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Preparation and characterization of aqueous dispersions of high amylose starch and conjugated linoleic acid complex

Tae-Rang Seo [a], Hee-Young Kim [b], Seung-Taik Lim [a,*]

[a] Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul 136-701, South Korea
[b] Department of Food Science and Human Nutrition, Iowa State University, Ames, IA 50011, United States

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ABSTRACT

Crystalline starch-CLA complexes were prepared by blending an alcoholic solution of conjugated linoleic acid (CLA) in an aqueous high-amylose maize starch dispersion. Recovery yield of CLA in the precipitates obtained by centrifuging the dispersion was dependent on reaction conditions such as temperature, time and pH. The CLA recovery reached a maximum when the reaction was performed at 90 °C for 6 h at neutral pH, with 67.7% of the initial CLA being co-precipitated with starch. The precipitates contained amylose-CLA complex exhibiting a V6I-type crystalline structure under X-ray diffraction analysis and a type II polymorph under DSC analysis. Ultrasonic treatment for the re-dispersed starch-CLA complex in water resulted in the reduction of hydrodynamic diameter of the complex particles to 201.5 nm. The dispersion exhibited a zeta potential of −27.0 mV and remained stable in an ambient storage without forming precipitates for more than 4 weeks.

1. Introduction

Along with the health benefits of poly-unsaturated fatty acids (PUFAs), conjugated linoleic acid (CLA) has drawn great attention from scientific and industrial communities. CLA includes several positional (9,11; 10,12) and geometric (*c*,*t*; *t*,*c*; *c*,*c*, and *t*,*t*) isomers of octadecadienoic acid (C18:2) with conjugated double bonds. It is naturally found in ruminant products such as meat, milk, and dairy foods; however, it may also be produced using biological (e.g., synthesis of linoleic acid by bacteria) or chemical processes (e.g., alkaline isomerization) (Kim, Park, Park, Kim, & Ha, 2000; Kishino, Ogawa, Omura, Matsumura, & Shimizu, 2002).

The health-promoting functions of CLA have been extensively studied by numerous researchers (Kelley, Hubbard, & Erickson, 2007; McLeod, LeBlanc, Langille, Mitchell, & Currie, 2004; Park & Pariza, 2007; Ryder et al., 2001); these include potential anti-carcinogenic, anti-atherogenic, anti-diabetic, and anti-adipogenic activity. Nevertheless, industrial application of CLA is limited due to its poor oxidation stability. CLA oxidation is considerably faster than that of other PUFAs such as linoleic, linolenic, and arachidonic acids because its conjugated double bonds are highly susceptible to oxidation (Zhang & Chen, 1997). Another obstacle in the application of CLA in foods and nutraceuticals is its hydrophobicity and low water solubility (0.01513 mg/L at 25 °C). Techniques such as encapsulation and emulsification have been used to overcome these drawbacks of CLA. For example, Park et al. (2002) used cyclodextrin to form inclusion complexes of CLA and reported that CLA oxidation was completely prevented. Microencapsulation of CLA in cyclodextrin using a freeze- or spray-dryer has also been studied (Jimenez, Garcia, & Beristain, 2006; Kim, Park, Kang, et al., 2000). For instance, Kim, Park, Kweon, and Han (2013) prepared a nanoemulsion of water-soluble CLA using a high-pressure homogenizer. The emulsified CLA effectively reduced body weight and decreased cholesterol and triglyceride levels in the blood and liver. Yet, nanoemulsion formation requires extensive use of emulsifiers which are generally chemically synthesized and cause off-flavor of the final products. Also, the cyclodextrin used for encapsulation is one of the most expensive polymers among encapsulating agents, and inclusion complexes of cyclodextrin tend to form large aggregates in aqueous media (Loftsson, Masson, & Brewster, 2004).

Starch is a biodegradable and renewable biopolymer which is naturally synthesized through photosynthesis. Amylose, the starch fraction composed of relatively linear molecules, has a strong tendency to form single helices by accommodating hydrophobic guest compounds (Conde-Petit, Escher, & Nuessli, 2006; Le Bail, Rondeau, & Buléon, 2005; Whittam et al., 1989; Zabar, Lesmes, Katz, Shimoni, & Bianco-Peled, 2009). The single helical configuration is referred to as V-amylose because of its specific crystalline arrangement. Similar to cyclodextrin, the central cavity of V-amylose single helices is hydrophobic whereas the outer surface

* Corresponding author.
E-mail address: limst@korea.ac.kr (S.-T. Lim).

