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Corn starch granules with enhanced load-carrying capacity via citric acid treatment

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ABSTRACT

This research investigated conditions by which maize starch granule porosity and load-carrying capacity (LCC) might be enhanced via treatment with varying citric acid concentrations (0.5–1.5 M), temperatures (40–60 °C), and lengths of treatment (1–8 h). At the lowest temperatures (40 and 50 °C), citric acid treatment induced minimal physicochemical changes to granules. In contrast, both aqueous and oil LCCs of starches treated at 60 °C (0.5 M citric acid, 2 h) were almost doubled (15.69 and 14.48 mL/10 g starch, respectively), recovering 92% of the granular starch after treatment. Such treatment increased starch hydration capacity (0.97–1.91) and reduced gelatinization enthalpy (10.6–7.4 J/g). More severe treatment conditions adversely impacted aqueous LCC (due to excessive granule swelling), but improved oil absorption. The basis for LCC enhancement by citric acid treatment was ascribed to leaching of starch material from granules and partial disruption of the granule crystalline structure, as opposed to starch hydrolysis or chemical substitution.

1. Introduction

Highly porous materials possessing exceptional specific surface areas find use as catalysts, sorbents, plating/carrying agents, etc. with surface structure being a primary factor determining their functionality and efficiency in technological applications (Szymonska & Wodnicka, 2005). Compared with inorganic porous materials such as mesoporous silica (Zhang et al., 2010), clay particles (Zhang et al., 2010) and lipid-inorganic hybrids (Tan, Simovic, Davey, Rades, & Prestidge, 2009), starch-based porous biomaterials offer the distinct advantages of non-toxicity, biocompatibility, biodegradability, renewability, and low cost (Wu et al., 2011). Thus, porous starch particles find extensive use in food, pharmaceutical, agricultural, cosmetic, pulp/paper, and other aligned industries (Glenn et al., 2010; Qian, Chang, & Ma, 2011).

Starch granules of certain botanical origin (i.e., maize, sorghum, millet, wheat, etc.) possess native internal channels, many of which extend inward from the external granule surface to the central cavity at the granule hilum (Huber & BeMiller, 2000). Both cavity and channel structures within starch granules may be enlarged to further increase granular porosity via limited erosion/hydrolysis of amorphous regions within starch granules using amylolytic enzymes (α-amylase, glucoamylase, etc.) under varied experimental conditions (Uthumporn, Zaidul, & Karim, 2010; Zhao, Madson, & Whistler, 1996). A higher degree of hydrolysis produces channels and cavities of greater size, but also decreases the ultimate recovery of porous starch material, due to more extensive erosion or removal of starch from granules (Qian, Chen, Ying, & Lv, 2011; Uthumporn, Zaidul, & Karim, 2010; Zhao, Madson, & Whistler, 1996). Alternatively, hydrolysis of starch within granules via lintnerization may also enhance porosity of corn starch granules, with preferential attack occurring within granule amorphous regions (Jayakody & Hoover, 2002). Chabot, Allen, and Hood (1978) observed two primary patterns of action for acid hydrolysis within waxy maize starch granules, one in which hydrolysis proceeded from the inner hilum region outward (most likely facilitated by channel structures), and the other in which the hydrolysis front progressed from the external granule surface inward. As the starch granule matrix is reported to be permeable to molecules possessing a hydrodynamic radius less than 0.6 nm (Planchot, Roger, & Colonna, 2000), the extent of diffusion (as well as hydrolytic action) within the granule matrix was suggested to be greater for mineral acid treatment relative to that of amylolytic enzymes (Jayakody & Hoover, 2002). Nevertheless, porous starch materials generated by either amylolytic enzyme or mineral acid hydrolysis must be labeled as modified food starch within the U.S. Though modified starches prepared for food use are considered both functional and safe ingredients (Singh, Kaur, & McCarthy, 2007), consumer demand for 'clean label' ingredients has renewed efforts to develop alternative starch products.

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One possible approach for generating alternative porous starch materials involves treatment of starch granules with organic acids, such as citric acid. Citric acid is common to citrus fruits, and is further utilized as a GRAS ingredient (e.g., acidulant, preservative) within processed foods. Hirashima et al. (2004) reported differing effects of citric acid on the pasting behaviors of corn starch granules depending on treatment conditions. Below pH 3.5, citric acid treatment induced fracture or collapse of starch granules, as well as extensive hydrolysis of starch chains, resulting in reduced pasting viscosities. Conversely, at less acidic pH values ($3.6 < pH < 5.5$), citric acid treatment promoted enhanced leaching of starch chains from granules (during heating/gelatinization), leading to increased pasting viscosities. In short, citric acid has potential to cleave glucose chains within starch granules (similar to common mineral acid or enzymatic hydrolysis) and/or accelerate leaching of starch chains from granules during heating. Based on the fact that the granule hilum is reported to be the least organized region within the granule (Baker, Miles, & Helbert, 2001), and that the hilum has direct access to the external granule environment via interconnected channels within corn starch granules (Huber & BeMiller, 1997, 2000), it was hypothesized that citric acid treatment could induce preferential erosion and/or leaching of starch from amorphous regions of granules to enhance granule porosity. Nevertheless, there are no prior reports detailing preparation of porous starch particles via citric acid treatment.

The primary objective of this research was to investigate the potential for increasing the load-carrying capacity of normal maize starch granules, utilizing multiple concentrations of citric acid in conjunction with varied treatment temperatures and times.

2. Materials and methods

2.1. Starch source and chemicals

Normal maize starch (Pure-Dent B700) was provided by Grain Processing Corporation (Muscatine, IA, USA). Citric acid was purchased from Sigma–Aldrich Corp. (St. Louis, MO, USA). Isoamylase (EC 3.2.1.68; 1000 U/mL) was purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland). Mercury dibromofluorescein (disodium salt, merbromin) was purchased from Fluka (Seelze, Germany). All other utilized chemicals were at minimum of analytical grade.

