

Cite this: *Sustainable Food Technol.*,
2023, 1, 228

Water chestnut starch: extraction, chemical composition, properties, modifications, and application concerns

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The increase in non-biodegradable waste has prompted research on the development of new biodegradable compounds that can act as substitutes for the prevalent synthetic materials used for packaging. In this regard, due to the advantages of starch including its biodegradable, abundant, cost effective, and non-texture nature, it has attracted attention in the food and pharmaceutical industries. Presently, numerous studies have been reported on the search for new sources of starch. Herein, we summarize research on water chestnut as a non-conventional source of starch. Advantageously, due to low protein, ash and lipid content in water chestnut, its processing requires little effort and a large pure starch fraction can be extracted. The large amount of carbohydrates in water chestnut makes it a tremendous reservoir of starch for the food and non-food industries and it can replace other commercial sources of starchy such as wheat and potato. The starch in water chestnut can be extracted using a series of methods including centrifugation, and subsequently, its purification results in shapes ranging from elliptical and globular to prismatic. However, the native form of starch is not stable with variations in temperature and pH, which restricts its use in specific applications. Thus, native starches are commonly altered to achieve improved characteristics such as solubility, swelling, texture, and thermal tolerance. Starch from water chestnut is known to show potential as a pharmaceutical excipient and also a potent candidate for the prevention of type II diabetes and obesity. This review highlights the physicochemical properties, modifications and applications of water chestnut starch in the food and non-food industries.

Received 11th November 2022
Accepted 6th January 2023

DOI: 10.1039/d2fb00041e

rsc.li/susfoodtech

1. Introduction

Water chestnut (WC) is a floating leaved annual, aquatic herb found in freshwater wetlands, ponds, and lakes.¹ According to its geographical distribution, higher rainfed areas contribute to its successful cultivation.² Primarily, it is considered a vegetable and used in the preparation of human food including sweet dishes and baked stuff as dry flour. Furthermore, it possesses a wide range of pharmacological activities including micro-bicidal, anti-inflammatory, pain killer, and anti-diabetic activities.^{3–6} Water chestnut has several bioactive constituents including cysteine, lectin, quercetin, proteinase inhibitor, fibers, vitamins, fatty acids, and minerals. The main constituent of this vegetable is starch (85–97.4% dry basis), which signifies its potential as a healthy food.^{7,8} Moreover, the starch industry is continuously searching for novel sources to counteract production costs and also meet the increasing demand for new

starches in food with pharma applications. Currently, the demand for starch has increased tremendously, which is widely employed for the production of ethanol and biodegradable plastics, besides its use in the food processing industry.⁹ In this case, water chestnuts are suitable candidates to supply a large quantity of starch given that they are widely available and economic. Furthermore, water chestnut starch has a unique characteristic compared to other sources of starch, *i.e.*, potato and rice. Specifically, water chestnut can maintain a brittle and crunchy texture after heat treatment during cooking or canning.¹⁰ According to its geographical allocation, Chinese water chestnut starch shows variable morphological attributes ranging from round to croissant-shaped such as irregular and triangular;¹¹ however, starches extracted from Indian chestnuts were observed to be irregular, oval, and cubic.¹⁰ Basically, starch is employed as a thickener, bulking, gelling, stabilizer, and water-retaining agent. Currently, most food processing industries utilize native and modified starches to give proprietary organoleptic properties to the finished product. Furthermore, presently modified food starches are considered critical and beneficial functional food additives.¹² The functional role of starch in several foods is based on its physicochemical characteristics.^{13–15} Presently, the food processing industry is

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focused on finding starch that can tolerate the extreme heat and shear conditions associated with processing. This is because native starches cannot be employed alone due to their many limitations such as low paste clarity, low shear stress resistance, and high retrogradation extent. Alternatively, the modification of starch by physical or chemical means or both enhances its native functional characteristics, thus widening its application.¹⁶ Furthermore, because native starches exhibit some limitation, it is necessary to alter them to match the requirements of the food industry. In the case of physical modification, mainly gelatinization or simple chemical modification is employed to meet the specific requirements of food and other allied industries. Modified food starches usually exhibit efficient pasting properties, stability, enhanced obstruction to retrogradation, and freeze-thaw stability. Acid modification is broadly employed in the starch industry to design thin-boiling starches for use in food, paper, textile, and other industries.¹⁷ Another major use of modified starches in regards to human health is as a fat replacer, which provides a gross range of benefits to product developers and also healthy status to consumers by reducing the proportion of fat. Thus, water chestnut starch is a potential substitute for traditional starch, which is increasingly in demand by the food sector to meet the increasing needs of the global population and utilized in a variety of ways. In this case, the various features of water chestnut starch, including its extraction, chemical composition, physicochemical qualities, modification, and applications in food items, need to be summarized to complement the knowledge that is currently available.¹⁸

2. Chemical composition of water chestnut starch

The chemical composition of starch extracted from water chestnut is presented in Table 1. Starch extracted from water chestnut has 0.2% w/w protein, ash (0.2% w/w) and lipids (0.1% w/w), therefore containing 99.5% w/w pure starch.¹⁹ The amount of amylose in starch isolated from water chestnut is found to be in the range of 21.8 to 32.10 g per 100 g.^{20–22} The water chestnut starch from the Dal Lake showed a lower protein (0.11% w/w) and ash (0.006% w/w) content compared to that from the Wular and Anchar Lakes, depicting its high purity.¹⁴ Alternatively, the amylose content was observed to be greater in the water chestnut starch from the Anchar Lake (30.5% w/w),

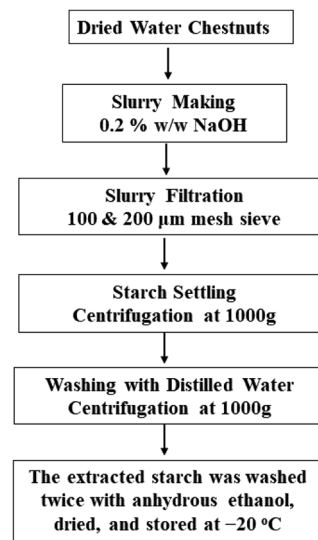


Fig. 1 Isolation of starch.

followed by that from Wular Lake (29.6% w/w) and Dal Lake (28.5% w/w). These differences in chemical composition may be due to the agro-climatic variations in different areas.⁷

3. Isolation of starch from water chestnut

Water chestnut fruit was peeled, washed, and blended with 0.2% w/w solution of NaOH. Firstly, the homogenate was sieved using a 100 μm mesh sieve, and then filtered through 170 μm mesh sieve followed by centrifugation. The upper brown-colored layer of protein was removed mechanically and the residue was washed several times with distilled water to remove the last traces of the brown layer. The extracted starch was washed twice with anhydrous ethanol, dried, and stored at $-20\text{ }^{\circ}\text{C}$ for further analysis. In another study, toluene was used for the removal of the protein layer to obtain pure starch^{22,28–31} (Fig. 1).

4. Properties of water chestnut starch

4.1. Thermal properties

Starch granules are practically insoluble in cold water, but they swell due to the absorption of water on heating in the presence

Table 1 Chemical composition of different starches^a

S. no.	Type of starch	Protein (%)	Ash (%)	Lipid (%)	Amylose (g per 100 g)	References
1	Chinese water chestnut starch	0.39	0.49	0.86	32.10	21
2	Native water chestnut starch	0.1	0.17	0.19	29	22–24
3	Native water chestnut starch	0.2	0.2	0.1	22.3	19
4	Native water chestnut starch	0.21	0.04	NR	NR	25
5	Native water chestnut starch	0.2	0.1	0.2	21.8	26
6	Native water chestnut starch	NR	0.69	NR	26.78	27

^a NR: Not reported.



of a sufficient amount of water. Consequently, the birefringence is lost due to the absorption of water in the amorphous regions of the starch granules, disrupting their crystalline structure. Gelatinization and the associated properties of starch can be well established by several methods, including electron microscopy, optical microscopy, X-ray diffraction, differential scanning calorimetry, Fourier transform infrared spectroscopy, and nuclear magnetic resonance spectroscopy.^{32,33} A researcher studied the gelatinization behavior of starch granules together with the disruption of their crystalline regions followed by their swelling using a polarising microscope combined with a hot stage. The distribution of amylose in the native starch granules was also studied *via* confocal laser scanning microscopy (CLSM) to explain the gelatinization process of starch granules. Specifically, 12 different starches were isolated from sweet potato tuberous root, lotus and yam rhizomes, potato tuber, water chestnut, and pea, barley, bean, wheat, lotus, and ginkgo seeds. Subsequently, four different patterns of gelatinization were observed. The gelatinization pattern in the water chestnut starch indicated that the disruption in its crystallinity started from the central hilum area, which then propagates along the central part and causes a small amount of swelling. With an increase in temperature, the crystallinity disruption continues and swelling of the starch granules occurs from the proximal to distal regions³⁰ (Table 2).

The thermal properties of water chestnut starch were identified by Xu and Shoemaker,³⁵ indicating an onset temperature (T_o) of 59.0 °C, peak temperature (T_p) of 70.4 °C, conclusion temperature (T_c) of 82.8 °C, and gelatinization enthalpy (ΔH_{gel}) of 3.16 cal g⁻¹. In another study,³⁴ the thermal properties of C-type starches of fava bean, water chestnut, pea, and yam were investigated using differential scanning calorimetry (DSC). A much lower gelatinization temperature (49.8 °C) and enthalpy of gelatinization (8.9 J g⁻¹) were reported for water chestnut starch in comparison to the previously reported values by Xu and Shoemaker,³⁵ indicating a lower amount of energy is needed to initiate gelatinization. Also, much higher values of T_o (58.5 °C), T_p (64.1 °C), T_c (68.4 °C) and ΔH_{gel} (10.8 J g⁻¹) were reported for WCS. In general, the thermal parameters of starch are dependent on its (i) granular ultrastructure, (ii) size, (iii) amylopectin branch chain, (iv) amylose content, (v) damaged starch content, (vi) phosphorus content and (vii) crystalline structure. Moreover, the presence of a large number of amylopectin short chains in water chestnut starch results in inefficient packing, and thereby a low gelatinization temperature and enthalpy.³⁶ Additionally, water chestnut starch contains a large proportion of damaged starch, and thus its ordered crystalline

structure is converted into a random amorphous structure, which is easily accessible to water.³⁷ Among the four starches investigated by Cai *et al.*,³⁴ water chestnut starch was associated with the lowest degree of structural order and amylose content, smallest granule size, largest damaged starch content, and highest amylopectin short-chain content, which are responsible for its lower gelatinization temperature and enthalpy. Singh *et al.*¹⁰ described the thermal properties of water chestnut starch and compared it with commercial maize and potato starch. The highest gelatinization temperature of 69.60 °C was observed in the case of water chestnut starch in comparison to 68.7 °C for corn and 59.9 °C for potato starch. The starch extracted from water chestnut demonstrated the maximum T_c of 75.20 °C compared to corn (74.4 °C) and potato (73.7 °C) starch. However, the enthalpy of gelatinization was minimum for water chestnut starch.

4.2. Morphological characteristics

Microscopic studies indicated that the starch granules of water chestnut are elliptical or globular in shape. In addition, a few prismatic granules were also observed. The granules of starch extracted from the water chestnut displayed a consistent “Maltese cross” with the hilum in the middle of the granules. Moreover, a few impaired granules were also observed in the water chestnut starch. Further, scanning electron microscopy (SEM) was employed to detect the sub-microscopic contour and surface structure of starch granules. The contour of starch showed an identical morphology as that observed under a light microscope. It was observed that the exterior of the granules was plain with negligible orifices and slits. Consistent with the findings of light microscopy, a few weakened granules were found in the starch extracted from water chestnut.³⁴ Singh *et al.*¹⁹ demonstrated the morphological characteristics of native, acid, and hydrothermally modified water chestnut starches through SEM. The starch remnants that existed in native water chestnut were found to be flat, ovoid to aberrant. The diameter of the granules varied from minute to average in the range of 5–30 μm. The acid-modified starch showed a negligible effect on the conformation of the granules because acid mainly destroys the amorphous domains, whereas has a minor impact on the crystal zone of the starch granules. However, 1 M HCl treatment demonstrated the generation of small furrows on the exterior of the starch granule. Contrary to this, treatment with HMT revealed fragmentation of the starch granules. It was found that the proportion of highly minute starch granules (5 μm) was enhanced from 2% in native starch

Table 2 Thermal properties of water chestnut starch

S. no.	Method of investigation	T_o (°C)	T_p (°C)	T_c (°C)	ΔH_{gel} (J g ⁻¹)	Reference
1	DSC	58.54	64.13	68.43	10.77	38
2	DSC	58.00	70.40	82.8	13.22	35
3	DSC	49.80	57.90	68.70	8.90	34
4	DSC	69.60	73.30	75.20	7.30	10
5	DSC	70.53	79.64	84.65	12.9	27
6	DSC	66.77	71.99	79.84	12.04	26



to 10% in hydrothermally modified starch. Gani *et al.*⁷ described that the starch purified from water chestnut from Dal Lake was observed to have expanded ovoid granules in comparison to that from Anchar and Wular Lakes. The starch extracted from Dal Lake exhibited several huge-sized granules as compared to that from Anchar and Wular Lake. This revealed that the starch extracted from water chestnuts from the different lakes display several materializations of granules. Various conformations of starch granules impact their physicochemical properties such as gelatinization temperature, amylose content, and pasting properties. Similarly, Guo *et al.*³⁹ demonstrated the morphological characteristics of starch of chestnut grown in various areas. It was observed that the starch from various regions displayed an analogous granule shape because of their identical botanic source, but varied in size. Most of the starch granules presented an ovoid to globular shape, with few ovoidal ones dispersed among the granules. Starch granules displayed plain exterior and their edges and did not show any furrows. In accordance with Koteswara Reddy *et al.*,⁴⁰ the morphology of starch granules is associated with the physiological characteristics of their botanic origin such as amylose content, transmittance, swelling, and water absorption capacity. Lutfi *et al.*²² demonstrated also globular or ovoid-shaped granules with “horns” bulging from the surface of water chestnut starch. The acid-thinned and acetylated starches showed granules with an uneven surface. Furthermore, it was observed that acetylation of starch caused remarkable surface erosion in comparison to the acid-thinned starch, depicting the rigidity of acetylated treatment. Pregelatinized water chestnut starch upon bifold modification by acetylation showed granules with a more uneven and wrinkled surface. The acetylation of pregelatinized starch changes the inter- and intra-granular interplay among its granules, which may alter the microarchitecture of the starch and create numerous surface slits and orifices. Thus, SEM studies depict the greater effect of double modification on the morphological and microarchitecture of starch in comparison to one-step chemical modification.

4.3. Physico-chemical properties of starch

The fundamental physicochemical properties of purified chestnut starches including swelling power (SP), water retention capacity (WRC), solubility, pasting, freeze-thaw stability, light transmittance, and syneresis.

