopectin phosphorylation in the N. *lotus* rhizome that reduces the retrogradation tendency. Starch that has higher setback viscosity, suggesting it could be used in foods such as noodles, soups, sauces where high gelation and retrogradation is desirable.

The final viscosity of *N. lotus* seed starch was higher ($p < 0.05$) than that of *N. lotus* rhizome. The final viscosity determines the stability of paste after cooking and cooling, which increases upon cooling due to the aggregation of amylose molecules (Dhull et al., 2022). A high final viscosity suggests that the paste may form a more rigid gel and is more resistant to mechanical shear. Overall, it was reported that the pasting properties of starches are likely affected by amylose content, lipid content, and the branch chain-length distribution of amylopectin and by degree of amylopectin phosphorylation.

3.6. *Solubility and swelling power*

The solubility of starch isolated from *N. lotus* rhizome, seed, potato,

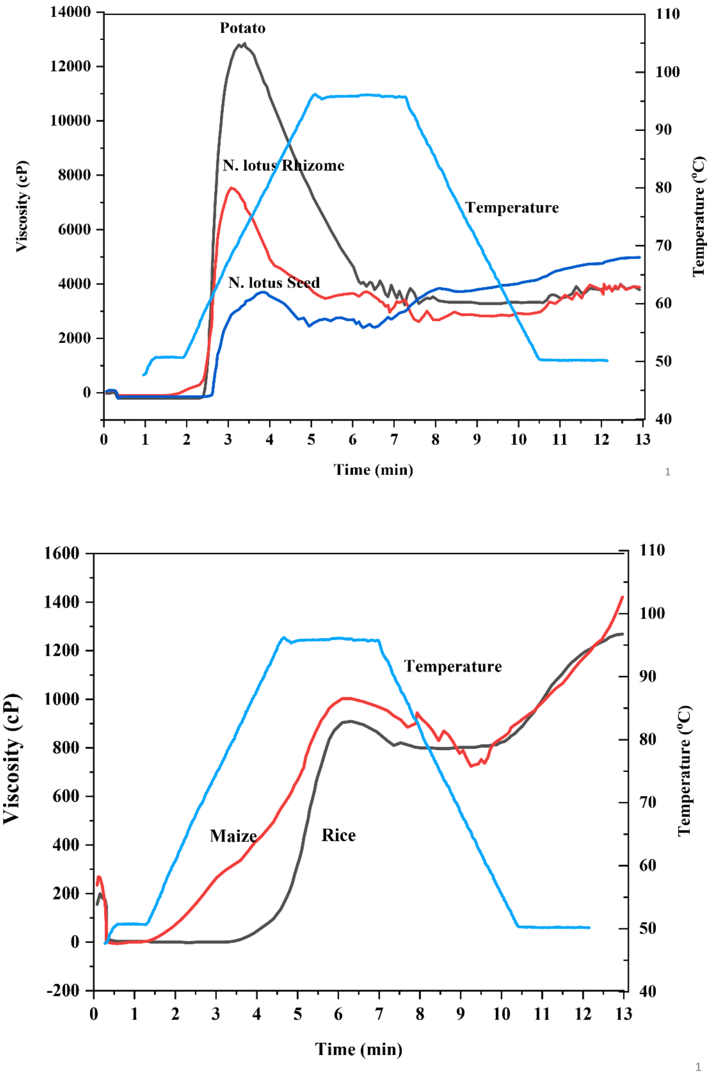

Fig. 7. RVA pasting properties of starches isolated from *N. lotus* rhizome, *N. lotus* seed, potato, rice and maize.

Table 3
The pasting properties of starch isolated from *N. lotus* rhizome, *N. lotus* seed, potato, rice and maize.

Pasting properties	*N. lotus* rhizome	*N. lotus* seed	Potato	Rice	Maize
Pasting temperature (°C)	71.90 ± 0.05^{c}	78.27 ± 0.02^{b}	67.02 ± 0.02^{d}	93.32 ± 0.42^{a}	79.47 ± 0.4^{b}
Peak viscosity (cP)	7201 ± 367^{b}	4105 ± 404^{c}	13,180 ± 285^{a}	895 ± 260^{d}	946.5 ± 25.5^{d}
Trough viscosity (cP)	2464 ± 121^{a}	2119.5 ± 252^{a}	2747 ± 494^{a}	702 ± 36^{b}	637.50 ± 58^{b}
Breakdown viscosity (cP)	4737 ± 246^{b}	1986 ± 656^{c}	10,433 ± 779^{a}	193 ± 62^{c}	309 ± 32^{c}
Setback viscosity (cP)	1292.5 ± 0.5^{ab}	3228.5 ± 617^{a}	1190.5 ± 617^{ab}	620 ± 87^{b}	769.5 ± 58^{b}
Final viscosity (cP)	3756.5 ± 122^{b}	5348 ± 365^{a}	3937.5 ± 123^{b}	1322 ± 51^{c}	1407 ± 0^{c}
Peak time (min)	3.93 ± 0.0^{d}	5.33 ± 0.06^{b}	3.33 ± 0.06^{e}	6.23 ± 0.03^{a}	5.07 ± 0.0^{c}