2.2. Citric acid treatment

Normal maize starch was treated with citric acid according to a factorial experimental design ($3 \times 3 \times 5$) consisting of three concentrations of citric acid solution (0.5, 1.0, or 1.5 M), three levels of temperature (40, 50, or 60 °C), and five lengths of treatment (1, 2, 3, 5, or 8 h). Normal maize starch (10 g, dry basis [db]) was dispersed in the appropriate concentration of citric acid solution (100 mL), and the dispersion was incubated at the designated temperature with stirring (440 rpm) for a set length of time (as defined by the factorial design). After citric acid treatment, treated starch was collected by centrifugation ($3000 \times g$, 10 min), and neutralized with 0.1 N NaOH. Neutralized starch was washed with 70% aqueous ethanol (100 mL × 2 times) followed by washing with absolute ethanol (100 mL) to remove residual citric acid and dehydrate the starch. Treated starch was dried in a vacuum oven (50 °C, 70 kPa, 8 h), and ground to pass through a No. 120 sieve. Control starch was prepared according to the same procedure (without addition of citric acid) as a reference. Recovery of starch material following citric acid treatment was calculated according to the equation below:

$$\text{Recovery}\,(\%) = \frac{\text{Weight of starch after citric acid treatment}}{\text{Weight of starch before citric acid treatment}} \times 100$$

2.3. Load-carrying capacity (LCC) of starches

Load-carrying capacity (LCC) of starch granules was assessed in both water and oil media using ASTM Standard Method D281-95. In general, starch (10 g, db) was transferred to a glass beaker (150 mL), after which a sufficient aliquot of water or oil was mixed into the starch with a spatula to yield a homogeneous powder. Additional water or oil was added to the starch (with further stirring) until a stiff, continuous, putty-like paste was formed (the endpoint was a continuous paste that did not break with stirring). If the paste became flowable (i.e., too much liquid had been added), the obtained value was discarded and the test was repeated. Load-carrying capacity was calculated as the total volume of fluid added to the starch and reported as mL of liquid per 10 g of starch (db).

2.4. Scanning electron microscopy (SEM)

Native, control, and citric acid-treated starch granules were mounted directly onto double-sided carbon tape attached to aluminum stubs. Mounted starches were carbon-coated, and viewed using scanning electron microscopy (SEM) (Amray 1830 Scanning Electron Microscope, Amray Inc. Bedford, MA, USA) at 10 kV.

2.5. Optical microscopy

Starch granules were placed in distilled water or immersion oil on a glass slide, overlaid with a cover slip, and observed with a Nikon Eclipse E600 microscope (Melville, NY, USA).

2.6. Merbromin treatment and fluorescence microscopy

Interior channels and cavities of native maize, treatment control and citric acid-treated starch granules were flooded with a methanolic solution of merbromin as described by Kim and Huber (2008). Merbromin solution was prepared by dissolving merbromin (0.1 g) in methanol (100 mL). Starch granules (200 mg, db) were dispersed in methanolic merbromin solution (20 mL) with stirring (440 rpm) for 4 h in the dark at ambient temperature. Merbromin-treated starch granules were recovered by vacuum filtration, lightly washed on the filter with ethanol (merbromin is insoluble in ethanol) to fix merbromin to starch granule surfaces, and allowed to air-dry.

Merbromin-treated starch granules were placed in immersion oil on a glass slide, overlaid with a glass cover slip, and observed with an Olympus upright BX51 microscope (Melville, NY, USA) equipped with a fluorescence source (excitation at 450–550 nm).

2.7. Hydration capacity of starch granules

Hydration capacity of starch granules was measured as described by AACC Method 56-20 (AACC, 2000). Starch (2.0 g, db) was suspended in distilled water (40 mL) and shaken vigorously to fully disperse the starch. The starch suspension was allowed to stand (10 min), during which time tubes were inverted after 5 and 10 min. After the 10 min suspension period, the dispersion was centrifuged ($1000 \times g$, 15 min), the supernatant was decanted, and the sediment was weighed. Hydration capacity of starch granules was calculated according to the equation below:

$$\text{Hydration capacity} = \frac{(\text{Weight of tube} + \text{sediment}) - (\text{weight of tube})}{\text{Sample weight}\,(\text{db})}$$

2.8. Differential scanning calorimetry (DSC)

Thermal transition properties of starch granules were carried out using differential scanning calorimetry (DSC) (2920

Modulated DSC, TA instruments, New Castle, DE, USA). Starch granules (10.0 mg, db) and distilled water (20 μL) were transferred to an aluminum pan, and the mixture was equilibrated at room temperature for 24 h. Sample pans were scanned from 20 to 180 °C at a heating rate of 10 °C/min, using an empty aluminum DSC pan as a reference.

2.9. Debranched starch chain profiles

Native, control, and citric acid treated starches were solubilized using the microwave dissolution method (Kim & Huber, 2010) with slight modification. Starch (50 mg, db) was dispersed in dimethyl sulfoxide (DMSO), vortexed mildly, and transferred to a 90 mL Teflon PFA (perfluoroalkoxy Teflon) jar (Savillex, Minnetonka, MN, USA), after which the mixture was irradiated for 35 s in a 2450 MHz microwave oven (Model AR732, Emerson Radio CO., North Bergen, NJ, USA). After cooling to room temperature, the resulting starch solution was precipitated by addition of absolute ethanol (25 mL), and collected by centrifugation (3000 × *g*, 20 min). Precipitated starch was washed with 85% (v/v) aqueous ethanol (25 mL), recovered by centrifugation (3000 × *g*, 20 min), and allowed to air dry (24 h).