4.3.1. Swelling property, solubility and water retention capacity (WRC). The swelling power of starch is a measure of the extent of the interactions present between the crystalline and amorphous regions of its chains. However, the swelling power of starch granules is primarily decided by their water-retention capability due to the formation of hydrogen bonds. During the gelatinization process, the hydrogen bonds that stabilize the double helices of the starch crystallites break, and new bonds are found among the exposed –OH groups of amylose, amylopectin, and water molecules. It has been reported that in comparison to intact granules, hydration of the impaired granules occurs smoothly, and therefore they possess greater swelling capacity. Besides the gelatinization temperature,

granule swelling is based on the extent of impairment and the nature of starches. Amylose inhibits the swelling of starch and preserves the existence of swollen granules. Water chestnut starch exhibits excellent swelling power, which is ascribed to the presence of less amylose and increased quantity of impaired starch. In comparison to other starches from beans, yam starch, and pea, water chestnut starch demonstrates higher swelling power, as investigated by Cai *et al.*³⁴ in the temperature range of 40 °C to 95 °C at 5 °C intervals. The water chestnut starch displayed an abrupt increment in swelling ability from 50 °C, whereas its swelling ability slightly increased with an increase in temperature before gelatinization. Lutfi *et al.*²² compared the swelling ability and solubility of water chestnut starch and its modified forms. Its swelling ability was found to vary linearly with an increase in temperature up to 50–90 °C. The acid modifications and pregelatinizing significantly decreased the swelling power of water chestnut starch in contrast to its native counterpart. Acid affects the amorphous domains first, and then the crystalline areas, attacking the surface of the granule first, and then its inner core. The increased roughness of the surface of starch due to acid thinning and the increase in crystalline domains may be the major cause for its restricted swelling. A significant increase in swelling power was observed in acetylated starch of water chestnut (aWCS) in comparison to native starch (WCS) mainly at 80 °C to 90 °C. Acetylated water chestnut starches (aWCS) demonstrated a remarkable increase in swelling power than their native counterparts due to the improved hydrophilicity of the starch granules. Yadav *et al.*²⁶ reported that annealing and heat moisture treatment decreased the swelling power and solubility of starch granules. The decreased swelling power of modified starches indicate the presence of robust forces of attraction in the crystallite regions. During annealing, the amorphous amylose content is transformed into helical chains, resulting in an increase in attractive forces between the amylose helices, and consequently a decrease in swelling power. Similarly, an increase in moisture content makes the amorphous starch chains more mobile and enables stronger interactions between the crystalline regions of the starch. When water formulations of starch granules are heated, hydration and swelling of the starch molecules occur with the release of a few soluble starch granules into the liquid. The swelling ability of water chestnut starch was determined at 10 °C intervals over a wide temperature range of 50–90 °C. It was observed that the starch extracted from water chestnut from Anchar Lake revealed a lower swelling power of 1.4% to 11% w/w in comparison to that from Wular Lake (2.2% to 15% w/w). The maximum swelling ability of starch extracted from water chestnut was found to be 4.2–18.3% w/w from Dal Lake. Similarly, Dal Lake also displayed the greater solubility of starch (3.4% to 17% w/w) in comparison to the starch from Wular Lake (1.4–14.4% w/w), while the starch from Anchar Lake showed 0.4–10% w/w solubility over the temperature range of 50–90 °C. This revealed that the starch granules from water chestnut withstand swelling at temperatures less than 70 °C. When the temperature increased from 70 °C to 90 °C, the starch granules showed swelling due to the rupture of the hydrogen bonding in their amorphous regions, which displayed inevitable and



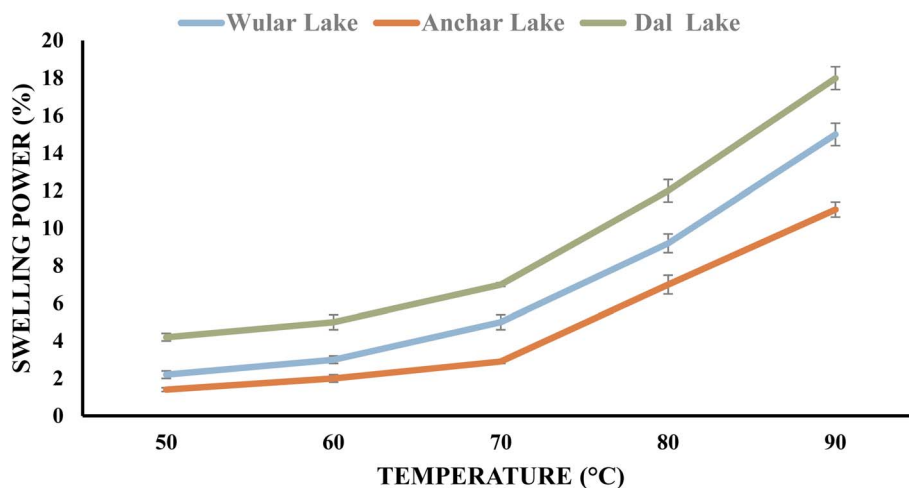


Fig. 2 Swelling power of water chestnut starches at different temperatures.⁷

dynamic water absorption.⁷ Similarly, an enhancement in the swelling of starch granules was observed in *T. natans* and *T. bispinosa* in a wide temperature range.²⁰

The starch extracted from water chestnut from Dal, Wular and Anchar Lakes showed an increment in water binding capacity from 13% to 60% w/w, 9% to 52% w/w, and 7% to 44% w/w with an enhancement in temperature from 50 °C to 90 °C (Fig. 2 and 3), respectively.⁷ The lowest increment in the water binding capacity of starch from Anchar Lake can be ascribed to its greater number of –OH groups for the formation of hydrogen and covalent bonding among the starch chains in contrast to water.⁴¹ However, Bello-Pérez *et al.*⁴² demonstrated that starch isolated from water chestnut possessed a similar water-binding capacity. The WRC values of the water chestnut starch were found to be 0.8–2.5 (g g⁻¹) in different areas. The water-binding sites were enhanced given that the number of –OH groups was greater with an increase in temperature. The greater water absorption capacity of starches can increase the functional applications of some food products, which may gain gradual water uptake and hinder the accumulation of lumps upon mixing with other powder forms of food.³⁹

4.3.2. Syneresis. Syneresis is the release of water from starch gel during its storage upon freezing. The rate of syneresis of chestnut starches was observed to be between 37.06% w/w (Fuzhou) and 56.40% w/w (Zhaotong).³⁹ According to Hermansson and Svegmak,⁴³ a greater amount of amylose causes greater interaction with the amylopectin chains, which leads to greater shrinkage of the gel and syneresis. It was found that a higher acid concentration leads to a reduction in the syneresis of starch gels.⁴⁴ The decrease in the amylose content may be the reason for the decreased starch syneresis during gel formation. However, heat-moisture treatment boosts the syneresis of the to a lower degree in comparison to the native starch. This can be ascribed to the reduced water absorption of the starch granules during the course of their disintegration and casehardening of the granules during HMT. Lutfi *et al.*⁴⁵ analysed the effect of salts on the syneresis of water chestnut starch and WCS-XG (xanthan gum) complexes. The proportion of syneresis for the native WCS was observed to be 38% w/w in comparison to that of corn, sweet potato starch, and mung bean starch, which was found to decrease.^{46,47} It was observed that the syneresis value of WCS was enhanced after treatment with a higher concentration

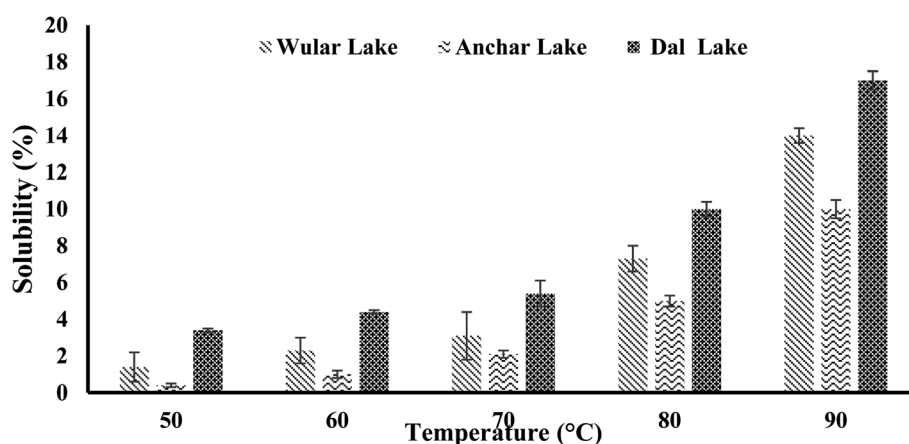


Fig. 3 Solubility of water chestnut starches at different temperatures.⁷



of NaCl at a lower temperature. The mode of action behind this may be due to the rearrangement of the starch molecules or enhanced molecular interactions among the starch molecules at a lower temperature, leading to the release of water from the starch. The syneresis value of these starch mixtures was observed to be low in the absence of sodium chloride, according to the results reported by.^{48,49}

4.3.3. Light transmittance. Light transmittance is the quantity of light passing through starch paste, which gives important data about its behaviour. Many factors influence the transmittance including granule size, percentage of amylose, swelling power, amylose/amylopectin ratio, and proportion of swollen and non-swollen granule remnants. The transmittance values of WCS alone and WCS-XG complexes with and without the addition of NaCl were studied.⁴⁵ It was found that the transmittance was enhanced in water chestnut starch with the addition of salt. The transmittance value of the starch paste increased at salt concentrations of 0.5% w/w and 1% w/w. The enhancement in transmittance was due to the breakdown of the hydrogen bonds among the starch molecules, and also between the starch and water molecules. These hydrogen bonds hindered the starch molecules from reorganizing during retrogradation. The lower rate of retrogradation enhanced the light transmittance in these starch gels in contrast to the native starch gels. Similarly, extrusion-polyphenol treatment of WCS caused a remarkable decline in retrogradation.⁵⁰ In addition, Singh *et al.*¹⁹ demonstrated that the transmittance of native, AM, and HMT water chestnut starches was reduced with an increase in their storage time. It was observed that acid-thinning caused an increase in the first part of light transmittance and HMT treatment decreased the ability of the starch suspension to transmit light compared to the native starch. A reduction in retrogradation is one of the reasons for the enhancement in light transmittance of the acid-thinned starch paste. The discharge of amorphous areas during acid-thinning increased the interactive bond formation among the amylopectin molecules, and hence boosted the light-transmitting efficiency.⁵¹ These results are consistent with previous research on banana starch.⁴² Similarly, Gani *et al.*⁷ demonstrated that water chestnut starch from Dal Lake showed higher

transmittance and swelling power compared to that from Wular Lake and Anchar Lake.

4.3.4. Pasting properties. The pasting properties of water chestnut starch include the parameters of pasting temperature (PT), peak viscosity (PV), trough viscosity (TV), final viscosity (FV), and setback viscosity (SV). Viscosity is one of the most crucial properties of starches and can provide important knowledge about their cooking behaviours during heating and cooling cycles.⁵² When a starch–water dispersion is shear heated at a higher gelatinization temperature, a phase change from ordered to random state occurs and the viscosity of the system enhances dramatically, resulting in the formation of starch paste. The characteristics of starch paste are affected by the amount and length of the amylose chain, as well as the width and position of the branched chains of amylopectin. Moreover, the pasting property of starches acts as an efficient mode to monitor their functionality with their structural characteristics and determining several potent applications for their use in industry as a condenser or binder.^{52,53} There were significant changes found in the PT, PV, TV, FV, and SV of the starch samples. Specifically, there was a constant enhancement in viscosity with an increase in temperature in the initial phase, as shown by the release of free water and reduced flow of water because of the enhancement in the amount of swollen starch granules, which occupy more space. PT is the temperature at which the viscosity starts to increase or the starch begins to gelatinize. The PT of starches was found to be between 68.67 °C (Kunming, Yunnan) and 77.76 °C (Yangjiang, Guangdong). According to Singh *et al.*,⁵⁴ a higher PT depicts more resistance to swelling. Thus, the starches purified from chestnuts grown in Zhejiang, Jiangsu, Guangdong, and Henan revealed greater resistance to swelling in contrast to others. The starch purified from chestnut observed in Yangjiang (Guangdong) also showed a greater amylose content. The PV of chestnut starch from Zhaotong (Yunnan) was observed to be 1120.67 cP, while that from Liuzhou (Guangxi) showed the maximum PV, *i.e.*, 4422.67 cP. Most of the starch samples showed PV values in the range of 3000 and 4000 cP. Singh *et al.*¹⁹ compared the pasting properties of water chestnut starch with corn and potato starch (Fig. 4). Potato starch showed the maximum PV of 8112 mPa s followed

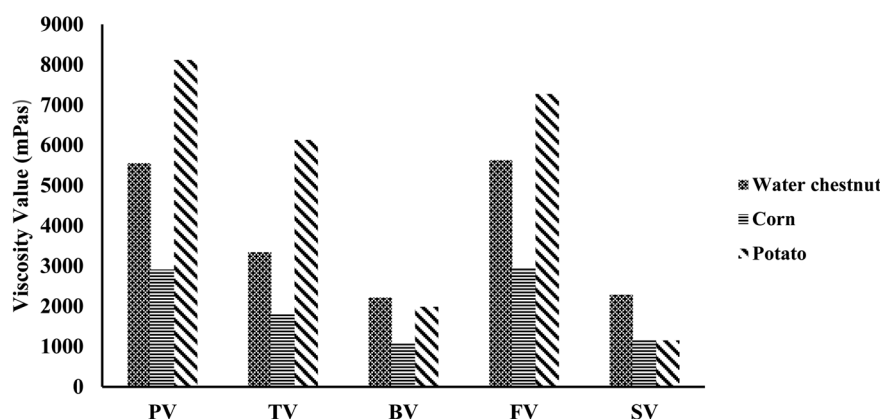


Fig. 4 Comparison of the pasting properties of water chestnut starch with corn and potato starch.¹⁹



by water chestnut with 5554 mPa s and corn with 2907 mPa s. The trough viscosity also displayed a similar pattern. The breakdown viscosity (BV) was found to be minimum in native corn starch, whereas maximum in native water chestnut starch. The final viscosity (FV) was more in potato starch at 7277 mPa s followed by water chestnut and corn starch at 5624 and 2956 mPa s respectively. The increment in final viscosity could be due to the clustering of the amylose molecules.⁵⁵ The setback (final viscosity minus trough viscosity) is the viscosity enhancement appearing from the movement of the amylose molecules that have been extracted from swollen starch granules during cooling and is usually employed to determine the gelling capability or retrogradation efficiency of starch.⁵⁶ Water chestnut starch revealed the highest setback viscosity, which was similar in corn and potato starches. The pasting temperature of native potato starch was found to be minimum (66.97 °C), while water chestnut and corn starches showed higher pasting temperatures (75 °C and 76.77 °C). Xu and Shoemaker³⁵ reported a greater gelatinization temperature for water chestnut starch compared to potato starch. Three potato cultivars were demonstrated to show pasting temperatures in the range of 68.2 °C to 74.5 °C.⁵⁷

The pasting properties are also based on the hardness of starch granules, which mainly affects the granule swelling potential and quantity of amylose released in solution.⁵⁸ Water chestnut starches displayed a constant enhancement in viscosity with an increase in temperature. The enhancement in viscosity with temperature can be ascribed to the expulsion of water from the loosened amylose in the granules when they swelled.⁵⁹ The PV was observed to be 2719 cP for the water chestnut starch from Anchar Lake, while 3714 cP in the case of that from Dal Lake. The final viscosity (FV) of water chestnut starch from Anchar Lake was found to be 3323; however, that from Dal Lake showed the maximum FV of 4385 cP.⁷ Miles *et al.*⁵⁵ observed that the increment in final viscosity may be due to the clustering of the amylose molecules. The pasting temperature for water chestnut starches was found to be between 71.4 °C to 84 °C and the highest pasting temperature was shown by the water chestnut starch from Anchar Lake. The higher pasting temperature of water chestnut starches from Anchar Lake (84 °C) and Wular Lake (81 °C) compared to that from Dal Lake (71.4 °C) showed their increased obstruction towards swelling.

