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Effect of Physical and Chemical Modifications on Purity, Proximate Composition, and Amylose Content of *Nymphaea Pubescens* and *Nelumbo Nucifera* Seed Starches: Relevance to Pharmaceutical Excipients

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Abstract

Manufacturing medicines in developing countries often face high cost due to high dependence on imported starches. Locally grown plants with high starch content such as *Nymphaea pubescens* (water lily) and *Nelumbo nucifera* (lotus) could serve as in-expensive natural excipients that support both modern and traditional medicine systems. To investigate the effect of pregelatinization, acetylation, and oxidation on proximate composition, purity, and amylose content of *N. pubescens* and *N. nucifera* seed starches. Starches were extracted using aqueous methods and were modified by pregelatinization, acetylation, and oxidation. Proximate composition (moisture, protein, fat, fiber, ash), carbohydrate content and purity were determined. Amylose content was measured by iodine colorimetry. *N. nucifera* (60.55 %) exhibited the highest starch yield upon extraction. Among native starches, *N. pubescens* starch exhibited high purity (90.27 %) and amylose content (30.57 %), while *N. nucifera* starch showed high protein (24.35 %) and ash (3.60 %) with lower purity (68.26 %) and amylose (18.70 %). Pregelatinization indicated improved starch purity in both *N. pubescens* (91.01 %) and *N. nucifera* (77.94 %) starches. Acetylated *N. pubescens* starch increased ash content (2.28 %) possibly due to reaction salts, whereas oxidation moderately increased ash content (1.10 %) with slightly improved purity. Carbohydrate and amylose content remained largely unchanged across all modifications. Physical and chemical modifications affected non-starch components such as protein, fat, fiber and ash contents rather than total carbohydrate content, purity and amylose content. *N. pubescens* starch demonstrated superior compositional suitability for development as a natural pharmaceutical excipient.

Keywords: Amylose Content, Chemical Modification, Lotus, Pregelatinization, Proximate Analysis, Water Lily

Introduction

Starch is a polysaccharide that has become popular in both in pharmaceutical and integrative medicine research. It has long been recognized as one of the most commonly used excipients in the formulation of tablets and capsules. It plays key roles as a binder, filler, disintegrant and a stabilizer in solid dosage forms such as tablets and capsules. It is more favourable and attractive over synthetic excipients due to its several benefits such as safe profile, biodegradability, cost-effectiveness, and wide availability [1].

The performance of starch in a formulation can depend on its botanical source and composition. Especially, the swelling, solubility and starch mechanical behavior are mainly controlled by the ratio of amylose and amylopectin content. In addition, minor non-starch components such as crude proteins, fibers, lipids, and inorganic minerals can also affect not only the major properties such as compressibility, flowability, stability, but also the quality and performance of the finished dosage form [2]. Therefore, when investigating locally-sourced plant starches, it is important carry out a comprehensive analysis of proximate composition and overall purity.

Nymphaea pubescens (water lily) and *Nelumbo nucifera* (lotus) are well-known aquatic plants in Asian countries and these plants have been used for many years in Ayurvedic medicine for health benefits. Although different parts of these *N. pubescens* and *N. nucifera* plants such as flowers and roots are commonly used in traditional remedies, their seeds are mainly rich in

carbohydrates, making them a potential natural source of starch. Recently, there is a growing interest in exploring these plant-based excipients that perform well and align with both western and traditional medicines.

Starch can be modified following different modification techniques such as physically (pregelatinization), or chemically (acetylation and oxidation). Pregelatinization use heat and water to disrupt the starch granule structure without introducing new chemical groups [3]. Acetylation introduces acetyl groups [4] while oxidation introduces carbonyl and carboxyl groups by replacing hydroxyl groups present in the starch chemical structure, altering swelling behaviour, molecular interactions and solubility [5]. These modification methods are often used to improve starch physicochemical properties such as flowability, compressibility, swelling ability, solubility, stability, and compatibility in pharmaceutical formulations. Therefore, it is important to evaluate the composition of these seed starches and their behavior after modification to investigate their potential as excipients within integrative medicine systems.

