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Preparation of aqueous alpha-lipoic acid dispersions with octenylsuccinylated high amylose starch

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ABSTRACT

Aqueous dispersions prepared with OSA-modified high amylose starch were investigated in comparison with native high amylose starch and beta-cyclodextrin using alpha-lipoic acid as a model substance. Alpha-lipoic acid (ALA), a lipophilic antioxidant essential for energy metabolism in human, was dispersed in gelatinized starch solutions (1.0% w/v) at different temperatures (50–90 °C) and times (3–12 h). High amylose starch modified with 3% OSA (dry starch base) was most favored in maximizing the dispersibility of ALA (84% recovery) under mild heating (70 °C for 3 h). The optimally prepared dispersion was milky white and contained particles with a narrow size distribution (200–300 nm). The precipitate isolated from the dispersion contained crystalline V-complexes of ALA and amylose while the supernatant contained free ALA accounting for 1/3 of total ALA, indicating OSA-modified high amylose starch stabilized ALA either by complexing with amylose or by retarding aggregation of ALA.

1. Introduction

Alpha-lipoic acid (1,2-dithiolane-3-pentanoic acid; ALA) is a naturally occurring sulphydryl compound, essential for the energy metabolism in human cells as a cofactor in mitochondrial dehydrogenase reactions (Reed, Debusk, Gunsalus, & Hornberger, 1951). One of the unique characteristics of ALA is that its oxidized and reduced forms create a potent redox couple and thus exhibit antioxidant properties with universal activities in biological system to scavenge reactive oxygen species and chelate transitional metal. In addition, it behaves as a recycle endogenous antioxidant like vitamin E and C (Packer, Witt, & Tritschler, 1995), which has been clinically proved (Bilska, Dubiel, Sokołowska-Jez, Lorenc-Koci, & Włodek, 2007; Odabasoglu et al., 2011). It is also used as a therapeutic intervention for several diseases associated with oxidative stress such as diabetes mellitus, hypertension and cardiovascular disease (Gorąca et al., 2011). Because of its unique activity to protect against oxidative stress, ALA has drawn much attention to its potential application as dietary supplements (Shay, Moreau, Smith, Smith, & Hagen, 2009) and cosmetics for anti-wrinkling and anti-aging as well (Beitner, 2003).

However, utilization of ALA is limited because of its poor water-solubility and low bioavailability. The distorted five membered dithiolane ring chromophore (Fig. 1), responsible for its antioxidant activity (Gorąca et al., 2011), readily absorbs light and decomposes to dithiyl radical and/or other activated species (Bucher, Lu, & Sander, 2005; Wada, Wakami, Konishi, & Matsugo, 2009). It is also vulnerable to thermolysis and polymerization with its ring being opened and interlocked upon heating, partially due to its low melting point (Kisanuki et al., 2010). Moreover, the unpleasant smell and irritating taste induced by the sulfide usually accompany photochemical and thermal reactions, resulting in degrading the quality of ALA-containing products. Therefore, improvement of its water solubility and stability against decomposition is imperative for the utilization of ALA.

Encapsulation is a practical way to stabilize and deliver various ingredients and nutraceuticals, especially with water-insoluble or poorly soluble compounds. Most studies involved in stabilizing ALA are dedicated to incorporate ALA in cyclodextrins (Ikuta et al., 2013; Racz et al., 2013; Takahashi, Bungo, & Mikuni, 2011). However, complexes with native cyclodextrins, especially with β-cyclodextrin, have often limited solubility mainly due to the tendency of aggregation (He, Fu, Shen, & Gao, 2008). Correspondingly, the bioavailability may not be improved by the complex formation with native cyclodextrins, which could be attributed to the decreased physical contact of guest compounds (Hörter & Dressman, 2001; Loftsson, Másson, & Brewster, 2004).

Alternatively, starch, either in native or modified form, has been introduced as an encapsulating and delivery agent for various hydrophobic ingredients (Kim, Kim, Chung, & Lim, 2012; Kim, Seo, & Lim, 2013). The starch modified with octenyl succinic anhydride

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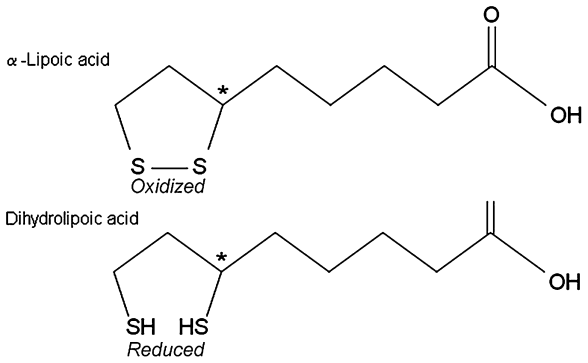

Fig. 1. *Chemical structure of α-lipoic acid and dihydrolipoic acid (Gorąca et al., 2011).*
* The structure contains a single chiral center.