4.3.5. Freeze-thaw stability. Freeze-thaw stability is a major factor to be appraised in refrigerated and frozen foods. Greater syneresis depicts the decreased freeze-thaw stability of starch. In this case, water chestnut starch showed greater freeze-thaw stability in a few mixtures and was used in the formation of frozen products and several industrial applications.²⁴ When starch-containing foods such as sauces, ice-creams, and soups undergo repeated freeze-thawed (FT) cycles, their morphological and physicochemical status may be significantly altered. A gelatinized starch gel is a looped homogeneous system. After freezing, the starch gel system becomes heterogeneous and is divided into starch-rich and starch-deficient ice stages. Repeated FT cycles of thickened pastes result in the formation of cryotropic gel, and the final products possess a sponge-like

texture.⁶⁰ Duplicating the FT cycle invokes phase separation and ice growth⁶¹ due to the syneresis in the starch gel and the separation of the water from the gel. The freeze-thawed starch gels form a honeycomb-like network, which is almost cracked at the 7th FT cycle. Freeze-thawed starch gels exhibited a classic B-type crystal structure according to their X-ray diffractogram, which is distinct from native starch (A-type crystal structure). The crystallinity was found to be enhanced by recasting the FT cycles. Moreover, the thermodynamic values of the freeze-thawed starch gels were also boosted with an increase in the number of freeze-thaw cycles, and the retrogradation ratio was determined to be 30% w/w in the 7th FT cycle. In addition, the textural characteristics such as hardness and pliability of the freeze-thawed starch gels were observed to be affected by repeating the FT cycles. Freeze-thawing hindered the digestive enzyme of starch gels, which is due to the fast retrogression of seeped amylose.⁶² The syneresis was observed to be remarkably enhanced with an increment in freeze-thaw cycles. It was revealed that after the 5th cycle, a low proportion of syneresis appeared in HMT starch, whereas it was the greatest in the annealed starch.²⁶ It has been observed that the liberation of water from starch gel systems preserved at low temperatures may be due to the enhanced amylose, amylose-amylopectin, or amylopectin interactions.⁶³ During annealing, the dissolution and case crystallization of the starch granules lead to lower water absorption by the granules, which may be the probable cause for the increased syneresis in annealed starch.²⁶ In another study, the freeze-thaw stability depicted that native water chestnut gel has a syneresis of 38% w/w, which is lower than that of sweet potato, corn, and bean starch.^{46,47} In contrast, the syneresis value of water chestnut gel is more than that of rice and banana starches.⁶⁴ The reduction in syneresis may be due to the extensively branched amylopectin, which grips extra water, and subsequently decreases the rate of retrogradation.⁶⁵

The freeze-thaw stability was boosted with the inclusion of gums and the highest increment was found with guar gum and acacia gum having values ranging from 38% w/w to 52% w/w. It was observed that the freeze-thaw stability increased upon the addition of hydrocolloids to starch because the hydrocolloids have good water absorption properties. Lee *et al.*⁴⁷ and Funami *et al.*⁶⁶ also demonstrated identical patterns. The insufficient hydration or swelling of starch granules is responsible for the decreased water gripping ability or enhanced syneresis in the aggregate phase. When starch is warmed in an aqueous condition, its amylopectin fraction disintegrates, resulting in the swelling of the starch; however, the amylose together with some of the amylopectin seeps out, resulting in moisture uptake.⁶⁷

5. Physical modification of water chestnut starch

Natural starch suffers from the limitations of thermal instability and easy retrogradation,⁶⁸ whereas modified starches possess distinct gelatinization and gel properties, and thus have been extensively used in food and related allied industries to fulfil the



requirements of consumers. Moreover, physical modes of modification are gaining increasing attention from researchers due to the cost-effective modification procedures and the absence of any chemical residues compared to the chemical modes of modification.⁶⁹ Physical modification techniques alter the functionality of starch in an environmentally acceptable mode without the use of any biological/chemical agents and production of any chemical waste.⁷⁰ Guo *et al.*⁷¹ physically modified chestnut starch by high-pressure homogenization and observed that its solubility, swelling and transmittance were enhanced with an increase in pressure. However, the morphology and crystalline architecture of the starch particles remained unchanged. Dry heat treatment (continuous and repeated dry heating) resulted in a significant improvement in the gelatinization, rheology and morphology of starch, whereas continuous dry heating was found to be more effective to decrease the pasting viscosity.⁷² Physical modifications of starch *via* heat-moisture treatment (HMT) and annealing (ANN) produce desirable physicochemical properties in native starch without changing its granular structure.^{73–75} Heat-moisture-treated and annealed starches play a vital role in the food industry owing to their enhanced thermal stability and reduced degree of retrogradation.⁷³ Both HMT and ANN are closely related processes, which can be controlled by various parameters including the starch: moisture ratio, heating period and temperature; however, they differ in the quantity of water required and temperature conditions.⁷⁶ Elevated temperatures in the range of 90 °C to 120 °C above the gelatinization temperature and limited moisture content ranging from 10–30% are essential for HMT, whereas annealing needs lower temperatures below the gelatinization point and higher than the glass transition temperature in the presence of an excess amount of water (50% to 60%).⁷⁵ HMT restricts the molecular mobility at elevated temperatures by controlling the quantity of water.⁷⁶ However, the structural and functional modifications of starches are dependent on the source of starch and amount of amylose present in them.⁷⁹ Annealing causes reorganisation in starches, giving them a well-organised configuration comprised of double helices of amylopectin.⁷⁷

Recently, the interest in the blending of starches with hydrocolloids to alter their rheological properties has been increasing. The properties of the blend depend on the concentration of the hydrocolloids and starches, conditions for the preparation of the blend and type and level of interactions between them.⁷⁸ The presence of hydrocolloids in starches primarily influences their flow, texture and viscosity, and also provides shear stability to the starch granules together with decreased syneresis.⁷⁹ Consequently, they provide stability and enhance the quality of food products. The treatment of starches with various hydrocolloids intensifies the outcomes of their physical modification, particularly the stability and gel formation ability of starch pastes.^{68,80,81} The addition of hydrocolloids is also a very effective method for the physical modification of starches, and simultaneously is more economical. Carboxy methyl cellulose (CMC), a derivative of chitosan, is the most employed cationic hydrocolloid, which contains carboxyl and amino groups attached to it and is extensively used in the food

industry owing to its good solubility in water.⁸² Similarly, xanthan gum (XG) is an anionic hydrocolloid having a rigid β -glucan backbone and polar water-loving trisaccharide side chains, which impart gel-thickening and emulsifying stability to starch pastes.⁸⁰ Likewise, the anionic hydrocolloid sodium alginate (SA) is also associated with excellent gel thickening and stabilization property. Water chestnut starch has potential to serve as a promising starch resource in the food processing industry but it has not been exploited fully due to the poor understanding of its modification. Also, thorough exploration of the properties of water chestnut starch can be helpful for its applications in the food and other allied industries and will permit its modification for application-oriented utility. Some researchers have explored the effects of hydrocolloids on the physicochemical properties of WCS. The prime aim of these modifications is to improve the properties of WCS to accomplish the requirements of starch-based foods. Gul *et al.*²⁷ studied the effects on the physicochemical, thermal, pasting and morphological properties of water chestnut starch (WCS) by modification through dry heating and addition of ionic gums, *i.e.*, carboxy methyl cellulose (CMC) and sodium alginate (SA). Native starch was modified by dry heating treatment (DHT) and with and without the addition of ionic gums using a reported method.⁸³ Initially, gum solutions of (0.4 g) SA and CMC were prepared separately by vigorous stirring them in distilled water (70 mL), and the dispersion formed by adding starch (39.6 g) to these prepared gum solutions was stirred continuously at room temperature for 30 min, and then transferred to a dish and dried in an oven at 45 °C. Similarly, Lutfi *et al.*²³ evaluated the functional properties of water chestnut (*Trapa bispinosa*) starch in the presence of four different gums, *i.e.*, xanthan, CMC, guar and acacia, by preparing aqueous solutions of isolated WCS with hydrocolloids including XG, gum acacia, gum guar and CMC having a moisture content of 10.1%, 14.3%, 13.7% and 11.5% w/w, respectively (Table 3).

5.1. Impact on morphology

Lutfi *et al.*²³ observed roughness on the surfaces of WCS granules containing carboxy methyl cellulose (CMC) *via* scanning electron microscopy prior to gelatinization due to the adherence of CMC on the surfaces of the starch granules. In contrast, after gelatinization, the starch granules were ruptured and formed a large number of smaller lumps, which provided compactness to the starch structure. Similar roughness in starch granules was also observed in another report.²⁷ The authors concluded that the granular size of WCS was affected to a lower extent and insignificant damage to its surface morphology occurred with the addition of CMC or SA. Additionally, the average size of the granules was found to be bigger when treated with SA (26 μ m) than that in the presence of CMC (21 μ m). The SEM images of the modified starches (with SA and CMC) indicated roughness and the presence of cracks on their granular surfaces because of the leaching of amylose in the presence of heat; also, the amount of cracks increased with an increase in temperature. Dry heating of WCS in the presence of sodium alginate for 4 h at 130 °C also resulted in similar



Table 3 Physical modifications of starch

Type of starch	Physicochemical characteristics					Thermal Characteristics					Physiochemical characteristics										
	Solubility (g per 100 g)	Swelling power (g g ⁻¹)	Water binding capacity (g g ⁻¹)	Oil binding capacity (g g ⁻¹)	Viscosity	Light transmittance	Moisture	Ash	Amylose	Total carbohydrates	References										
Native starch	19.5	8.67	106	122.3	PV = 3402 cP BV = 1980 cP SV = 2748 cP	6.9	9.12	0.34	28.77	70.1	Gul <i>et al.</i> , 2014 ²⁷										
												19.6	7.78	120.2	150.2	PT = 81.7 °C PV = 3867 cP BV = 2429 cP SV = 2988 cP	5.8	4.71	0.36	28.81	74.56
Native starch + heating (130 °C for 2 h)	19.6	7.78	120.2	150.2	PT = 81.7 °C PV = 3867 cP BV = 2429 cP SV = 2988 cP	5.8	4.71	0.36	28.81	74.56											
											Native starch + Heating (130 °C for 4 h)	18.2	7.39	134	153.4	PT = 81.21 °C PV = 4327 cP BV = 2798 cP SV = 3423 cP	6.4	4.60	0.35	29.69	74.14
Native starch + CMC (130 °C for 2 h)	19.4	7.45	122	154.3	PT = 81.65 °C PV = 4317 cP BV = 2875 cP SV = 3822 cP	4.9	4.31	0.68	27.37	74.91											
											Native starch + CMC	18.8	7.22	138.1	151.1	PT = 79.75 °C PV = 3950 cP BV = 2303 cP SV = 3567 cP	4.6	4.24	0.69	24.43	74.66
Native starch + SA (130 °C for 2 h)	17.2	6.97	148.2	163.2	PT = 81.5 °C PV = 4299 cP BV = 2786 cP SV = 3738 cP	6.8	4.69	0.73	25.6	76.56											
											Native starch + SA (130 °C for 4 h)	16.5	6.26	151.1	167.1	PT = 80 °C PV = 4404 cP BV = 2742 cP SV = 3778 cP	5.4	3.77	0.73	24.77	75.45
Native starch + XG	60 °C = 9.9 70 °C = 17.5 80 °C = 17.7 90 °C = 7.5	60 °C = 3.99 70 °C = 5.45 80 °C = 8.53 90 °C = 6.14	109.0	—	4.3	—	—	—	—	—											



Table 3 (Contd.)

Type of starch	Solubility (g per 100 g)	Swelling power (g g ⁻¹)	Water binding capacity (g g ⁻¹)	Oil binding capacity (g g ⁻¹)	Viscosity	Thermal Characteristics	Light transmittance	Physiochemical characteristics						
								Moisture	Ash	Amylose	Total carbohydrates	References		
Native starch + guar gum	60 °C = 13.8 70 °C = 11.8	60 °C = 2.30 70 °C = 3.13 80 °C = 13.5	72.7		PV = 79.5BU BV = 2.1 BU 80 °C = 6.67		4.6	—	—	—	—	—	—	
90 °C = 11.6		SV = 34.2 BU												
Native starch + CMC	60 °C = 15.8 70 °C = 13.8 80 °C = 9.7	90 °C = 6.69 60 °C = 2.51 70 °C = 2.85 80 °C = 5.22	$T_g = 77.5$ °C 72.7		PV = 66.2U BV = 1.4 BU SV = 32.1 BU $T_g = 74.2$ °C		4.1	—	—	—	—	—	—	
Native starch + gum acacia	60 °C = 15.8 70 °C = 13.6 80 °C = 17.5 90 °C = 13.6	90 °C = 2.06 60 °C = 2.65 80 °C = 5.97 90 °C = 6.86	36.3		PV = 65.4BU BV = 0BU SV = 20.5 BU $T_g = 79.2$ °C PV = 72.3BU BV = 0		3.6	—	—	—	—	—	—	
Native starch			98.2		SV = 25.2 BU $T_g = 77.2$ °C PV = 70.1BU BV = 2.1		3.5	—	—	—	—	—	—	
Native starch + NaCl (0.5%)			76.19		SV = 17.2 BU $T_g = 78.2$ °C PV = 78.5BU BV = 1.1		3.9	—	—	—	—	—	—	
Native starch + NaCl (1%)			95.45		SV = 18.2 BU $T_g = 77.8$ °C		4.4	—	—	—	—	—	—	
Native starch + NaCl (2%)			70.83		PV = 61.2BU BV = 1.4BU SV = 30.1 BU $T_g = 84.2$ °C		3.1	—	—	—	—	—	—	
Native starch + XG			176.92		PV = 170.2BU BV = 52.0BU SV = 37.2 BU $T_g = 70.6$ °C		3.2	—	—	—	—	—	—	
Native starch + XG + NaCl (0.5%)			370.3		PV = 172.1BU BV = 27.1BU SV = 42.2 BU $T_g = 71.9$ °C		0.3	—	—	—	—	—	—	
Native starch + XG + NaCl (1%)			300.8		PV = 176.5BU BV = 16.1BU SV = 41.2 BU $T_g = 75.2$ °C		1.0	—	—	—	—	—	—	



Table 3 (Contd.)

Type of starch	Solubility (g per 100 g)	Swelling power (g g ⁻¹)	Water binding capacity (g g ⁻¹)	Oil binding capacity (g g ⁻¹)	Viscosity	Thermal Characteristics	Light transmittance	Physiochemical characteristics				References
								Moisture	Ash	Amylose	Total carbohydrates	
Native starch + XG + NaCl (2 h)			211.3		PV = 185.2BU BV = 10.4BU SV = 39.1 BU T _g = 80.1 °C		1.8	—	—	—	—	
Native starch + Heating (2 h)			1.66		PV = 780.63 RVU BV = 719.56 RVU SV = 200.34 RVU T _g = 64.65 °C ΔH = 12.34 (J g ⁻¹)	T _o = 65.34 °C T _p = 75.76 °C T _c = 80.56 °C	3.18	9.73	0.37	28.90	74.36	Lutfi <i>et al.</i> , 2021 ⁸⁴
Native starch + Heating (4 h)			2.01		PV = 817.76RVU BV = 730.08RVU SV = 267.03RVU T _g = 65.14 °C	T _o = 62.14 °C T _p = 74.12 °C T _c = 81.80 °C ΔH = 11.03 (J g ⁻¹)	3.17	9.34	0.36	27.80	74.25	
Native starch + SA			1.501		PV = 518.56RVU BV = 164.54RVU SV = 86.14RVU T _g = 68.40 °C	T _o = 67.90 °C T _p = 77.89 °C T _c = 82.54 °C ΔH = 21.14 (J g ⁻¹)	3.05	9.61	0.71	29.54	76.14	
Native starch + SA + Heat(130 ° C, 2 h)			1.66		PV = 480.56RVU BV = 141.84RVU SV = 85.31RVU T _g = 67.10 °C	T _o = 68.10 °C T _p = 77.51 °C T _c = 81.84 °C ΔH = 17.31 (J g ⁻¹)	2.94	8.68	0.70	28.84	76.31	
Native starch + SA + Heating (130 °C, 4 h)			2.16		PV = 450.45RVU BV = 147.34RVU SV = 118.12RVU T _g = 66.56 °C	T _o = 68.56 °C T _p = 77.23 °C T _c = 81.34 °C ΔH = 21.12 (J g ⁻¹)	2.84	8.38	0.69	27.81	75.40	
Native starch					PV = 2731 cP BV = 347 cP SV = 2291 cP PT = 76 °C	T _o = 66.77 °C T _p = 71.99 °C T _c = 79.84 °C ΔH = 12.04 (J g ⁻¹)	—	—	—	—	—	
Native starch + HMT (20 °C)					PV = 1576 cP BV = 194 cP SV = 968 cP PT = 80.9 °C	T _o = 75.53 °C T _p = 78.85 °C T _c = 89.01 °C ΔH = 19.18 (J g ⁻¹)	—	—	—	—	—	Yadav <i>et al.</i> , ²⁶ 2013
Native starch + HMT (25 °C)					PV = 1864 cP BV = 182 cP SV = 1088 cP PT = 85.1 °C	T _o = 81.09 °C T _p = 85.48 °C T _c = 90.25 °C ΔH = 12.65 (J g ⁻¹)	—	—	—	—	—	
					PV = 1637 cP	T _o = 82.98 °C	—	—	—	—	—	



Table 3 (Contd.)

Type of starch	Solubility (g per 100 g)	Swelling power (g g ⁻¹)	Water binding capacity (g g ⁻¹)	Oil binding capacity (g g ⁻¹)	Physicochemical characteristics								
					Viscosity	Thermal Characteristics	Light transmittance	Moisture	Ash	Amylose	Total carbohydrates	References	
Native starch + HMT (30 °C)					BV = 57 cP SV = 856 cP PT = 87.3 °C	$T_p = 87.35\text{ °C}$ $T_c = 91.87\text{ °C}$ $\Delta H = 12.84$ (J g ⁻¹)							
					PV = 662 cP BV = -223 cP SV = 764 cP PT = 85.8 °C	$T_o = 81.58\text{ °C}$ $T_p = 84.96\text{ °C}$ $T_c = 88.12\text{ °C}$ $\Delta H = 12.59$ (J g ⁻¹)							
Annealed starch													

rupturing of the starch granules.⁸⁴ In another study, the effect of NaCl on WCS and WCS-XG blends was investigated and Lutfi *et al.*⁴⁵ observed that in comparison to the oval, bulging, undamaged granules having smooth surfaces present in native WCS, the presence of NaCl at different levels of concentration yielded defective and ruptured starch granules, which had lost their original shapes and exhibited an increase in porosity. In the WCS-XG blend, the SEM images indicated presence of both intact swollen starch granules and gum penetrated inside the starch granules. The addition of NaCl to the WCS-XG combination produced more defective granules with reduced sizes and increased porosity.