Dried starch was dispersed in boiling water (9 mL), and stirred for 30 min in a boiling water bath to fully dissolve starch molecules. Starch solution was cooled to ambient temperature, after which 40 mM sodium acetate buffer (1 mL, pH 4.0, containing 0.2% sodium azide) was added to the mixture. For starch debranching, isoamylase solution (12.5 μL, 2.5 μL/10 mg starch) was added to the starch solution, which was then incubated at 40 °C for 24 h. The debranched starch solution was then boiled for 15 min to inactivate the isoamylase. To remove impurities (e.g., inactivated enzyme, salts, etc.), an ion-exchange resin (1 g, IONAC NM-60 H^+/OH^- form, J.T. Baker Phillipsburg, NJ, USA) was added to the debranched starch solution, after which the mixture was shaken for (1 min) on a Wrist Action Shaker (Model 75, Burrell Co., Pittsburgh, PA, USA). The purified debranched starch solution was filtered through a porous syringe filter (5.0 μm, National Scientific Co., Pittsburgh, PA, USA), and injected onto an intermediate-pressure size-exclusion chromatography (IPSEC) system. The IPSEC system (Waters Corp., Milford, MA, USA) consisted of a model 1525 binary HPLC pump, Rheodyne 7725i manual sample injector with a 200 μL loop, and a 2410 refractive index (RI) detector (operating at an internal temperature of 30 °C). Debranched starch chains were eluted at ambient temperature on two Tricorn 10/300 columns packed with Superdex 75 and 30 prep grade gels, respectively (Amersham Bioscience, Piscataway, NJ, USA). The mobile phase consisted of deionized water containing 0.02% sodium azide; flow rate for the IPSEC system was 0.4 mL/min.

Pullulan standards of differing molecular weights (M_w 47,300, 22,800, 11,800, and 5900; Shodex Standard P82, JM Science, Inc, NY, USA) were used to estimate the molecular weights of fractionated starch chains. A semi-logarithmic plot of the standard molecular weights versus K_{av} at the maximum RI index values for pullulan standards was used for molecular weight calculations.

2.10. Fourier transform infrared spectroscopy (FT-IR)

Absorbance spectra of starch granules were recorded on a Thermo Nicolet Avatar 370 spectrometer (Thermo Scientific, Waltham, MA, USA) using attenuated total reflectance (ATR) with a zinc selenide crystal as described by van Soest et al. (1995). Samples were analyzed directly after pressing starch powder on the crystal, while spectra were obtained at a resolution of 4 cm^{-1} following 64 scans.

2.11. Experimental design and statistical analysis

The experiment consisted of two full treatment replications, while individual replicate analyses of starch particles were conducted in at least duplicate. Experimental data were analyzed using Analysis of Variance (ANOVA), while Duncan's test was used to determine differences among experimental mean values ($p < 0.05$). All statistical analyses were conducted using SAS version 9.1 for Windows (SAS Institute, Cary, NC).

3. Results and discussion

3.1. Starch recovery and load-carrying capacity following citric acid treatment

For all citric acid treatment combinations evaluated, starch granule recoveries ranged from approximately 44% to 99%. Recovery of granular starch generally decreased with increasing citric acid concentration, treatment temperature, and/or length of treatment (Fig. 1A). However, ANOVA analysis of the main effects revealed that only treatment temperature and citric acid concentration significantly impacted starch recovery following treatment, whereas treatment time was not significant (Table 1). Nevertheless, significant two-way (temperature × citric acid concentration, temperature × treatment time) and three-way interactions were noted, indicating more complex relationships amongst the main effects. Data interaction plots revealed that starch recoveries at treatment temperatures of 40 or 50 °C were impacted very little by either citric acid concentration or treatment time. However, at a treatment temperature of 60 °C, increasing molar concentrations of citric acid and/or lengths of treatment led to much greater losses of starch material from granules (Fig. 1B and C). Thus, a treatment temperature of 60 °C greatly magnified the impact of citric acid concentration and treatment time, resulting in greatly reduced starch granule recoveries (Fig. 1). Ohishi et al. (2007) reported that addition of acetic acid (0.2 M) to granular rice starch increased both starch swelling power and solubility at temperatures above 60 °C, attributing these observed effects to accelerated water absorption of amylopectin chains within granules. Citric acid treatment ($3.6 < pH < 5.5$) of corn starch granules was further demonstrated to promote leaching of starch chains from granules during heating/gelatinization (Hirashima, Takahashi, & Nishinari, 2004). In our experiments, pH values of the 0.5, 1.0, and 1.5 M citric acid solutions used for treatments were 1.93, 1.71, and 1.56, respectively, which values were somewhat lower than those ($3.6 < pH < 5.5$) reported to promote starch leaching by Hirashima et al. (2004) (Above-noted citric acid concentrations reported in this cited paper exerted minimal impact on starch recoveries within our treatment scheme, and were consequently increased to achieved desired effects.). In short, it was hypothesized that a temperature of 60 °C (in combination with citric acid treatment) together promoted a higher degree of granule swelling, facilitating enhanced leaching and/or hydrolysis of starch chains, as well as relatively lower recoveries of granular starch following treatment.

Load-carrying capacities (LCC) of native (7.71 ± 0.08 mL/10 g starch), treatment control (subjected to treatment conditions without addition of citric acid), and citric acid-treated maize starches were assessed in an aqueous medium, with the general observation that citric acid treatment largely improved maize starch load-carrying attributes. The aqueous LCC of the treatment control did not significantly differ from that of the native starch at lower treatment temperatures (40 and 50 °C), though at a treatment temperature of 60 °C, a very slight LCC increase was observed for the treatment control (9.59–10.42 mL/10 g starch) relative to

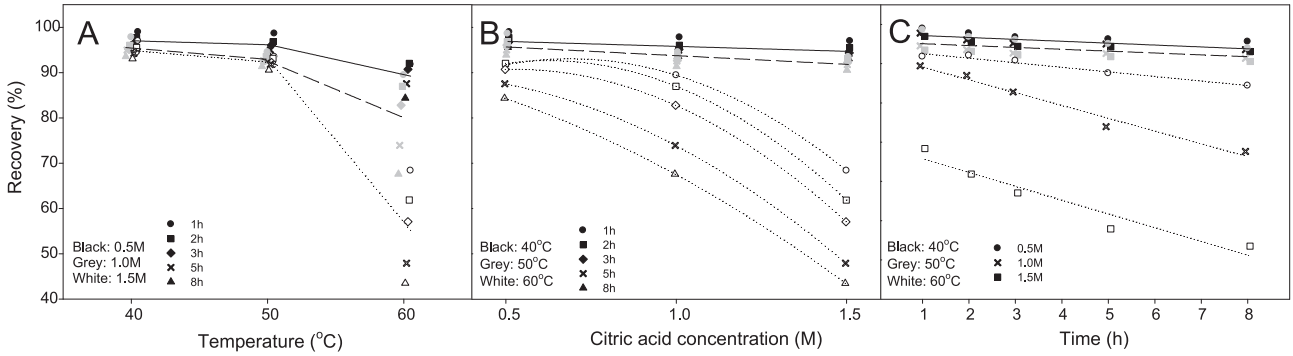

Fig. 1. Starch recovery following citric acid treatment plotted in relation to: (A) treatment temperature, (B) citric acid concentration and (C) treatment time.

that of native starch. Thus, heating of starch granules at 60 °C alone slightly increased LCC.

