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Preparation and characterization of gelatinized granular starches from aqueous ethanol treatments

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ABSTRACT

In order to modify the properties of native starch granules, the formation of gelatinized granular forms (GGS) from normal, waxy, and high amylose maize, as well as potato and tapioca starches was investigated by treating granules with aqueous ethanol at varying starch:water:ethanol ratios and then heating in a rotary evaporator to remove ethanol. The modified starches were characterized using bright field, polarized and electron microscopy. Short/long range molecular order and enthalpic transitions on heating were also studied using infrared spectroscopy, X-ray diffractometry and differential scanning calorimetry respectively. A diffuse birefringence pattern without Maltese cross was observed for most GGS samples. Treatment with aqueous ethanol resulted in starch-specific changes in the surface of granules, most noticeably swelling and disintegration in waxy maize, surface wrinkling in normal maize and tapioca, swelling and opening-up in potato starches, and swelling and bursting in high amylose maize. The ratio of ethanol to water at which original granular order was disrupted also varied with starch type. GGS had less short range molecular order than native granules as inferred by comparing 1047/1022 wave number ratio from infrared spectroscopy. Similarly, A- and B-type diffraction reflections were either reduced or completely lost with evolution of V-type patterns in GGS.

1. Introduction

Semi-crystalline native starch granules are water insoluble which often makes them undesirable for food, feed and industrial applications. To enhance water solubility, starches can be physically modified by drum drying of a granular slurry and grinding to fine powder to produce pre-gelatinized cold-water-soluble (CWS) starches. Due to the consequent loss of granular integrity, the resulting cold water dispersible starches are inferior in terms of pasting quality compared to freshly cooked starches (Rajagopalan & Seib, 1992a). To overcome this limitation, several technologies have been developed for producing CWS starch with granular integrity (granular cold water soluble (GCWS) starch) such as injection and nozzle-spray drying of starch slurry (Pitchon, O'Rourke, & Joseph, 1981), thermal treatment in aqueous alcohol at high temperature and pressure (Eastman, 1987; Eastman & Moore, 1984; Jane, Craig, Seib, & Hoseney, 1986a), alcoholic–alkaline treatment (Bello-Perez, Romero-Manilla, & Paredes-Lopez, 2000; Chen & Jane, 1994; Jane & Seib, 1991; Singh & Singh, 2003) and polyhydric alcohol treatment (Rajagopalan & Seib, 1991; Rajagopalan & Seib, 1992a). In common, during preparation of GCWS starches, starches are heated in the presence of water as a gelatinization inducer with other chemicals such as alcohols to inhibit the swelling of granules resulting in the formation of less (or non-) crystalline starch (gelatinized granular starch, GGS) with granular integrity. These starches contain elevated levels of V-type crystallinity due to complexation of amylose and small ligands such as alcohols (Jane et al., 1986a; Jane, Craig, Seib, & Hoseney, 1986b; Rajagopalan & Seib, 1992b). Alcoholic–alkaline treatment was found to give a better result for some waxy and high amylose starches, as waxy starches tend to gelatinize more quickly during aqueous alcohol treatment whereas strong alkali facilitates the swelling of high amylose starches (Jane & Seib, 1991). In a different approach, Wang, Zhai, and Zheng (2011) prepared crystalline GCWS starch by surface modification of corn starch with poly-ethylene glycol. Reviews of the preparation, food applications and morphology of CWS and GCWS starches can be found elsewhere (Jane, 1992; Yan & Zhengbiao, 2010). In the current paper, a simple and novel method is described for preparation of GGS in which granule slurries in aqueous ethanol are heated to evaporate ethanol. The effect on starch granules of changes in starch to water and ethanol ratios is studied, aiming to elucidate the morphological and supra-molecular changes accompanying formation of GGS for A- and B-type polymorphic starches. The microscopic morphology, short and long range molecular order and thermal

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properties of three forms of starches viz. native starch, GGS, and conventional gelatinized starch (GS) were further studied. The use of a simple production methodology, a wide range of starches, and comparison of GGS with native and gelatinized starches allows a detailed mechanistic understanding of GGS formation.

2. Material and methods

2.1. Materials

Starches, normal maize (MS), waxy maize (WMS), potato (PS), tapioca (TS) and high amylose maize (Gelose 50; G50) were provided by National Starch (Lane Cove, NSW, Australia) and were used as received. Compositional analysis (Dhital, Shrestha, Hasjim, & Gidley, 2011; Shrestha et al., 2012) showed apparent amylose contents by iodine colorimetry of 27.6% (MS), 1.2% (WMS), 36.7% (PS), 23.5% (TS), and 57.5% (G50). All starches contained less than 1% of protein and lipid and had moisture contents of 10–12% (w/w).

2.2. Methods

Starches (50 g, dry basis) were suspended in water at varying starch:water ratios (1:1, 1:2, 1:2.5, 1:3, 1:4, 1:5, 1:6, w/w) with the final weight adjusted to 500 g with addition of absolute ethanol, except for Gelose 50 where the starch:water ratio was 1:20 without addition of ethanol. The dispersion was transferred to a round bottomed flask and heated in a water bath set at 95 °C, at 40 rpm using a rotary evaporator (Buchi Rotavapor R-114, Switzerland) under ambient pressure until all ethanol was evaporated (confirmed by the absence of ethanol entering the collection flask of the evaporator). As soon as ethanol had ceased entering the collection flask, the collected ethanol was added to the starch slurry in the round-bottomed flask to prevent the formation of an aqueous starch gel. After cooling to room temperature, the starch slurry was filtered with filter paper (Whatman 22). The recovered cake was further dispersed in fresh 500 mL absolute ethanol and recovered by filtration as above. The residual starch was dried in a vacuum oven (Thermoline Scientific, NSW, Australia) at 40 °C overnight, finally ground and passed through a 125 μm sieve. The resulting starches contained 8–10% moisture (db) and were stored in a sealed container until further use. Gelatinized starches were prepared by boiling starch suspensions in water (1%, w/v) for 10 min on a hot plate, followed by evaporation to a film in a fan-forced oven at 150 °C to a moisture content of 5–8% (dry basis), and grinding through a 150 μm screen.