Many studies have investigated changes in starch functional and physical properties at different modifications but fewer studies have focused on how modifications affect to proximate composition and purity. It is important to know if modifications change moisture, protein, lipid, fiber, or ash content, because these can affect quality, stability, compatibility, and regulatory approval. Therefore, to address this gap, the present study investigated changes in the proximate composition, purity, and amylose content of *N. pubescens* and *N. nucifera* seed starches following pregelatinization, acetylation and oxidation, focusing on whether these modifications change their basic composition in ways that could affect pharmaceutical applications.

Materials and Methods

Collection and authentication of plant materials

Dried seeds of *N. pubescens* and *N. nucifera* and their fresh plant materials were collected from the local vendors who source the plants from natural aquatic habitats located in Hambanthota District (Southern Province, 6.311630° N, 80.993561° E), Sri Lanka. Each plant type was authenticated by the Bandaranaike Memorial Ayurvedic Research Institute, Sri Lanka (Accession No. 3078-3079).

Isolation of Starch

Dehusked-seeds of *N. pubescens* and *N. nucifera* were cleaned, dried, and ground into fine powder. The powder was suspended in distilled water and allowed to soak. The slurry was filtered through muslin cloth, and the filtrate was allowed to settle for sedimentation of starch. The supernatant was discarded, and the sedimented starch was repeatedly washed to remove soluble impurities. The starch was then dried at 40°C for 24 hours, pulverized, and stored in airtight containers [6].

Pregelatinization

Starch was dispersed in water and heated with continuous stirring until gelatinization occurred. The paste was dried at 40 °C for 48 hours and milled into powder [7].

Acetylation

Starch was suspended in distilled water under alkaline conditions. Acetic anhydride was added gradually while maintaining pH at 4.5 with 0.5 M hydrochloric acid. After completion of reaction, the mixture was filtered, washed with distilled water, and air-dried at 30 °C for 48 hours [8].

Oxidation

Starch was treated with sodium hypochlorite solution under controlled pH. After reaction, the starch was neutralized with 1 M sulphuric acid. It was washed with distilled water, and air-dried at 30 °C for 48 hours [8].

Determination of acetyl group content

Acetylated starch (5 g) was dispersed in distilled water and titrated with 0.1 M sodium hydroxide using phenolphthalein. After adding 0.45 M sodium hydroxide and shaking for 30 minutes, excess alkali was back-titrated with 0.2 M hydrochloric acid. A blank was prepared using native starch. Acetyl content (%) was calculated from the difference between blank and sample titrations [9].

Determination of carboxyl group content

Oxidized starch (2 g) was treated with 0.1 M hydrochloric acid, filtered, washed with distilled water until there were no chloride ions in the filtrate, and titrated with 0.01 M sodium hydroxide to pH 8.3. A blank was performed using native starch. Carboxyl content (%) was calculated from the difference between sample and blank titrations [10].

Evaluation of moisture content

Moisture content was determined using an Infrared Moisture Analyzer (Kern DLB 160-3A, Germany). Approximately 1 g of dried starch sample was evenly spread on the sample tray and heated until the end-point signal was obtained. The percentage moisture content was automatically recorded [11].

Evaluation of crude fat content

Crude fat content was determined using a solvent extractor. Five grams of starch sample were extracted with petroleum ether for 4 hour. After solvent recovery, the residue was dried at 105 °C to constant weight, and fat content was calculated [12].

Evaluation of crude protein content

Crude protein content was determined by the Kjeldahl method. Nitrogen content was estimated from titration data and converted to crude protein using a conversion factor of 6.25 [13].

Evaluation of ash content

Ash content was determined by incinerating 2 g of starch sample in a muffle furnace at 550 °C for 4 h. The residue obtained was weighed and expressed as a percentage of the initial sample weight [13].

Evaluation of crude fiber content

Crude fiber content was determined by sequential acid and alkaline digestion of defatted starch, followed by drying, ashing, and calculation based on weight differences [12].

Determination of carbohydrate content

Carbohydrate content was calculated by subtracting all the other proximate determinations, including percentage of moisture content, crude fat, crude protein, crude fiber, and ash content from 100 % [14].

Determination of starch purity

Starch purity was calculated on a dry basis by correcting carbohydrate content for moisture content [15].

Evaluation of amylose content

Amylose content was determined using the iodine binding colorimetric method described by Juliano [16]. Absorbance was measured at 620 nm, and amylose content was calculated using a calibration curve prepared with potato amylose standards [17]. Based on the amylose content, starches were classified into low amylose (12-20 %), intermediate amylose (20-25 %) and high amylose (>25 %) groups [18].