Values in the same row with different letters are significantly different ($p < 0.05$). cP- centipoise.

rice, and maize is shown in Fig. 8. The solubility of all starches increased with an increase in temperature. In the past studies the solubility at 95 °C was found to be 16 % for lotus seed (Chandak et al., 2022), and 13.5 % for maize starches (Lan, Zhihua, Yun, Bijun, & Zhida, 2008). When heated in water, it has been observed that big-size starch granules have higher solubility than small-sized starch granules (Lin et al., 2015). The higher phosphate group in potato starch increases hydration by weakening the extent of bonding within the crystalline domain Hoover, (2001).

The swelling power of *N. lotus* rhizome, *N. lotus* seed, potato, rice, and maize starches is shown in Fig. 9. It was observed that the increase in temperature from 45 °C to 95 °C increased the swelling power of all starches. The swelling power of *N. lotus*, maize, and seed starches was similar at 55 °C. Compared to rhizome starch, *N. lotus* seed starch has a little higher amylose concentration, which could account for its slightly reduced swelling capacity (Man et al., 2012). Because *N. lotus* seed starch has small granules, it tends to pack tightly, increasing the degree of association and decreasing swelling. These characteristics of *N. lotus* seed starch may be investigated for possible application in the pharmaceutical industry as a binder.

The phosphate monoesters found on the amylopectin of potato starch carry negative charges that cause ionic repulsion, finally expand, and weaken the starch chains, and make it easier for water molecules to enter the starch granules (Singh et al., 2003; Waterschoot et al., 2015). Nevertheless, the phospholipids found in grain starch (rice and maize) tend to combine with amylose to produce a complex that includes long, branching long chains of amylopectin, which limits swelling (Singh

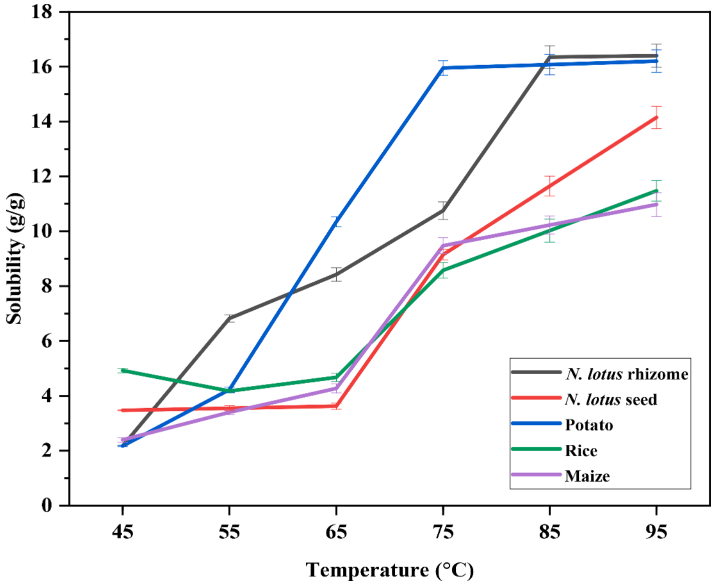

Fig. 8. Effects of temperature on solubility (%) of *N. lotus* rhizome, *N. lotus* seed, potato, rice and maize starches.

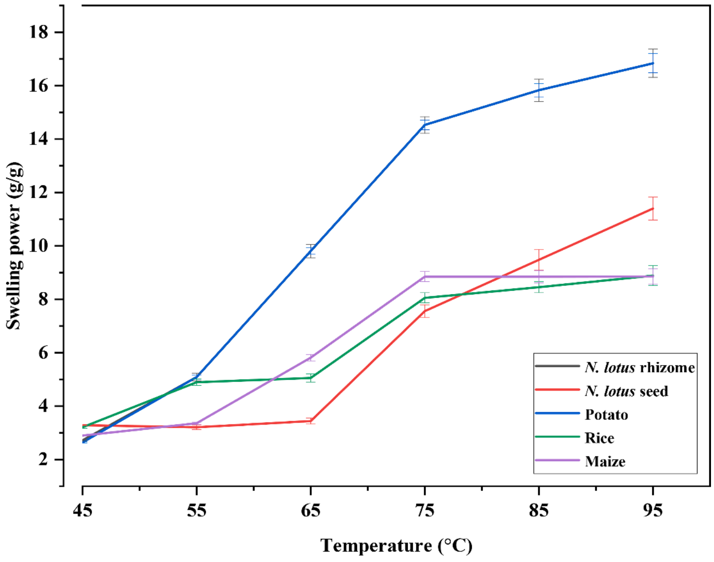

Fig. 9. Effect of temperature on swelling power (g/g) of *N. lotus* rhizome, *N. lotus* seed, potato, rice and maize starches.

et al., 2003). It is advantageous to use *N. lotus* rhizome starch as thickeners and binders in gravies, soups, canned food, sauces, bread, pancakes, noodles, cake, muffins, and cookies because it has higher swelling and solubility than *N. lotus* seed.