(OSA) has been extensively used to make emulsions or dispersions of hydrophobic compounds (Drusch, Serfert, & Schwarz, 2006; Liang, Shoemaker, Yang, Zhong, & Huang, 2013). The octenyl part of substituents provided hydrophobic nature to the starch, improving the miscibility with hydrophobic compounds. Moreover, it was reported that microcapsules made from OSA-modified starches exhibited better retention performances than those prepared with β-cyclodextrin (Jeon, Vasanthan, Temelli, & Song, 2003), maltodextrin, and whey protein (Brückner, Bade, & Kunz, 2007).

Amylose, which is a relative linear fraction of starch molecules, has a function of encapsulating guest compounds in similar way to cyclodextrins (Cheng, Luo, Li, & Fu, 2015; Yang et al., 2011). The hydrophobic cavity of amylose single helix allows hydrophobic guest molecules to be incorporated by forming V-amylose complex, and thus makes the guest compounds water-dispersible and protective against oxidation and physical treatments. As a consequence, utilization of a starch containing high amount of amylose was expected to be effective in protecting labile ALA. Hence, it could be assumed that high amylose starch would provide a synergic effect by OSA modification available high amylose starch with OSA modification and possess more acceptable dispersing and stabilizing ability than native high amylose starch and beta-cyclodextrin.

In the present study, ALA served as a model substance to investigate the performance of OSA-modified high amylose starch in comparison with native high amylose starch and β-cyclodextrin. Aqueous dispersions of ALA in the presence of β-cyclodextrin and high amylose starches, either native or modified with OSA, were prepared under identical conditions. The ALA recovery (percent dispersibility) was compared in terms of degree of starch modification and conditions for dispersion preparation. In addition, the ALA in dispersions was characterized in its physical status including size distribution, crystalline structure and melting property.

2. Materials and methods

2.1. Materials

High amylose maize starch (Hylon VII, 70% amylose) was obtained from Ingredion Incorporated (Bridgewater, IL, USA). Alpha-lipoic acid (ALA), beta-cyclodextrin (β-CD) and 2-octen-1-ylsuccinic acid anhydride (OSA) were purchased from Sigma-Aldrich Company (St. Louis, MO, USA). All other chemicals used were of analytical grade.

2.2. Preparation of OSA-modified high amylose starch

The OSA-modified high amylose starch (OS) was prepared as described by Zhang et al. (2011) with minor modification. The high amylose starch (100 g, dry weight) was suspended in distilled water (35% starch solids, w/v) with agitation, and pH of the dispersion was adjusted to 8.0 by adding aqueous NaOH solution (0.5 M). While being stirred magnetically, the dispersion was heated to 35 °C and the pH was maintained at 8.0. Within these conditions, OSA (1.5, 3.0 or 10.0% based on the dry starch solids) was gradually added dropwise for 2 h. The reaction was allowed to proceed for total 3 h and then the pH was adjusted to 6.5 with aqueous HCl solution (0.5 M). Then the OSA-modified high amylose starch was recovered by centrifugation (2890 × *g*, 15 min) at 20 °C, washed twice with distilled water and once with absolute ethanol (100 ml each), and then oven-dried at 40 °C for 12 h. The degree of substitution (DS), defined as the average number of hydroxyl group substituted by OSA per every glucose unit, was determined by titration. The high amylose starches treated with 1.5, 3.0, and 10.0% OSA (w/w, dry starch base), which were named OS 1.5, OS 3.0 and OS 10.0, had respective DS values of 0.010, 0.019 and 0.039. High amylose starch subjected to same processing without addition of OSA was used as the control.

2.3. Preparation of ALA dispersion

The OSA-modified high amylose starch (OS, 600 mg, dry weight) was gelatinized by dispersing in NaOH solution (1.0 M, 3 ml) and then the solution was diluted by adding distilled water (54 ml). The pH of the starch solution was adjusted to 7.0 with 0.1 M HCl solution and the final volume was adjusted to 60 ml by adding distilled water. Here pH 7.0, greater than the p*K*a value of ALA (4.8), was applied to allow the full ionization of ALA in the dispersion and to avoid any undesired changes in starch structure at the elevated temperatures during the reaction. After neutralization the starch solution was purged with nitrogen gas and was autoclaved for 20 min at 121 °C for complete gelatinization. Then ALA pre-dissolved in absolute ethanol (60 mg in 0.5 ml) was added to the starch solution which had been adjusted to a desired temperature for dispersion preparation (50, 70 or 90 °C). The mixture, in a container covered with aluminum foil to prevent ALA oxidation, was continually stirred with magnetic bar (1200 rpm) at the constant temperature (50, 70 or 90 °C) for different periods (3, 6, and 12 h). The dispersions were then allowed to cool to room temperature with continuous stirring for 12 h, and then treated by a ultrasonication (VCX 500, Sonics & Materials Inc., CT) for 3 min at 40% amplitude with on-off cycles (3 s each) in an ice bath. Exposure to light was minimized during the process by covering the glass container with aluminum foil. The ALA dispersions in native high amylose starch and β-CD solutions were separately prepared under the same procedure for comparison. The dispersed particles were isolated and then freeze-dried for morphological and thermal characterization by centrifuging the dispersion (25,000 × *g*, 30 min) at 4 °C.

