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Fabrication of Charged Self-Assembling Patchy Particles Templated with Partially Gelatinized Starch

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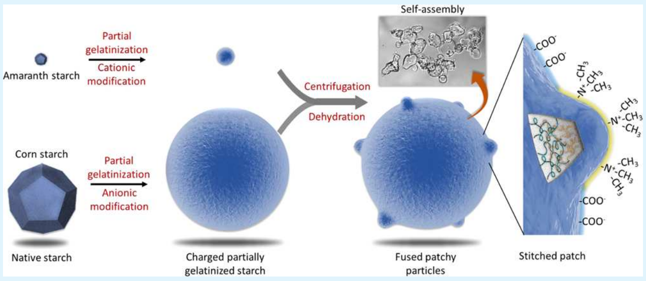

ABSTRACT: Starch, as a staple carbohydrate, is frequently used as a thickener to enhance food texture. As such, there is an increasing interest in studying starch modification to improve its thickening ability. Instead of the conventional mechanism of swelling-based thickening, the present work presents an alternative using starch-based patchy particles as a texturizer prepared through a bottom-up method by physically grafting small amaranth starch granules (∼1 μm) onto corn starch granules (>10 μm). After thermal treatment in aqueous ethanol, starches were partially gelatinized, and the particle stiffness was reduced. The corn starch and amaranth starch were modified to carry a negative charge and a positive charge, respectively. The hydrated swollen starch granules were centrifuged and dehydrated, which stitched particles together, forming a corona-shaped patchy structure with a negatively charged core and positively charged patches. The electrostatic interaction allowed particles to associate, and the pockets created in the flocs were able to trap more water. The enhanced water-holding capacity consequently contributed to a significantly higher storage modulus, loss modulus, and viscosity compared to the native starch and the mixed charged starch with the same blending ratio between amaranth and corn starch. The enhanced viscoelasticity was not affected by cooking and mechanical stress, which could be used as a shear-reversible thickener to modify texture with less raw ingredients, thus helping to reduce the amount of energy-dense starch in diets. This is the first time that the concept of patchy particles has been extended to food-grade ingredients with a facile and scalable method.

KEYWORDS: *patchy and Janus particles, starch, cationic and anionic charge modification, rheology, self-assembly*

■ INTRODUCTION

The research area of patchy particles, surfaces with non-homogeneous patterns, has gained considerable interest recently.[1] The patches exhibit different properties than the isotropic particle body and produce materials with different characteristics such as self-assembly.[2] Patchy particles self-assemble through favorable free-energy mechanisms such as electrostatic and hydrophobic interactions and have found applications in fabricating photonic crystals,[3] targeted delivery of drugs,[4] as surfactants,[5] and in electronics.[6]

These smart self-assembling particles have been made using numerous creative approaches to achieve the desired morphological engineering. For example, to prepare patchy particles, polystyrene and silica particles have been used to stabilize emulsions by residing at the oil−water interface, and with the internal solvent being evaporated, droplets shrink and eventually form well-defined clusters.[7] Particle lithography was also reported for patterning particle surface utilizing the contact area between particles to avoid chemical adsorption to the surface.[8] For example, by attaching the positively charged polystyrene particles to a negatively charged glass surface, a mask forms underneath the particles thus avoiding surface absorption of the negatively charged polystyrene sulfate.[8] The approach of glancing-angle deposition, which uses two-step vapor deposition on a monolayer of particles on a tape forming particles with two poles, was also adapted to manipulate surface morphology.[9] This method allows the patches to be accurately positioned with a single patch as small as 3.7% of the total surface area of polystyrene beads. Besides approaches based on surface modification, bottom-up methods were also reported.[10,11] The particles produced in this manner exhibited anisotropy not only on the surface but also inside the body, which used an electrohydrodynamic cojetting capillary apparatus to produce a laminar flow of three immiscible streams carrying different polymers. After the solvent evaporated, the polymers precipitated as triphasic particles with three compartments formed with immiscible liquid,[11]

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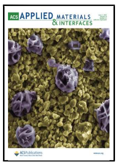

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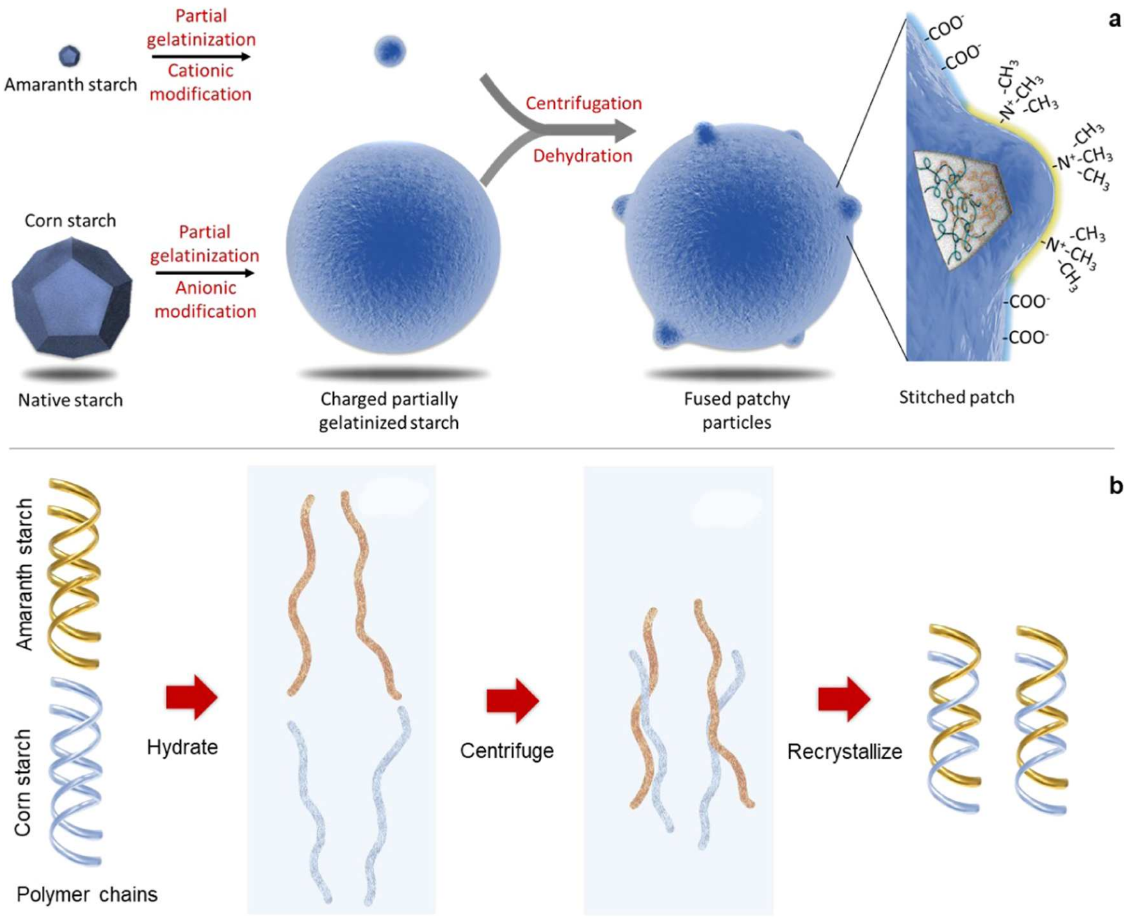