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is hydrophilic allowing guest compounds to disperse in aqueous media (Eliasson, 2004). The reduction of starch complex particle size to nanoscale dimensions by post treatments such as ultrasonication could also improve water dispersibility and storage stability for extended periods (Kim, Kim, Chung, & Lim, 2012; Yoon, Seo, & Lim, 2014). Recently, several studies have been carried out on the formation and properties of amylose-CLA complexes (Lalush, Bar, Zakaria, Eichler, & Shimoni, 2005; Yang, Gu, & Zhang, 2009; Zabar et al., 2009). Most of these studies have focused on the characterization of the V-amylose complexes, their thermal transition, and crystalline structure. However, the detailed investigation is required for the preparation of water dispersible starch-CLA complexes on the large scale.

In this study, starch-CLA complexes were prepared by blending CLA in an aqueous starch dispersion under different reaction conditions varying in time, temperature, and pH followed by characterization of it crystalline structure and degree of complex formation. In addition, mild ultrasonication as a post-treatment was applied to reduce the particle size and to increase the storage stability of the dispersed starch-CLA complexes.

2. Materials and methods

2.1. Materials

High-amylose maize starch (Hylon VII, 70% amylose) was provided by Ingredion Inc. (Westchester, IL, USA). Prior to complex formation, defatting of starch was performed by dissolution in aqueous dimethyl sulfoxide (DMSO, 90% w/w) and precipitation in ethyl alcohol (95%) following the method of Lee, Han, and Lim (2009). Conjugated linoleic acid (a mixture of *cis*- and *trans*-9,11 and 10,12-octadecadienoic acids, ⩾99% purity) was purchased from Sigma-Aldrich Company (St. Louis, MO, USA). All other chemicals were analytical grade.

2.2. Preparation of starch-CLA complexes

Formation of starch-CLA complexes in an aqueous system was performed according to a previously described method (Seo, Kim, & Lim, 2015) with minor modification. Defatted starch (500 mg, dry basis) was dissolved in 1.0 M NaOH solution (5 mL) by vortexing, diluted with distilled water (40 mL), and pH adjusted to 5, 6, 7, or 8 by the addition of 1.0 M HCl solution at room temperature. The starch solution was purged with nitrogen gas for 3 min to remove oxygen and was autoclaved in a sealed container (121 °C, 20 min). CLA (25 mg) was dissolved in absolute ethanol (2.0 mL) under mild stirring at room temperature, and the mixture was added dropwise to the starch solution under vigorously stirring (1200 rpm) at different temperatures (i.e., 50, 70, or 90 °C). The resultant solution was purged with nitrogen gas for 3 min to prevent oxidation of CLA, and the dark container was tightly sealed. After a designated period (i.e., 3, 6, 24, or 48 h), the starch-CLA solution was cooled to ambient temperature for 6 h with continuous stirring (1200 rpm). The starch-CLA complex obtained as precipitates were recovered by centrifugation (25,000×*g*) at 4 °C for 30 min. Successive washings under identical centrifugation conditions were carried out with hot distilled water (80 °C, 50 mL) and diethyl ether (room temperature, 50 mL) to remove uncomplexed starch and CLA, respectively. The residual diethyl ether in the precipitates was removed by purging with N_2 gas for 5 min.

2.3. Starch and CLA contents

The amount of CLA in the precipitates was analyzed by gas chromatography (HP 5890 series II instrument, Sunnyvale, CA, USA). Quantitative analysis of CLA followed previously described methods, partially modified (Seo et al., 2015). Accordingly, the complex precipitates (15 mg, wet basis) obtained immediately after centrifugation were used for CLA extraction and methyl esterification. The CLA methyl ester was extracted by boiling for 2 min in n-heptane (5 mL) followed by the addition of a saturated, aqueous NaCl solution (50 mL) to the mixture. The heptane layer was used for gas chromatography analysis which was performed with a flame ionization detector and Omagawax™ 320 capillary column (30 m × 0.32 m × 0.25 μm, Supelco, Bellefonte, PA, USA). The CLA methyl ester content was calculated using heptadecanoic acid methyl ester as an internal standard (Sigma-Aldrich Company, St. Louis, MO, USA).