Surface morphology of native, GGS and GS was observed using a light microscope (Olympus BX61, Olympus Optical Co. Ltd., Tokyo, Japan) operating in bright field and polarization modes and a scanning electron microscope (JSM 6300, JEOL Ltd., Tokyo, Japan). For light microscopy, 40 μL of starch suspension (1%, w/v) in water was placed on the glass slide and covered with a cover slip before observing through a 60× objective lens except for PS which was observed through a 20× objective lens. For electron microscopy, starch powders were sprayed on circular metal stubs previously covered with double-sided adhesive and platinum coated at 5 nm thickness using an Eiko Sputter Coater (Eiko Engineering Co. Ltd., Tokyo, Japan) under vacuum, with observation through the microscope at 5 kV accelerating voltage.

Infra-red spectra were obtained using a FTIR 100 series Perkin-Elmer spectrometer fitted with a DTGS (deuterated triglycine sulphate) detector using the Universal attenuated total reflectance (ATR) single reflectance cell with a diamond crystal. For each spectrum, 32 scans were recorded at room temperature (about 22 °C) at a resolution of 4 cm^{-1}, co-added and Fourier transformed. An aliquot of sample was placed over the diamond crystal and pressed gently with a small steel cylinder until an optimum force of 120 (machine arbitrary scale) was reached and the spectrum recorded over the range 1200–800 cm^{-1}. The background spectrum was recorded in air (without samples) and subtracted from sample spectra. All spectra were baseline-corrected using the in-built programme in the Spectrum FTIR 100 system. The ratio of absorbance at wave numbers 1047/1022 was calculated as an index of ordered short range starch structures (vanSoest, Tournois, deWit, & Vliegenthart, 1995).

In order to study the long-range molecular order, X-ray diffraction patterns of native, GGS and GS samples were recorded on a PANalytical X'Pert Pro diffractometer (PANalytical, Almelo, The Netherlands). The instrument was equipped with a Cu long fine focus tube, programmable incident beam divergence slit and diffracted beam scatter slit (both fixed at 0.125°), and an X'celerator high speed detector. The samples were examined over the angular range of 4–60 degrees 2θ with a step size of 0.03° and a total count time of 800 s.

The thermal characteristics of the samples were studied using a differential scanning calorimeter (DSC 1, Mettler Toledo, Greifensee, Switzerland). Each sample (4 ± 0.1 mg) was mixed with (12 ± 0.3 mg) deionized water in a DSC pan (aluminium-low pressure, 40 μL), which was then hermetically sealed. The pans were held at 10 °C for 5 min and then heated to 100 °C at 5 °C/min. The onset (T_o), peak (T_p), and conclusion temperatures (T_c), and the enthalpy of gelatinization (ΔH) were determined using the built-in software (STARe System, Mettler Toledo, Greifensee, Switzerland).

3. Results

A simple methodology for preparation of GGS was developed using rotary evaporation to facilitate the uniform mixing of the starch dispersion in aqueous ethanol without using harsh chemicals such as NaOH. This method was found to be suitable for generating GGS from diverse starches. For starches such as MS, PS, and TS, a starch:water:ethanol ratio of 1:3:6 was found to be the most appropriate of those tested, whereas for waxy and high amylose starch:water:ethanol ratios of 1:2.5:6.5 and 1:20:0 respectively gave the best results. Due to rapid swelling and gelling during heating, preparation of GGS from WMS was difficult compared to other starches whereas addition of any ethanol inhibited the formation of GGS in G50.

3.1. Morphological studies

Observed by light and electron microscopy, native MS appeared to be round to polygonal in shape with a distinct Maltese cross in each granule under cross polarization. The morphological changes to MS at varying starch:water:ethanol ratios from 1:1:8 to 1:5:4 are shown in Fig. 1. Swelling of granules was positively correlated with the amount of added water. At a starch:water:ethanol ratio of 1:1:8, granules started to burst from the hilum region (dark area in the centre of most of the granules in Fig. 1B1) leading to substantial wrinkling on the surface, with a slight increase in the granule size at starch:water:ethanol ratio of 1:3:6 (Fig. 1D2). Under the polarized microscope, the majority of granules were non-birefringent (Fig. 1D3). The further increase in water amount caused deformation of granules and the agglomeration of granule fragments at starch:water:ethanol ratio of 1:5:4 (Fig. 1F2).