Statistical analysis

All analyses were performed in triplicate. Results are expressed as mean ± standard deviation. Statistical significance among groups was evaluated using one-way ANOVA followed by Tukey's multiple comparison test, with $p<0.05$ considered significant.

Results and Discussion

Extraction yield of native starches

The aqueous extraction method produced a starch yield 55.69±0.40 % for *N. pubescens* and 60.55±0.41 %for *N. nucifera*. The starch yield from *N. nucifera* was significantly higher ($p< 0.01$) than yield of *N. pubescens*. These results indicate that both botanical sources provide considerable starch recovery under aqueous extraction, with *N. nucifera* demonstrating highest extract yield.

Yield of modified starches

The extracted starch yield of *N. pubescens* and *N. nucifera* seed starch after each modification are summarized in Table 1. It highlights how pregelatinization, acetylation, and oxidation affect the total yield.

According to Table 1, the percentage yield after modification remained high (>94 %) for all modification methods. pregelatinization showed the highest yield in both starches.

Table 1: Percentage yield of each modified starch after pregelatinization, acetylation, and oxidation

Starch type	% Yield of starch ± SEM under each modification		
	Pregelatinization	Acetylation	Oxidation
Nymphaea pubescens	97.13±0.98	95.62±0.93	96.12±0.79
Nelumbo. nucifera	98.10±0.76	94.81±0.16	95.68±1.03

Values are expressed as mean ± Standard Error of Mean (SEM); n=3.

Table 2: Percentage of acetyl content and carboxyl contents in modified starches

Plant	% w/w of Acetyl groups in acetylated starch ± SEM	% w/w of Carboxyl groups in oxidized starch ± SEM
Nymphaea pubescens	1.20±0.19	0.09±0.02
Nelumbo. nucifera	1.34±0.19	0.08±0.01

Values are expressed as mean ± Standard Error of Mean (SEM); n=3.

Acetyl group content and carboxyl group content

The acetyl content in acetylated starches and carboxyl content in oxidized starches are tabulated in Table 2.

According to Table 2, acetyl content varies among the starches indicating highest level of acetylation by *N. nucifera*. The level of acetylation and oxidation is indicated in both starches are in the safe limit according to the purity and safety criteria of food and drug administration below 2.5% and below 1.1%, respectively.

Proximate composition

Table 3 summarizes the moisture, protein, fat, fiber, ash, carbohydrate, and purity of native and modified starches comparing how modification changes the basic composition.

Table 3: Descriptive analysis of proximate composition of native and modified starches

Type of Starch	% Content (w/w) ± SEM						
	Moisture % w/w	Protein % w/w	Fat % w/w	Fiber % w/w	Ash % w/w	Carbohydrate % w/w	Purity % w/w
Native *Nymphaea pubescens*	9.38± 0.18	7.09± 0.10	0.54± 0.10	0.63± 0.13	0.47± 0.03	81.89± 0.38	90.27± 0.24
Pregelatinized *Nymphaea pubescens*	6.41± 0.29	7.07± 0.06	0.27± 0.11	0.57± 0.07	0.50± 0.03	85.18± 0.39	91.01± 0.23
Acetylated *Nymphaea pubescens*	7.74± 0.12	6.47± 0.15	0.52± 0.08	0.54± 0.04	2.28± 0.03	82.45± 0.13	90.16± 0.23
Oxidized *Nymphaea pubescens*	9.89 ± 0.18	6.23± 0.30	0.42± 0.12	0.41± 0.09	1.10± 0.04	81.95± 0.13	90.94± 0.35
Native *Nelumbo. nucifera*	7.58± 0.17	24.35± 0.08	0.18± 0.11	1.20± 0.10	3.60± 0.03	63.09± 0.23	68.26± 0.33
Pregelatinized *Nelumbo. nucifera*	9.07± 0.18	16.71± 0.04	0.14± 0.21	0.76± 0.08	2.45± 0.07	70.87± 1.04	77.94± 0.51
Acetylated *Nelumbo. nucifera*	8.55± 0.15	19.12± 0.08	0.14± 0.08	0.90± 0.10	0.90± 0.04	70.39± 0.16	76.94± 0.25
Oxidized *Nelumbo. nucifera*	8.52± 0.06	19.02± 0.22	0.18± 0.20	0.96± 0.05	1.00± 0.07	70.32± 0.33	76.87± 0.53

Values are expressed as mean ± Standard Error of Mean (SEM); n=3.