Prior to the analysis of starch, the precipitates (15 mg, wet basis) were dispersed in water (50 mL) and autoclaved to disrupt the complex between the CLA and starch. The amount of residual starch in the precipitates was analyzed using the phenol-sulfuric acid method (DuBois, Botelho, Bedell, Marshall, & Comroe, 1956).

Recovery yield of CLA and starch was calculated as a percent ratio of the weight of CLA and starch recovered in the precipitates to the initial weight of CLA and starch in the reaction mixture.

2.4. Thermal transitions

Thermal transition behavior of the complexes was analyzed using a differential scanning calorimeter (DSC 6100, Seiko Instruments, Chiba, Japan). Wet precipitates (10 mg, wet basis) containing about 30–35% dry solids were weighed and hermetically sealed in an aluminum pan that was equilibrated in a cold chamber (4 °C, 2 h) before analysis. The sample was scanned under a pressurized container from 20 °C to 160 °C at a rate of 5 °C min^{-1} and quickly cooled to 20 °C. Rescanning was also performed by reheating the sample immediately after cooling. The DSC instrument was calibrated with indium using an empty pan as a reference. The melting temperatures and enthalpies of the complexes were determined using the EXSTAR 6000 Thermal Analysis System (Seiko, Chiba, Japan).

2.5. X-ray diffraction analysis

The crystalline structures of the complexes were determined using an X-ray diffractometer (XPERT MPD, Philips Analytical, Almelo, Netherlands) at a target voltage of 40 kV and a target current of 30 mA. For the analysis, the precipitates collected after the centrifugation and washing process were freeze-dried. The scanning range and rate were 5–30° (2θ) and 1.0°min^{-1}, respectively.

2.6. Ultrasonication treatment

The wet precipitates isolated by centrifugation were redispersed in 80 °C filtered water (50 mg/50 mL). Ultrasonication (probe type, 500 W/cm^2, 20 kHz, VCX 500, Sonics & Materials Inc., Newtown, CT) was applied to the dispersion for 3, 5, 7 and 10 min in an iced container. The sonication power was 300 W/cm^2 (amplitude of ultrasonication: 60%) and pulsed on and off for 10 s each.

2.7. Particle size distribution and zeta potential

The complex dispersion obtained after ultrasonication treatment was analyzed for particle size and zeta potential. The dispersion was diluted to an appropriate concentration of 0.1–0.5% (w/v) for each analysis. An aliquot (1 mL) was injected into the cell and analyzed using a 90 Plus nanoparticle size and zeta potential analyzer (Brookhaven Instruments Corporation, NY, USA). The measurements were carried out at 20 °C and pH 6.5; and the

refractive index and viscosity of water were, respectively 1.333 and 1.00 cP at 20 °C.

2.8. Statistical analysis

All measurements were performed triplicate for each dependent variables, and the results were presented as the average values. The data was statistically analyzed using Duncan's multiple range tests ($p < 0.05$) using the SAS software system (version 9.2 SAS Inst., Cary, North Carolina, USA).

3. Results and discussion

3.1. Complex formation between starch and CLA

The reaction mixture of high-amylose maize starch and CLA was opaque white, whereas the controls – CLA in ethanol solution (control 1) and a starch dispersion containing pure ethanol (control 2) – were relatively translucent (data not shown). The opaqueness indicated that CLA in starch dispersion existed as an insoluble suspension. Cooling to ambient temperature resulted in the formation of precipitates that could be readily recovered by centrifugation. There are other studies that have reported on the recovery of complexes of starch and hydrophobic compounds as precipitates (Biliaderis & Galloway, 1989; Karkalas & Raphaelides, 1986; Raphaelides & Karkalas, 1988). Without starch, the addition of the CLA/ethanol solution in pure water resulted in the rapid separation of CLA from solution (data not shown), whereas, the presence of starch led to homogeneous CLA dispersion. Tapanapunnitikul, Chaiseri, Peterson, and Thompson (2007) suggested that hydrophobic ligands could interact with amylose when those were added in a dissolved state. When a flavor compound was used to form a complex with starch, the extent of complex formation was related to its water solubility (Kuge & Takeo, 1968). Flavor compounds with higher water solubility tended to form greater amounts of complex precipitate which would, in turn, enmesh more flavor compounds with starch (Tapanapunnitikul et al., 2007).