Potato starch showed similar morphological behavior to that of MS during aqueous-alcohol treatment at varying starch:water:ethanol ratios (Fig. 2). The smooth, round to oval shaped, PS granules with distinct birefringence pattern started bursting from the inside towards the outside on changing the starch:water:alcohol ratios from 1:1:8 to 1:2:7 associated

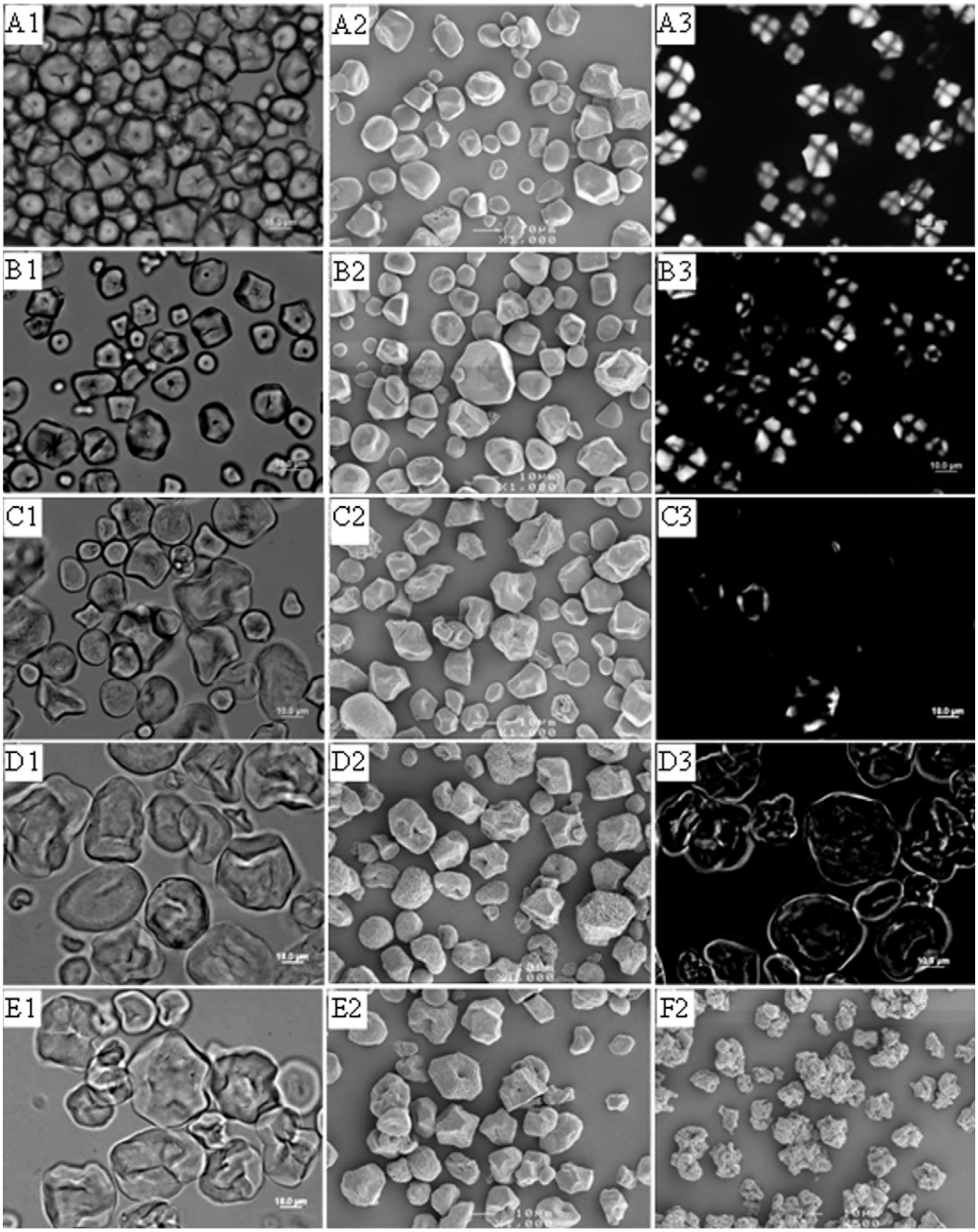

Fig. 1. Optical and electron micrographs of MS and GGS samples produced with different starch:water ratios. Native starch (A), GGS at starch:water ratio – 1:1 (B), 1:2 (C), 1:3 (D), 1:4 (E), 1:5 (F). 1, 2 and 3 after (A)–(E) refer to bright field, electron and polarized light micrographs respectively.

with a slight increase in size of granules (Fig. 2B1 and C1). At starch:water:alcohol ratio of 1:3:6 (Fig. 2D1), the majority of granules were opened up without a distinct Maltese cross. However, a diffuse birefringence pattern was observed in the outer part of granules (Fig. 2D3). The majority of granules were swollen with a hollow centre at starch:water:alcohol ratio of 1:4:5 (Fig. 2E2) and were disintegrated at a starch:water:alcohol ratio of 1:5:4 (Fig. 2F2).

Morphological changes were also observed for WMS, TS and G50 following aqueous alcohol treatment. Round to polygonal shaped WMS granules enlarged slightly with loss of granular integrity and Maltese crosses in some granules (Fig. 3A4–A6) at a starch:water:ethanol ratio of 1:2.5:6.5. However, almost all granules were disintegrated with loss of birefringence pattern (micrographs not shown) at starch:water:ethanol ratio of 1:3:6. Thus, there was no water to ethanol ratio that allowed full conversion of WMS to a swollen granule form. Smooth and disk shaped tapioca starch granules (Fig. 3B1 and B2) swelled (Fig. 3B4) with a wrinkled surface (Fig. 3B5) and diffuse birefringence around the granule surface (Fig. 3B6) at starch:water:ethanol ratio of 1:3:6. G50 had a more irregular granule shape compared to other starches used in the experiment. At a starch:water ratio of 1:20 (no ethanol), most of the granules swell in water (Fig. 3C4) with loss of birefringence pattern (Fig. 3C6). Under electron microscopy, some of