Based on ANOVA analysis of the experimental main effects (i.e., temperature, citric acid concentration, treatment time), only treatment temperature significantly impacted the load-carrying capacity of maize starch granules, though significant two-way interactions (temperature × citric acid concentration, temperature × treatment time) were also noted (Table 1). The presence of these interactions suggested that the impact of both citric acid concentration and treatment time varied according to treatment temperature. From data interaction plots, it was observed that the lowest treatment temperatures (40 or 50 °C) exhibited minimal impact on LCC, whereas a treatment temperature of 60 °C greatly enhanced LCC of starch granules, regardless of treatment time or citric acid concentration (Fig. 2A). For treatments conducted at 40 or 50 °C, LCC of starch granules gradually trended upward with increasing citric acid concentrations (Fig. 2B) or treatment times (Fig. 2C), whereas LCC of starch granules treated at 60 °C exhibited the reverse trend in both cases. Excessive treatment conditions (higher citric acid concentrations and/or longer treatment times) at 60 °C produced starch granule suspensions that became undesirably sticky when hydrated, adversely impacting LCC. Of all test parameters evaluated, a treatment temperature of 60 °C in combination with a 0.5 M citric acid concentration and a treatment period of 2 h yielded starch granules with the highest aqueous LCC (15.69 mL/10 g starch) (Fig. 2 C). From this point forward, particular experimental focus will be directed to this specific treated starch (60 °C, 0.5 M citric acid concentration, 2 h) due to its enhanced aqueous LCC.

Load-carrying capacities of select citric acid-treated starches were also assessed within an oil medium to differentiate effects associated with starch hydration/swelling from those strictly due to an internal volume increase within granules. Fig. 3 depicts non-aqueous LCC of maize starch granules treated with varied concentrations of citric acid (0.5, 1.0. or 1.5 M) at 60 °C for 2 h. In all cases, treated starches exhibited significantly higher oil absorption values than native maize starch. The citric acid-treated starch previously demonstrated to possess the highest aqueous LCC (15.69 mL/10 g starch; 60 °C, 0.5 M citric acid concentration, 2 h) exhibited a similar LCC in oil (14.48 mL/10 g starch), suggesting that LCC enhancement by citric acid treatment likely involved some degree of internal volume increase within granules. Nevertheless, treated starches exhibiting the highest LCC in water did not necessarily possess the greatest LCC in oil. Thus, it is possible that citric acid-treated starches subjected to the most severe treatment conditions (i.e., those that became sticky under hydrated conditions) might still have relevant application in non-aqueous systems.

3.2. Morphology of citric acid-treated starch granules

Surface structures of citric acid-treated maize starch granules were investigated via SEM to gain additional insight into their enhanced LCC. Fig. 4 illustrates citric acid-treated granules previously shown to possess the highest aqueous LCC (0.5 M citric acid concentration, 2 h treatment, 60 °C) in comparison to native maize starch granules. Citric acid-treated granules did not appear to be greatly altered at their granule surfaces relative to native maize starch granules, though in some cases, citric acid treatment did induce some granule deformation (i.e., inward collapse, shrinkage) or occasional development of an enlarged opening extending to the granule surface (i.e., circled feature depicted in Fig. 4). However, no consistent and obvious observations at the surfaces of granules consistently explained the enhanced LCC of these citric acid-treated maize starches.

Internal features of citric acid-treated maize starch granules were observed via light microscopy in both water (hydrated conditions) and immersion oil (non-hydrated conditions). Maize starch granules subjected to citric acid treatment at either 40 or 50 °C exhibited very minimal morphological changes when viewed in either water or oil media (Fig. 5A), which observation is consistent with the relatively high starch recoveries (≥90%) previously noted for these treatment conditions (Fig. 1). While granule structures of

Table 1
Analysis of variance and level of significance for the effects of treatment temperature, citric acid concentration, and treatment time on load-carrying capacity and recovery after citric acid treatment.

Factor	df	Load-carrying capacity			Recovery		
		Mean square	*F*-Value	*p*-Value	Mean square	*F*-Value	*p*-Value
Temp.	2	131.40	311.40	<0.0001*	1957.50	21.10	<0.0001*
Citric acid concentration (CA)	2	0.14	0.02	0.979	673.00	4.37	0.019*
Time	4	0.92	0.13	0.969	103.00	0.56	0.694
Temp. × CA	8	33.80	117.80	<0.0001*	872.10	37.50	<0.0001*
Temp. × time	14	19.30	57.70	<0.0001*	331.00	3.10	0.004*
Time × CA	14	0.37	0.04	1.000	128.00	0.64	0.812
Temp .× CA × time	44	87.00	1.10	0.416	324.10	164.60	<0.0001*

* Denotes statistical significance ($p < 0.05$).