3.2. Recovery yields

The formation of amylose-lipid complex is affected by various parameters including amylose chain length, solution pH, reaction temperature, structure of lipids, concentration and ratio, reaction time, and water content (Godet, Bizot, & Buléon, 1995; Jovanovich, Zamponi, Lupano, & Anon, 1992; Karkalas & Raphaelides, 1986). This study focused on the effects of reaction conditions (varied in temperature, time, and pH) on crystalline complex formation between starch and CLA. To begin, different ratios of starch and CLA in the initial reaction mixture were examined in regards to the residual amount of CLA in the precipitated complex. Recovery yield was defined as the percentage of recovered starch and CLA in the precipitates after the complex formation reaction and centrifugation (Table 1). Maximum recovery was achieved when the reaction (90 °C, 6 h, and pH 7) was carried out with a mixture of 500 mg starch and 25 mg CLA in 50 mL solutions. Additions of CLA greater than 25 mg resulted in higher losses of CLA; i.e. lower recovery yield (data not shown).

At constant time and pH for the complex formation reaction (24 h and pH 7), the data showed that CLA recovery was significantly greater at 70 °C and 90 °C than at 50 °C (Table 1). This result suggests that the thermal treatment enhances the complex formation between amylose and CLA. Presumably, the increased mobility of amylose chains by heating may have raised the possibility for the interaction with CLA. On the other hand, the recovery yield of starch (26–34%) appeared less affected by reaction temperature compared to that of CLA. The recovery yields of starch suggest that a large portion of the starch added (more than 2/3) was not precipitated after the reaction. Re-association of starch, especially among linear amylose chains, could be a major cause for the formation of insoluble matrices in aqueous dispersions. However, the presence of hydrophobic compounds such as CLA, under the controlled conditions, may preferentially induce the complex formation with amylose producing insoluble matrices. Accordingly, there was a positive relation between the two recovery yields of CLA and starch measured at different reaction temperatures. Additionally noteworthy was the lack of difference in the recovery yields of CLA and starch between 70 °C and at 90 °C. The mild heating at 70 °C might provide sufficient mobility for the starch to interact with CLA. In a previous research (Seo et al., 2015), it was found that the recovery yield of polyunsaturated fatty acids became lowered through oxidation when reaction temperature was high. Despite the nitrogen purging applied for the inhibition of CLA oxidation, excessive thermal treatment might not be desirable.

Table 1
Recovery yields (%) of CLA and starch in precipitated complexes, under different reaction conditions.

Reaction parameters	Yields of CLA (%)	Yields of starch (%)
Temperature (pH 7, 24 h)		
50 °C	38.3 ± 3.3^{e}	26.2 ± 3.2^{cde}
70 °C	66.4 ± 6.2^{ab}	34.2 ± 2.8^{ab}
90 °C	63.5 ± 4.2^{abc}	33.4 ± 4.4^{ab}
Time (pH 7, 90 °C)		
3 h	48.3 ± 3.1^{d}	32.2 ± 3.9^{bce}
6 h	67.7 ± 5.2^{a}	39.4 ± 3.2^{a}
24 h	63.5 ± 4.2^{abc}	33.4 ± 4.4^{ab}
48 h	39.5 ± 3.4^{e}	30.7 ± 4.2^{bcd}
pH (90 °C, 24 h)		
pH 5	34.6 ± 5.8^{e}	25.3 ± 2.1^{de}
pH 6	57.2 ± 3.9^{c}	30.2 ± 3.6^{bcd}
pH 7	63.5 ± 4.2^{abc}	33.4 ± 4.4^{ab}
pH 8	58.6 ± 3.3^{bce}	22.5 ± 4.3^{e}

All data are presented as mean ± SD of triplicate determinations. Values with different alphabets in the same column are significantly different ($p < 0.05$).