2.4. ALA recovery

The ALA recovery, which was defined as the percentage of ALA remaining in starch dispersions based on the ALA initially added, was analyzed by measuring the residual ALA contents in the dispersions or by measuring the precipitates using a capillary gas chromatography. Methylation was carried out by hydrolyzing an aliquot of the dispersion or a portion of the precipitates with methanolic NaOH (0.5 M, 5 ml) at boiling temperature for 10 min, methylating with boron trifluoride-methanol solution (14% v/v, 5 ml), and extracting it with hexane (5 ml).

The methylated samples were then analyzed by a Hewlett Packard 5890 gas chromatography equipped with an Omegawax capillary column 320 (30 m × 0.32 mm × 0.25 μm film thickness), and a FID. The column temperature was initially 140 °C for 7 min, and was increased to 250 °C at a rate of 5 °C/min, and then was maintained at 250 °C for 3 min. Both injector and detector temperatures were equally 280 °C.

2.5. Particle size analysis

Size distribution of the dispersed particles was evaluated by dynamic light scattering using a BIC 90 plus particle size analyzer equipped with a Brookhaven BI-9000AT digital correlator (Brookhaven Instrument Corp., New York). The ALA-starch dispersions were diluted 10 times with distilled water for the measurement. The analysis was done at a fixed scattering angle of 90° at 25 °C. The light source of the particle size analyzer was a red diode laser operating at 659 nm with 35 mW power. The mean hydrodynamic diameters of the dispersions were determined by frequency analyses of the intensity-intensity autocorrelation functions.

2.6. Differential scanning calorimetry (DSC)

A DSC (DSC 6100, Seiko Instruments Inc., Chiba, Japan) was employed to examine the thermal transition properties of the precipitates obtained from the ALA-starch dispersions. The freeze-dried precipitates (2.0 mg, dry weight) were mixed with distilled water (6.0 μl) in sealed aluminum pan. After equilibrated at 4 °C for 2 h, the samples were analyzed by scanning from 30 to 140 °C at the rate of 5 °C/min.

2.7. X-ray diffraction (XRD)

The crystal structure of the precipitates was analyzed using an X-ray diffractometer (Philips XPERT MPD, Almelo, The Netherlands) equipped with Cu-*K*α radiation at a 40 kV and 30 mA. The scanning was carried out from 5° to 30° at the rate of 0.8°/min. For comparison, untreated ALA, starch, and β-CD were respectively analyzed under the same procedure.

2.8. Statistical analysis

Experiments were carried out in triplicate, and data were presented as the average and standard deviation. Statistical analysis was performed by the one-way analysis of variance (ANOVA), followed by the Duncan's test for multiple comparisons when the F value was significant. Statistical Package for the Social Sciences (SPSS17.0, IBM Corp., New York, USA) was employed to determine the differences within statistical significance ($p < 0.05$).

3. Results and discussion

3.1. Stability of ALA dispersions

Aqueous dispersions of ALA, prepared with or without starch, are shown in Fig. 2. Without starch, the ALA quickly precipitated at the bottom of aqueous dispersion. The dispersion of OSA-modified high amylose starch (OS) without ALA appeared hazy although the starch was fully gelatinized in the alkaline solution. The ALA dispersion containing the native (Hylon VII) or OSA-modified high amylose starch (OS) appeared opaque but remained homogeneous (Fig. 2). Although the ALA dispersion containing Hylon VII was similar to that containing OS, the precipitates appeared in the Hylon VII dispersion when stored at room temperature for 2 days. The ALA dispersion prepared with OS, however, remained homogeneous during an ambient storage for up to 2 weeks (data not shown), indicating that using the OSA-modified high amylose starch was effective in stabilizing the ALA dispersion. The dispersion stability might be attributed to the hydrophobicity of high amylose starch induced by the OSA modification. The hydrophobic substituents provided high amylose starch an amphiphilic nature (Bao, Xing, Phillips, & Corke, 2003) and made the starch possible to interact with ALA which sterically stabilized in the aqueous dispersion. In addition, the bulky succinyl groups of OSA-modified high amylose starch are likely to retard retrogradation by preventing the formation of an ordered structure of starch, which might contribute to the preferable dispersing and stabilizing performance. Further, the ultrasonic treatment after blending might be effective in disintegrating the cluster aggregates possibly formed during dispersion preparation (Rajan & Pandit, 2001), resulting in stabilizing the dispersions. It has been effectively used to disperse nanoparticles homogenously, and been proved better than other conventional dispersing techniques (Nguyen, Rouxel, Hadji, Vincent, & Fort, 2011). Nevertheless, from a separate experiment using beta-cyclodextrin (β-CD), the ALA dispersion prepared under the identical procedure exhibited precipitates within two days, indicating that β-CD was not so effective in stabilizing the ALA dispersion than OS (data not shown). The ALA particles in CD dispersion might readily form aggregates to clusters shortly after ultrasonication.