5.2. Impact on physico-chemical properties

Annealed- and heat-moisture-treated (HMT) water chestnut starches exhibited no change in amylose content (20.1–21.5 g per 100 g) in contrast to the 21.8 g per 100 g present in the native starch, supporting the fact that no chemical modifications in starch takes place after heat moisture treatment and annealing.^{19,26,85}

The physicochemical properties of water chestnut starch (WCS) are significantly affected by the addition of ionic gums in the absence and presence of heating. Gul *et al.*²⁷ investigated the physicochemical properties of native and heat-treated WCS in the presence of ionic gums including SA and CMC and noticed that the moisture content in native starch, WCS-CMC and WCS-SA blends exhibited a remarkable decrease on heating and the decrease in moisture content became more significant on increasing the duration of heating to 2 h and 4 h. Heat penetrated the interior of the starch granules and decreased their moisture content. Also, starch tailored with sodium alginate gum displayed a higher ash content (0.73–0.74 g per 100 g) in contrast to (0.68–0.69 g per 100 g) in starch modified with CMC, whereas the ash content in native starch was observed to be 0.34 g per 100 g. Similarly, an increase in ash content due to the addition of XG and heating the blend at 130 °C for a duration of 2–4 h was also observed. The starches modified with ionic gums, *i.e.*, CMC, SA and XG, were observed to have a higher amylose content (28.77–24.43) g per 100 g and higher carbohydrate content (70.01–76.56) g per 100 g in comparison to that present in native starch. However, starch modified by gums (CMC, XG and SA) exhibited a significant decrease in carbohydrate content when subjected to heat treatment.²⁷

5.2.1. Impact on water binding capacity and oil binding capacity. The water absorption capacity (WAC) signifies the degree of association between polymeric chains of amylose and amylopectin present in starch granules.⁸⁶ In comparison to other available starches, WCS has a larger WAC owing to the large size of its granules²⁷ and the weaker forces of attraction between the amylose and amylopectin chains of native starch.⁸⁷ Modification of WCS with sodium alginate and CMC in the presence of heating is associated with remarkable changes in the WAC of its granules. Upon DHT, starch granules exhibited an increase in water absorption capacity in comparison to native unmodified starch, which increased with an increase in the duration of heating from 2 to 4 h. Heat treatment exposes



the hydrophilic groups of the granules and increases their water absorption tendency. Starches modified with ionic gums have increased concentration of carboxyl groups, which significantly increase the absorption of water molecules. Heating further intensified the starch–alginate interactions, thereby increasing the surface area for water absorption. Dry heating of water chestnut starch caused an increase in its oil binding capacity from 122.32 g g⁻¹ in the native starch to 167.1 g g⁻¹ when heated for a duration of 4 h. Hydrocolloid-modified WCS with CMC and SA increased its OAC significantly. Heating of WCS for a longer time and addition of CMC and SA increased its lipophilic character. Moreover, the effect of sodium alginate (SA) on OAC of WCS was more prominent than the effect of carboxy methyl cellulose (CMC) with an increase in oil binding capacity from 153.4.1 g g⁻¹ in native starch to 167.1 g g⁻¹ in the sodium alginate–water chestnut starch blend on heating at 130 °C for a duration of 4 h. In contrast, the oil binding capacity of the CMC-containing starch only marginally changed from 140.1 g g⁻¹ to 154.3 g g⁻¹ when heated for a period of 2 h. In another study performed by Lutfi *et al.*,²³ the addition of xanthan gum (XG) to native WCS increased its water absorption capacity considerably, which is probably due to the increase in hydrophilicity and increased possibility of interactions of water molecules *via* hydrogen bonding with the larger number of hydroxyl groups attached to XG.⁸⁸ Contrarily, the presence of guar, acacia or CMC in WCS decreased its WAC possibly due to the electrostatic nature of the water-polymeric starch chain interactions, which is inhibited by the presence of these gums. However, WCS modified with sodium alginate gum exhibited an increase in WAC when the temperature was increased from 2 to 4 h.⁸⁴ The presence of NaCl in WCS significantly affected its WAC and the inverse relationship existed between the two.⁴⁵ The movement of water molecules inside the starch granules is prevented in the presence of salt due to the formation of an electrostatic barrier. The addition of salt to the combination of WCS and XG caused a remarkable increase in the WAC of the blend due to the synergistic effect of the salt and XG.⁸⁹

5.2.2. Impact on swelling power and solubility. The amylose content influences the water solubility, while amylopectin affects the swelling power of starches. The swelling power of starches is influenced by the aggregated structure of their granules and the extent of interactions in the starch chains prevailing in both the crystalline and amorphous parts of starch.⁹⁰ Both annealing and HMT bring about a significant decrease ($p < 0.05$) in swelling power and solubility in water chestnut starch, which is probably due to the increase in crystallinity, increased interactions between amylose and amylopectin molecules, stronger intramolecular forces of attraction, transformations in the crystalline areas of starch,⁹¹ lower hydration of the amorphous regions of starch⁹² and increased organisation of its molecules. However, the swelling capacity was found to be a function of temperature and its average value increased appreciably with an increase in temperature ($p < 0.05$). Both the increased crystalline organisation and extent of interaction of amylose with itself and with amylopectin due to annealing prevent the hydration of the amorphous parts of starch, thereby decreasing the swelling ability of starch

granules.²⁶ Both annealing and heat-moisture-treatment result in decreased water solubility in water chestnut starch. The increased organisation of the WCS molecules due to annealing results in the lower solubilization of starch granules.⁹³ Additionally, annealing results in the strengthening of the amylose bonds with amylose itself and with amylopectin and inhibits the leakage of the starch granules. In contrast, the decreased solubility of starch molecules due to HMT may be because of the decreased stability of the starch granules and disentanglement in the double helical structure of the native starch.⁷⁷

Gul *et al.*²⁷ noticed that water chestnut starch (WCS)-modified by heat treatment exhibited a decrease in swelling power, which is attributed to the damage caused by heating. The inhibited swelling of hydrocolloid (CMC and SA)-modified starches is due to the formation of a complex matrix with amylose present outside the starch granules.⁹⁴ CMC-modified starches exhibit a considerable decrease in solubility probably due to the formation of ester linkages between the starch and CMC on dry-heating. However, more severe inhibition of swelling was caused by CMC than that produced by sodium alginate. Similarly, reduction in solubility was mentioned by Lutfi *et al.*⁸⁴ on the addition of SA to WCS due to the reduced water availability caused by the hydrophilic character of the gum and formation of a complex matrix with the previously leached amylose, which prohibits the swelling of the granules and more leaching of amylose.⁹⁵ In another study, Lutfi *et al.*²³ evaluated the swelling power of blends of water chestnut starch-gums (acacia, xanthan, and guar) at different temperatures. The swelling of these blends was found to be temperature dependent and took place before gelatinization. Swelling increased quickly with an increase in temperature after gelatinization. Maximum swelling of water chestnut starch was recorded at 90 °C. However, its swelling power was reduced considerably with the addition of different gums. The reduction in swelling power can be attributed to the strong interaction of gums with leached amylose, which restricts the swelling of the starch granules.⁹⁶ At the onset of heating, the gelatinized granules of starch approach each other and are held by the gums, which increases the forces of attraction between the two and increases the diffusion of water and subsequent leaching of amylose. When the temperature further increases (beyond 80 °C and 90 °C), the leached amylose and gums form a covering around the starch granules, restricting the leaching process. Moreover, the presence of hydrocolloids alters the rheology of water, which prevents the entry of water molecules in the swollen granules. Among the four hydrocolloids, gum acacia produced the largest decrease in the swelling power of WCS due to its lower viscosity observed at lower concentrations (<10% w/w).⁹⁷ Native starch exhibited the highest solubility of 5.7 g g⁻¹ at 90 °C. An increase in solubility was observed with the addition of all the gums but the maximum effect was exhibited by CMC, which dramatically increased the solubility of WCS to 15.4 g g⁻¹. Likewise, the swelling power of WCS was observed to decrease when subjected to a high temperature of 130 °C for a duration of 2–4 h, which was further reduced upon the addition of sodium alginate.⁸⁴

The swelling power of WCS decreased in the presence of NaCl at the 0.5% w/w level from a value of 12.33 g g⁻¹ in native



starch to 5.60 g g^{-1} ⁴⁵ and an increase in the concentration of NaCl to 2% w/w further reduced its swelling power to 4.65 g g^{-1} . The most probable cause for this reduction in swelling power is the competition between the starch and NaCl salt for water molecules. Due to the decrease in swelling power, the leaching of amylose also decreases.⁹⁸ Additionally, the swelling power of a water chestnut starch–XG mixture was lowered with the 2% level of salt concentration. Lufti *et al.*⁴⁵ reported a lower solubility value (5.7% at 90 °C) for WCS than that reported by Gul *et al.*²⁷ This could be because of the difference in cultivars of WCS. With an increase in the concentration of NaCl, the solubility of WCS also increased. The large-sized chloride ion, due to its good polarization power, disturbs the hydrogen bonding interactions between starch–water and starch–starch molecules and increases the access of more water molecules, thereby increasing the solubility of WCS.⁹⁹ An increase in the solubility of WCS in the presence of XG was also reported. The addition of NaCl at the 2% w/w concentration level dramatically increased the solubility of the WCS–XG blend and a maximum solubility of 21.2 g g^{-1} was detected.⁴⁵

5.2.3. Impact on paste clarity. In comparison to native starch, annealed starches exhibit higher paste clarity, while HMT starches have comparatively lower paste clarity. The paste clarity varies with time and significantly decreases with an increase in the storage time of chestnut starch paste.²⁶ Upon annealing, the swollen starch granules become fragile and starch pastes comprised of more damaged granules leads to better transmittance of light in contrast to that containing larger amounts of intact swollen granules.¹⁰⁰ An increase in storage period led to a decrease in % transmittance (*T*), similar to the effect reported for banana starch.⁴²

As investigated by Gul *et al.*,²⁷ the paste clarity of modified starches varied significantly ($p < 0.05$) in comparison to native starch. When water chestnut starch (WCS) was heated to a temperature of 130 °C, the paste clarity was reduced from 6.9% w/w in native starch to 4.6% w/w. Heating of a starch suspension to a high temperature causes rupturing of the starch granules, which results in the leaching of amylose and formation of shorter chains of amylose with an increase in temperature and duration of heating. Cooling initiates the reassociation of amylose to form a network during the process of retrogradation. The paste clarity of WCS modified by adding gums varied significantly ($p < 0.05$) in comparison to that of native WCS.²³ The %*T* of native WCS was reported to be 3.5% w/w, which is much lower than the previously reported values⁷ probably due to the heating of starch suspensions as explained by Gul *et al.*²⁷ The presence of selected hydrocolloids had insignificant effects on the paste clarity of native water chestnut starch. Additionally, the gums bind with the leached amylose and cause opacity in the starch pastes. Likewise, a noticeable decrease in paste clarity of WCS occurred upon the addition of sodium alginate gum in comparison to the native unmodified WCS.⁸⁴ In contrast, the addition of NaCl at the 0.5% w/w and 1% w/w levels resulted in a noticeable increase in the paste clarity of WCS. The addition of salt cleaves the hydrogen bonds present between starch–starch and starch–water systems to certain extent and prohibits the rearrangement of the starch molecules

during retrogradation, subsequently increasing the paste clarity. Conversely, the addition of NaCl to the WCS–XG blend decreased the paste clarity.⁴⁵

5.2.4. Impact on pasting properties. Both heat-moisture treatment (HMT) and annealing alter the pasting properties of water chestnut starch considerably. Hydrothermal treatment of starches causes alterations in their crystallinity and association of chains present in the amorphous areas of starch and these transformations increase with an increase in the moisture content. Due to the increase in the strength of these intra-granular bonds and presence of cross-links, more heat is needed for the disintegration of the starch structure and for paste formation.¹⁰¹ Therefore, an increase in pasting temperature takes place on HMT. According to Yadav *et al.*,²⁶ native-unmodified water chestnut starch exhibited a pasting temperature of 76 °C, while the hydrothermally modified and annealed starch displayed an increase in pasting temperature ranging from 80 °C to 85 °C. Both the annealed and heat-moisture-treated water chestnut starch displayed reduced values for PV, SB and BD viscosities although the annealed starch exhibited the lowest values for all the viscosities. However, a decline in peak viscosity, breakdown viscosity, final viscosity and retrogradation was observed after HMT, which is probably due to the increased reorganisation and reduced swelling capacity of the water chestnut starch granules, resulting in the restricted leaching of amylose in the starch medium.²⁶ The decrease in breakdown viscosity and SB viscosity on hydrothermal treatment indicated more thermal and shear stability of water chestnut starch pastes. The decrease in SB viscosity on annealing and HMT can find potential applications in frozen and canned food. The influence of annealing on the pasting characteristics of starch is still under debate. In the case of potato starch, it causes an increase in pasting temperature and final viscosity but it reduces the peak viscosity,^{102,103} while in the case of pea, rice and wheat starch, annealing increases both their peak and final viscosities.¹⁰² However, the structural characteristics and analysis conditions during annealing affect the pasting properties of starch. Annealed starches offer better resistance to heat and mechanical agitation than native starches based on the heating rates.¹⁰⁴ Annealing also promotes the strengthening of bonds, resulting in higher gelatinization temperatures.¹⁰⁵ Annealing of water chestnut starch resulted in an increase in the pasting temperature and better shear stability due to the lower swelling and leaching of amylose and decrease in the peak viscosity and viscosity at the termination of the cooling cycle.⁸⁵

The heating effects of starches are enhanced in the presence of hydrocolloidal gums, which increases the paste viscosities.²⁷ Additionally, heating disrupts the granular structure of starches, which also increases the paste viscosity. On increasing the duration of heating from 2 h to 4 h, the amorphous region of WCS was altered, which increased the values of the breakdown viscosity, final viscosity and setback viscosities. However, the addition of gums caused decrease in peak viscosity, which is probably because the heat treatment and the addition of CMC and SA altered the packing arrangement of the starch chains, accompanied by some melting of the crystal structure in the



presence of high temperature.²⁷ Starch modified with CMC in the absence of heating displayed the lowest peak viscosity, which is probably due to the sticking of ionic gums on the surface of the starch granules, creating an overall negative charge on the surface and restricting the approach of water molecules. This causes delayed swelling of the granules. Heating of a WCS-CMC blend to 130 °C for 2 h resulted in a remarkable increase in the peak and final viscosities. The paste viscosity increased prominently when heating was performed after the addition of CMC owing to the faster impregnation of the reactive carboxy methyl cellulose (CMC) into the starch aqueous solution.^{83,106,107} A marginal increase in PV and FV occurred on the addition of SA in the absence of heat treatment in comparison to the control starch. However, the increase caused by SA was comparatively less than that caused by CMC. Heating the starch–sodium alginate blend for 2 h caused a significant increase in peak viscosity due to the increased interactions between the carboxyl groups of sodium alginate and –OH groups of starch on the removal of moisture on heating. Likewise, among the tested gums including acacia, xanthan, acacia and CMC, early gelatinization could only be achieved in the case of CMC-containing WCS.²³ The addition of carboxy methyl cellulose created new interactions between the hydroxyl groups of CMC and water chestnut starch, which reduced the pasting temperature of the blend. The peak viscosity of the starch–gum mixture significantly increased in the presence of xanthan and guar gums (from 72 to 98 and 79 BU, respectively), whereas a decrease in peak viscosity was observed in the presence of gum acacia and CMC probably due to the restricted leaching of amylose from the starch granules. WCS exhibited zero breakdown, which suggested high paste stability and excellent shearing resistance. The addition of different gums had no significant effect on the breakdown viscosity of WCS. However, the breakdown viscosity increased in the presence of xanthan gum, which decreased the stability of water chestnut starch during cooking.²³ The addition of gums is supposed to increase the shear forces and breakdown of the granules, causing easy disruption of the granular integrity of starch. However, the interaction of gums (xanthan, acacia, guar and CMC) with WCS had no effect on swollen granules of starch and rendered the breakdown tough. The setback (SB) viscosity of WCS was found to be enhanced in the presence of all the tested gums of guar, CMC and xanthan with the maximum increase detected in the presence of XG, whereas the minimum was observed with gum acacia. The increase in SB viscosity caused by xanthan, CMC and guar signified the enhancement in swelling power of the starch granules due to the restricted oozing amylose during gelatinization. In contrast, the decrease in SB viscosity in the presence of gum acacia was perhaps due to the interaction of gum with the leached amylose, which delayed the re-association of the amylose chains, and consequently decreased the setback ability of water chestnut starch.