Figure 1. Procedures (a) and schematic diagrams (b) of the colloidal-fusion method for preparing starch-based patchy particles.

which provided the proof of concept for using a bottom-up method to prepare self-assembling patchy particles.

Despite the creativity of the methods for fabricating patchy particles, the majority of methods are limited to the laboratory production scale. That is, the cost of producing these materials is too high to be practically scaled up for industrial purposes. Future research needs to explore additional approaches that can prepare patchy particles economically. Another gap related to patchy particles is that the materials reported in the literature are made with hazardous organic chemicals, which suggests that future studies could explore alternatives based on ingredients that are biodegradable and food-grade.

Starch, as a staple source of carbohydrates, is ubiquitous in foods, not only as a calorie source but also as clean-label additives, such as a thickeners.[12] The use of starch as a conventional thickener is based on gelatinization by hydrothermal treatment, which cleaves the hydrogen bonds in a semicrystalline region of the starch thus providing space in the structure to absorb water.[13] The swollen granules provide a larger volume, which results in a stronger resistance to flow thus a higher viscosity and a thicker solution. Meanwhile, amylose molecules are released from gelatinized granules due to the disrupted structure and are able to form a network as a settled gel upon cooling.[13] However, a conventionally cooked starch slurry is susceptible to mechanical stress as the high shear of processing disintegrates the soft, swollen granules and the amylose gel network, thus causing issues with food texture.[14] It would be meaningful, therefore, to develop a new shear-reversible approach beyond swelling-based thickeners.

Instead of trapping water inside the swollen granules, a possible alternative to enhance thickening is to retain the water molecules between starch particles. To achieve this, patchy particles would be a potential platform to render the starch granules with self-assembling features. To the best of our knowledge, starch has not been used to fabricate patchy particles though it is a food-grade, affordable, and naturally available ingredient. There are more than 20 different modification methods for starch geared toward improving starch functionality,[15] but none of the conventional approaches has been able to produce starch granules showing a heterogeneous surface with patches providing different properties compared with the native particle body. Previous work from our lab successfully fabricated starch-based Janus particles with two faces using a two-dimensional (2D) masking method.[16] The Janus particles exhibited hydrophobic properties on the hemisphere esterified by octenyl succinic anhydride and allowed the starch granules to assemble into worm-like super micelles.[15] Our work presented here describes a new approach to develop a scalable modification method to yield a heterogeneous surface on corn starch granules.

The granular size of starch depends on the botanical sources and can vary from 1 μm (amaranth starch) to over 50 μm (potato starch).[17] We were inspired by the corona-shaped patchy particles that can be prepared by grafting the small starch particles on the surface of large starch granules. Therefore, a possible approach to prepare a fused starch particle is partial gelatinization. Alcohols precipitate starch from aqueous solutions and have been widely used to limit the degree of gelatinization.[18] With the hydrothermal treatment in aqueous alcohols, the granules would still maintain their intact shape, even though the double helices of starch dissociate.[19] Alcohols have also been shown to enter the single helix of amylose, forming metastable inclusion complexes.[20] When the partially gelatinized starch is redispersed in water, the granules swell and exhibit lower particle stiffness and higher deformability due to the decreased crystallinity.[21] We hypothesized that the swollen starch would fuse particles together as the conformational entropy of the polymer chains increases with partial gelatinization and the tangled chains would fuse after dehydration, reforming crystallized particles (Figure 1). In addition to surface modifications, this project investigated methods for creating charged starches to fabricate starch-based patchy particles with a negatively charged core and positively charged patches. Our design aims to allow starch granules to interact and associate with superstructures that would be able to trap water and improve viscoelasticity. The starch with the enhanced rheological properties would act as a stronger thickener, and as such, the food industry could use less starch to achieve the same textures and consumers can reduce the calorie intake from the energy-dense carbohydrates.

MATERIALS AND METHODS

Materials. High amylose corn starch (50%) was purchased from a commercial vendor. Anhydrous ethanol (>99% purity) was purchased from BioPlus (New York). Chloroacetic acid, sodium hydroxide, epoxypropyltrimethylammonium chloride (ETMAC), sodium chloride, and calcium chloride were purchased from Sigma-Aldrich (Missouri).