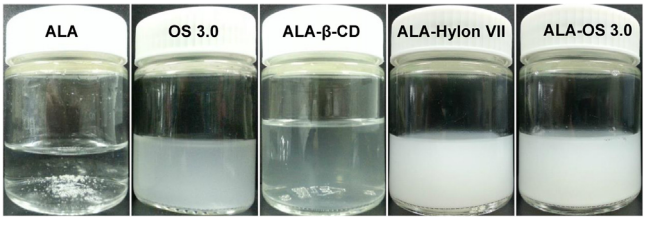

Fig. 2. Aqueous dispersions of α-lipoic acid (ALA), OSA-modified high amylose starch (OS 3.0), ALA with β-CD (ALA-β-CD), ALA with native high amylose starch (ALA-Hylon VII) and ALA with OSA-modified high amylose starch (ALA-OS 3.0) prepared by blending at 70 °C for 3 h. The ALA concentration in each model reaction system was 1.0 mg/ml.

3.2. ALA recovery under different conditions

The ALA recovery was measured for both the dispersions and the precipitates isolated from the dispersions under different DS values for starch modification and reaction conditions. Based on the preliminary factorial experiments regarding the effects of reaction times (3, 6, 9 and 12 h) and temperatures (50, 60, 70, 80, 90 °C), the more representative parameters of reaction time (3, 6 and 12 h) and temperature (50, 70, 90 °C) were selected for the systematic approach in this study. As shown in Fig. 3a, the ALA recovery in the dispersion containing OS 3.0 (starch modified with 3.0% OSA) was 84.2% when the reaction was carried out for 3 h at 70 °C, indicating that most of the ALA added could be dispersed when the OSA-modified high amylose starch co-existed. However, the recovery decreased with statistical significance ($p < 0.05$) as the reaction time increased to 12 h (77.5%). Ahmadi and coworkers found that a prolonged incubation was not effective in forming the complex between amylose and lipids, especially when the reaction was done at around or above starch gelatinization temperature (Ahmadi-Abhari, Woortman, Oudhuis, Hamer, & Loos, 2014). With a continuous stirring while heating, it was hypothesized that the ALA in the dispersions underwent thermal and/or oxidative degradation (Kisanuki et al., 2010). The similar trends observed for both dispersions and precipitates, indicated that the loss of ALA occurred not only in the dispersions but in the precipitates as well.

There were substantial differences in ALA recovery between the dispersions and the precipitates, which were greater than 20% (Fig. 3a). These differences indicated that a part of the ALA dispersed in aqueous media remained in the supernatant, not in the precipitates obtained by centrifugation. With an assumption that the ALA in the complexes with amylose in Hylon VII or OS was isolated mostly in the precipitates, the ALA dispersed in the supernatant might exist in its free form. The hydrophobic interior of single