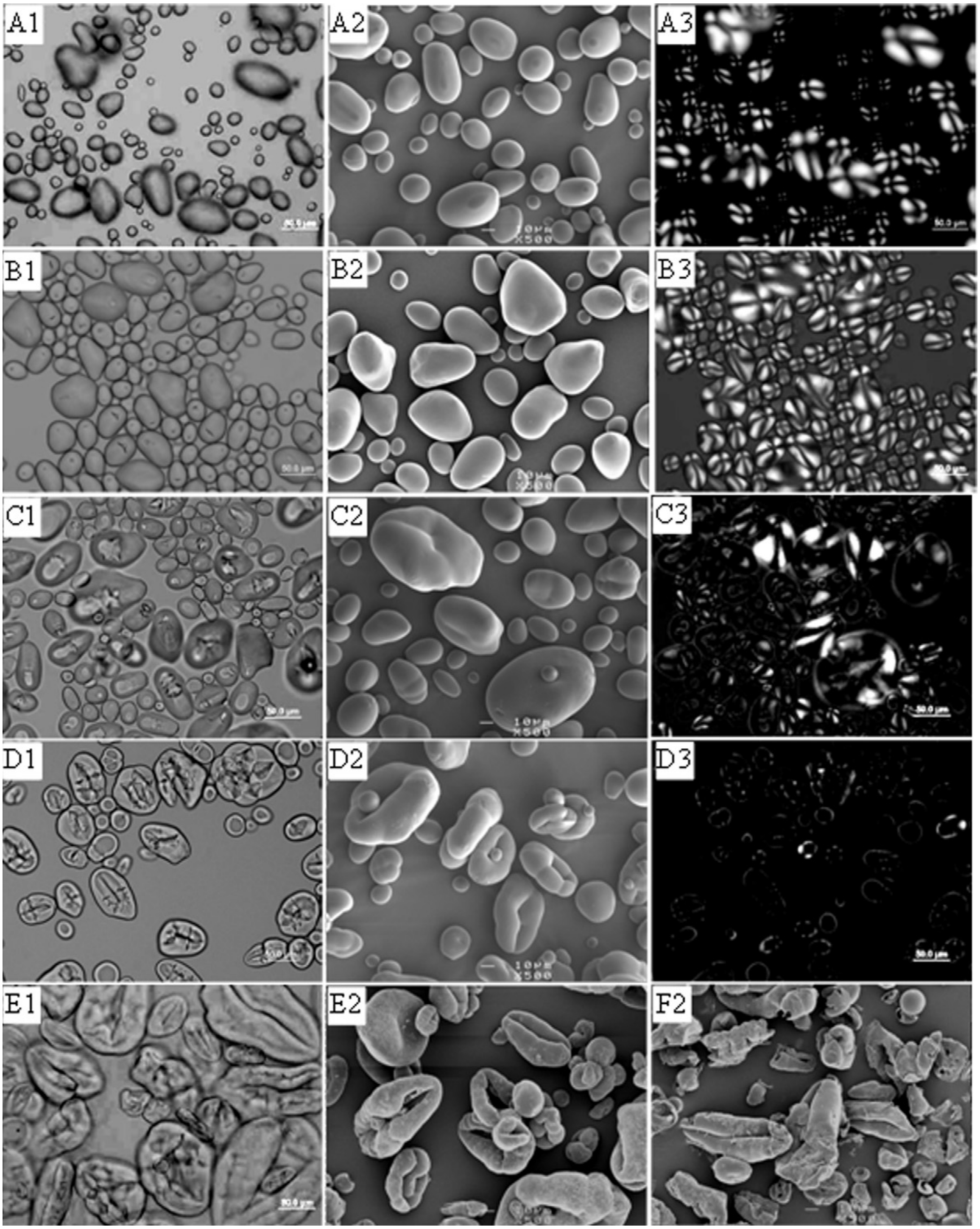

Fig. 2. Optical and electron micrographs of potato starch and GGS samples produced with different starch:water ratio. Native starch (A), GGS at starch:water ratio – 1:1 (B), 1:2 (C), 1:3 (D), 1:4 (E), 1:5 (F). 1, 2 and 3 after (A)–(E) refer to bright field, electron and polarized light micrographs respectively.

the granules 'burst' from the surface whereas the majority of other granules were intact with a slightly roughened surface (Fig. 3C5) and with fine particles adhering to the surface.

3.2. Short-range molecular order

The infra-red spectra of native, GGS with varying starch:water:ethanol ratio, and GS were recorded and bands at 1047 and 1022 cm^{-1} associated with ordered (organized) and amorphous (less organized) structures are shown in Table 1. As expected, the 1047/1022 wave number ratio followed the order native starch > GGS > GS for all starches, and decreased with the starch:water ratio, further signifying the loss of molecular order at short range scale.

3.3. Long-range molecular order

The X-ray diffraction pattern of native starches, GGS (produced from different starch:water:ethanol ratios), and GS are shown in Fig. 4. As expected, MS, WMS and TS exhibited the A-type