The starch-CLA complex was prepared by simple blending at various reaction times up to 48 h. The data show that maximum recovery of starch-CLA complex was obtained after the reaction for 6 h (at 90 °C and pH 7). In a previous study (Seo et al., 2015), increasing reaction time for the complex formation between starch and polyunsaturated fatty acids (PUFA) resulted in a steady rise in starch-PUFA recovery yield until 6 h, after which the yield gradually decreased. This result indicated that adequate period of thermal reaction improved the reaction efficiency of complex formation. However, excessive treatment might lead to the structural decomposition of the complexes and possibly the oxidation of PUFA as well as CLA. Under the fixed temperature and time (90 °C and 24 h), slightly acidic (pH 5) and alkaline (pH 8) conditions induced the decreases in the recovery yield of CLA in the precipitates when compared to the reaction in neutral pH. Acidic conditions of aqueous media limited solubility and accelerated the degradation of starch molecules (Han & Lim, 2004), resulting in decreased reactivity of starch chains with CLA. At pH 8.0, starch was relatively well dissolved due the breakage of its intermolecular hydrogen bonds induced by alkali (Suortti & Pessa, 1991). It was hypothesized that the solubility of starch-CLA complexes was also increased by alkali, resulting in the decrease in CLA and starch recovery yields (Table 1). In addition, the solution pH was close to the pKa of the CLA, and thus a portion of CLA could be ionized (Kanicky & Shah, 2002). The ion-dipole interaction between the ionized and unionized CLA groups might be negatively effected.

As shown in the results of temperature effect, the change in CLA recovery yield at different pH values was similar to that of starch recovery yield. It proved that the interaction (possibly complex formation) between starch and CLA induced the formation of insoluble matrices in the dispersions.

In summary, the maximum yield of CLA (67.7%) was obtained when the reaction was performed at 90 °C and pH 7 for 6 h. It indicates that more than 16 mg CLA was recovered in the starch co-precipitates out of the 25 mg CLA initially added. Under the same conditions, the recovery yield of starch in the precipitates was 33.4%. Based on the initial amounts of starch and CLA, the weight ratio of starch and CLA in the recovered precipitates was approximately 10:1 (CLA 95.8 mg/g starch).

3.3. Crystalline structures of complexes

Fig. 1 exhibits the XRD patterns of the freeze-dried precipitates obtained by centrifuging the starch-CLA dispersions prepared under various reaction conditions. The patterns proved that the precipitates had a crystalline structure of amylose-CLA inclusion complex, and the crystallinity was influenced by the reaction parameters such as temperature, time, and pH. The samples showed equally V6I-type pattern having two main reflection peaks at Bragg angles (2θ) of 13.0 and 20.0° and one minor reflection peak at approximately 7.0°. The V6I complexes consisting of six D-glucosyl residues per turn (Godet et al., 1995; Lalush et al., 2005; Le Bail et al., 2005; Yang et al., 2009; Zabar et al., 2009) have been proposed for its application with various linear guest molecules such as emulsifiers (Brisson, Chanzy, & Winter, 1991), alcohols, (Whittam et al., 1989) and fatty acids (Godet et al., 1995).

The intensity of the XRD peaks gradually increased with the increase in reaction temperature (50–90 °C). The XRD data agreed well with previous reports (Godet et al., 1995; Zabar et al., 2009) in which amylose-fatty acids complexes formed at higher temperatures (at least 90 °C) exhibited higher crystallinities in XRD patterns. Also, there were minor peaks (about 17, 18, and 23°) presented only when the reaction temperature was 90 °C. This phenomenon was considered that a small quantity of different type crystals, such as B-type, was formed. It was possible due to the increased chain mobility of starch induced by adequate heating. The data also demonstrated that V-type crystals could even be formed at relatively low temperatures (50 and 70 °C). It was noteworthy that the crystallinity of the isolated starch-CLA precipitates was not proportionally changed by the recovery yield of CLA in the precipitates (Table 1). It indicates that a portion of CLA existing in the precipitates might not form the long-ranged crystalline complex with amylose.