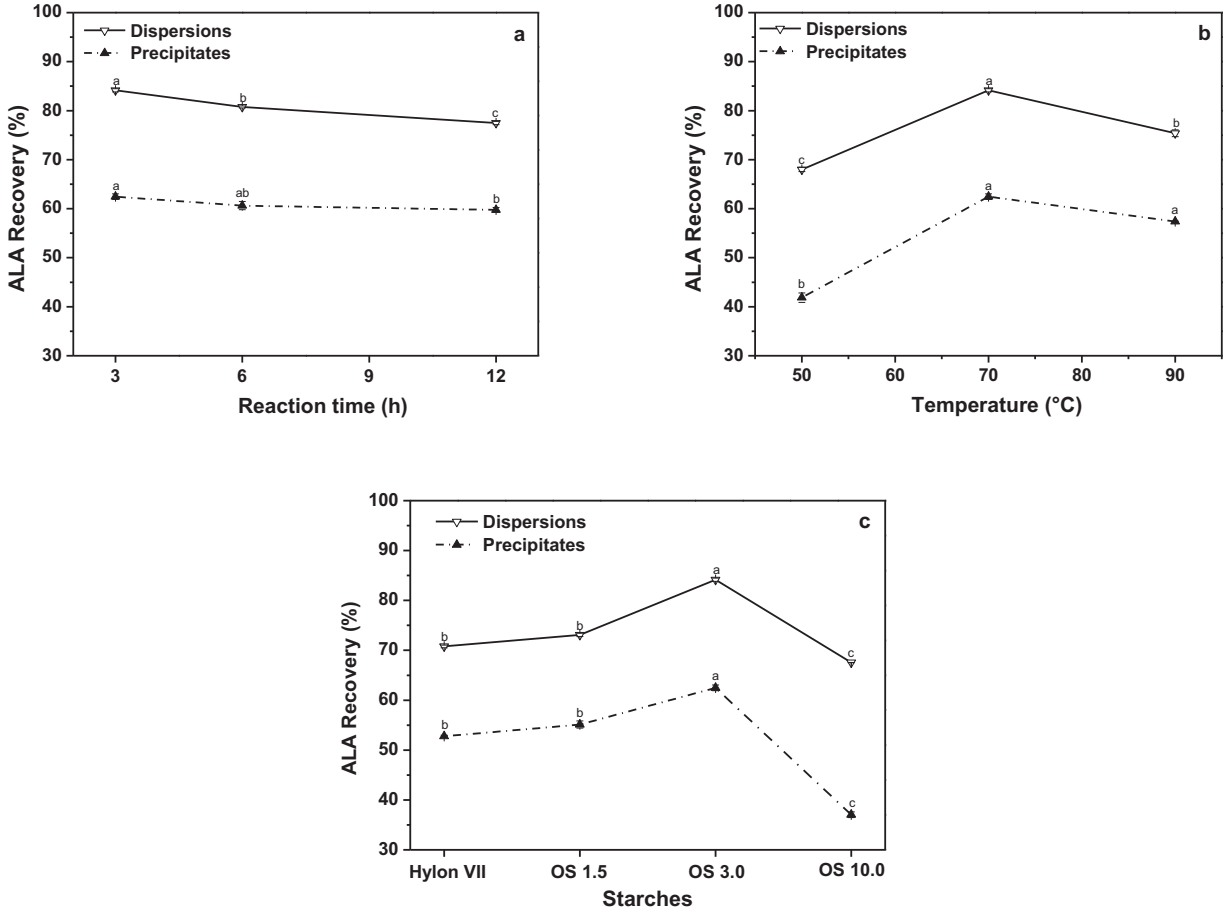

Fig. 3. Effects of reaction conditions [(a) time and (b) temperature], and (c) degree of starch modification on the ALA recovery in dispersions and precipitates. Data of different alphabets were different with statistical significant ($p < 0.05$).

helices produced by the linear fraction of amylose may accommodate the hydrophobic ALA. The amylose single helical complexes exist typically in crystalline structure with high melting temperature (>100 °C), and thus the ALA-amylose complexes might be insoluble being readily isolated in the precipitates by centrifugation. It was also expected that some of ALA in the precipitates might be entrapped in the interstitial space between the amylose helices (Sweedman, Tizzotti, Schäfer, & Gilbert, 2013). In the supernatant, steric stabilization was required for the free ALA to be freely dispersed but not being precipitated by the centrifugation. The hydrophobic nature of the starch induced by the modification with OSA might provide this stabilization effect, possibly by increasing the interactions with ALA molecules.

The ALA recovery values in the dispersions containing OS 3.0, but prepared at different temperatures (50, 70 and 90 °C) are shown in Fig. 3b. The maximum recovery (84.2%) in the dispersion was obtained when the reaction temperature was 70 °C, which was significantly ($p < 0.05$) higher than those obtained at 50 and 90 °C. The ALA recovery for the precipitates displayed a similar trend to that of the dispersions. For the complex formation of amylose and hydrophobic components, the solubility of each component was one of the determining factors for the level of complex formation because the process involves the physical contact of both components (Eliasson, 2004). Because the melting point of ALA in pure water dispersion was observed to be around 48 °C (data not shown), the ALA in the dispersions heated at 70 or 90 °C was expected to remain amorphous having molecular mobility, and thus be readily available for the complex formation. Therefore, the mild heating was preferable for the complex formation which could be ascribed to increased molecular mobility and availability (Ahmadi-Abhari et al., 2014). Accordingly the data revealed that most of the ALA dispersed remained in the supernatant when the reaction temperature was 50 °C, but transformed to the complexes (precipitates) when the temperature increased to 70 °C (Fig. 3b). Because ALA is readily oxidized and polymerized under thermal conditions, the ALA both in the dispersions and in the precipitates might be decomposed as the temperature increased to 90 °C. Therefore, thermal treatment should be carefully controlled to maximize the dispersibility (recovery) of the substances susceptible to heat like ALA in aqueous media.

The starches of different degrees of modification were compared on the ALA recovery in the dispersions and precipitates (Fig. 3c). Considering that starch modified with high OSA level could be used for non-food applications such as cosmetics, paper sizing and biodegradable plastics, the excess amount (10%) of OSA was included the systematic approach to find out the effect of OSA concentration, although exceeding the maximum amount of OSA allowed for modification by FDA (3%, dry starch base). For comparison, the ALA dispersions containing unmodified starch (Hylon VII) and β-CD were also prepared under the same procedure. For the ALA dispersion containing β-CD, the ALA recovery values measured highly fluctuated among the measurements, possibly suggesting that the ALA in β-CD dispersion was not stable and heterogeneously distributed (data not shown). This result was in good agreement with the previous study showing that cyclodextrins had little effect in increasing the solubility of ALA, even with increasing cyclodextrin concentrations (Takahashi et al., 2011). The low water solubility and high tendency of self-association of β-CD might be

responsible for the low efficiency for ALA dispersion (Connors, 1997). As shown in Fig. 3c, native starch (Hylon VII) exhibited a lower recovery compared to those obtained with the modified starches, OS 1.5 and OS 3.0. However, OS 10.0 which had the highest degree of substitution (DS 0.039) showed the lowest recovery value which was even lower than that of native starch. Especially the recovery difference between the dispersions and precipitates became substantial when OS10.0 was used. It was hypothesized that the complex formation became retarded when the degree of modification increased, possible due to the steric hindrance induced by the substituents. In addition, octenyl chains in the modified starch were supposed to compete with ALA to interact with hydrophobic interior of amylose helices and even to form the inclusion complexes, which might be responsible for the decreased ALA recovery in the precipitates at excessive modification. Overall, the experimental results revealed that the OSA-modified high amylose starch with DS of 0.019 was most favorable in improving the dispersibility of ALA, which showed the high ALA recovery of 84.2%. The substituents provided by OSA modification might improve the dispersibility of amylose chains and decrease the tendency of chain association, allowing the amylose accessible for complex formation with ALA (Zhang et al., 2011). Consequently, the hydrophobic and bulky substituents of starch retarded the chain association of amylose and assisted the interactions between amylose and ALA, which resulted either in the complex formation or in stabilization of the suspended ALA particles.