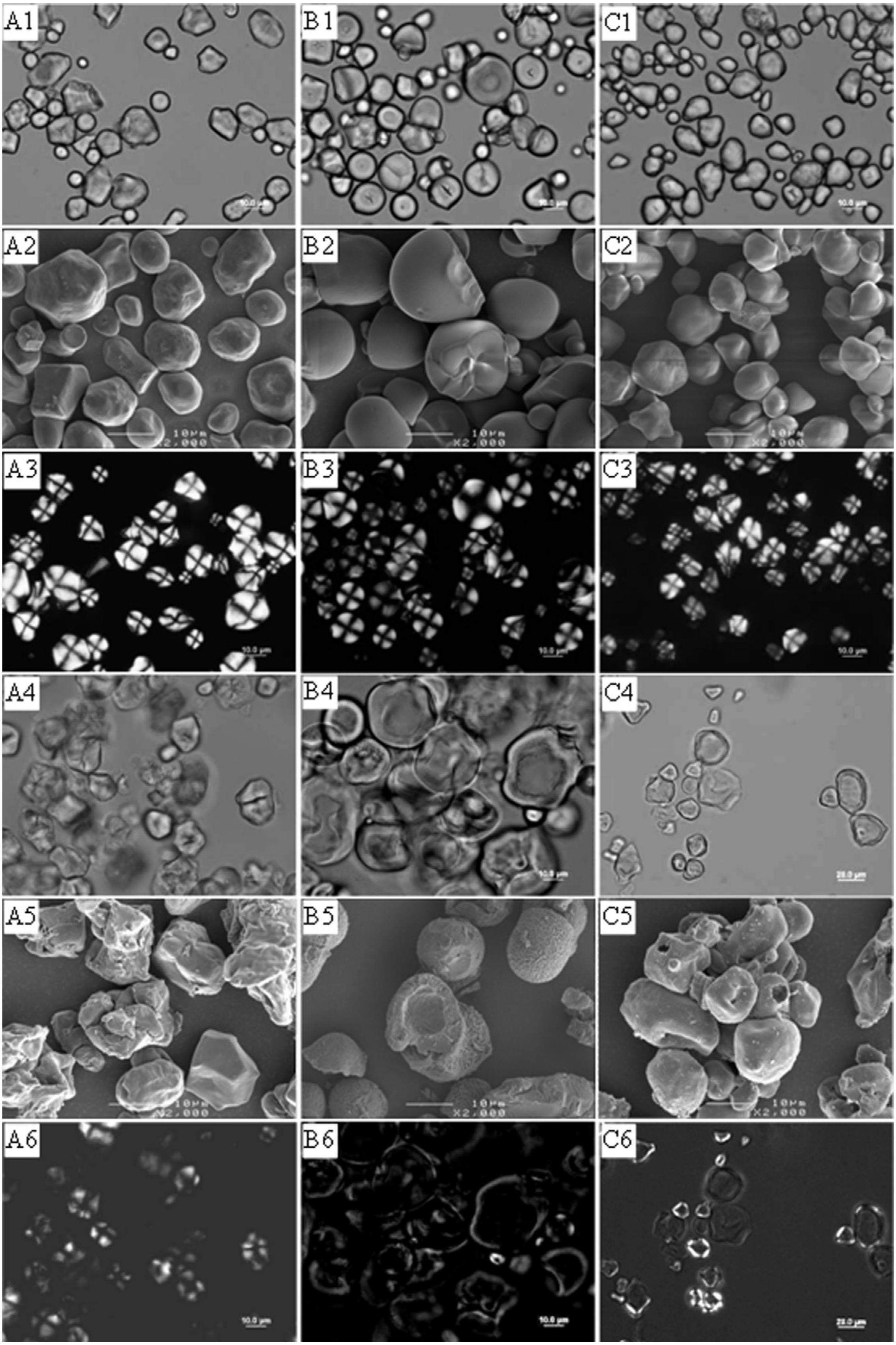

Fig. 3. Optical and electron micrographs of native and GGS starches. WMS (A), TS (B), G50 (C). Numbers 1, 2 and 3 refer to bright field, electron and polarized light micrographs of native starches. Numbers 4, 5, and 6 refer to bright field, electron and polarized light micrographs of GGS produced at starch:water ratio of 1:2.5, 1:3 and 1:20 for WMS, MS and G50 respectively.

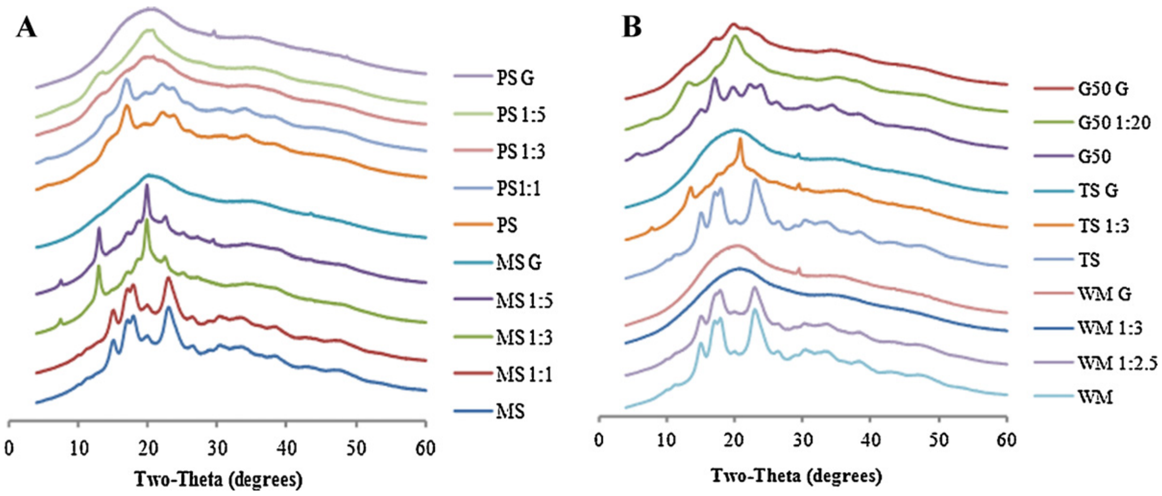

Fig. 4. X-ray diffraction patterns of native, GGS (produced at varying starch:water:ethanol ratios) and gelatinized starches. MS and PS (A), WMS, TS and G50 (B).