3.7. Syneresis

The syneresis of starches isolated from *N. lotus* rhizome, *N. lotus* seed, potato, rice, and maize is shown in Table 4. It was observed that, as storage duration increased from zero day to 120 h, the syneresis of all starches gradually increased. After five days storage, the lowest synaeresis was observed in *N. lotus* rhizome. It was reported that the syneresis of maize and potato starches at first day storage was 76.5 % and 41.6 % and progressively increased to 82.3 and 61.7 % respectively (Singh et al., 2009). The syneresis percentage of *N. lotus* seed occurred to a higher extent than that of *N. lotus* rhizome, potato, and maize during the storage. Chen and Zhu (2024) observed the syneresis of lotus seed gels occurred faster and to a higher extent than that of maize starch and

Table 4
Effects of storage duration at 4 °C on syneresis (%) of *N. lotus* rhizome, *N. lotus* seed, potato, rice, and maize starch pastes.

Storage duration (h)	*N. lotus* rhizome (%)	*N.lotus* seed (%)	Potato (%)	Rice (%)	Maize (%)
First day	68.41 ± 0.38^{b}	78.88 ± 0.88^{e}	63.87 ± 0.85^{e}	79.68 ± 0.34^{e}	76.20 ± 0.34^{e}
24	71.36 ± 0.15^{a}	82.89 ± 0.23^{d}	69.36 ± 0.10^{d}	82.10 ± 0.13^{d}	78.61 ± 0.18^{d}
48	71.36 ± 0.15^{a}	83.59 ± 0.39^{cd}	71.58 ± 0.27^{c}	83.79 ± 0.33^{c}	80.00 ± 0.55^{c}
72	71.36 ± 0.15^{a}	84.79 ± 0.20^{bc}	73.37 ± 0.17^{b}	85.40 ± 0.41^{b}	83.41 ± 0.21^{b}
96	71.36 ± 0.15^{a}	86.29 ± 0.49^{ab}	74.86 ± 0.17^{a}	87.83 ± 0.19^{a}	83.41 ± 0.21^{a}
120	71.36 ± 0.15^{a}	87.05 ± 0.11^{a}	74.86 ± 0.30^{a}	88.48 ± 0.15^{a}	83.41 ± 0.11^{a}

Values in the same column with different letters are significantly different ($p < 0.05$).

potato gels.

When starch molecules cool down, with time they re-associate in a complex re-crystallization of amylopectin chains process called retrogradation, which is frequently linked to the separation of water from the gel. After five days of storage at a low temperature refrigeration at 4 °C, the syneresis of *N. lotus* rhizome starch shown lowest (71.36 ± 0.15 %) syneresis, which implies it could be best suited for refrigeration food processing. These starch granules are important for emulsifying and stabilizing frozen desserts like ice cream in order to avoid the formation of ice crystals and improve whipping qualities. Starch that shows higher syneresis has higher tendencies to retrograde on low storage temperature. Since *N. lotus* seed starch has a high syneresis (87.05 ± 0.11 %) and is not suitable for use in food systems that involve refrigeration food processing.

4. Conclusions and recommendations

In this study, the structural and physicochemical properties of starches isolated from *N. lotus* rhizome and seed were described and compared with commercial starches (potato, rice, and maize) to predict their potential for applications in the food and non-food industries. Isolation of starch from *N. lotus* was feasible and pure that the protein, fat, and ash contents were very small (<1 %) and the fiber content was negligible. The amylose content of starches isolated from *N. lotus* was comparable to that of conventional potato starch. The granules of starch isolated from potato, *N. lotus rhizome*, maize, and *N. lotus* seed are categorized as large, medium, small, and very small, respectively hence starch isolated from seed can be used as filler and as fat mimetic where small size starch granules are required. Starches isolated from *N. lotus* rhizome and seed were shown type A- X-ray diffraction patterns. The pasting temperature of starch isolated from *N. lotus* seed was comparable to maize indicating its potential to incorporate in products requiring higher temperature to cook, such as tropical cereal based foods. The syneresis of *N. lotus* rhizome starch was the lowest, which implies it could perform better in refrigerated products. However, *N. lotus* seed starch has a high syneresis and is not suitable for use in food system that involves refrigeration. There is a limitation that the present study did not addressed total starch content, *in vitro* digestibility, thermal properties, macromolecular structure, mechanism of modifications like thermal, microwave, high pressure, chemical or enzymatic processes for starches isolated from *N. lotus* rhizome and seed are suggested for functionality improvement. In the future, it is suggested to determine these properties for potential applications of starch isolated from *N. lotus*.

CRediT authorship contribution statement

Alemu Lema Abelti: Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Tilahun A. Teka:** Conceptualization, Methodology, Formal analysis, Supervision, Writing – original draft. **Geremew Bultosa:** Conceptualization, Methodology, Formal analysis, Supervision, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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