3.3. Size distribution of ALA particles

Average size and size distribution of the dispersed particles determine the behaviors of dissolution, agglomeration and degradation which are critical to the stability of dispersions and the efficacy of delivery (Mohanraj & Chen, 2007). According to Stokes' law, the velocity at which a small sphere settles through a viscous fluid is proportional to the square of its radius (Kemper, Rosenau, & Klute, 1986). As shown in Fig. 4, dispersions containing the native starch (Hylon VII) or modified starch (OS 3.0) exhibited relatively narrow size distributions of submicrometer particles. When β-CD was used for ALA dispersion, however, the particle size exhibited a much wider distribution compared to those observed with the starch dispersions (Fig. 4c). Micro-sized particles in dispersions containing β-CD accounted for a major portion might indicate the presence of aggregates while the fraction around 155 nm might correspond to β-CD agglomerates or ALA-β-CD complexes which were decreased into small size by ultrasonication but did not agglomerate. Unmodified β-CD has a tendency of aggregation in water, causing its low water solubility (He et al., 2008), which might be responsible for the wide size distribution with primarily large particle size of β-CD dispersions. The mean diameter of the dispersed particles in ALA-OS 3.0 dispersion was 288.5 nm whereas that in ALA-Hylon VII dispersion was 498.3 nm, indicating that the modification positively affected on decreasing the size of dispersed particles. Decreasing particle size favorably contributes to increasing the dissolution rate and thus the dispersibility and bioavailability of poorly soluble bioactive compounds. Not only the increased hydrophobicity by the modification with OSA but also the ability of producing small-sized particles thus enhanced the dispersibility and stability of the ALA particles in the aqueous starch dispersions.

3.4. Thermal transition properties

Thermal transition behavior of the precipitates isolated from the ALA dispersions was analyzed to characterize the crystalline complex formation between amylose and ALA (Fig. 5). Pure ALA dispersed in water (1:3 ratio) exhibited a sharp melting transition

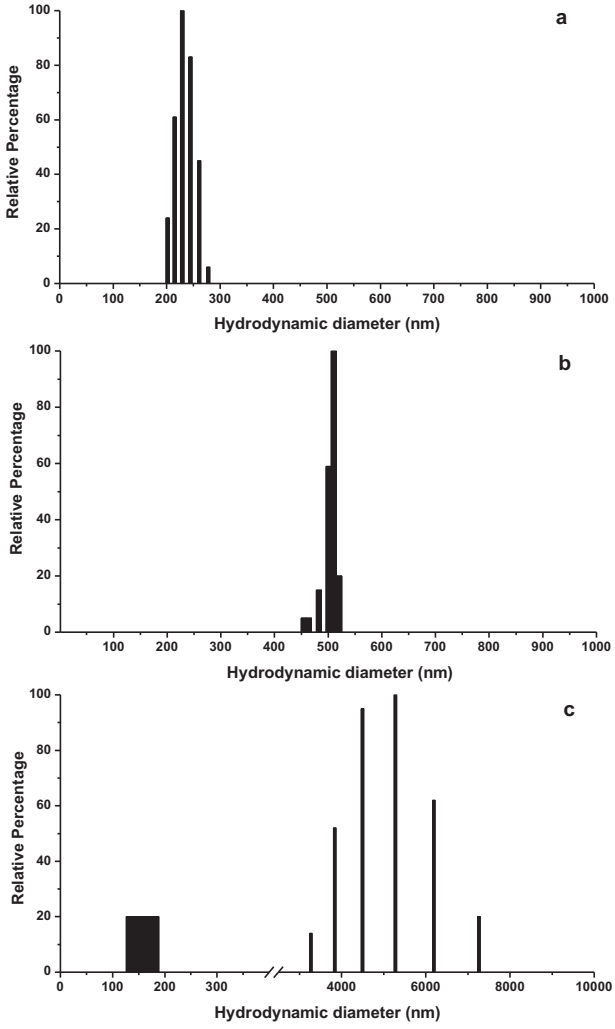

Fig. 4. Particle size distribution of ALA dispersions containing (a) OSA-modified high amylose starch (OS 3.0), (b) native high amylose starch (Hylon VII), and (c) β-cyclodextrin (β-CD). The ALA concentration in each model reaction system was 1.0 mg/ml.

ranging from 42 to 56 °C, whereas native (Hylon VII) and modified starch (OS 3.0) in the aqueous dispersion showed broad transitions ranging from about 70 to 105 °C. The broad and high temperature endotherm for high amylose starches resulted from the association