Extraction of Amaranth Starch. The amaranth starch was isolated from amaranth flour by a previously published method.[16] Specifically, amaranth flour (100 g) was mixed with 500 mL of a 0.15% w/v NaOH solution using an overhead stirrer at ambient temperature for 1 h. The slurry was then sieved (53 μm mesh opening, VWR, Illinois) with a laboratory shaker (Derrick Mfg. Co., Buffalo, NY). The fine starch granules dispersed in liquid were passed through the sieve and the remaining solid was redispersed in a fresh NaOH solution (100 mL) followed by another 10 min of mixing. The extract was filtered using the sieve with a 53 μm mesh opening and combined with the first batch, which was then centrifuged at 3000*g* for 20 min. The supernatant was discarded, and the top brown protein layer was removed using a spatula. The sediments were redispersed in deionized water with pH adjusted to 6.0 ± 0.1 using 1 M HCl. The yellow protein layer that formed after pH adjustment was removed again to purify the starch. The remaining lower white layer was freeze-dried and ground into a powder. The isolated starch contained 2.4% protein and 2.2% fat.[16]

Preparation of Partially Gelatinized Starch. The partially gelatinized starch was prepared by thermal treatment in alcohol as previously published.[12] Specifically, we mixed 100 g of native starch with 500 mL of aqueous ethanol (50 v/v % for amaranth starch and 30 v/v % for corn starch). The starch-in-solvent suspensions were incubated in a water bath at 80 °C for 30 min. After thermal treatment, an additional 100 mL of ethanol was added to the dispersion, and the starch was then filtered (Grade 1, Waterman). The filtered starch was washed three times with absolute ethanol and then dried in an oven at 80 °C for 8 h to remove the residual solvent. The dry starch was ground using a blender (Oster 2095357, Florida) for 2 min, and the powder was sieved (#50 mesh) and then stored in an air-tight plastic bag.

Preparation of Negatively Charged Corn Starch. The partially gelatinized corn starch was modified to carry a negative charge based on carboxymethylation by chloroacetic acid.[22] Specifically, we dissolved 50 g of NaOH in 266 mL of deionized (DI) water, and 1066 mL of ethanol was added to the alkaline solution. Chloroacetic acid (81.3 g) was then added, and the mixture was stirred for 1 h, after which, 100 g of partially gelatinized corn starch was mixed with the reaction solution. After the final mixture was stirred for 2 h at room temperature, the resulting slurry was filtered using a Whatman Grade 1 filter paper and the solid collected was washed with 80% ethanol. The washed starch was subsequently centrifuged at 3000*g* for 3 min and then washed with additional 80% ethanol and centrifuged twice more. The starch was then dispersed in 200 mL of DI water and 0.1 M HCl was used to adjust the pH to 7 to neutralize residual NaOH. The neutralized starch was centrifuged (3000*g* for 3 min) and washed twice to remove residual salts. The starch pellet was freeze-dried for further use.

Preparation of Cationic Amaranth Starch. The partially gelatinized amaranth starch was positively charged by a modification method previously established.[23] Specifically, we dispersed 1 g of amaranth starch in 2 mL of water containing 1.91 g of ETMAC. The pH of the slurry was adjusted to 10.5 using 0.1 M NaOH. The mixture was then stirred at room temperature for 24 h after which the slurry was centrifuged (5000*g* for 3 min) and the pellet was further washed with DI water twice, and the collected starch was further washed using ethanol. The starch was dried in a ventilated oven at room temperature.

Preparation of Patchy Starch via Particle Fusion. Separately, partially gelatinized corn starch and amaranth starch were hydrated with excess water (starch/water ratio = 1:5). Then, these solutions were mixed (with an amaranth-starch-to-corn-starch ratio at 10:1 or 50:1) and incubated at different temperatures (25, 40, or 50 °C) for 5 or 20 min. After incubation, the solutions were centrifuged (8000*g*, 12,000*g*, or 15,000*g*) for different durations (5 or 15 min). When the granules were swollen with water, the starch granules were less rigid, and the conformational freedom of polymer chains increased owing to less hydrogen bonding. Therefore, the swollen granules interpenetrated the other granules while being centrifuged, and the polymer chains tangled together (Figure 1). After centrifuging, the clear supernatant was decanted, and the starch was dried in a ventilated oven at room temperature for 96 h and then stored at 4 °C for further characterization.

Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR). The FTIR spectra of starches were measured using an ATR-FTIR (Affinity-1S, Shimadzu) to identify the chemical composition of the charge modification of starch. Samples were scanned from 400 to 4000 cm^{-1} in a transmittance mode with a 2 cm^{-1} resolution and 128 scans.

ζ-Potential. The ζ-potential of starches was measured with a NanoZS90 Zeta-Sizer (Malvern Instrument Ltd., U.K.) with a He/Ne laser (λ = 633 nm) at 25 °C. Starch was homogenized in water (0.1%); the ζ-potential was calculated from electrophoretic mobility based on the Smouluchowski model and was reported as an average value from triplicates.

X-ray Diffractometry (XRD). The XRD patterns of the starch samples were collected using a Bruker D8 Advance ECO powder diffractometer (Massachusetts) operating at 40 kV and 25 mA with Cu Kα radiation. The samples were examined for the angles 2θ between 5° and 40°.

Scanning Electron Microscopy (SEM). Samples were coated with Au−Pd using a sputter coater (Denton Desk V, New Jersey). The coated samples were examined with a SEM (Zeiss Gemini 500, Jena, Germany). Objects were scanned with 1 keV and imaged by a high-efficiency secondary electron detector with a 20.0 μm aperture.

Confocal Laser Scanning Microscopy (CLSM). To visualize fused patches, CLSM was employed to examine patchy particles after the amaranth starch was stained using a fluorescence probe. The probe, fluorescein isothiocyanate (FITC), was mixed in DI water to obtain a 25 μg/mL solution. Partially gelatinized amaranth starch (1 g) was added in 10 mL of an FITC solution and stirred overnight to saturate the swollen starch with the fluorescent dye. The resulting mixture was centrifuged at 3000*g* for 10 min and the starch pellet was washed three times with DI water to remove excessive FITC. The stained starch was then freeze-dried. The fluorescent-dyed amaranth starch was mixed with nondyed corn starch to prepare the patchy particles per the method described above. The patchy starch was then dispersed in water and examined by a CLSM (LSM 710, Carl Zeiss, Göttingen, Germany) with excitation/emission wavelengths at 488/515 nm. To visualize the three-dimensional (3D) structure, a z-stack was employed.