A hydrocolloid–starch mixture displayed significantly improved pasting properties in the presence of salts. Recently, the influence of different concentrations of NaCl salt (0.5% w/w, 1% w/w and 2% w/w) on the pasting characteristics of water chestnut starch (WCS) alone and WCS-xanthan gum (XG)

mixtures at fixed concentrations of water chestnut starch (5% w/w) and xanthan gum (0.3% w/w) has also been investigated. A linear relationship was observed between the pasting temperatures of both the native WCS and WCS-XG complex and concentration of NaCl, whereas the peak viscosity, breakdown values and setback values decreased in WCS alone and a pronounced increase in peak viscosity, breakdown and setback values of WCS-XG complex was observed. The increase in peak viscosity was a synergistic effect of the biphasic system and presence of XG in the entire volume of the continuous phase, causing lesser accessibility due to the swelling of starch during the course of pasting. The breakdown values and setback values of the WCS-XG blend increased due to the reduced stability and increased retrogradation, respectively, promoted by hydrocolloids.⁴⁵

5.2.5. Impact on gelatinization/thermal characteristics.

The heating of WCS results in a decrease in its gelatinization temperature and enthalpy of gelatinization due to the disruption of its granular structure.²⁷ Accordingly, the minimum gelatinization temperature ($T_o = 31.51$ °C) was detected when WCS was heated for a duration of 2 h. Additionally, heating starch for a longer period of 4 h resulted in an increased gelatinization temperature (69.46 °C), whereas a much higher gelatinization temperature of 70.53 °C was observed for its non-heated native starch counterpart. A marginal increase in the gelatinization temperature was observed in the presence of sodium alginate (SA) gum without heating WCS. However, and increase in heating duration from 2 to 4 h increased T_o , which is probably due to the inhibition of gelatinization caused by the restricted interactions between the large-sized gum molecules and comparatively smaller starch molecules. The onset temperature (T_o) attained its lowest value (31.26 °C) when CMC was added to WCS and the heating period was 4 h. The enthalpy of gelatinization (ΔH_{gel}) significantly increased on heating WCS for a period of 4 h (12.9 J g⁻¹ to 17.30 J g⁻¹). However, the addition of CMC and SA gums resulted in a further intensification in the ΔH_{gel} values, which exhibited a decrease with an increase in the heating duration from 2 to 4 h. A similar decrease in gelatinization temperature was also reported by Lutfi *et al.*,⁸⁴ where on heating the native water chestnut starch for a period of 4 h (from 65.34 °C to 62.14 °C) due to the disruption of its cell walls at elevated temperatures. Additionally, T_p and T_c decreased due to the weakened starch granules. However, when sodium alginate was added to WCS before heating, a small increase in T_o (67.90 °C) and a significant increase in the enthalpy of gelatinization (21.14 J g⁻¹) was observed. The restricted gelatinization indicated the presence of strong interactions between starch–sodium alginate, which caused the starch granules to become compact.

In comparison to native chestnut starch, annealed and heat-moisture-treated (HMT) starches exhibited an increase in T_o , T_p and T_c of gelatinization together with a significant decline in the gelatinization temperature range.²⁶ Moreover, the gelatinization temperature range ($T_c - T_o$) varied inversely with the moisture content of the water chestnut starch. However, the lowest gelatinization temperature range was displayed by the annealed starch (88.12 °C to 81.58 °C). During the gelatinization process,



the crystalline parts and double helices of starch melt, which is decided by the hydration and swelling abilities of the amorphous parts of the granules. Swelling of the amorphous regions puts a pressure on the crystalline regions. Post HMT, the new interactions between amylose–lipid and amylose–amylose restrict the mobility of the amylopectin chains present in the amorphous areas, and consequently a comparatively higher temperature is required for swelling and breaking of the crystalline areas.⁹¹ This leads to an increase in the onset temperature (T_o), peak temperature (T_p) and conclusion temperature (T_c) of gelatinization. Annealing influences the gelatinization characteristics of native water chestnut starch, with a remarkably increase in T_o (from 66.77 °C to 81.58 °C), T_p (from 71.99 °C to 84.96 °C) and T_c (from 79.84 °C to 88.12 °C). However, a marginal decrease in T_o (from 69.58 °C to 68.72 °C) and ΔH_{gel} (from 7.29 to 7.21 J g⁻¹) was reported by Singh *et al.*¹⁹

5.2.6. Impact on freeze thaw stability. Native WCS has a syneresis value of 37.8% w/w²² and this low value is perhaps due to the presence of substantially branched amylopectin, which has greater water holding capacity and lower rate of retrogradation.⁶⁵ The addition of gums substantially increased the freeze-thaw stability of WCS with highest increase observed with acacia and guar gums (52.0% w/w syneresis value). Generally, the addition of hydrocolloids to starch increases its freeze-thaw stability because of the good WAC of hydrocolloids. However, Lutfi *et al.*²³ reported the opposite results, probably due to the incomplete swelling of water chestnut starch, which decreased the water holding capacity of the granules. The addition of gum acacia or guar gum to WCS restricted the swelling of the starch granules due to the generation of a polysaccharide barrier, which partially or totally encloses the starch granules and prohibits moisture intake.

The evaluation of the syneresis of WCS and WCS-XG blend in the absence and presence of NaCl was performed. With the incremental addition of salt, the syneresis value (37.8% w/w) of WCS exhibited an increase in cold storage possibly because of the stronger molecular interactions between the WCS chains or due to the re-organization of starch at lower temperatures, facilitating water leakage from the starch pastes. Moreover, the addition of 2% w/w concentration of sodium chloride to the WCS-XG blend caused a remarkable increase in syneresis (62.0% w/w) due to the probable starch–salt interactions.⁴⁵

6. Chemical modification of water chestnut starch

Native starch is associated with problems of insolubility in cold water and easy retrogradation. Therefore, with the intention of overcoming the unsuitable physicochemical properties of native starch, chemical modification is the most appropriate approach for introducing different functionalities in its backbone to alter its morphological and physicochemical properties. The chemical modification of starch can be achieved using diverse methods including grafting, etherification, esterification, oxidation, acidification and dual modification. The properties of modified starches are governed by factors including source of

starch biopolymer, conditions and methods exploited for their modification.¹⁰⁸ Hydroxypropylated starches (HPS) are known to exhibit enhanced free thaw stability, cold storage stability and high clarity,¹⁰⁹ whereas succinylated starches (SS) due to their polyelectrolytic nature possess outstanding swelling ability in cold water, acetylated starches with high degree of substitution behave like thermoplastic materials, although low degree-substituted acetylated starches display excellent freeze thaw stability, lower gelatinization temperatures, improved clarity, binding and film-forming capabilities.³⁸ Carboxymethylated starches (CMS) are quantified to have increased solubility with an increase in the degree of substitution, where the highly substituted ones are soluble even in cold water.¹¹⁰ Xiao *et al.*¹¹¹ attempted the chemical modification of Chinese water chestnut starch by acetylation, succinylation and carboxymethylation owing to the presence of a large number of hydroxyl groups in its backbone. The isolation of starch from the corms of Chinese water chestnut was conducted according to the method reported by Wang *et al.*¹¹² with a few modifications, which was subsequently acetylated, succinylated and carboxymethylated by reaction with acetic acid, succinic acid and chloroacetic acid, respectively.^{113–115} Subsequently, the physicochemical and structural properties of chemically modified acetylated water chestnut starch (AWCS), succinylated water chestnut starch (SWCS) and carboxymethylated water chestnut starch (CMWCS) were investigated and compared with that of native water chestnut starch. The degree of substitution of AWCS, SWCS and CMWCS was found to be 0.087, 0.575 and 0.972, respectively. The appearance of new peaks in the FT-IR spectra of the modified water chestnut starches substantiated the successful incorporation of new functional groups in the backbone of starch.^{113,115,116}

Similarly, Ansari *et al.*¹¹⁷ attempted the succinylation and acetylation of water chestnut starch by using esterifying agents, *i.e.*, succinic anhydride and acetic anhydride, and hydroxypropylation and carboxymethylation by employing propylene oxide and chloroacetic acid, respectively, as etherifying agents and all reactions were carried out under alkaline mediums. The degree of substitution in AWCS, SWCS and HPWCS was detected to be 2.4%, 2.5% and 4.1% w/w, respectively (Table 4). In another study, Lutfi *et al.*²³ attempted the chemical modification of WCS by acetylation, acid-thinning and dual modification employing acetylation and acid-thinning of the pregelatinized starch for obtaining pregelatinized acid-thinned and pregelatinized acetylated WCS using the methods proposed in ref. 118.

6.1. Impact on morphology

The morphology of native and chemically modified starches as examined by SEM demonstrates oval, polygonal or round starch granules with a particle size ranging from 5–20 μm. In contrast, AWCS and SWCS reveal considerable rough surfaces with small changes in shapes.¹¹¹ Acetylation causes larger surface erosion, which is attributed to the harsh reaction conditions used in the acetylation process.²³ In another study, acetylation and phosphorylation caused irregular concavities on the surface and clustering of starch granules.¹¹⁹ This deformation in AWCS or SWCS



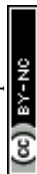


Table 4 Chemical modifications of water chestnut starch

Types of starch	Degree of substitution	Relative crystallinity %	Thermal properties	Swelling power (g g ⁻¹)	Solubility (%)	Pasting properties	Paste clarity (%)	Water absorption capacity (%)	Water retention capacity (%)	% Functional groups	References	
Native starch	ND	30.6	$T_o = 52\text{ }^\circ\text{C}$	19	13	25.1	—	—	—	—	Xiao <i>et al.</i> , 2018 ¹¹¹	
			$T_p = 57.4\text{ }^\circ\text{C}$									
			$T_c = 64.6\text{ }^\circ\text{C}$									
Acetylated starch	0.087	28.9	$\Delta H = 9.2\text{ J g}^{-1}$	28.5	23	28.6	—	—	—	—	—	
			$T_o = 51.4\text{ }^\circ\text{C}$									
			$T_p = 56.8\text{ }^\circ\text{C}$									
Succinylated starch	0.575	29.1	$T_c = 65.5\text{ }^\circ\text{C}$	67.3	18	31.6	—	—	—	—	—	
			$\Delta H = 5.2\text{ J g}^{-1}$									
			$T_o = 52.7\text{ }^\circ\text{C}$									
Carboxymethylated starch	0.972	ND	$T_p = 57.5\text{ }^\circ\text{C}$	89.7	35.3	81.5	—	—	—	—	—	
			$T_c = 70.5\text{ }^\circ\text{C}$									
			$\Delta H = 5.7\text{ J g}^{-1}$									
Native starch	—	—	ND	9.6	0.17 g g ⁻¹	PT = 72.6 °C PV = 264.5BU BD = 49BU SB = 199.5 BU	—	—	—	—	Ansari <i>et al.</i> , 2016 ¹¹⁷	
			$T_o = 52.7\text{ }^\circ\text{C}$									
			$T_p = 57.5\text{ }^\circ\text{C}$									
Succinylated starch	—	—	—	14.3	0.72 g g ⁻¹	PT = 61.7 °C PV = 330BU BD = 122.5BU	—	—	2.5	—		
Acetylated starch	—	—	—	11.1	0.22 g g ⁻¹	SB = 87.5BU PT = 69.1 °C PV = 330.5BU BD = 110.5BU	—	—	2.4	—		
Hydroxypropylated starch	—	—	—	8.4	0.46 g g ⁻¹	SB = 137.5BU PT = 57.6 °C PV = 355.5BU BD = 239BU SB = 93 BU	—	—	4.1	—		
Native starch	—	—	—	50 °C = 224 60 °C = 261 70 °C = 558 80 °C = 960 90 °C = 1223	50 °C = 2.8 60 °C = 2.8 70 °C = 3.8 80 °C = 5.6 90 °C = 5.7	$T_g = 77.2\text{ }^\circ\text{C}$ PV = 72BU BD = 0BU SB = 25 BU	3.5	98.2	50 °C = 71.5 60 °C = 69.8 70 °C = 201.4 80 °C = 588.5 90 °C = 772.9	—	—	Lutfi <i>et al.</i> , 2017 ²²
			—									
			—									
			—									
			—									
Acetylated starch	—	—	—	50 °C = 205.1 60 °C = 205.1 70 °C = 670.1 80 °C = 1144.1 90 °C = 1262.1	50 °C = 4.3 60 °C = 7.8 70 °C = 11.3 80 °C = 21.6 90 °C = 25.4	$T_g = 72.8\text{ }^\circ\text{C}$ PV = 39BU BD = 0BU SB = 27 BU	25.7	85.1	50 °C = 40.6 60 °C = 81.4 70 °C = 318.1 80 °C = 822.2 90 °C = 1136.1	—	—	
			—									
			—									
			—									
			—									

Table 4 (Contd.)

Types of starch	Degree of substitution	Relative crystallinity %	Thermal properties	Swelling power (g g ⁻¹)	Solubility (%)	Pasting properties	Paste clarity (%)	Water absorption capacity (%)	Water retention capacity (%)	% Functional groups	References
Acid thinning	—	—	—	50 °C = 222.2 60 °C = 224.8 70 °C = 267.1 80 °C = 783.1	50 °C = 7.1 60 °C = 9.3 70 °C = 10.9 80 °C = 13.8	$T_g = 33.9$ °C PV = 7BU BD = 0BU SB = 0 BU	2.4	96.06	50 °C = 100.7 60 °C = 100.5 70 °C = 129.4 80 °C = 537.2	—	—
	—	—	—	90 °C = 986.1	90 °C = 19.3	$T_g = 77.2$ °C	3.4	366.2	90 °C = 690.1	—	—
	Pregelatinized acetylated starch	—	—	50 °C = 656.7 60 °C = 740.1	50 °C = 9.8 60 °C = 10.1	PV = 72BU	—	—	50 °C = 406.9 60 °C = 459.3	—	—
		—	—	70 °C = 820.1 80 °C = 1058.2 90 °C = 1235.6	70 °C = 11.1 80 °C = 15.3 90 °C = 17.1	BD = 0BU SB = 25 BU	—	—	70 °C = 619.5 80 °C = 773.1 90 °C = 822.4	—	—
Pregelatinized acid-thinned starch	—	—	—	50 °C = 518.1 60 °C = 578.1 70 °C = 653.3 80 °C = 804.6 90 °C = 991.4	50 °C = 9.6 60 °C = 10.3 70 °C = 9.3 80 °C = 17.1 90 °C = 17.3	$T_g = 25.3$ °C PV = 5 BU BD = 0 BU SB = 0 BU	1.9	315.05	50 °C = 350.9 60 °C = 379.9 70 °C = 402.3 80 °C = 625.7 90 °C = 627.6	—	—

may be due to the introduction of functional groups in their backbone.¹¹¹ However, Ansari *et al.*¹¹⁷ reported that these chemical modifications hardly affect the shape, size and smoothness of starch granules. The destruction of starch granules resulting from their deformation was reported during the carboxymethylation process. In contrast, dual-modified, acetylated-pregelatinized starch (pgAWCS) exhibited extremely uneven and rough surfaces due to the alteration of the microstructure of water chestnut starch resulting from the changed intra- and inter-granular interactions existing in the starch granules.²³

6.2. Impact on swelling power and solubility

Generally, chemically modified water chestnut starch exhibits higher swelling power and solubility in comparison to native water chestnut starch. This difference in the swelling powers of chemically modified and native water chestnut starches reveals differences in their water absorption capacity. The replacement of the hydroxyl groups in native starches with bulkier organic substituents probably reduces the intermolecular forces of attractions existing between starch granules and increases the steric hindrance caused by the hydrophilic acetyl and succinyl groups in the glucan chains of AWCS and SWCS, resulting in the formation of steady hydration of amorphous areas and water percolating into starch granules causing them to swell.^{111,117}

Moreover, a linear relationship existed between the swelling power and solubility of native and modified water chestnut starches in the temperature range of 50–90 °C. In comparison to native water chestnut starch (WCS), a prominent increase in the swelling power of AWCS in the temperature range of 80–90 °C was reported. The incorporation of acetyl group improved the hydrophilicity and played a vital role in increasing the swelling power of AWCS in comparison to native WCS. Remarkably, SWCS displayed higher swelling power than AWCS probably due to much higher degree of substitution of SWCS in comparison to AWCS¹¹⁷ and the bulkier succinyl groups stimulated the starch chains to swell to a larger extent than the relatively smaller acetyl groups.¹²⁰ Moreover, phosphorylated WCS revealed greater swelling power compared to the native WCS.¹¹⁹

Xiao *et al.*¹¹¹ observed that in comparison to AWCS and SWCS, the highest swelling powers and solubility were exhibited by CMWCS, which is probably due to its highest hydrophilicity owing to the amorphous state of carboxymethylated starches.¹⁰⁹ However, Ansari *et al.*¹¹⁷ noticed that hydroxypropylation reduced the swelling power of WCS, which can be attributed to its higher solubility at elevated temperatures. Chemical modification of starches significantly increases their solubility in comparison to native starch. However, the solubility of SWCS was found to be lower than that of AWCS, indicating that succinylation was not very effective in enhancing the solubility of water chestnut starch.¹²¹