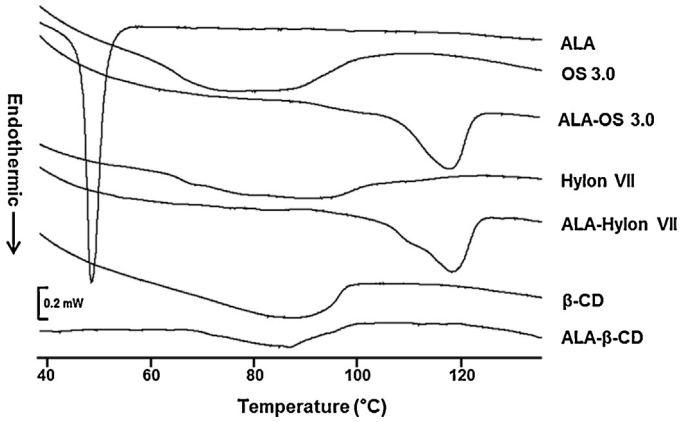

Fig. 5. DSC thermograms of pure ALA, native high amylose starch (Hylon VII), OSA-modified high amylose starch (OS 3.0), β-cyclodextrin (β-CD) and the precipitates isolated from ALA dispersions prepared by blending at 70 °C for 3 h. DSC samples contained 25% solids.

among linear amylose chains (Liu, Yu, Xie, & Chen, 2006). The thermal transition of OS 3.0 was observed in a slightly lower temperature range compared to that of Hylon VII, which indicated that the modification allowed the starch granules to be more readily swollen and gelatinized.

Thermograms of the precipitates from the ALA dispersions containing native and a modified high amylose starch (ALA-Hylon VII, and ALA-OS 3.0) exhibited melting peaks at a temperature range from 110 to 125 °C. The helical complexes of amylose may be defined by observing their melting temperatures: randomly oriented type I complexes melting at lower temperature as opposed to lamellarly ordered type II counterparts melting at higher temperature (Tufvesson, Wahlgren, & Eliasson, 2003). The type II complexes can be further subdivided into type IIa which melts around 115 °C and more thermostable type IIb complexes melting at a higher temperature (Putseys, Lamberts, & Delcour, 2010). Therefore, the melting peaks with T_p around 120 °C in the thermograms observed for the ALA-OS 3.0 and ALA-Hylon VII dispersions indicated that the ordered type IIa complexes were formed between ALA and amylose. The enthalpy values of those endothermic peaks were slightly different: 18.13 J/g for OS-ALA and 17.22 J/g for ALA-Hylon VII samples. It proved that the modified starch had a higher tendency of forming the inclusion complex with ALA, as found with the difference in ALA recovery for the precipitates (Fig. 3c).

B-CD alone revealed a broad endotherm peak in a temperature range from 60 to 100 °C, which was probably due to the dehydration of heavily hydrated β-CD molecules which formed a cage crystal structure with water molecules. That is, with increasing temperature, water molecules induced in the cavity, also located in the interstices between β-CD, evaporated (Connors, 1997; Mura, Maestrelli, Cirri, Furlanetto, & Pinzauti, 2003). A broad endotherm peak was observed on the DSC thermogram of β-CD-ALA at the similar location with that of β-CD alone. In contrast, however, the endothermic peaks on the thermograms of ALA-OS 3.0 and ALA-Hylon VII involving melting of crystalline complexes were located at much higher temperatures than the peaks of pure OS 3.0 and Hylon VII corresponding to gelatinization. In addition, the enthalpy (10.19 J/g) of the endothermic peak for β-CD-ALA dispersion was less than half of that for pure β-CD sample (22.83 J/g). This could be a proof of interaction between ALA and β-CD, possibly indicating the replacement of water molecules included in β-CD cavity with ALA. Accordingly, the more decrease of enthalpy of β-CD-ALA compared with that of pure β-CD, the more replacement of water molecules thus the more β-CD complex formation. However, the lower peak temperatures and enthalpies of endothermic peaks of ALA-OS 3.0 and ALA-Hylon VII indicated less stable complexes formation. Therefore, it could be concluded that thermal behaviors of starch complexes with CD complexes on DSC thermograms implied different rules involved in complex formation.

It was noteworthy that all the dispersions tested showed no ALA melting peak (T_p 48 °C) in their precipitates, confirming that the ALA dispersed had no longer its original crystalline structure. It suggested that the ALA in aqueous dispersions interacted with starch or β-CD to remain stable in its amorphous state. The complex formation with these carbohydrate polymers contributed for the stabilization.