Water-Holding Capacity. To study the water-holding capacity, 0.5 g of dry starch was weighed in a 15 mL tube and 5 mL of water was added. The slurry was mixed vigorously for 1 min. The self-assembling sedimentation occurred overnight, and the volume of the sediment was recorded. The water held in the slurry was calculated by deducting out the supernatant volume. Experiments were carried out in triplicate.

Quantification of Flocculation. To visualize the flocculation of the patchy particles, a diluted starch slurry (2%) was prepared. The slurry was vortexed and homogenized with a high-shear mixer at 5000 rpm for 1 min, and 20 μL of each sample was transferred to a glass slide and covered with slip. The suspension was examined using a Leica Model DMIL LED Inverted Phase Contrast Microscope at 100×. For each sample, 10 images were taken that contained >10,000 particles in total, and the particle size was analyzed by ImageJ software (v1.51, National Institute of Health) based on pixel calculation. The mean size (μm^2) for each sample was recorded. Objects were classified as individual particles (<100 μm^2), small aggregates (100−2000 μm^2), and large clumps (>2000 μm^2).

Examination of the Assembling Process. Patchy particles (25 mg) were mixed in 5 mL of DI water using a high-shear homogenizer (T25 digital Ultra-Turrax, IKA Works, North Carolina) at 5000 rpm for 1 min. An aliquot of the slurry (20 μL) was placed on a glass slide, and the image was observed with a Leica Model DMIL LED Inverted Phase Contrast Microscope at 200×. Another 20 μL of the slurry was pipetted onto a plate, which induced a slight flow causing the added patchy particles to interact with the particles already on the slide. Immediately, 2000 photos were recorded using a camera control system (Andor Solis, U.K.) at a 40 Hz frame rate and a 200 MHz pixel readout rate with low noise to capture the assembling process.

Rheological Measurements. The dynamic oscillation study was conducted using a rheometer (Anton Paar MCR301, Graz, Austria) with a 25 mm parallel plate and the gap set at 0.5 mm. A slurry containing 25 wt % starch was prepared. The storage modulus (G') and the loss modulus (G'') were recorded using the amplitude sweep (1 Hz, 0.1−100% strain) to scan the linear region. A 0.3% strain was chosen, and the frequency sweep was conducted from 0.5 to 100 Hz. The rotational shear study was also carried out from 0.01 to 100 s^{-1} and viscosity was plotted against the shear rate. A rheological study was carried out on native starch, charged mixed starch (mixing ratio = 1:50), and charged patchy starch (patchy ratio of 1:50). The rheological behavior of cooked starch was also studied on 10 wt % slurries of charged mixed starch and patchy starch, which were gelatinized at 90 °C for 10 min. The stability against mechanical processing was tested using the 10 wt % slurries sheared by a homogenizer (5,000 rpm for 1 min).

Statistical Analysis. Analyses were conducted in triplicates, and results are presented as mean ± standard deviation, which were tested by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison using JMP Pro (Version 14, SAS Institute) to identify significant difference ($p < 0.05$).

RESULTS AND DISCUSSION

Fabrication of Starch-Based Patchy Particles. The hydrogen bonding in starch can be disrupted by thermal processing at temperatures higher than its gelatinization temperature in aqueous alcohol.[19] Ethanol as a precipitant of starch is able to inhibit amylose leaching and the original granular shape of the starch is maintained.[18] This partial gelatinization allows the starch to absorb the water and the swollen starch becomes softer and less crystalline.[21] The XRD curves (Figure 2) indicate that native corn starch exhibits typical B-type crystals with peaks at 5.5, 15, 17, 20, and 22.5°, which were in agreement with the data reported previously.[24] After partially gelatinized starch was produced by hydrothermal processing in aqueous alcohol, however, these peaks diminished, suggesting the dissociation of the hydrogen bonding between polymer chains and thus lower crystallinity.

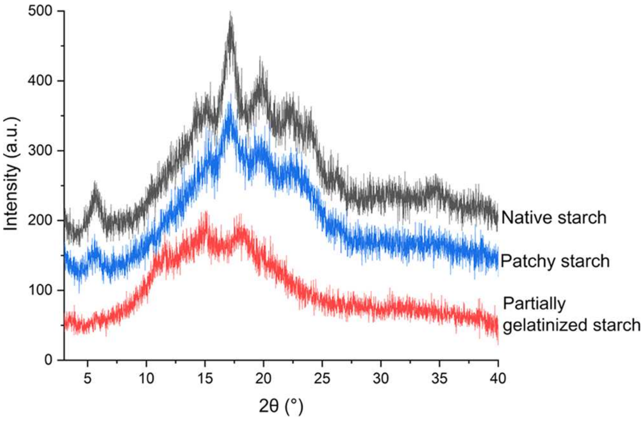

Figure 2. XRD curves of native starch, partially gelatinized starch, and fused patchy starch.

The partially gelatinized starch undergoes retrogradation, which refers to the spontaneous process by which disordered polymer chains recrystallize.[25] The patchy starch after dehydration showed recovered crystallization compared with the partially gelatinized starch (Figure 2). During storage, water evaporates, allowing the hydroxyl groups on the polymer chains to get close enough to reform the hydrogen bonds. Although retrogradation is usually considered an undesirable process for food products causing bread staling,[26] it can also be utilized as an approach to construct patchy particles.

To test the robustness of the colloidal-fusion method, we first prepared patchy starch without charge modification (Figure 3). After hydration, the swollen starch particles were less crystalline and thus less stiff. Patchy starch formed after centrifuging the partially gelatinized starch under the mild processing conditions of incubation at room temperature, followed by centrifugation at 8000g for 5 min (Figure 3a,b). The soft swollen starch granules interpenetrate each other during centrifugation. Upon drying, the polymer chains of the starches tangle together and the dehydrated hydroxyl groups spontaneously form hydrogen bonds with the chains of the neighboring starch, thus stitching multiple particles together. However, the depth of the connection was less than one-quarter of an amaranth starch granule (Figure 3b). When the patchy starch was redispersed in water (Figure 3c), a considerable amount of amaranth starch granules detached as free particles in water, and only a small number remained attached to the main corn starch particles. The weak connection, less than 0.25 μm, could be the reason for the free particles observed (Figure 3c).