6.3. Impact on light transmittance

Chemically modified starches (AWCS, SWCS, CMWCS, atWCS, pgsAWCS, and pgAWCS) exhibit better paste clarity in comparison to native starches,^{23,111} which can be attributed to the presence of swollen granules in water chestnut starch, resulting



in the refraction of light to different extents and blurred images.³⁸ Due to the increased steric hindrance and electrostatic repulsions owing to the introduction of bulky functional groups, the re-association of the starch granular chains is prevented, which gives better light transmittance and better clarity to starch pastes. Especially, CMWCS presented the highest paste clarity among the chemically modified water chestnut starches, owing to the introduction of carboxymethyl groups, which facilitate the decomposition of the starch granules.¹¹⁰ Consequently, CMWCS is more suitable than AWCS and SWCS for the preparation of clear beverages and pie fillings.¹²² Furthermore, both singly chemically modified acid-thinned water chestnut starch (atWCS) and acetylated water chestnut starch (AWCS) as well as dual-modified, pregelatinized acid-thinned (pgatWCS) and pregelatinized acetylated starches (pgAWCS) showed enhanced paste clarity than native WCS. The noticeable increase observed in acetylated water chestnut starch (AWCS) compared to native and other single and dual modifications was principally due to the presence of newly added acetyl groups.²³

6.4. Impact on thermal stability

The comparison of the stability of chemically modified starches with native starches can be performed using thermal gravimetric analysis (TGA) curves. Native and AS display two stages of weight loss in comparison to three in the case of SS and CMS. The first stage corresponds to the loss of water molecules, whereas the second stage represents the thermal decomposition of starch itself. In accordance with the TGA curves, the weight loss in various modified starches follow the order of SWCS > Native starch > AWCS > CMWCS, showing that the highest stability is exhibited by CMWCS.^{111,123}

6.5. Impact on pasting properties

The peak viscosities of acid-thinned water chestnut starch (atWCS), acetylated water chestnut starch (AWCS), and dual-modified pregelatinized acetylated water chestnut starch (pgAWCS) and pregelatinized acid-thinned water chestnut starch (pgatWCS) were studied by Lutfi *et al.*,²³ which exhibited a significant reduction as compared to the native WCS (Fig. 5). This can be attributed to the partial cleavage of the glycosidic bonds as a result of chemical modification and the damaged

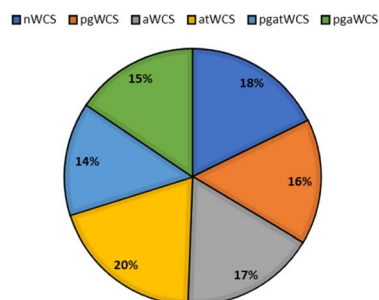


Fig. 5 Freeze thaw stability of nWCS, pgWCS, aWCS, atWCS, pgatWCS, and pgaWCS.²³

network being more susceptible to shear forces. Moreover, there was a reduction in the time needed to attain the peak viscosities in AWCS, pgWCS and atWCS due to the unhindered penetration of water molecules inside the starch granules. The time taken to reach the peak viscosity further decreased in acid-thinned WCS (atWCS) because of the easier penetration of water owing to the cleavage of longer starch chains into dextrins.

A similar observation of a decrease in the pasting temperatures of acetylated water chestnut starch (AWCS), succinylated water chestnut starch (SWCS) and hydroxypropylated water chestnut starch (HPWCS) was reported by Ansari *et al.*,¹¹⁷ however, the peak viscosities were observed to increase with respect to the native starch. Simultaneously, not much difference was seen in the peak viscosities of the differently chemically modified starches (AWCS, SWCS, and HPWCS). Chemical modifications disrupted the inter- and intra-molecular forces existing between the different macromolecules, causing larger percolation of water molecules into the starch granules, and thus an increase in the peak viscosity values. The hot paste viscosity (HPV), which was measured after holding the starch paste for 10 min at 95 °C, was found to be the lowest for HPWCS compared to AWCS, SWCS and native water chestnut starch.¹¹⁷ The lowest hot paste viscosity exhibited by HPWCS may be due to the structural weakening and disruption at elevated temperatures.¹²⁴ The breakdown viscosity (BD) of starches signifies their instability against heat and shear. In comparison to AWCS or SWCS, HPWCS exhibited the highest breakdown viscosity (BD) value, signifying that etherification causes greater weakening of the starch granular structures in comparison to esterification in the case of AWCS and SWCS.¹¹⁷ However, insignificant changes in the BD viscosities of native starch upon chemical modifications such as acid thinning, acetylation, pregelatinized acid-thinning and pregelatinized acetylation of water chestnut starches have also been reported.²³

HPWCS also exhibited lower values of both cold paste (CPV) and set back (SB) viscosities, showing short-term gelation or retrogradation tendency of starch granules on cooling in comparison to other modified and native starches. The decline in viscosity in comparison to native starch during cooling reveals that the short-term retrogradation or reassociation affinity of starches declined significantly in the modified starches.¹¹⁷ Acid thinning also caused a similar decline in the SB viscosity. Starches with lower SB viscosities can be applied in the preparation of puddings and custards, where they can prevent syneresis and provide cold temperature stability.²³

6.6. Impact on thermal properties

Acetylated water chestnut starches (AWCS) have a lower onset temperature (T_o) and peak temperature (T_p) but higher conclusion temperature (T_c) values than the native water chestnut starch, whereas succinylated starch (SWCS) possesses higher values of conclusion and onset temperatures but have the same peak temperature values.¹¹¹ Both AWCS and SWCS displayed much lower gelatinization enthalpy values in comparison to native starch, signifying that a lower energy is needed to start the gelatinization of AWCS and SWCS.^{120,123} The



probable cause for this is the decrease in the degree of crystallinity exhibited by acetylated and succinylated WCS. However, carboxymethylation results in the total disruption of the crystalline structure of the starch granules, transforming the starch into a total amorphous moiety, which makes it difficult to determine its gelatinisation temperature and enthalpy of gelatinization.¹¹⁰ In addition, phosphorylation disorganizes the molecular arrangement of the double helices in chestnut starch, which leads to a reduction in the gelatinization temperature and enthalpy.¹¹⁹

6.7. Impact on crystallinity

Generally, starch granules can be categorised into A-, B- or C type of crystallinities based on their XRD patterns.¹²⁵ A-type starch crystals exhibit strong diffraction peaks at 2θ values of around 15° and 23° together with an unresolved doublet at 2θ values of about 17° and 18° . In contrast, crystalline B-type starches show an intense diffraction peak at about 17° , a characteristic peak at the 2θ value of 5.6° , together with a few smaller peaks at 2θ values of 15° , 20° , 22° and 24° . C-type starches are a combination of A- and B-type crystallinities, which can be further classified as C_A type and are closer to A type, C type or C_B type, while resembling more B-type crystallinities based on the amount of A- and B- polymorphs. A typical C-type starch granule exhibits strong diffraction peaks at 2θ values of around 17° and 23° , C_A type shows intense diffraction peaks at 2θ of around 15° and 23° together with a shoulder at 2θ near 18° , which is characteristic of an A-type crystal structure. The XRD pattern of C_B -type crystal granules is comprised of a doublet of shoulders at 2θ of around 22° and 24° , in addition to a weak peak at a 2θ of about 15° . Cai *et al.*³⁰ investigated the XRD pattern of twelve starches isolated from a wide variety of plant sources comprised of corms, tuberous roots, rhizomes, tubers and seeds. The starches isolated from water chestnut were established to have C_A -type crystallinities based on their XRD patterns. Due to fact that the crystalline characteristics of A- and B-type crystal starches are not altered by acid modification, they can be exploited for the investigation of the allomorphic distribution of C-type starches.¹²⁶ The XRD pattern of acid-modified water chestnut starches indicate the disappearance of the characteristic peak at the 2θ value of 5.6° , appearance of a peak at 18° , and sharpening of the peak at 23° , revealing the presence of A-type crystallinities in acid-modified starches.³⁴ This is probably due to the early or faster degradation of the B-type allomorph present in C- or C_A -type starches in comparison to their A-type counterpart during acid hydrolysis. The direction of acid hydrolysis of starch granule by HCl is generally from the inner to outer region.¹²⁶ This change in the crystallinity of acid-modified starches suggests the existence of B-type allomorphs in the interior part of C-type starch granules, accompanied by the presence of A-type allomorphs at the periphery. Xiao *et al.*¹¹¹ assigned the A-type crystallinity pattern to water chestnut starches owing to the presence of strong diffraction peaks at 2θ values of 15.36° and 22.63° together with an unresolved doublet at about 17° . The X-ray diffraction pattern of native water chestnut starch exhibited insignificant changes upon

acetylation and succinylation. However, the degree of crystallinity of AWCS and SWCS showed a decline due to the destruction of the crystalline regions of starch upon chemical modification. However, CMWCS displayed complete disruption of its crystallinity due to the harsh alkaline reaction conditions of the carboxymethylation reaction.¹²⁷ However, phosphorylation and acetylation had little effect on the C-type crystalline architecture of starch of water chestnut.¹¹⁹

6.8. Impact on rheological properties

The thickening potential of starches for numerous applications is determined by their rheological properties. Factors such as the chemical structure of starch, pasting and storage conditions and concentration determine the rheological characteristics of starch pastes. The viscoelastic properties of starches are analysed based on their oscillatory rheological parameters including storage modulus (G'), loss modulus (G'') and loss tangent ($\tan \delta$), which is the ratio of the loss modulus and storage modulus. The viscoelastic properties of starches are significantly affected by chemical modification. Xiao *et al.*¹¹¹ reported an increase in the values of storage modulus and loss modulus for SWCS and CMWCS with an increase in frequency from 0.01 Hz to 10 Hz in comparison to the native WCS. Moreover, the relative increase in storage modulus was greater than the increase in loss modulus, indicating that the succinylated and carboxymethylated water chestnut starches exhibited greater elasticity and less solid/gel-like characteristics.^{128,129} An increase in temperature had an inverse effect on the viscoelastic parameters (G' and G''), which declined with an increase in temperature from 25°C to 85°C . The $\tan \delta$ value was never greater than one, signifying that all the modified starches (AWCS, SWCS and CMWCS) displayed solid-like behaviour even at elevated temperatures. The viscoelastic properties of chemically modified water chestnut starches increased in the order of SWCS > CMWCS > native WCS > AWCS. The superior gelatinization behaviour of succinylated and carboxymethylated water chestnut starch pastes increases their applicability as thickening agents in hot beverages.¹³⁰

6.9. Impact on water retention and water absorption capacity

As reported by Lutfi *et al.*,²³ the water retention capacity (WRC) of modified WCS significantly increased in comparison to native WCS when heated from 50°C to 90°C . Acetylated water chestnut starch (AWCS) and pregelatinized acetylated water chestnut starches showed a remarkable enhancement in water retention capacity with an increase in temperature from 50°C to 90°C . The accessibility of the water binding sites due to the disruption of the hydrogen bonds at elevated temperatures may be the reason for the increase in the WRC of these starches.

The water absorption capacity of dual-modified pgAWCS substantially increased in contrast to the native water chestnut starch.²³ The observed increase can be attributed to the electrostatic repulsions between the starch molecules, enabling the easy penetration of water molecules in the matrix. In contrast, AWCS and atWCS exhibited a decrease in WAC due to the lower



availability of binding sites in the starch granules for water molecules.¹³¹

6.10. Impact on freeze-thaw stability

Lutfi *et al.*²³ reported that the freezing of starch gel leads to the creation of starch-rich areas in the matrix and presence of unfrozen water in the matrix. Subsequently, syneresis occurs in the starch gel upon thawing and leaves it with a sponge-like form. In the case of acetylated starch, the acetyl groups present on the amylose chain hindered the re-joining of the starch chains and formation of a proper gel network. Acid-thinned starch exhibited lower strength, which may be due to the less intermolecular binding forces among the low molecular weight starch chains. Acid-modified starch from water chestnut showed greater freeze-thaw stability, and thus can be utilized in the formation of frozen food products and non-food industrial applications.

7. Nanoparticle formation with water chestnut starch

Currently, the synthesis of starch nanoparticles (SNPs) has revolutionised starch-related research owing to their wider applications in the bio-medical field as nanocarriers, in water treatment plants, catalytic nanosystems and food industry. SNPs have gained considerable attention due to their distinctive functional properties such as nano-meter size, non-toxic nature, better biocompatibility and biodegradability, increased surface area and excellent absorptivity in comparison to its natural form.^{132,133} Starch nanoparticles can act as fillers in starch composites and exhibit exceptional mechanical properties and biodegradability, which may be of significance in the future for packaging purposes.¹³⁴ Recently, naturally occurring starch polymers have also been employed in the synthesis of nano-structured materials associated with excellent biocompatibility and biodegradability in various fields such as drug carriers and agricultural and packaging applications.¹³⁵ Starch has also been utilized in the preparation of biodegradable and non-toxic edible coatings and films that contain antimicrobial agents for increasing the shelf life of food products, preventing rancidity, moisture loss and flavour loss from food products.¹³⁶ Dularia *et al.*²⁹ synthesized a water chestnut starch (WCS) film *via* the solution casting method and nano water chestnut starch-based composite films by adding native WCS to nano starch (5% w/w, dry basis) in varying concentrations including 0.5% w/w, 1% w/w, 2% w/w and 10% w/w. The thickness and flexibility of the nanocomposite films increased with an increase in the concentration of WCSNPs. Alternatively, the moisture content of the native starch film was found to be the maximum, which decreased with an increase in the concentration of WCSNPs to starch for the formation of composite films. The water vapour transmission rate (WVTR) decreased from $4.678 \times 10^{-3} \pm 0.42 \text{ g m}^{-2} \text{ s}^{-1}$ in native starch film to $2.221 \times 10^{-3} \pm 0.42 \text{ g m}^{-2} \text{ s}^{-1}$ in nano starch composite film at 10% concentration of WCSNPs. This decrease in WVTR in the composite film is possibly due to the decrease in the size and

increase in the surface to volume ratio, which inhibited the mobility of the polymeric chains and increased the compactness of the composite film, thus increasing the water vapour resistance of the film.¹³⁷ The native water chestnut starch film exhibited the highest solubility in comparison to the nano starch composite films containing different concentrations of WCSNPs.²⁹ The burst strength of the water chestnut starch-based composite films increased with an increase in the concentration of WCSNPs, whereas the native WCS based film exhibited the lowest burst strength. The nano starch-based composite film at 10% w/w concentration required the maximum amount of force to burst it ($1488.5 \pm 12.14 \text{ g}$) in comparison to $976.4 \pm 12.47 \text{ g}$ for the native water chestnut starch film.

Numerous methods have been reported for the preparation of starch nanoparticles (SNPs), such as acid hydrolysis, enzymatic hydrolysis, nanoprecipitation, ultrasonication, physical methods including reactive extrusion, high-pressure homogenization, stirred media milling and gamma irradiation, and the combination of methods such as heat-moisture treatment (HMT) and homogenization under mild acidic conditions as well as HMT under mild acidic conditions.^{138–143} The preparation of SNPs by physical methods is more advantageous compared to other chemical methods given that it is safer and more environment friendly. A combination of nanoprecipitation and ultrasonication acts is considered a simple, reliable, effective and environment-friendly method, which involves the slow addition of a dilute solution of starch to a solvent, and then breaking of the covalent bonds of starch due to the strong shear forces or mechanical forces of sound waves.^{142,144,145} SNPs can also be prepared *via* the ultrasound method in the absence of chemical additives. However, to obtain nanoparticles with desirable properties, a combination of physical and chemical methods is more desirable.¹³² Ahmad *et al.*¹⁴⁶ prepared SNPs from water chestnut starch (WCS) *via* the ball milling process, resulting in the modification in the crystalline structure and morphology of the starch granules.^{147–149} In another study, Ahmad *et al.*²⁸ prepared water chestnut starch nanoparticles (WCSNPs) *via* a combination of alkaline hydrolysis under mild reaction conditions and ultra-sonication method, which served as a simple, trustworthy and environment-friendly method for synthesizing nanoparticles with a desirable size (Table 5).