Table 1
FTIR 1047/1022 wave number ratio of granular gelatinized starches produced at varying starch:water ratios (ethanol added to make starch concentration 10%).

	MS	PS	WMS	TS	G50
Native	0.65	0.65	0.67	0.66	0.64
1:1	0.64	0.65	N/D	N/D	N/D
1:2	0.62	0.64	N/D	N/D	N/D
1:2.5	N/D	N/D	0.66	N/D	N/D
1:3	0.61	0.63	0.65	0.63	N/D
1:5	0.60	0.60	N/D	N/D	N/D
1:20	N/D	N/D	N/D	N/D	0.59
Gelatinized	0.58	0.59	0.60	0.59	0.58

ND, not determined.

diffraction pattern with strong reflections at about 15 and 23° 2θ, and an unresolved doublet at 17 and 18° 2θ, whereas high amylose maize starch (G50) and PS showed the strong diffraction peak at 17° 2θ and smaller peaks around 5, 22 and 24° 2θ, characteristic of a B-type diffraction pattern (Zobel, 1964).

For MS, the A-type pattern was similar at a starch:water:ethanol ratio of 1:1:8. At increased water content, e.g. starch:water:ethanol ratio of 1:3:6, the A-type pattern was more diffuse and new peaks evolved at 7, 13 and 20° 2θ characteristic of V-type crystallinity (Zobel, 1964). The gelatinized MS had a broad peak showing lack of crystalline order (Fig. 4A). Similarly, for PS, B-type crystallinity was lost at starch:water:ethanol ratio of 1:3:6. However compared to MS, V-type crystallinity was less prominent for PS. The gelatinized PS, similar to MS, only showed a shallow hump without distinct reflections (Fig. 4A). Similarly, the intensity of A-type reflections decreased at a starch:water:ethanol ratio of 1:2.5:6.5 for WMS and further increasing the water content to a starch:water:ethanol ratio of 1:3:6 resulted in broad peaks resembling those of gelatinized WMS. V-type crystallinity was absent in gelatinized granular WMS. On the other hand, for TS at starch:water:ethanol ratios of 1:3:6, all A-type reflections were lost with evolution of V-type reflections similar to that of MS. The B-type reflections of G50 were lost at starch:water ratio of 1:20 (no ethanol) with evolution of broad V-type peaks at 7, 13 and 20° 2θ (Fig. 4B). In contrast to other gelatinized starches, the 'gelatinized' G50 showed minor B-type peaks at 17 and 22° 2θ suggesting that not all native granule order had been lost.

3.4. Thermal properties

DSC thermograms of native starches and their corresponding GGS produced at varying starch:water:ethanol ratios are presented in Fig. 5 and measured values given in Table 2. MS showed a sharp melting peak with onset and conclusion temperature at around 63 and 72 °C respectively. As expected, the intensity of the subsequent melting endotherm for MS GGS reduced as water

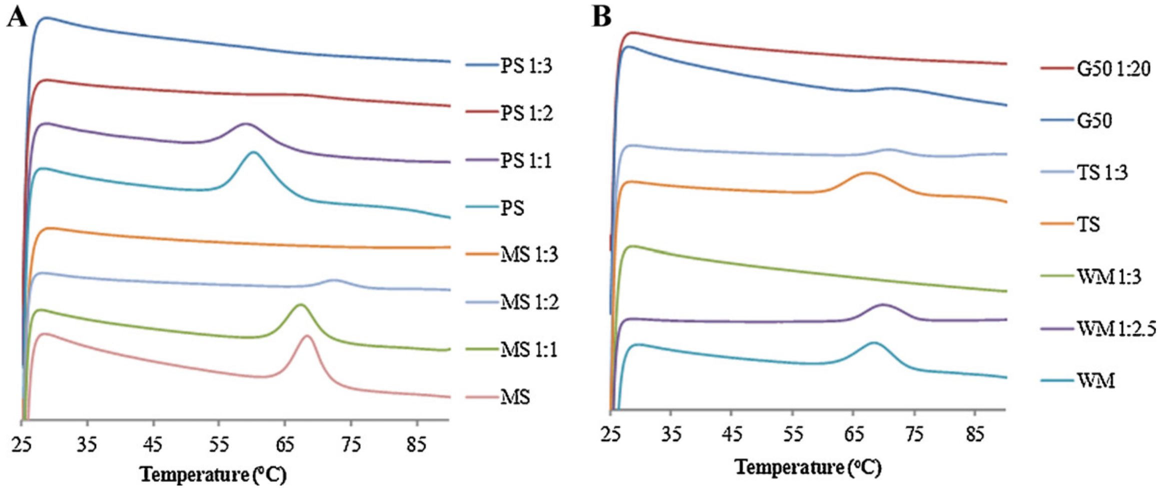

Fig. 5. DSC thermograms of native, GGS (produced at varying starch:water:ethanol ratios) and gelatinized starches. MS and PS (A), WMS, TS and G50 (B).

Table 2
Gelatinization parameter of native and GGS starches produced at varying starch:water ratios (ethanol added to make 10% total starch concentration).