When starch was prepared by incubation at 50 °C for 20 min followed by centrifugation at 15,000g for 20 min, the morphology changed significantly, and there was almost no observable amaranth starch (Figure 3d) although the mixing ratios used were the same. The image captured at higher magnification (Figure 3e) shows that the connection is much stronger such that almost the entire body of the small particle is fused into the large particle, suggesting that the higher incubation temperature and stronger centrifugation strengthened the connection. It would be reasonable to hypothesize that the amaranth particles could be completely engulfed as

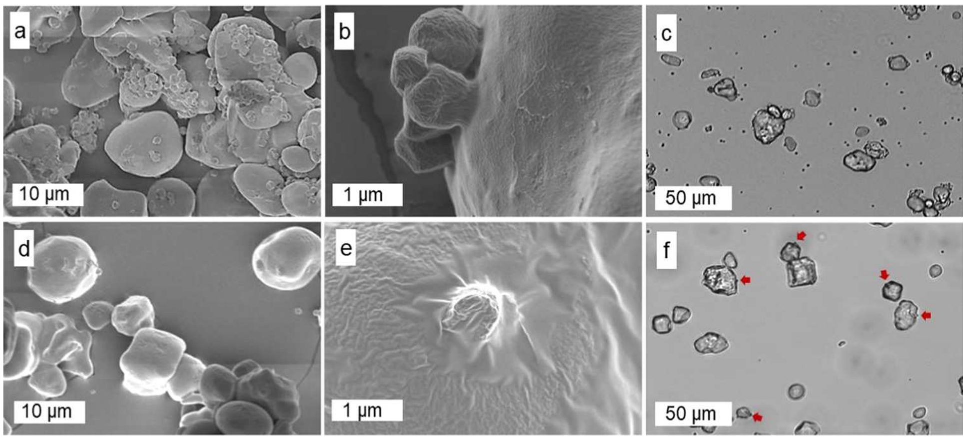

Figure 3. SEM images (a, b) of dry uncharged patchy particles prepared by incubation at 25 °C followed by centrifugation at 8000*g* for 5 min, and their water suspension (c). SEM images (d, e) of dry uncharged patchy particles prepared by incubation at 50 °C followed by centrifugation at 15,000*g* for 15 min, and their water suspension (f). The arrows indicate visible patches on the surface of corn starch granules. The starches shown in the figure are uncharged to demonstrate that the particle−particle association was not due to electrostatic interaction. The ratio between amaranth starch and corn starch was 1:10.

many of the small particles were missing (Figure 3d). When the patchy starch was redispersed in water (Figure 3f), only a few free amaranth starch granules were observed. It should be noticed the starches presented in Figure 3 were all uncharged, suggesting that the majority of the small particles were physically grafted onto the corn starch rather than being attracted by the electrostatic interaction, which is also attributed to the stronger fusion of particles.

The origin of the patches is not detectable by SEM; further, due to strong fusion, sometimes the patches were difficult to distinguish from surface irregularities in the native corn starch. Therefore, we used CLSM to examine the patches by staining the amaranth starch using FITC for fluorescent contrast (Figures 4 and S2). As confocal microscopy can only image monolayers, samples were scanned using the z-stack mode and combined to simulate a 3D structure. We observed that the fluorescent patches are distributed on the surface of the nonstained body, suggesting that the small amaranth particles were physically fused to the surface of the larger corn starch particles.

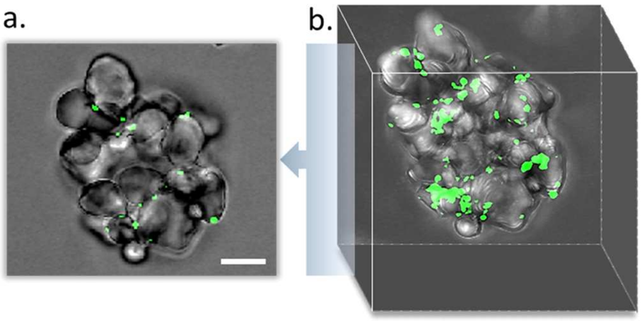

Figure 4. CLSM planar image (a) and the z-stack (b) of patchy particles. The patches (amaranth starch) were stained with FITC shown in green, and the main body (corn starch) was left unstained and visualized under a bright field. The scale bar is 15 μm.

The principle behind fabricating starch-based patchy particles is attributed to the partially gelatinized and hydrated starch granules. The softening of the starch granule is an indication of the increased degree of freedom of polymer chains and then the strong centrifugation helps them penetrate into the other granules. The polymer chains tangle together, and during the processing of dehydration and refrigeration, they can recrystallize through hydrogen bonding. This retrogradation allows multiple particles to fuse as a single particle (Figure 1).

Although the intense treatment enhanced the connection between the patches and the primary granule, we found that after treatment, there was a small amount of amaranth starch granules extremely gelatinized, which was not entirely unexpected due to the elevated incubation temperature. During centrifugation, these extremely swollen granules rise to the top of starch sediments due to their lighter density. This thin layer of gelatinized starch on the top retained moisture well and slowed down recrystallization. Therefore, we switched to an intermediate processing condition: incubation at 40 °C for 15 min followed by centrifugation at 12,000*g* for 15 min. The patchy starch prepared under these conditions showed that about half of the particle body of the amaranth starch had fused (Figure S1a). A patch where a small particle detached from indicated that two particles were physically stitched together via molecular entanglement (Figure S1b). Because the intermediated conditions (incubation at 40 °C for 15 min, centrifugation at 12,000*g* for 15 min) were able to produce fused particles with a shorter dehydration process, our studies focused on these samples.