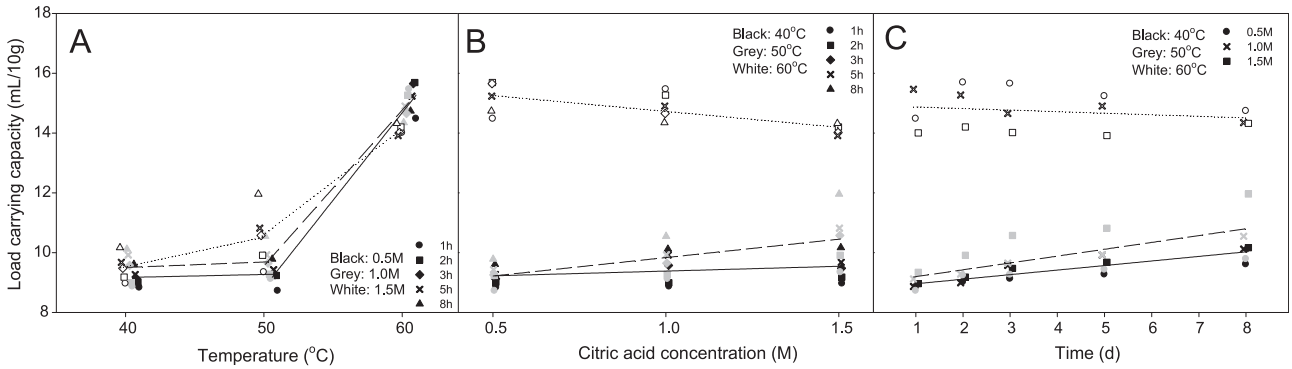

Fig. 2. Aqueous load-carrying capacity of the citric acid-treated starch granules plotted in relation to: (A) treatment temperature, (B) citric acid concentration and (C) treatment time.

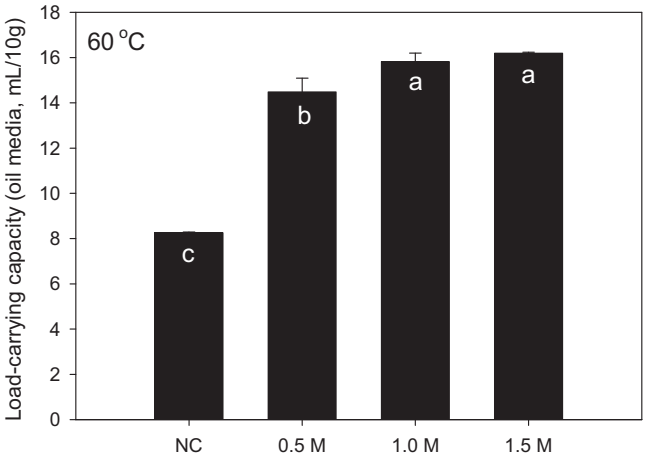

Fig. 3. Load-carrying capacity (oil medium) of select citric acid-treated (60 °C; 0.5, 1.0, or 1.5 M citric acid concentration; 2 h) starch granules relative to native normal maize starch (bars along the *x*-axis denoted by the same letter are not significantly different, $p < 0.05$).

treated starches primarily remained intact, some granules developed slight visible fissures/cracks, especially those treated for extended periods of time (8 h).

In contrast, citric acid treatment conducted at 60 °C caused much more significant morphological changes to starch granules (relative to those treated at 40 or 50 °C), particularly as the length of treatment (8 h, data not shown) and/or citric acid concentration (Fig. 5B) increased. Citric acid-treated granules previously shown to possess the highest aqueous LCC (60 °C, 0.5 M citric acid concentration, 2 h) retained both granular shape (though slightly swollen) and birefringence, while granules treated with higher concentrations of citric acid (1.0 or 1.5 M) at 60 °C became increasingly swollen and translucent (Fig. 5B), losing birefringence (data not shown) even after relatively short treatment times (2 h). These latter observations account for the reduced aqueous LCC, as well the observed stickiness of starch dispersions, for granules treated with the highest levels of citric acid (1.0 or 1.5 M) at 60 °C (Fig. 5B). Viewing these same starches in immersion oil (non-swelling conditions) allowed the central cavity regions within granules to be clearly observed (Huber & BeMiller, 1997). Increasing levels of citric acid (treatment conditions: 60 °C, 2 h) significantly increased the volume of internal cavity regions within granules (Fig. 5B), suggesting that leaching and/or hydrolysis of starch occurred preferentially at the hilum region of granules. The preferential loss of starch from inner granule regions is attributable to the hilum being the least organized region within the granule (Baker et al., 2001) and its direct connection to the extragranular environment via pores and channels (i.e., likely facilitate access of citric acid to the inner granular regions). The most severe treatment conditions (60 °C; citric acid concentrations greater than 0.5 M, treatment times in excess of 2 h) that resulted in excessive swelling and loss of granular order in aqueous systems (Fig. 5B; 1.0 and 1.5 M citric acid treatments) promoted enlargement of central cavity regions (without loss of granular structure) when suspended within a non-aqueous (i.e., oil) medium (Fig. 5B). Thus, the same treatment conditions that produced granules with poor aqueous LCC due to excessive hydration/swelling of granules (Fig. 2B, 60 °C temperature) actually improved oil LCC (Fig. 3) due to a retained granular structure (i.e., lack of excessive granule swelling).

Under starch non-swelling conditions (i.e., methanol), merbromin (fluorescent dye) has been shown to absorb at granular surfaces (including those of granule channels and interconnected

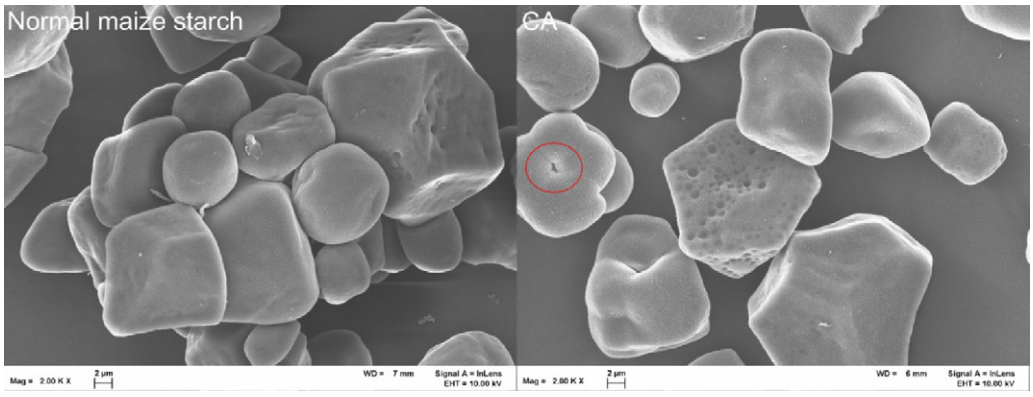

Fig. 4. SEM micrographs of citric acid-treated starch (CA; 60 °C, 0.5 M citric acid concentration, 2 h) relative to native normal maize starch.