Sample	Onset (°C)	Peak (°C)	End (°C)	ΔH (J/g)
MS	63.0 ± 2.2	67.6 ± 0.8	71.6 ± 0.2	11.7
MS 1:1	61.3 ± 0.7	67.2 ± 0.1	71.4 ± 0.6	10.3
MS 1:2	69.4 ± 1.1	72.9 ± 0.4	77.3 ± 0.2	2.0
PS	53.8 ± 1	59.3 ± 0.2	65.7 ± 0.3	10.1
PS 1:1	55.5 ± 1.2	60.2 ± 0.4	66.2 ± 0.6	3.6
PS 1:2	59.4 ± 0.7	67.6 ± 0.1	75.5 ± 1.2	1.9
WMS	62.3 ± 1.6	68.5 ± 0.0	74.1 ± 0.0	13.0
WMS 1:2.5	64.5 ± 0.2	69.8 ± 0.2	76.2 ± 0.1	8.5
TS	61.2 ± 0.8	67.8 ± 0.5	75.1 ± 0.4	15.6
TS 1:3	65.5 ± 0.7	71 ± 0.3	75.5 ± 0.5	1.7

content during preparation increased (Fig. 5A and Table 2) to starch:water:ethanol ratios of 1:2:7, and the thermogram was flat without any detectable melting enthalpy at starch:water:ethanol ratio of 1:3:6. The gelatinized MS also showed a similar featureless thermogram (data not shown). In contrast to MS, the gelatinization enthalpy of PS (10.1 J/g) was reduced significantly for GGS produced at a starch:water:ethanol ratio of 1:1:8 (3.6 J/g). The enthalpy value decreased to 1.9 J/g on increasing water content to a starch:water:ethanol ratio of 1:2:7 and the melting endotherm was totally absent at a starch:water:ethanol ratio of 1:3:6. For MS and PS, the gelatinization onset and conclusion temperatures of GGS at starch:water:ethanol ratios of 1:1:8 were similar to native starches, however on further increase of water at starch:water:ethanol ratios of 1:2:7 and 1:3:6, the gelatinization temperatures shifted to higher values. A similar pattern of decrease in gelatinization enthalpy, and slight increase in onset and conclusion temperature was also observed for WMS and TS at starch:water:ethanol ratio of 1:2.5:6.5 and 1:3:6 respectively. For G50, the conclusion temperature was not attained, however, the GGS of G50 produced at starch:water ratio of 1:20 showed a flat thermogram without any distinct gelatinization peak.

4. Discussion

The differing effects of water (a swelling agent) and ethanol (a precipitation and/or complexation agent) on starch allow for a balance to be struck in the preparation of (partially) gelatinized but still granular starches. This is conceptually the same balance as found for gelling biopolymers dispersed in an amorphous particulate form into a gelling solution (Burey, Bhandari, Howes, & Gidley, 2009), where the relative rates of solubilization/swelling and gelation determine whether the particles dissolve or form expanded particles. In the case of starches heated in the presence of aqueous alcohol, it is clear from the current and previous results (Jane et al., 1986a) that amylose is required for the complete formation of stable swollen granule particles (GGS or GCWS). The observation of V-type crystallinity in GGS suggests that single helical structures are important in maintaining granular integrity. However, the observation of V-type crystallinity for G50 GGS produced in the absence of ethanol (Fig. 4B) shows that the presence of ethanol during the formation of GGS is not critical for V-type helix formation. The novelty in the current treatment is to control granule swelling through concurrent ethanol evaporation (boiling point 82 °C) and starch gelatinization brought about by heating to 95 °C. At high water contents, swelling is unrestrained leading to break-up of granular structure, whereas at high ethanol contents, swelling is restricted so much that gelatinization is incomplete. A particular practical benefit of the current method is that the ethanol evaporated during preparation can be recovered and re-used.

Treatment with aqueous ethanol brought specific surface changes in all varieties of starch used in the study, most noticeably swelling in WMS, surface wrinkling in MS and TS, and swelling and opening-up in PS. The variety-specific surface changes are presumably due to the difference in molecular (amylose and amylopectin) and supra-molecular (packing of amylose and amylopectin) structure of these starches. Waxy maize was able to retain granular integrity at a starch:water:ethanol ratio of 1:2.5:6.5 (Fig. 3A5). At a starch:water:ethanol ratio to 1:3:6, granules were highly swollen with loss of birefringence pattern. However for other starches (NM, PS and TS) granular integrity was well conserved at a starch:water:ethanol ratio of 1:3:6 (Figs. 1D2, 2D2 and 3B5). Rapid swelling and loss of granule integrity in commercially prepared (cooking and drying of an aqueous starch slurry on a hot roll) GCWS has been previously reported (Jane et al., 1986a), whereas those prepared by alcoholic alkaline treatment (Chen & Jane, 1994) displayed highly swollen but intact granules. Amylose is proposed to hold the amylopectin molecules together during the thermal transition consequently preserving granular integrity (Jane et al., 1986a). Thus due to lack of amylose, waxy starches are fragile and tend to disperse during processing at high temperature (Debet & Gidley, 2007). Maize (Fig. 1D2) and tapioca (Fig. 3B5) GGS have wrinkled surfaces with the shape and size of the granules similar to that of raw starches. Surface wrinkling is most likely due to concurrent swelling and shrinkage driven by water and ethanol respectively, with water inducing and alcohol inhibiting the further swelling of granules either by decreasing the effective water concentration or by complexing with dissociated starch chains (Chen & Jane, 1994). In contrast to the present aqueous ethanol treatment, during alcoholic-alkaline treatment, MS tends to swell with indention on granule surfaces (Chen & Jane, 1994) whereas TS is swollen with internal fragmentation (Yan & Zhengbiao, 2010). Larger granules of PS were swollen and opened-up, however small granules were apparently less affected by aqueous-alcohol treatment (Fig. 2C2–E2) as previously reported for alcoholic–alkaline treatment (Chen & Jane, 1994; Singh & Singh, 2003). This variation with granule size might be due to greater contact of alcohol with the larger available surface area of small granules to counteract the swelling of granules, but it should also be noted that both molecular compositions and polymer molecular sizes vary with potato granule size (Dhital et al., 2011). In G50, the granules were swollen to a limited extent with numerous granules showing burst-holes on the surface (Fig. 3C5). Such burst holes however were not observed by Chen and Jane (1994) during preparation of GCWS from alcoholic–alkaline treatment of high amylose starch (HAMS). The longer branches in the amylopectin of HAMS are expected to enable firmer association of the amylopectin molecules with each other and/or with amylose, thereby increasing resistance of the granule to swelling and gelatinization (Montgomery, Sexon, & Senti, 1961). Adherence of small particles on the granule surface was observed for GGS of PS (Fig. 2E2 and F2) and G50 (Fig. 3C5). These micro-particles are most probably surface fragments dislodged from granules or molecules leached during heating in aqueous ethanol, and re-precipitated by ethanol. A similar phenomenon was also reported by Chen and Jane (1994) during preparation of GCWS from alcoholic–alkaline treatment, where 0.01, 0.4 and 1.9% starch was leached out in supernatants for waxy, normal and high amylose maize respectively.