Nain *et al.*¹⁵⁰ in a separate study, reported the synthesis of starch nanoparticles from different botanical sources such as water chestnut, wheat, potatoes, mango kernels and mung beans and nanocomposites of isolated starches by coupling SNPs with zinc oxide (ZnO). The same research group also explored the biocompatibility of nanocomposites of ZnO-coupled water chestnut starch against human HeLa cells, and their cell viability was ascertained using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. The nanocomposites of WCS were found to be biocompatible to HeLa cells and non-toxic in nature. Therefore, they can potentially be used as drug delivery vehicles. The morphological, thermal, rheological and functional properties of starch



Table 5 Nanoparticle modifications of water chestnut starch

Type of starch	Water absorption capacity	Oil absorption capacity	Average particle size (nm)	Polydispersity index	Zeta potential (mV)	Relative crystallinity (%)	Thermal characteristics	Water vapour transmission rate ($\text{g m}^{-2} \text{s}^{-1}$)	Swelling power (g g^{-1})	References
Native water chestnut starch	1.06	1.21	—	—	—	26	—	—	—	Ahmad <i>et al.</i> , 2020 ²⁸
Water chestnut nanoparticles	1.32	1.09	271.5	0.378	-20.45	7.96	—	—	—	—
Water chestnut nanoparticles	—	—	535.21	0.37	-41.29	5.56	$T_0 = 52.76$ °C $T_c = 134.15$ °C $T_p = 110.23$ °C	—	—	Ahmad <i>et al.</i> , 2020
Native starch	—	—	—	—	—	26	$T_0 = 49.26$ °C $T_c = 124.23$ °C $T_p = 87.68$ °C	—	—	—
Native starch composite film	65.2%	25.1%	5559	—	—	—	—	4.673×10^{-3}	—	Dularia <i>et al.</i> , 2019 ²⁹
Nanostarch composite film	—	—	396	—	—	—	—	2.221×10^{-3}	—	—

nanoparticles exhibit remarkable changes in comparison to native starch.

7.1. Impact on particle size and zeta potential

Ahmad *et al.*¹⁴⁶ reported that water chestnut starch nanoparticles had an average size of 271 nm, polydispersity index (PDI) of 0.378 and zeta potential of -20.45 mV. In another study, Ahmad *et al.*²⁸ reported that WCSNPs had an average particle size of 535.21 nm, PDI of 0.37 and zeta potential of -41.29 mV. The large negative zeta potential value for WCSNPs signifies the presence of negative charges on their surfaces and electrostatic repulsive interactions between the starches, thus endowing them with stability and hindering their agglomeration due to the reduced van der Waals forces of attraction.¹⁴⁹ Nain *et al.*¹⁵⁰ observed that the particle size and distribution of ZnO-coupled nanocomposites of water chestnut starch was in the range of 506–1209 nm.

7.2. Impact on water and oil absorption capacity

The water absorption capacity (WAC) and oil absorption capacity (OAC) of water chestnut starch was found to be $65.2\% \pm 0.25\%$ w/w and $25.1\% \pm 0.10\%$ w/w, respectively.²⁹ The WAC exhibited an increase with a decrease in the size of WCSNPs, as reported by Ahmad *et al.*¹⁴⁶ The WAC of water chestnut starch showed an increase from 1.06 to 1.32 after ball milling. In contrast, its oil absorption capacity (OAC) showed the opposite trend, where it decreased from 1.35 to 1.15 after ball milling. The WAC exhibited an increase possibly due to the increase in surface area and exposure of the water-loving binding sites of starch granules produced by the disruption of amylose and amylopectin chains on ball milling.⁵¹ Ahmad *et al.*²⁸ reported that WCSNPs showed a much higher WAC than the native starch, whereas the OAC of these nanoparticles was much lower than that of the native unmodified WCS. The alkaline hydrolysis and ultrasonication conditions used in the preparation of WCSNPs break or reduce the crystalline regions of amylopectin, and also permit the hydrogen bonding sites created by cleavage of intermolecular bonds of starch to interact with a greater number of water molecules, thereby increasing their WAC. Similarly, the OAC decreased upon ball milling owing to the reduction in capillary forces caused by the disruption of the water chestnut starch granules.¹⁵¹ According to Ahmad *et al.*,²⁸ the decrease in the oil absorption capacity of WCSNPs was because of their smaller sizes and the variations caused in the polymeric framework, which affected the hydrophobicity of the water chestnut starch nanoparticles.

7.3. Impact on morphology

The micrographs of native water chestnut starch particles as taken by SEM indicated oval/round shapes together with smooth/porous surfaces.²⁸ Forces such as shear, compression and impact used in the ball milling process make the granules smaller with cracked and broken surfaces.^{28,152} A couple of starch granules also adhered to each another. WCSNPs formed by the combined effect of alkaline hydrolysis and ultrasonication were smaller in size and had eroded surfaces



containing grooves with broken cell walls.¹⁵³ The SNPs had irregular shapes and no uniform pattern in their sizes. However, the native WCS granules were observed to have smooth polygonal surfaces and size in the range of 2 to 5 μm .²⁸ In contrast, Dularia *et al.*²⁹ monitored the different shapes of native and nano starch granules employing field emission-scanning electron microscopy (FE-SEM). The WCS granules were found to have oval, ellipsoidal and spherical shapes with smooth and crack-free surfaces. In contrast, water chestnut starch nanoparticles (SNP) having diameters of 100 μm and 10 μm were agglomerated and had rough and irregular surfaces. It was also reported by Sun *et al.*¹³⁰ that starch nanoparticles having average diameters in the range of 10–700 nm exhibit irregular morphologies.

Nain *et al.*¹⁵⁰ observed that morphologically, water chestnut starch-coupled nanocomposites existed as agglomerates of irregular shapes. The agglomeration resulted from the initial complex formation of starch with divalent Zn^{2+} metal ion. This was followed by the formation of supramolecular assemblies, supplemented by the formation of intramolecular and intermolecular hydrogen-bonds, which served as a template for NP growth.

7.4. Impact on crystallinity

The XRD diffraction patterns of native water chestnut starch and starch nanoparticles were compared. Both the native WCS and WCSNPs were observed to possess an A-type crystalline pattern. The size reduction in ball milling was accompanied by a decrease in peak intensity and decrease in relative crystallinity (RC) from 26.16 to 7.98 in the water chestnut starch nanoparticles. The decrease in relative crystallinity is probably due to the formation of a larger proportion of amorphous regions upon the breakdown of the crystalline regions of amylopectin during ball milling. Starch molecules generally exist in two crystalline forms, *i.e.*, A and B. The crystalline form A is a monoclinic unit cell associated with 8 inter-helical water molecules inside it, whereas the crystalline form B is an open packed structure containing 36 interhelical water molecules.^{146,150} The larger number of water molecules in the latter makes it resistant to mechanical stress, as reported by Tan *et al.*¹⁵⁴ Thus, the crystalline form A is damaged upon ball milling. However, in another study, Ahmad *et al.*²⁸ observed a decrease in RC from 26 in native WCS to 5.56% w/w in water chestnut starch nanoparticles. The decrease in RC was attributed to the increased amorphous content of starch in the small-sized starch particles, with diminished or narrow diffraction peaks shown by the nano-sized water chestnut starch particles.

7.5. Impact on thermal properties

Ahmad *et al.*¹⁴⁶ investigated the thermal properties including phase transitions of starch nanoparticles (SNPs) of water chestnut starch by differential scanning calorimetry (DSC). The starch nanoparticles of water chestnut exhibited a broad endothermic peak at 85.51 at a higher transition onset temperature (T_o) compared to the native starch particles, which is associated with the melting of the smaller crystallites. The

increase in the transition temperature of SNPs can be attributed to their smaller granular size because of the ball-milling process. Similar reports of an increase in gelatinisation temperature with a decrease in granular size are also available in the literature.¹⁵⁵

Comparatively, Ahmad *et al.*²⁸ performed the thermal characterization of native starch and starch nanoparticles from different plant varieties and significant differences in initial temperature (T_o) and final temperature (T_c) were observed. WCS and WCSNP exhibited peaks between 49.26–124.23 $^{\circ}\text{C}$ and 52.76–134.15 $^{\circ}\text{C}$, with the peak transition temperatures at 87.68 $^{\circ}\text{C}$ and 110.23 $^{\circ}\text{C}$, respectively. The sharp peak at 135.80 $^{\circ}\text{C}$ for water chestnut starch nanoparticles (WCSNP) well established their crystalline nature. However, the lower transition temperatures for water chestnut native starch can be attributed to its gelatinization enthalpy. They also concluded that ultrasonication disrupted the crystalline character of amylopectin clusters and led to increased stability of hydrogen bonds and van der Waals forces of attraction. Subsequently, the melting points increased because of the increased hydrocarbon chains, which led to reduced crystallinity in WCSNPs.

7.6. Impact on rheological properties

The influence of starch granular size on rheological properties was reported by Ahmad *et al.*¹⁴⁶ The reduction in the size of WCSNP upon ball milling demonstrated the solid-like behaviour of all the isolated nanoparticles in contrast to the gel-like behaviour of native starch. The viscoelastic behaviour of WCSNPs was evaluated after the reduction in their size. WCSNPs were found to have higher viscosity than the native starch. The possible reasons for this behavioural transformation can be the variation in surface area, charge, distribution of granules, amylose content and interaction between the molecules.

Ahmad *et al.*²⁸ compared the storage modulus (G') and loss modulus (G'') of water chestnut starch nanoparticles (WCSNPs) with native unmodified starch as a function of frequency. The values of storage modulus (G') were much higher than the loss modulus (G'') values and the difference between these two values indicated that the gel-like behaviour of the WCSNPs increased with an increase in frequency. Also, WCSNPs exhibited more solid-type behaviour in comparison to the native starch. The water chestnut starch nanoparticles showed much higher viscosities compared to native starch, confirming the effect of the size of the granules on the rheological profile of starch. The possible reasons for this behavioural transformation can be the variation in surface area, charge, distribution of granules, amylose content and interaction between the molecules.

7.7. Structural elucidation of water chestnut starch nanoparticles by infrared (IR) and energy dispersive X-ray spectroscopy (EDX)

The structural elucidation of native starch and water chestnut starch nanoparticles (SNP) by attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy was



performed by Ahmad *et al.*,¹⁴⁶ displaying similar IR peaks for O–H stretch, $-\text{CH}_2-$, C–O, C–O–H and C–O–C vibrational modes of anhydrous glucose ring, and the characteristic IR peak at 1643 cm^{-1} signifies the presence of water molecule attached to starch; however, insignificant changes in intensity after size reduction were also observed.¹⁵⁶ The IR peaks in the range of $3200\text{--}3300\text{ cm}^{-1}$ signify the O–H stretching frequency, whereas the bands in the range of $2920\text{--}2933\text{ cm}^{-1}$ indicate the $-\text{CH}_2-$ stretching frequency.²⁸ Additionally, the shift in the O–H stretching peak towards a higher wavelength for WCSNPs can be ascribed to the loss of crystallinity and exposure of their O–H groups on the addition of alkali and ultrasonication.^{157–159} The existence of β -glycosidic linkages in WCSNPs was ascertained by the presence of characteristic absorption peaks at 855, 856 and 867 cm^{-1} .^{160,161} FTIR spectroscopy was helpful in determining the crystallinity of starch by exploring the changes in the IR bands in the semi-crystalline and amorphous areas of starch granules. Moreover, the impact of the addition of alkali and ultrasonication on the physico-chemical properties of starch was further corroborated by the decrease in the ratio of absorbance bands at $(1047/1018)$ and $(995/1018)\text{ cm}^{-1}$ after a reduction in its size, implying a decrease in its crystallinity and generation of an amorphous phase. However, Ahmad *et al.*¹⁴⁶ observed a similar decrease in the ratio of the IR absorption bands at $(1047/1018)$ and $(995/1018)\text{ cm}^{-1}$ with a decrease in the size of WCSNPs due to the ball-milling process. Nain *et al.*¹⁵⁰ confirmed the presence of starch nanoparticles by energy dispersive X-ray spectroscopy (EDX) based on the appearance of peaks at 0.3 keV and 0.6 keV, signifying the existence of C and O respectively. Additionally, the existence of Zn as coupled molecule was also ascertained by EDX studies.

8. Application concerns

8.1. Food utilization

Starch is a macro-constituent in many foods, and thus its properties and interactions with other constituents are of interest in the food industry and for human nutrition. It is widely used as a food additive to maintain the consistency and smooth texture of sauces and soups. Moreover, starch is employed to hamper the breakdown of gels during processing and to enhance the shelf-life of food products. The bakery industry in India is considered one of the largest food processing industries and is ranked as the second biggest manufacturer of biscuits after the USA. Celiac disease is an autoimmune reaction caused by the interaction of gluten in a genetically inclined population,¹⁶² which is very frequent in North India where wheat is a staple food.¹⁶³ In this case, only gluten-free food can restore their health completely and better the quality of life in patients with celiac disease.¹⁶⁴ Therefore, there is a requirement for the creation of a wide range of gluten-free products, and the number of these products is increasing worldwide with the increase in the number of individuals diagnosed with celiac disease.¹⁶⁵

Water chestnut (*Trapa natans*) is employed as an alternative food to cereals in the Indian subcontinent during fasting and it can be a better substitution for wheat flour (WF) regarding

Celiac disease caused by the difficulty in the digestion of gluten (wheat protein).^{27,166} Cookies made from water chestnut flour are used as a specialized dish during Navratri and other fasting days in India. Water chestnut flour (WCF) is devoid of gluten and contains a high content of starch, which makes this product promising for the development of gluten-free bakery products. Moreover, it has a high fiber content and multiple nutritional values, which authenticates its use in the foods. Cookies made from water chestnut show a greater spreading ratio in comparison to WF cookies, causing an increase in the demand for water chestnut flour. This may be due to the higher quantity of starch present in water chestnut flour.^{27,166,167} Lutfi and Hasnain¹⁶⁸ investigated the effect of modified water chestnut starch on the volume index, symmetry index, and uniformity index of sponge cake, which revealed that the addition of acetylated starch boosted the volume index of sponge cake to a remarkable extent. Acid-thinned starch at 1% w/w concentration caused an increment in the symmetry index of sponge cake. At 4–5% w/w concentration, pregelatinized and acid-thinned starch exhibited an excellent uniformity index. In another investigation, Malik *et al.*¹⁶⁹ studied the physicochemical and sensory parameters of yogurt supplemented with water chestnut starch and compared them to that of yogurt enriched with stabilizer gelatin (0.5% w/w). The use of water chestnut starch gave good outcomes because of the decreased syneresis and enhanced water carrying capacity, viscosity, and all-inclusive appropriateness for all sensory characteristics. Water chestnut starch showed no effect on the taste and quality of yogurt. It was observed that water chestnut starch at 1.25% w/w and 0.75% w/w displayed efficient results for water holding capacity, synergies, viscosity, and all sensory attributes. Moreover, it was found that the shelf-life of yogurt was enhanced by up to four weeks.

Starches play a vital role in food and industrial applications because of their condensing ability. Water chestnut starch is employed in several foods to enhance their textural qualities, such as sustaining sauces and soups. The amylose content of water chestnut starch is 22.3% w/w, which it is firmly associated with amylopectin. However, it is not employed as a stabilizing agent in milk products.²⁵ The starch purified from water chestnut (*Trapa bispinosa* Roxb) showed a moisture content of 7–8% w/w, protein content of 0.21–0.22% w/w, ash content of 0.03–0.04% w/w, carbohydrate content of 81–85% w/w, and energy of 385 calories per 100 g. Isolated starch was added to dahi at various concentrations and it was found that there was a remarkable alteration in the attributes of dahi. The addition of water chestnut starch to dahi caused alterations in its physical, chemical, and sensory properties based on the concentration incorporated. It was observed that water chestnut starch as a stabilizer in dahi exhibited proficient results in view of the decreasing syneresis and acceptability, even producing creamy and adhesive dahi. The incorporation of water chestnut starch revealed no adverse effects on the taste and quality of dahi. Water chestnut starch gave good results regarding pH, acidity, syneresis, and all sensory attributes. It was found that the dahi could be used for about 1 month at cold temperatures. Water-chestnut starches (native and modified) can be used as a fat



alternative in mayonnaise and may remarkably decrease the calorie load given that carbohydrates possess half the calories of fats. Mayonnaise consisting of succinylated water-chestnut starch was observed to be better in view of sensory and textural properties compared to the mayonnaise containing acetylated starch.¹¹⁷

New bio-based packaging materials such as edible and biodegradable films have been employed for managing environmental issues and enhancing the shelf-life of food. This packaging can be ingested together with the food, giving extra nutrients, increasing sensory characteristics and quality. An edible film with microbicidal agents is one of the ways to stop the contamination of microbes on the surface of food products.^{170–172} Starch films and coatings have been employed for several food and medicinal applications. The film-forming capability of water chestnut starch shows a worthy future for exploration as a packaging material for food and non-food purposes.¹⁹ Mei *et al.*¹⁷³ conducted research on improving the microbicidal activity of water chestnut starch–chitosan (WSC) films *via* the addition of *C. officinalis* extract (COE), glycerol monolaurate (GML), nisin, and pine needle essential oil (PNEO). The addition of COE reduced the pH value of the film-preparing solution, the water content, and the water absorption expansion ability (WAEA). The GML-incorporated film showed a lower WAEA, tensile strength, elongation, and puncture strength. In contrast, the nisin films revealed strong mechanical properties. All the treated films displayed lower transparency and greater water vapor permeability values. The pine needle essential oil (PNEO) caused discontinuities with the lipid droplets or holes embedded in a continuous network and the addition of GML led to abaisse-like structures. The COE, GML, nisin, PNEO, and their combinations incorporated in WSC films were observed to be potent in hindering the growth of *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes* at various concentrations. In another study, a biodegradable composite film was prepared using water chestnut starch and polyvinyl alcohol. It was observed that the addition of plasticizer caused a reduction in the swelling power of the films; however, it enhanced the solubility of films. The tensile strength of the composite film was found to be greater at a low concentration compared to a higher concentration of plasticizer, where the tensile strength of the film was found to be low. Furthermore, the use of plasticizers resulted in an enhancement in the biodegradability of films and made them superior for food packaging without any harmful consequences.¹⁷⁴ Moreover, Lai and Wong¹⁷⁵ prepared clusteroluminogenic films of starch from maize, potatoes, and water chestnut by solution casting. All the films showed homogeneity with a width of 55–65 μm . The Water chestnut starch films revealed the maximum proportion of transmittance compared to the potatoes and maize. There was no marked variation in the clusteroluminescence intensity when a starch-based packaging bag was utilized for the packing of fresh or frozen food. However, a significant decrease in the luminescence intensity was observed upon the thawing of the frozen food in the bag. This study depicted the probability of utilizing the clusteroluminescence of starch as a pointer to expose the condition of packed frozen food.