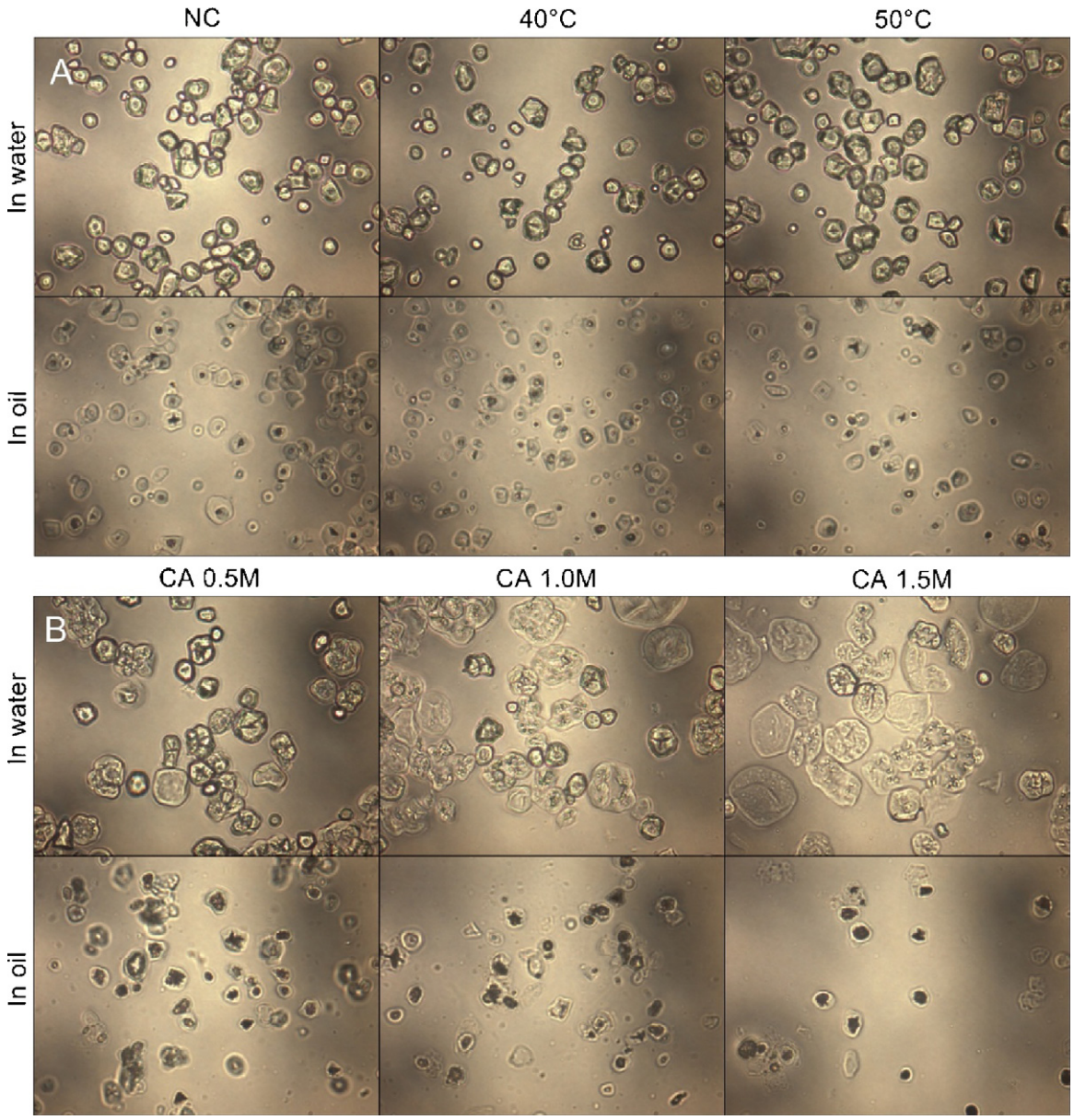

Fig. 5. Morphologies of citric acid-treated starch granules produced, (A) via a 0.5 M citric acid concentration at varied treatment temperatures (40 or 50 °C) for 8 h (relative to native maize starch (NC), and (B) under conditions of varied citric acid concentration (0.5, 1.0, or 1.5 M) at 60 °C for 2 h. All starches are depicted in both aqueous and non-aqueous (immersion oil) media (×400).

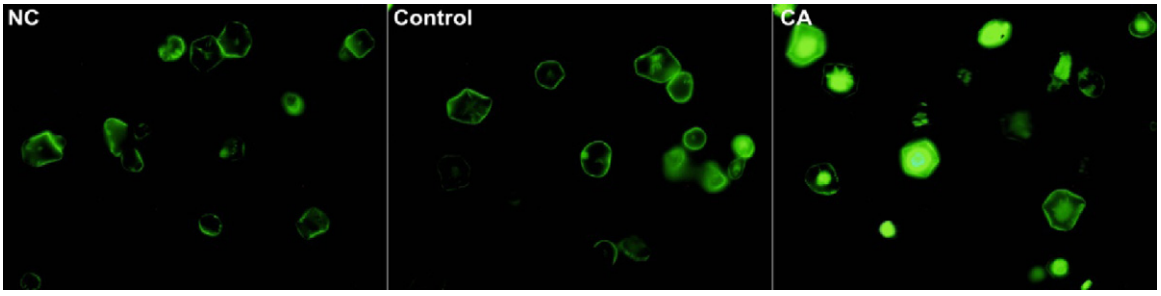

Fig. 6. Photomicrograph images of native maize (NC), treatment control (control), and citric acid-treated (CA; 60 °C, 0.5 M citric acid concentration, 2 h) starch granules treated with a methanolic solution of merbromin (fluorescent dye) to visualize internal granule porosities. Starches were mounted in immersion oil, and viewed by fluorescence microscopy.