All native starches showed a strong Maltese cross when observed with cross polarizers, characteristic of a high degree of radial molecular order. This radial orientation is lost in the majority of GGS granules. WM GGS at starch:water:ethanol ratios of 1:2.5:6.5 (Fig. 3A6) showed a few granules with Maltese crosses, however for the rest of the starches, diffuse birefringence as previously reported (Rajagopalan & Seib, 1992b) without distinct Maltese crosses was observed. The birefringence, mostly at the outer granule surface (Figs. 1D3, 2D3 and 3B6), signifies some molecular order, possibly V-type ordered amylose molecules. The gelatinized starches however were deprived of any Maltese cross consistent with more complete disruption of molecular order. GGS prepared from aqueous alcohol treatment was thus found to be intermediate between native and gelatinized starch in regards to molecular order.

Intensity ratios at wave number 1047 and 1022 cm^{-1} of FTIR spectra were used as an index to characterize the short range alignment of helices (vanSoest et al., 1995). The ATR-IR technique can give spectral information up to 2 μm in depth of starch granules representing several growth rings (Sevenou, Hill, Farhat, & Mitchell, 2002). The peak at 1047 cm^{-1}, which was distinct for raw starches, was broadened for GGS and GS (FTIR spectra not shown). Similarly, the 1022 peak which was indistinct for raw starches was sharpened for GGS and GS, again consistent with disordering of the granules.

When starch granules are heated in aqueous ethanol, ethanol not only restricts the swelling of granules due to a dehydration effect, but can also form complexes with amylose (single helical conformation) generating a V-type diffraction pattern. Due to the low amount of amylose, no V-type pattern was observed for WMS GGS (Fig. 4B). However, Jane et al. (1986a) concluded that as well as amylose, amylopectin complexes with aliphatic alcohols also contribute to V-type diffraction, as the intensity of V-type crystallinity (ca 50%) for maize starch (27% amylose) was too great to be due solely to amylose. Similarly, the present results (Fig. 4A) show strong V-type crystallinity for MS GGS. Although the V-type pattern appears not to be formed in the absence of amylose, once formed with amylose, it appears that amylopectin chains can also adopt the same conformation. Weaker V-type patterns were observed for B-polymorphic starches (PS and G50) compared to A-polymorphic starches (MS and TS) (Fig. 4). For, G50 the broad V-type pattern was developed during heating in the absence of ethanol, although ethanol was used during the recovery of GGS. The weaker V-type diffraction in PS compared to MS is proposed to be a consequence of differences in packing of amylose and amylopectin molecules in starch granules. Amylose and amylopectin are largely separated in corn starch granules while in potato starch they are more intimately associated (Zobel, 1984). Thus, segregated amylose in MS is more prone to form longer-range lateral assemblies of helical complexes required for more perfect crystals (and sharper X-ray diffraction peaks) than the more tightly bound amylose in PS. As a result, the V-complex in gelatinized granular potato starch has an insufficient degree of long range order to generate a sharp X-ray diffraction pattern.

The changes in the crystal structure as shown by the X-ray diffraction studies are consistent with enthalpic transitions in DSC thermograms. In parallel with the reduction in A- and B-type crystallinity of MS and PS with increase in starch:water ratio (Fig. 4A), the gelatinization enthalpy (ΔH) was lowered significantly at higher water concentration e.g. starch:water ratio of 1:3 (Fig. 5A). The melting of V-type structure was not observed on the endotherms as it occurs at higher temperature, e.g. 127 °C (Jane et al., 1986a), beyond the range of the present DSC protocol. Similar decreases in gelatinization enthalpy were also observed for other starches e.g. WMS, TS and G50 during aqueous ethanol treatment (Fig. 5B). The shift of gelatinization temperature for all starches reflects an annealing effect where the gradual melting of helical structure is also associated with perfection of the remaining helices.

5. Conclusion

GGS were prepared by aqueous-ethanol treatment with varying starch:water:ethanol ratios for A-type (MS, WMS, TS) and B-type starches (PS, G50). Starch:water:ethanol ratio of 1:3:6 provided the optimum GGS for MS, PS and TS whereas starch:water:ethanol ratios of 1:2.5:6.5 and 1:20:0 were suitable for WMS and G50. GGS lacked the distinct birefringence pattern of native granules as shown by polarized light microscopy. Though GGS had lesser short and long range molecular order compared to native starches, the granules were still intact when observed through electron microscopy. Thus, aqueous-ethanol treatment with evaporation of ethanol under ambient pressure could be a simple and practical method for modification of starches with granular integrity.

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