The patchy particles are noticeably nonuniform. Natural materials, unlike latex or polystyrene spheres, present unique challenges to fabricate uniform patchy particles. As the large particles (corn starch) are naturally nonuniform, with a wide size distribution (10−20 μm) and nonspherical shape, patchy particles may not appear significantly different from the native starch. Additionally, the smaller particles (amaranth starch), though fairly uniform in size (~1 μm), tend to aggregate extensively when mixed by a homogenizer prior to fabrication (Figures 3 and 4). This aggregation causes a nonuniform distribution of patches, where a part of the surface is densely patchy and a part of the surface is almost nonpatchy. We postulate that this could be due to the residual protein (~2.5%), which is largely water insoluble, in the extracted amaranth starch. Due to this hydrophobic association between amaranth starch, the particles tend to be aggregated. To solve

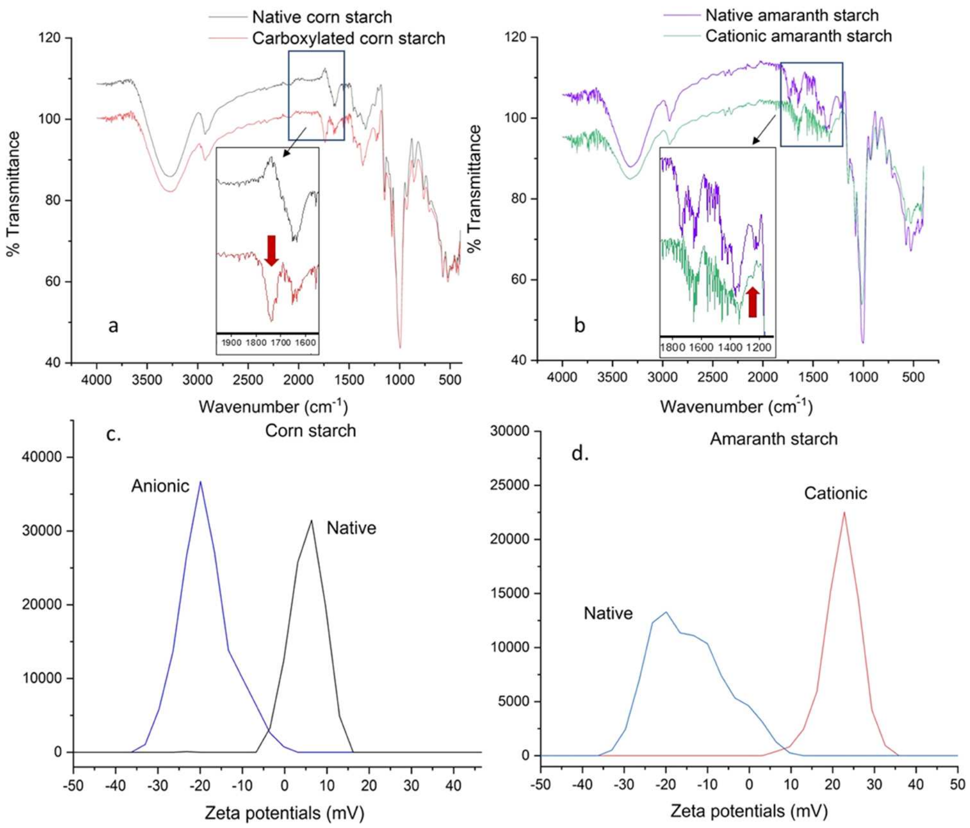

Figure 5. (a) FTIR spectra of native and anionic corn starch, (b) FTIR spectra of native and cationic amaranth starch, (c) ζ-potentials of native and anionic corn starch, and (d) ζ-potentials of native and cationic amaranth starch.

the issue of starch aggregation and perhaps obtain more evenly dispersed patches, starch can be purified with a basic solution as proteins tend to be more soluble in aqueous solutions at high pH. Alternatively, nonionic surfactants could be used to inhibit hydrophobic associations.

Effect of Charge and Patchy Modification on Starch Functionality. The FTIR spectra of modified corn starch have a band at 1750 cm^{-1}, which is attributed to the C═O stretching from carboxyl groups (Figure 5a).[27] The carboxymethyl modification gives the starch a negative charge, which we observe at −19.1 mV at neutral pH (Figure 5c). The FTIR spectra show that the native amaranth starch exhibits a band at 1750 cm^{-1} (Figure 5b), suggesting the presence of carbonyl groups, which can be attributed to the minor protein content (∼2.5%) after extraction from flour.[21] The negative ζ-potential (−19.4 mV) of the native amaranth starch was also rendered by the minor protein content (Figure 5d). After cationic modification using ETMAC, the amaranth starch had a ζ-potential of +19.6 mV (Figure 5d) and the carbonyl band on FTIR spectra was diminished, confirming that the ETMAC reacted with the carboxyl group of the proteins. Additionally, the new band at 1240 cm^{-1} can be attributed to the C−N stretch, indicating the presence of a trimethylammonium group.[26]

The charged starches were physically fused together and exhibited a negatively charged core and positively charged patches on the surface. When the charged patchy starch was redispersed in water, the particles were able to assemble via electrostatic interactions (Figure 6a), resulting in a heterogeneous opacity, and the flocculated starch sedimented to the bottom after 10 min settling. The amount of water held in the sediments was quantified. The rheological moduli and viscosity of uncharged, mixed charged, and patchy charged starches were also examined (Figure 6). The control group of uncharged starch held 2.1 g water per unit of starch in the slurry sediment, but when starch particles were oppositely charged (corn starch negatively charged and amaranth positively charged), the mixture was able to hold 3.3 g water per unit of starch (Figure 6b). When the charged particles were physically fused into patchy starch, the water-holding capacity increased further to 5.7 g per unit starch. The rheological study shows that the slurry of uncharged mixed starch exhibits a weak storage modulus, which was below detection limits when the angular frequency was higher than 10 s^{-1}, suggesting that the control group behaves fluid-like (Figure 6c). However, the charged groups showed an increase in rheological moduli, and the G' was higher than G'', indicating that the behavior was dominated by the storage modulus and the slurries showed gel-like behavior (Figure 6c). Improvement was also observed in the viscosity of the physically fused patchy starch with a heterogeneously charged surface, which was two orders of magnitude higher compared with the uncharged starch (Figure 6d). The viscosity-enhancing effect was more pronounced when particles were physically stitched together when compared with the mixed charge particles. The higher flexibility of mixed particles is most likely why they were not as effective at trapping water. The slurry of native starch was a Newtonian fluid with a constant viscosity under shear, but the charged groups exhibited shear-thinning with low viscosity at higher shear rates, which can be attributed to the dissociation of short-distance electrostatic interactions when the shear rate increased.