8.2. Textile industry

Starch is used in its raw state or processed by fermentation with bacteria to give bio-origin monomers, which are polymerized into bioplastics. Starch is employed in fine fabrics and the cosmetic industry. Moreover, it is used for increasing the strength of paper and printing characteristics in the paper industry and binder in the packaging industry.^{176,177} In addition, currently, bioplastics are made from plant-origin raw materials, such as starch. Gupta¹⁷⁸ reported the use of starch-origin bioplastic as a packaging material. Pilla¹⁷⁹ investigated the use of bioplastics, which is becoming widespread in the packaging sector, biomedical field, automotive industry, civil engineering, and general engineering, but mainly in consumer goods. There are two main significant facts regarding bio-origin plastic derivatives compared to their conventional versions. Firstly, they avoid the use of fossil sources because of the use of biomass, which gives the distinctive ability of carbon neutrality. Secondly, bioplastics play a major role in the decline of food waste, CO₂ emissions, and dependency on fossil raw materials and CO₂.¹⁸⁰ Starch from water chestnut, *Manihot esculenta*, cornflour, and rice showed a yield of bioplastics of 12 g, 9 g, 8 g, and 11 g respectively.¹⁸¹ Starch blends derived from potatoes and water chestnut were used in water-soluble chips as spacers to save the contents of the packages and other expanded materials as an alternative to polystyrene, shopping bags, bio-waste storage bags, food packaging, and medical and cosmetics products.¹⁸²

8.3. Pharmaceutical innovations

Starch is one of the most strongly demanded pharmaceutical excipients worldwide due to its abundance, biostability, and multi-faceted behaviour. In this case, maize and potato starch are the most commonly used excipients in tableting, while many scientists have attempted to design several botanical starches as tablet excipients.^{183–186} Binders are the compounds that provide the required adherence among the granules for adequate bonding during squeezing. The addition of binders to the formulation of a tablet gives plasticity, and hence enhances the inter particulate bonding among the tablet granules, and also boosts the rate of compaction; however, they reduce the brittle fracture liability during tableting.¹⁸⁷ In previous studies, some researchers determined their disintegration potential in tablet dosage form.¹⁸⁸ Diclofenac was used as the standard drug, which is used as a potent anti-inflammatory and pain-relieving agent in the cure of arthritis and acute injury. Singh *et al.*¹⁸⁹ aim to determine the binding potential of water chestnut starch in diclofenac sodium-based tablets and also compare it with native starch. The diclofenac tablets formed with a greater concentration (15% w/w) of starch revealed an increase in disintegration time value, which can be due to the low diffusion of the solvent in the tablet pores due to the formation of robust bonding among the granules. The dissolution of a drug in the stomach environment is also indirectly based on the disintegration time of the tablet formulation. It was found that the drug dissolved fast at a low starch concentration, whereas at higher concentrations, the native starch resulted in the



formation of a viscous mass, which hindered the seepage of the dissolution medium into the pores of the dosage form. The diclofenac sodium tablets formed with the chestnut starch exhibited favourable and comparable characteristics such as firmness, durability, high drug content, disintegration, weight variation, and dissolution compared to the native starch binders. Therefore, it was observed that water chestnut starch (*T. bispinosa*) displays excellent quality to be employed as a natural binder in pharmaceutical formulations. Similarly, Venkatesh *et al.*¹⁹⁰ studied starch extracted from water chestnut as a substitute pharmaceutical excipient to maize starch. The tablets were designed by dry and wet granulation using paracetamol and aspirin, respectively, with water chestnut starch. Furthermore, maize starch was employed as an alternative excipient for comparison by conducting a series of pre-formulation and formulation tests. Pre-formulation studies such as flow properties, sieve analysis, micrometry, moisture content, swelling index, and compatibility studies were performed. The results observed for water chestnut (*T. bispinosa*) starch were found to be comparable with maize starch, indicating that it can be employed in the preparation of tablets as a pharmaceutical excipient. Similarly, Rao *et al.*¹⁹¹ monitored the starch extracted from water chestnut (*Trapa natans*) as a binder in the preparation of tablets. Diclofenac granules were made by wet granulation and starch was isolated from *Trapa natans* by maceration. The physicochemical characteristics of *Trapa natans* were determined and the pre-compression characteristics such as tapped density, bulk density, and Carr index revealed that the diclofenac granules formed by using *Trapa natans* were detected to be in the limits and similar to that formed by using native starch. Their post-compression attributes such as hardness, weight variation, disintegration, thickness, and dissolution were estimated. Water chestnut starch formulations of diclofenac showed greater hardness in comparison to the native starch. A significant increment in disintegration time was observed in the tablets prepared with the water chestnut starch in contrast to the native starch. In another study, Bae *et al.*¹⁹² made biodegradable films by using starches from different herbal sources, and their physical and

mechanical characteristics were investigated. Among the examined samples, the potato, mung-bean, and water chestnut starches showed comparatively better mechanical and physical characteristics in contrast to the gelatin and HPMC (hydroxy propylated methyl cellulose) films. The starch films displayed better O₂ barrier and enhanced H₂O blockade characteristics in comparison to the gelatin and HPMC films. The water chestnut starches were employed in the design of hard capsules for the pharmaceutical industry. These capsules showed efficient molding characteristics and high quality. The starch capsules were observed to be clear, with a plain surface, and not rigid. In addition, the antioxidant activity of water chestnut starch (WCS) and its nanoparticles (WCSNP) was investigated by the DPPH scavenging assay. The DPPH radical scavenging potential of WCS and WCSNP was observed to be 17% w/w and 19.6% w/w. According to the literature, low molecular weight and mono-saccharide compositions can play a major role in the antioxidant potential of polysaccharides.¹⁹³ Many studies indicated that starch consists of flavonoids, which show strong radical scavenging potential.^{194,195} Ahmad *et al.*¹⁴⁶ also demonstrated that WCSNPs displayed greater inhibitory activity against lipid peroxidation in comparison to WCS. Similarly, the curative activity of enzymatically (amylase) modified WCS was evaluated in a murine model of obesity. Modification of starch by amylase enhanced the percentage of resistant starch, making it inaccessible for catalysis by minor-intestinal mucosal glucosidase. The amylose/amylopectin ratio in chestnut starch was boosted through enzymatic modification. Amylase-modified chestnut starch at the dosage of 1500 mg kg⁻¹ per b wt was given orally for ten weeks to high-fat diet stimulated obese mice. It was observed that the modified chestnut starch caused a marked reduction in obesity in the mice in comparison to the control group. However, it was observed that the administration of modified chestnut starch in the mice showed a remarkable increment in cecum weight, depicting the secretion of fatty acid in the cecum. Moreover, the modified starch activated the fatty acid receptor GPR43-mediated annihilation of carbohydrate signaling (especially insulin). Thus, amylase-modified starch enhances the indigestible-

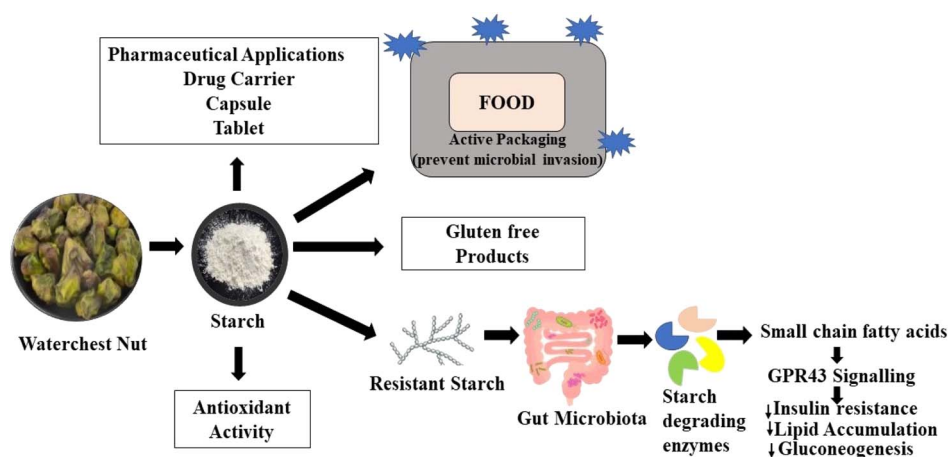


Fig. 6 Applications of water chestnut starch.



resistant starch and it mitigates food-stimulated obesity by the GPR43-induced decline of carbohydrate signaling, therefore causing a reduction in the accumulation of fat in white adipose tissue (Fig. 6).¹⁹⁶ In another investigation, acetylated WCS showed a greater percentage of resistant starch (81–84% w/w) in comparison to the native WCS (58% w/w) and phosphorylated WCS (62–76% w/w). However, the amount of rapidly and slowly digested starch was found to be low in the acetylated and phosphorylated WCS. Moreover, dry heat treatment with XG of WCS showed a marked quantity of resistant starch in contrast to that by dry heat treatment or XG alone. Resistant starch can be utilized in various foods to lower the caloric value and restrain the cases of diabetes.^{119,197}

8.4. Miscellaneous utilization as emulsions and delivery system development

In encapsulation, the inner material is filled in the socket of the wall material, which serves to save its inner components from physical, chemical, and biological degradation.¹⁴² Resveratrol is widely employed for encapsulation in several small polymeric materials such as hyaluronic acid, pectin, cyclodextrin, gelatin, albumin, gliadin, and chitosan.^{198–200} Recently, researchers have focused on finding economic and easily available wall materials for encapsulation. Thus, the efficiency of starch extracted from horse chestnut and water chestnut as encapsulation material was determined. The starch from these herbs will be more appealing in the future at the financial level given that it is consistent with the need for application as a food ingredient due to its gross existence at a lower price and the constitution of starch. Starch nanoparticles of resveratrol (HCSR and WCSR) enhanced the biopotential under imitated gastrointestinal environment. These nanoparticles were designed by employing the ultrasonication method without the use of any chemicals, and then screened for their encapsulation potential, zeta potential, and particle size. SEM, XRD, ATR-FTIR, and DSC methods were used for the characterization of the resveratrol-starch nanoparticles. The release nature and potential of the unbound and nano-encapsulated starch resveratrol were also investigated against diabetes and obesity. The size of HCSR and WCSR was 419 and 691 nm with a zeta potential of -16.09 and -15 and encapsulation ability of 81.5% and 79.4% w/w, respectively. The nanoparticles exhibited a film-like or porous configuration with reduced crystallinity and greater thermal transformation temperatures. The enhancement in thermal transformation temperatures indicates the efficient stability of the nano-encapsulated resveratrol. The protective efficacy of HCSR and WCSR against diabetes and obesity was monitored by determining the % inhibition of pancreatic lipase (PL), α -glucosidase, and cholesterol esterase enzymes (CE). The inhibition of pancreatic lipase was found to be 47% and 52% by HCSR and WCSR, respectively, after mimicking the gastrointestinal environment. Moreover, the percentage inhibition of cholesterol esterase was detected to be 76% and 73.2% by HCSR and WCSR, respectively, in the gastrointestinal environment. However, the free resveratrol displayed a decreased % inhibition for PE and CE, which was observed to be 26% and 53%,

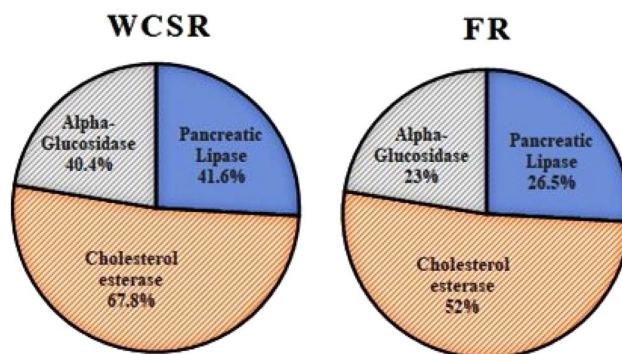


Fig. 7 Bioactivity of free encapsulated and nano-encapsulated resveratrol under simulated gastrointestinal conditions.²⁰³

respectively, under the same gastrointestinal environment. This depicted the efficient anti-obesity efficacy of the nano-encapsulated form of resveratrol. Both formulations of resveratrol showed a similar pattern for the hampering of α -glucosidase. The proportion of suppression of α -glucosidase from HCSR and WCSR was observed to be 45% and 40.4%, respectively, in comparison to the free resveratrol molecules, where it was found to be 23% (Fig. 7). The PL and CE enzymes play a vital role in the control of fatness,²⁰¹ and the inhibitory potential of resveratrol is associated with its high antioxidant properties.²⁰² The nano-encapsulated resveratrol displayed high inhibitory potential against cholesterol esterase compared to PL and α -glucosidase. This depicted the restoration of the potential of resveratrol in the nano-encapsulated form in contrast to the free form. Thus, the starch chestnut nanoparticles showed better nutraceutical potential and may be used to avoid the degradation of resveratrol from the outmost environment and to enable its absorption in the colon in its pure form.²⁰³ Previous studies also displayed similar results with the nanoencapsulation of starch catechin nanoparticles.¹⁴² Thus, it can be concluded that nano-encapsulation may be a useful way to circumvent the limitation of bioavailability by employing new starch-based nanoparticles. The nanoparticles showed good bioaccumulation and cell penetrability and may protect the catechin during digestion when mixed with functional foods. It was observed that horse chestnut starch (HSC) revealed the maximum encapsulation efficiency and reduction in particle size. Alternatively, water chestnut starch (WSC) had the highest stability and also allowed the slower release of catechin from its core. Nano-encapsulation using starch-based nanoparticles protected catechin against the harsh gastric environment and helped in retaining its bioactive properties during the *in vitro* digestion process. Hence, we suggest that HSC and WSC are more suitable for nanoparticle preparations and the encapsulation of catechin.¹⁴²

The cytotoxic potential of water chestnut starch extract was determined on the macrophage RAW 264.7 cell line and fibroblast NIH 3T3 cell line. In addition, the acute dermal toxic effect of water chestnut starch was checked in rats at a single dose of 200, 1000, and 2000 mg kg⁻¹ per b wt, and its oral toxic potential was monitored in rats at the concentration of 300 and



2000 mg kg⁻¹ per b wt The *in vitro* results revealed that water chestnut starch was nontoxic towards macrophages and fibroblasts at the dosage of 6.3–200 µg mL⁻¹. The acute dermal and oral toxicity studies indicated that the use of water chestnut starch is safe and showed no adverse effects up to a concentration of 2000 mg kg⁻¹ per b wt in animals. Thus, water chestnut starch can be employed as a pharmaceutical excipient or ingredient in cosmetic products. However, its long-term toxic effects should be studied to detect its chronic safety upon continuous consumption.²⁰⁴ In another investigation, the cytotoxic potential of nanoparticles of WCS against HeLa cells was determined using the MTT assay. After 52 h, 92% cells were found to be viable among the cells treated with nanoparticles of WCS. This indicated the non-toxic potential of nanoparticles of WCS, which can be utilized in the biomedical field for various applications.²⁰⁵

9. Conclusion

This review presented an extensive literature survey on water chestnut starches and their wide application. Accordingly, it was found that starch is a good reservoir of high-molecular-weight carbohydrates, which completes the elementary dietary requirements of people. It has been utilized in the textile, paper, pharmaceutical, and food industries. However, the use of native starch is frequently restricted by its intrinsically flawed nature such as insolubility in water at room temperature, high retrogradation, and syneresis. These imperfections can be solved by various modifications such as physical, chemical, and enzymatic modifications. Thus, more studies should be conducted to evaluate the potential of starch as an important component in the food and pharmaceutical industry.

Conflicts of interest

There are no conflicts to declare.

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