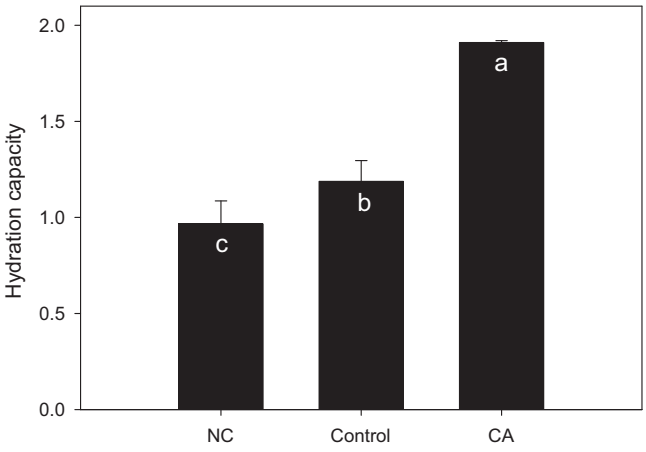

Fig. 7. Hydration capacities of native maize (NC), treatment control (control), and citric acid-treated (CA; 60 °C, 0.5 M citric acid concentration, 2 h) starches (bars along the *x*-axis denoted by the same letter are not significantly different, $p < 0.05$).

cavities) without penetrating the granule matrix, (Huber & BeMiller, 1997), providing a qualitative assessment of granule porosity. Both native maize starch granules, as well as those of the treatment control starch (subjected to treatment conditions, but without citric acid addition) exhibited fine channels and highlighted cavity regions (through fairly limited in size) (Fig. 6). These observations are consistent with the presence of pores/channels within native maize starch granules (Huber & BeMiller, 1997). While fluorescence intensity within the central cavity spaces of starch granules was increased by citric acid treatment (0.5 M citric acid concentration, 2 h treatment at 60 °C), channel structures within granules did not appear to be obviously enlarged by citric acid treatment. These observations corroborate light microscope evidence presented in the previous paragraph, suggesting that the increased LCC of citric acid-treated starches is due primarily to enlargement of cavity spaces in the hilum region of granules.

3.3. Physicochemical properties of citric acid-treated starch

Ambient temperature hydration capacities for native maize starch, treatment control (native maize starch subjected to conditions of citric acid treatment without citric acid addition), and citric acid-treated starches (60 °C, 0.5 M citric acid concentration, 2 h) are depicted in Fig. 7. The citric acid-treated starch exhibited nearly a two-fold higher hydration capacity than native maize starch, implying greater water absorption by the starch matrix – explaining in part the enhanced aqueous LCC of citric acid-treated starch granules. While the treatment control starch (60 °C treatment) also exhibited a slightly increased hydration capacity and LCC (9.59 mL/10 g starch) relative to that of native maize starch, the magnitude of the increase was much less than that observed with citric acid treatment (Fig. 7). While a 60 °C treatment temperature alone provided some enhancement of granule hydration, the enhanced hydration and aqueous load-carrying capacity of treated granules was only fully realized in the presence of citric acid, validating the primary, yet synergistic, role of citric acid (in conjunction with temperature treatment).

Thermal properties of native maize, treatment control (60 °C, 2 h), and citric acid-treated (60 °C; 0.5, 1.0, or 1.5 M citric acid treatment; 2 h) starches are compared in Table 2. Relative to native maize starch, the treatment control and all citric acid-treated starches exhibited slightly increased onset gelatinization temperatures, which effect was attributed to starch annealing during temperature treatment (60 °C). In addition, the gelatinization enthalpies of citric acid-treated starches also decreased in response to an increasing treatment citric acid concentration (Table 2), indicating that the native crystalline structure was partially disrupted by citric acid treatment in conjunction with heating (however, heat treatment alone did not lower gelatinization enthalpy). While annealing would be expected to decrease starch granule hydration and swelling due to crystallite perfection (Waduge, Hoover, Vasanthan, Gao, & Li, 2006), the partial disruption of the native granule crystalline structure (resulting in more amorphous starch) by addition of citric acid likely explains the increased hydration capacity of citric acid-treated starches. Higher concentrations of citric acid (1.0 and 1.5 M) in combination with 60 °C heating induced greater disruption of the crystalline structure, resulting in excessive granule swelling and starch leaching (Fig. 5B), both of which were likely responsible for the decreased aqueous LCC (as well as the perceived stickiness of the test dispersions) of these treated starches.

3.4. Molecular characterization of citric acid-treated starch

Debranched native maize starch consisted of three primary chain fractions defined according to chain-length (Table 3). The F-1 fraction comprised the largest chains, consisting largely of amylose, while fractions F-2 and F-3 comprised amylopectin long/intermediate (B1, B2, B3, etc.) and short (short B1 and A) chains, respectively. For the citric acid-treated starch previously shown to exhibit the greatest aqueous LCC (60 °C, 0.5 M citric acid concentration, 2 h), as well as the corresponding treatment control, the weight average chain-length (CL_w) of the F-1 fraction increased slightly relative to that of native maize starch, though the relative proportions of the F-1 fraction were not significantly altered by the treatments. In addition, the relative proportions of the F-2 fraction were slightly decreased (relative to that of the native maize starch), whereas neither the relative proportions nor the CL_w of the F-3 fraction were significantly changed by treatments (Table 3). It is significant to note that the treatment control starch, which was not subjected to citric acid treatment (but was subjected to 60 °C heating), exhibited similar (though less extensive) chain profile trends as the citric acid-treated starch. This observation, as well as the finding that the F-1 CL_w of the citric acid-treated and treatment control starches increased (rather than decreased, as might be expected for acid hydrolysis) after treatment, suggests that the relatively smaller starch F-1 (amylose) chains were leached from starch granules during the 60 °C heat treatment. Thus, citric acid treatment did not appear to induce significant decreases in chain-length profiles, but instead accelerated leaching of starch chains. Citric acid ($pH \leq 3.5$) was reported to hydrolyze starch chains at temperatures above gelatinization, leading to the fracture of starch granules (Hirashima et al., 2004). In our experiments, pH values of the 0.5, 1.0 and 1.5 M citric acid solutions (1.93, 1.71 and 1.56 respectively) were below those previously reported to induce hydrolysis of starch chains. However, hydrolysis of starch chains was minimal for the 0.5 M citric acid concentration (60 °C, 2 h) (Table 3), as well as the higher citric acid concentrations (1.0 and 1.5 M) (data not shown). Similarly, Shogren (2000) reported that treatment of starch granules in the presence of high concentrations of acetic acid (81–100%, w/v) at 180 °C failed to induce hydrolysis of starch chains. In short, citric acid treatment was observed to primarily promote leaching of starch chains from granules (preferentially from the hilum region of granules) rather than hydrolysis of starch chains. Though citric acid treatment (60 °C, 0.5 M citric acid concentration, 2 h) did not impart significant changes to starch molecular structure, it did induce structural changes to the crystalline regions of granules that promoted leaching of starch chains, enhancing the LCC of starch granules without significant loss of starch substrate (less than 8%).