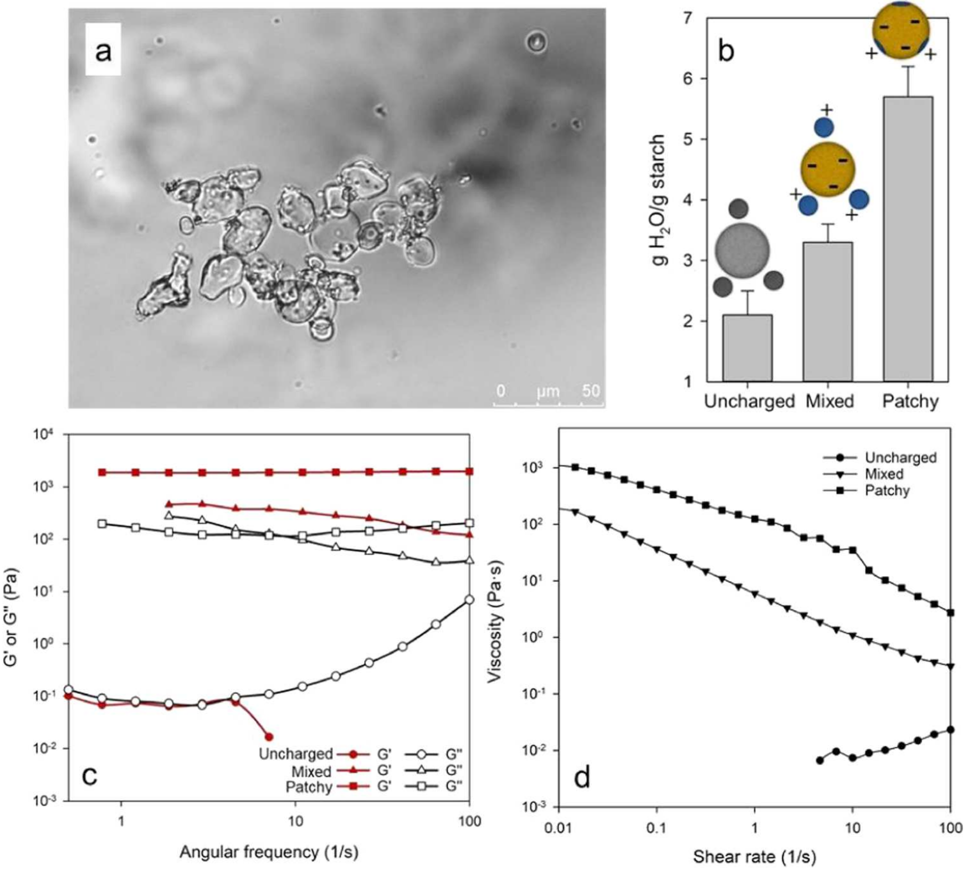

Figure 6. Self-assembly of charged patchy starch in water (a); the water-holding capacity (b); rheological moduli (c); and viscosity (d) of uncharged, mixed charged, and patchy charged starch. The ratio between amaranth starch and corn starch was 1:50.

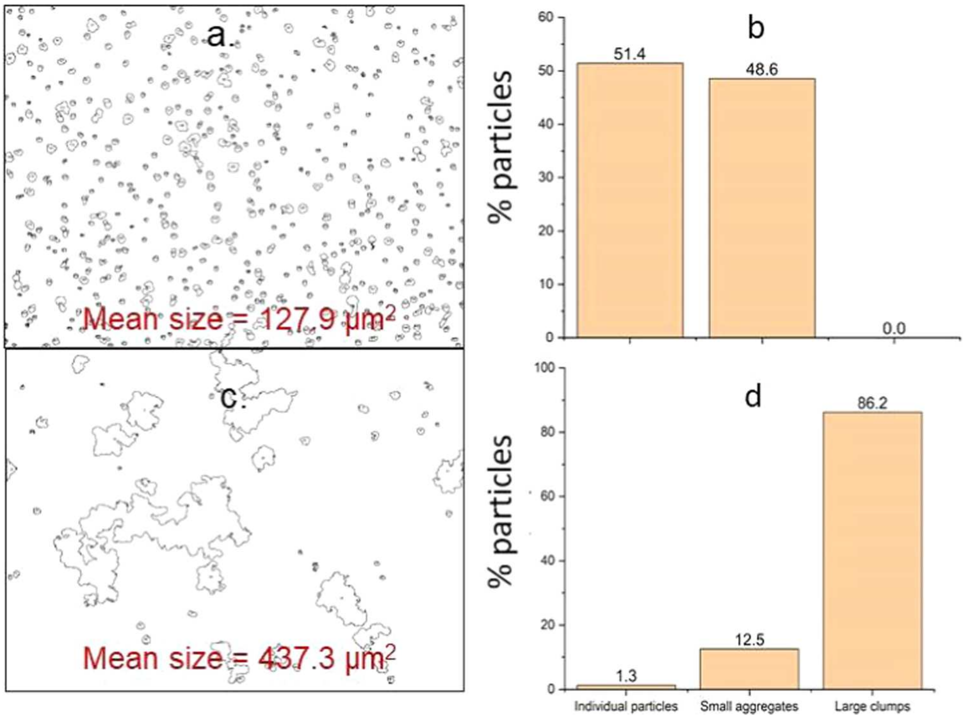

Figure 7. (a) Processed images of the 2% slurry of native corn starch; (b) distribution of native starch as individual particles (<100 μm^2), small aggregates (100−2000 μm^2), and large clumps (>2000 μm^2); (c) processed images of the 2% slurry of patchy starch; and (d) distribution of patchy starch as individual particles, small aggregates, and large clumps. The ratio between amaranth starch and corn starch was 1:50.