3.5. Crystalline structure of complexes

The X-ray diffractograms (XRD) of the precipitates isolated from various ALA dispersions are presented in Fig. 6. The pure ALA powders exhibited several distinct diffraction peaks indicating the presence of crystallinity. Native high amylose starch displayed a strongest diffraction peak at around 17° (2θ) and a few small peaks at around 22° and 23°, which basically correspond to a characteristic B type pattern. Similar diffraction peaks appeared for the

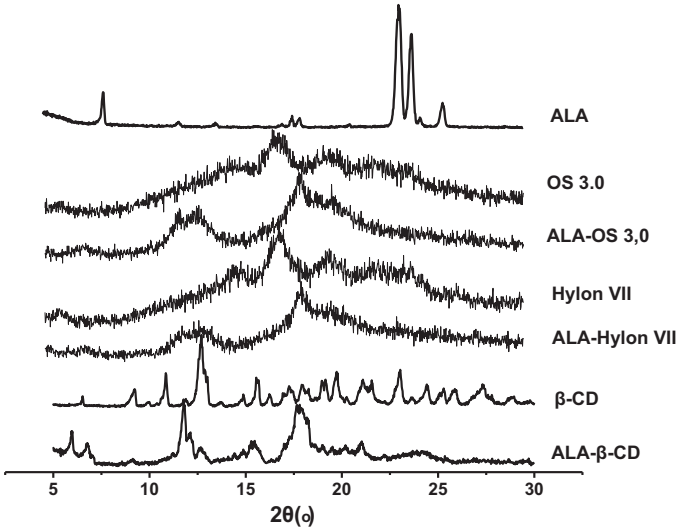

Fig. 6. X-ray diffractograms of pure ALA, native high amylose starch (Hylon VII), OSA-modified high amylose starch (OS 3.0), β-cyclodextrin (β-CD) and the precipitates isolated from ALA dispersions prepared by blending at 70 °C for 3 h.

modified starch (OS 3.0), but the peak intensity was slightly attenuated, probably due to the disruption of crystalline regions during octenylsuccination (Zhang et al., 2011). The precipitates isolated from ALA-Hylon VII and ALA-OS dispersions displayed two broad diffraction peaks at around 13° and 19° (2θ), corresponding to the V-type crystalline structure. As the DSC results showed, all characteristic XRD peaks of pure ALA disappeared in the ALA samples isolated from the dispersions, as a proof of complex formation. It was also noteworthy that the peak intensity for ALA-OS 3.0 sample was higher than that for ALA-Hylon VII sample, suggesting that the complex formation was facilitated by the modification. This finding was agreed with the results in ALA recovery and DSC thermograms (Figs. 3 and 5).

B-CD alone showed a strong diffraction peak at around 13° and other small peaks. After blending ALA in aqueous CD medium, a strong diffraction peak at about 18° (2θ) and unresolved doublet at around 12° (2θ) appeared, whereas the characteristic peaks of pure ALA and β-CD disappeared. It was worth mentioning that the amount of ethanol used to dissolve ALA was very minor compared to the starch solution, and thus its effect during the addition might be negligible. In combination with the absence of the absence of ALA melting peaks on the thermograms of ALA mixtures (Fig. 5), it was expected that ALA remained amorphous in the hot starch solution under the continuous heating and stirring condition and recrystallization occurred during cooling. Different crystalline packing of CD after blending with ALA was indicative of crystalline structure changes through authentic interactions between ALA and β-CD. The two major diffractions at 12 and 18° (2θ), similar to those found with the sample of ALA and starch dispersions, might suggest that β-CD also formed the single helical complex with ALA.

Considering the DSC and XRD results, it could be concluded that the high amylose starch, regardless of modification, readily formed the inclusion complex with ALA through simple blending with mild heating followed by ultrasonic treatment. The octenylsuccinyl groups, at DS 0.019, improved the complex formation as well as the dispersibility of free ALA molecules. It was reported that, during complex formation, some hydrophobic guest molecules might be trapped in the interstitial space between the helices (Putseys et al., 2010). Meanwhile, the hydrophilic exterior of the helix facilitated the miscibility and dispersibility of the complex in aqueous medium (Eliasson, 2004). The OSA-modified high amylose starch (OS) was found to be an effective encapsulating agent

for hydrophobic substances simply through its steric stabilizing ability (Sweedman et al., 2013). Likewise, it was expected that the hydrophobic property induced by the octenyl chains which might be attached on the exterior of amylose helices facilitated the incorporation or trapping of ALA in the space between the helices. Moreover, steric effects imposed by OSA modification retarded the retrogradation of high amylose (Sweedman et al., 2013), and thus allowed OSA-modified high amylose starch to disperse and stabilize ALA preferably compared with native high amylose starch and beta-cyclodextrin after cooling dispersions to the room temperature. In turn, it would be of great interest to construct a systematic comparison between the OSA-modified high amylose starch and water-soluble hydroxypropyl-beta-CD regarding the performance of increasing dispersibility and bioavailability of ALA or other lipophilic compounds in further study.

4. Conclusions

Aqueous dispersions of alpha-lipid acid (ALA) could be prepared with homogeneity and stability by using octenylsuccinylated high amylose starch under a simple blending process followed by mild ultrasonication. When 0.1% (w/v) ALA was dispersed as an example, approximately 84% of the ALA added was dispersed when the octenylsuccinylated starch of DS 0.019 was used (1.0% solids). The ALA dispersed was either included in the complex of amylose single helices having V-type crystals, or freely dispersed with stabilization by the starch. The OSA modification enhancing the hydrophobicity as well as high amount of amylose rendered synergic effects on improving the dispersibility of water-insoluble ALA in aqueous media, which may be desirable for the industrial application of ALA.

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