At elevated temperatures (120–170 °C), citric acid is reported to react chemically with starch to yield starch citrate (via dehydration

Table 2
Thermal characteristics[a] of citric acid-treated starches (0.5, 1.0, or 1.5 M citric acid concentration; 60 °C; 2 h) relative to native normal maize and treatment control (normal maize starch subjected to conditions of citric acid treatment, without citric acid addition) starches.

Sample	Thermal transition temperature[b] (°C)			Gelatinization enthalpy, ΔH (J/g)
	T_o	T_p	T_c	
Normal maize starch	70.45 ± 0.68b	76.13 ± 0.88b	85.63 ± 0.31a	10.64 ± 0.56a
Treatment control	72.05 ± 0.84a	76.13 ± 0.43b	85.43 ± 0.52a	9.96 ± 0.37a
CA 0.5 M[c]	72.83 ± 0.15a	76.11 ± 0.38b	85.10 ± 0.25a	7.37 ± 0.51b
CA 1.0 M[c]	72.69 ± 0.11a	76.16 ± 0.18b	84.43 ± 0.15a	5.59 ± 0.21c
CA 1.5 M[c]	73.37 ± 0.35a	77.55 ± 0.42a	84.42 ± 0.73a	2.53 ± 0.29d

[a] Values within the same column sharing a common letter are not significantly different ($p < 0.05$).
[b] T_o, T_p and T_c are the onset, peak and conclusion gelatinization temperatures, respectively.
[c] CA = citric acid.

Table 3
Chain profiles of citric acid-treated starch relative to native normal maize and treatment control (normal maize starch subjected to condition of citric acid treatment, without citric acid addition) starches.

Sample		Fraction (F) of dextrin chains[a,b]		
		F-1	F-2	F-3
Normal maize starch	CL_w[c]	315.40 ± 0.04C	43.20 ± 0.90A	9.60 ± 0.19A
	%	25.71 ± 0.30a	16.82 ± 0.00a	57.47 ± 0.31a
Treatment Control	CL_w[c]	321.10 ± 0.73B	44.30 ± 1.53A	9.50 ± 0.03A
	%	24.13 ± 1.24a	16.32 ± 0.14b	59.55 ± 1.40a
Citric acid-treated starch[d]	CL_w[c]	328.80 ± 2.01A	44.40 ± 1.13A	9.45 ± 0.42A
	%	24.00 ± 0.91a	16.18 ± 0.20b	59.82 ± 1.11a

[a] Values within the same column sharing a common upper or lower letter are not significantly different ($p < 0.05$).
[b] Fractions are defined as follows: F-1 = amylose; F-2 = amylopectin long/intermediate chains; and F-3 = amylopectin short chains.
[c] CL_w = weight average chain length.
[d] Citric acid-treated starch using 0.5 M citric acid concentration at 60 °C for 2 h.

of citric acid to yield an anhydride, which may react with starch hydroxyl groups) (Jyothi, Moorthy, Sreekumar, & Rajasekharan, 2007; Ma, Jian, Chang, & Yu, 2008; Salam, Pawlak, Venditti, & El-tahlawy, 2010; Xie & Liu, 2004). Starch citrate derivatives possess an ester group that can be detected by the presence of a peak at 1738 cm^{-1} using FT-IR analysis (Ma et al., 2008). The absence of such a peak in FT-IR spectra of our citric acid treated starches, as well as their respective treatment controls (data not shown), suggests that the relatively lower treatment temperatures (40–60 °C) utilized in our experiments were not likely sufficient to induce dehydration of citric acid for chemical reaction with starch. Thus, the enhanced LCC of citric acid treated starches in this study were not likely derived from starch chemical substitution.

4. Conclusions

Treatment of maize starch granules with citric acid (0.5, 1.0, or 1.5 M) at subgelatinization temperatures (40–60 °C) for various lengths of time (0–8 h) successfully increased granule porosity and LCC (relative to native maize and treatment control starches). The enhanced LCC of citric acid-treated starch was primarily ascribed to enlargement of central cavity spaces within the hilum region of granules, as well as partial disruption/rearrangement of the granule crystalline structure (resulting in increased granule hydration/swelling properties). Enlargement of granule cavity spaces was primarily attributed to the leaching of starch material from granules (most likely through native pores and channels), with minimal, if any, evidence to support a starch hydrolysis or chemical substitution mechanism. Thus, citric acid-treated starches represent a unique, distinct starch product, and should not be considered equivalent to starches modified via mineral acid (i.e., HCl, H_2SO_4) treatment, which promotes hydrolysis of starch chains within granules.

A temperature of 60 °C was critical to the effectiveness of citric acid treatment, minimizing both the concentration of citric acid and the length of treatment required to enhance aqueous LCC. At this temperature, the highest concentrations of citric acid and the longest treatment times led to excessive swelling and loss of granular order, adversely affecting load-carrying capacity within an aqueous medium. Conversely, more severe treatment conditions generally enhanced starch load-carrying capacities in non-aqueous media (due to conditions favoring minimized granule swelling and retention of the granule structure). Relative to native maize starch, a treatment temperature of 60 °C, a citric acid concentration of 0.5 M, and a treatment time of 2 h improved LCC in aqueous and oil media by 2.0 and 1.75 fold, respectively. Under this treatment condition, the total recovery of granular starch following citric acid treatment was approximately 92%, suggesting that LCC can be significantly improved (almost doubled relative to native maize starch) by citric acid treatment without incurring excessive losses of starch material from granules.

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