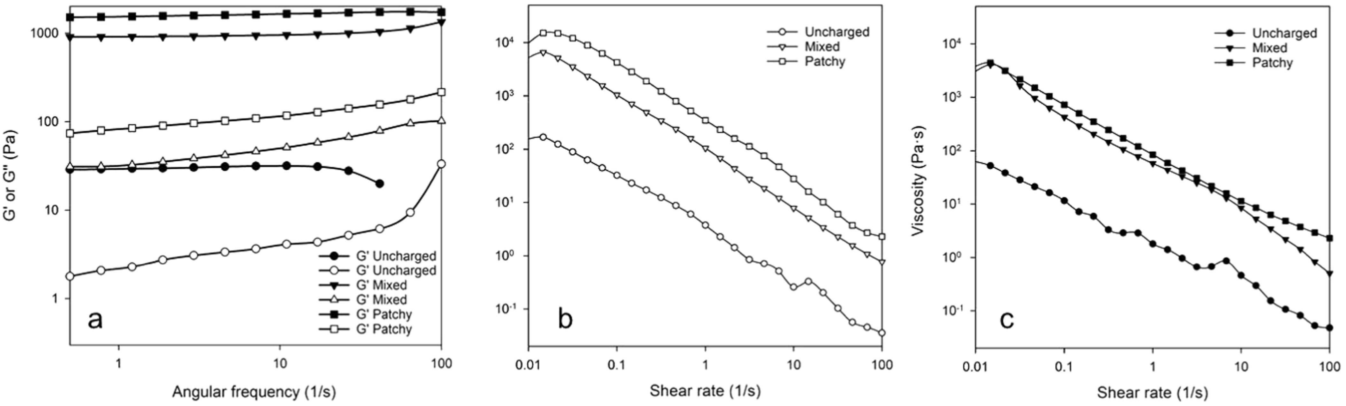

Figure 8. (a) Storage modulus and the loss modulus of the gelatinized slurry containing 10% uncharged, mixed charged, and patchy starch; (b) viscosity of the gelatinized slurry containing 10% uncharged, mixed charged, and patchy starch; and (c) viscosity of the sheared gelatinized slurry containing 10% uncharged, mixed charged, and patchy starch.

The charge-induced flocculation formed pockets that trapped water molecules and inhibited the movement of liquid, which led to enhanced functionalities. Starch granules were dispersed individually in the slurry of native corn starch (Figure S3a), while the charged patchy starch in water showed significant flocculation (Figure S3b). When the patchy particles encounter each other in the suspension, electrostatic interactions between particles attract each other (Figure S4). The assembling process was very rapid (<1 s) due to the strong electrostatic interaction, which provides the system with the ability to recover from shear. The images examined by a microscope were processed to quantitatively characterize the interaction between particles. The suspension of native corn starch in water showed that particle size calculated by pixels was 127.9 μm^2 based on image processing of over 10,000 particles (Figure 7a). The particle distribution indicated that 51.4% of starch in native corn starch samples is present as individual particles (Figure 7b). However, when starch was charged and physically fused (Figure 5c), particles flocculated to a large extent, 86.2% of starch particles formed large clumps (>20 particles) and only 1.3% of particles were in the individual state, with the average size of all particles in the sample as high as 437.3 μm^2 (Figure 7d).

Effect of Charge and Patchy Modification after Gelatinization and Mechanical Processing on Starch Functionality. Starch present in foods is usually gelatinized. The viscoelasticity of a starch slurry after gelatinization at 90 °C for 10 min showed that the charge modification and patchy fusion enhanced both storage and loss moduli, which is similar to the observation of the nongelatinized starch (Figure 8a,b). This means that the heating process did not impair the charged-induced interaction. As mechanical stress is usually involved in food processing, the viscosity of gelatinized starch was tested after blending with a homogenizer at 5000 rpm for 1 min. The shearing process after gelatinization was found to not be an issue for the charged-induced interaction, indicating that this platform could be used as a shear-reversible thickener in the food industry. The difference between a mixed group and a fused group was less obvious after homogenization (Figure 8c), which is likely due to the dissociation of the patchy construction at a high shear rate.

■ CONCLUSIONS

The present work demonstrated a facile method for preparing patchy particles using two different sized starches as food-grade ingredients, and it is the first time a study of the heterogeneous surface model has been extended to biodegradable natural materials. After partial gelatinization, the swollen starch granules were centrifuged and dehydrated, which yielded a corona-shaped patchy structure where the smaller amaranth starch grafted onto the larger corn starch particles. Further, the patchy starch exhibited an anionic core and cationic patches after charge modification. The patchy particles also showed higher water-holding capacity as the interparticle interactions allowed water to be trapped in pockets of the superstructure formed by granules. This impacted the rheological properties with the enhanced storage modulus, loss modulus, and viscosity as compared to the native starch and the mixed charged starch with the same blending ratio between amaranth and corn starch. The enhanced functionality of the patchy starch particles was stable to cooking and mechanical stress as the enhanced viscoelasticity was not impaired by the processing, showing potential as a shear-reversible texturizer in food products. It could be used to reduce the amount of calorie-dense starch in diets. To gain a more holistic understanding of our novel starch-based patchy particles, future work in this area includes investigating the impact of architecture, including the number of patches and the charge per patch, on the self-assembling properties, as well as the effects of different environmental factors such as pH and ionic strength on the overall functionality of patchy starch, specifically within a complicated food matrix.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsami.2c04738.

> SEM images of patchy starch prepared by incubation at 40 °C, CLSM image of the single patchy particle, suspension of corn starch and patchy starch, and time-lapsed images of the assembly process (PDF)

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Notes

The authors declare no competing financial interest.

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