Proximate analysis in Table 3 showed low moisture, ash, fat, and crude fiber contents in both native and modified starches, indicating high purity. No major compositional deviations were observed following modification. Native *N. pubescens* starch exhibited high carbohydrate content (81.89 %) and purity (90.27 %) with low ash (0.47 %). In contrast *N. nucifera* starch showed higher protein (24.35 %) and ash (3.60 %) content, resulting in lower purity (68.26 %). Acetylation of *N. pubescens* starch reduced moisture and slightly decreased protein and fiber content. However, ash content increased markedly to 2.28 %. Carbohydrate content remained relatively stable (82.45 %), and overall purity did not significantly change. Oxidation resulted in moderate reduction in protein and fiber, with ash increasing to 1.10 %. A slight improvement in purity (90.94 %) was observed in oxidized *N. pubescens* starch compared to its native starch.

Amylose content and classification

Table 4 shows the amylose content of each starch type and classifies them as high or low amylose content.

Table 4: Percentage of amylose content of native and modified starches and their classification

Starch type	Amylose (% w/w) (Mean± SEM)	Amylose category
Native *Nymphaea pubescens*	30.57 ± 0.18	High
Pregelatinized *Nymphaea pubescens*	29.35 ± 0.16	High
Acetylated *Nymphaea pubescens*	30.03 ± 0.17	High
Oxidized *Nymphaea pubescens*	30.14 ± 0.53	High
Native *Nelumbo nucifera*	18.70 ± 0.10	Low
Pregelatinized *Nelumbo nucifera*	15.08 ± 0.44	Low
Acetylated *Nelumbo nucifera*	17.48 ± 0.17	Low
Oxidized *Nelumbo nucifera*	15.68 ± 0.17	Low

Values are expressed as mean ± Standard Error of Mean (SEM); n=3.

As per Table 4, *N. pubescens* exhibited amylose content around 30 %, classifying it as high-amylose starch whereas *N. nucifera* showed amylose content between 15–19 %, classifying it as low-amylose starch. Modification resulted in slight variations in amylose values but did not alter the overall classification.

The findings of the present investigation clearly show that the botanical source of the starch played a major role in determining both the extraction yield and the amylose content. Among the two species studied, the higher extraction yield of *N. nucifera* may be due to the structural differences in starch granule distribution and reduced fiber entrapment. Previous studies have also shown similar differences in starch yield between plant sources, confirming that the type of plant strongly affects how much starch can be extracted [19, 20, 21]. Also, the recovery after modification was very high over 94% showing that little material was lost during processing. Among the modification methods evaluated, pregelatinization resulted in the highest yield. As reported by Karima *et al*., (2000), that physical modification methods generally do not involve chemical substitution or significant removal of soluble components, thereby keeping most of the original material unchanged [22]. Moreover, the slightly lower yields after acetylation and oxidation are possibly due to the additional washing steps and breaking down of starch chains occurring during chemical modifications. Similar observations with lower yields have been reported in other studies on chemically modified cereal and tuber starches [23, 24].

The acetyl group content of the acetylated starch produced in the present study indicated a successful acetylation. As reported by Wurzburg (1986) [25], acetyl groups are attached to the starch molecules mainly by weakening the hydrogen bonds between starch chains. The modified starch swells more easily and forms clearer pastes because of the weaker hydrogen bonds [25]. Food and drug administration purity and safety criteria of modified starches recommend the percentage of acetyl group content in acetylated starches below 2.5 %, and the percentage of carboxyl content in oxidized starches below 1.1 % for safe food applications. Increased acetyl or carboxyl substitution makes the starch chemically unstable, harder to digest, and unsafe to consume [26, 27]. As explained by Tharanathan (2005) [28], it is important to control level of acetylation at a moderate level because too many acetyl groups can damage the starch structure. Excessive acetylation can make the granules weaker, less stable, and reduce their performance in formulations [28]. The acetylated levels measured in this study exhibited a well-controlled acetylation without damaging its structure that could improve the starch's functional properties. Similarly, oxidation adds carboxyl groups to the starch, which can increase surface charge and make pastes more stable, while keeping the granules intact at mild oxidation conditions [29]. Zhang *et al*. (2022) reported that oxidized botanical starches usually contain carboxyl content between 0.03 and 0.2 %, depending on the strength of the oxidizing agent and reaction time [30]. The values observed in the present study match those observed with mild oxidation. Too much oxidation, however, can break starch chains, reduce molecular weight, and lower viscosity. Therefore, the moderate carboxyl levels found in the present study show that the oxidation was done under suitable conditions, improving starch functionality without damaging its structure [31].

The proximate analysis showed that both starches were very pure, with very low ash, lipid, and fiber content. Moisture levels were below 14%, which is acceptable for pharmaceutical-grade starch, indicating that they would remain stable during storage [32]. Keeping moisture low is important because too much moisture can encourage microbial growth and reduce flow and compressibility. The low ash content shows that there was very less mineral or inorganic contamination, indicating better the extraction and purification process. Similarly, the minor quantity of lipid and fiber show that non-starch materials were effectively removed. These qualities are important in pharmaceuticals because excipients need to have minimal impurities to ensure stable, compatible, and reliable performance in dosage forms [33]. Importantly, the present study exhibited no major changes in the proximate composition of the tested starches after the chemical modifications. This agrees with a previous study indicating that acetylation and oxidation mainly affect specific functional groups of starch without changing its basic starch structure [25]. Overall, the high purity of these starches makes them suitable for use in food and pharmaceutical formulations.

The amylose analysis indicated a clear difference between the amylose contents of two starches. *N. pubescens* had high amylose, around 30 %, while *N. nucifera* had low amylose, between 15–19 %. This difference in amylose content can affect the starch functionality in pharmaceutical

formulations. Starches with higher amylose content have a strong gel forming ability and less swell ability [33]. In contrast, starches with lower amylose content usually have high swelling ability and soft gel forming ability. These characteristics are useful in formulations that require rapid water absorption and faster tablet disintegration, especially when starch is used as a binder or disintegrant in immediate release and controlled release tablet formulations [34]. Minor reduction in amylose content after modification may be due to the changes in the starch structure or partial loss of amylose during modification as reported in past studies conducted on acetylated and oxidized starches [27, 30]. These compositional variations between these two plant species indicates that plant source is an important factor in starch functionality affecting its starch performance even before any modification.

Overall, the findings of the present study reveal that improvement in the purity of both starches and controlled level of incorporation of functional groups after modification, while maintaining their original amylose profiles. Although acetylation and oxidation caused considerable changes to the starch structure by adding functional groups, but these changes did not harm the basic structure of the starch granules. Basically, both proximate analysis and amylose test indicate that the starch modifications can improve its functionality and purity without damaging the core structure of the starch, especially amylose-amylopectin ratio.

It is essential to maintain a balance between modifying the starch and preserving its original structure when developing excipients to be used in integrative medicines. The consistent composition, distinct amylose profiles, and well-controlled modifications indicate that both starches could serve as better natural alternatives in pharmaceutical, food and neutraceutical applications. Since the integrative medicine more prefers plant-based ingredients, these findings would highlight the potential of *N. pubescens* and *N. nucifera* seed starches for use in formulation development.

Conclusion

The findings of the present study revealed that physical (pregelatinization) and chemical modifications (acetylation and oxidation) changed the non-starch components such as protein, fat, fiber and ash content, while keeping the total carbohydrate content mostly the same and improving the starch purity. Among the modifications, pregelatinization mostly improved the purity, possibly due to the non-use of chemicals for the modifications. Further, the present study reported distinct amylose profiles that remained unchanged after modifications, indicating that it did not significantly affect the core structure of starch especially amylose-amylopectin ratio. When comparing the two botanical sources, *Nymphaea pubescens* seed starch exhibited more favorable compositional characteristics compared to *Nelumbo nucifera* seed starch. Based on these findings, these starches could be considered as potential natural excipient for pharmaceutical and integrative medicine applications. Therefore, further studies are recommended for comprehensive physicochemical and functional evaluations to investigate their suitability in the formulation development.

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