The peak intensity representing crystallinity gradually increased as reaction time increased up to 24 h, but did not significantly change as the reaction continued up to 48 h. During the initial stage of the reaction, the complexes of starch and CLA were individually formed in an aqueous dispersion which may exist as independent units. For the complexes to develop a long arranged crystal matrix, which appeared as peaks in XRD pattern, prolonged reaction time was possibly required. Therefore, the crystallinity data observed in XRD for the starch-CLA precipitates prepared under different reaction times was not in accordance with the CLA recovery yield data (Table 1). Compared to the temperature effects, the reaction time (3–48 h) did not show a significant effect on the crystallinity of the starch-CLA complexes.

Effect of reaction pH on the XRD patterns of the starch-CLA complexes is shown in Fig. 1. Crystallinity maximized when the reaction was carried out at neutral pH (6 or 7) but decreased under acidic or alkali conditions (pH 5 and 8). The dissociation of fatty acids increases with a pH increase and a previous study has shown

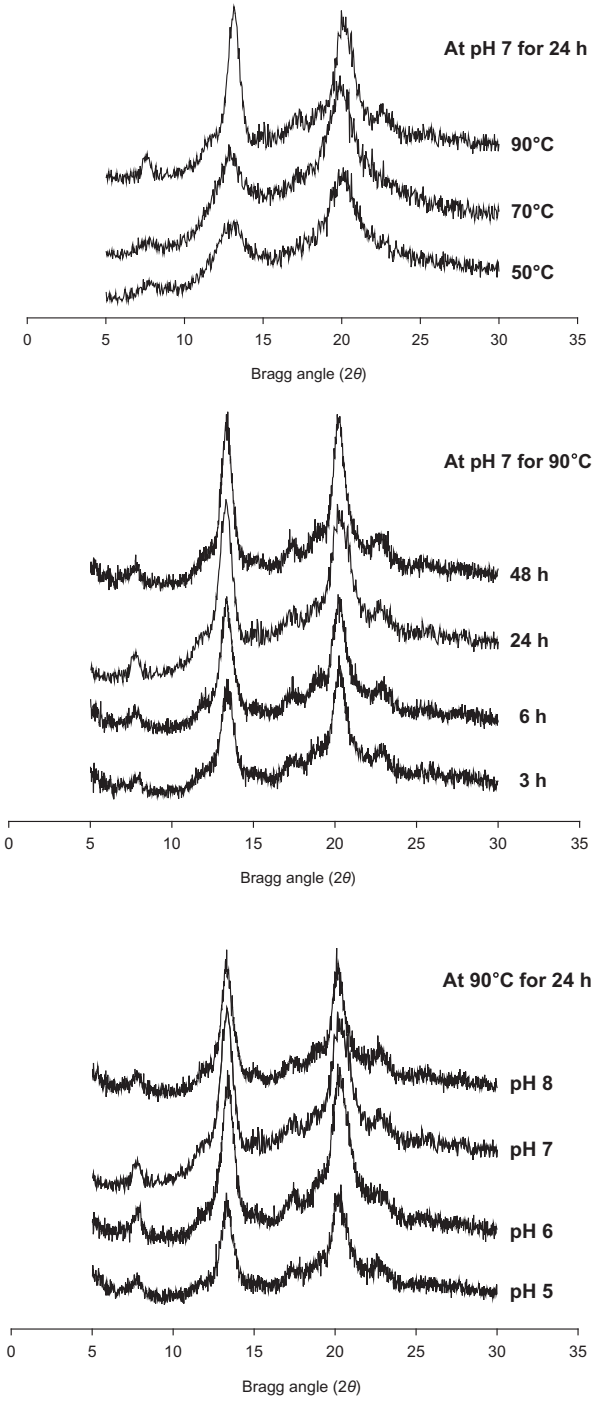

Fig. 1. X-ray diffraction patterns of the freeze-dried precipitates from starch-CLA dispersions prepared under different reaction conditions (temperature, time and pH). The defatted high amylose maize starch solution (1%, w/v) was adjusted to pH 7 and mixed with CLA solution (2.5% w/v) dissolved in ethanol.

that the dissociated form of CLA favored complex formation with starch (Yotsawimonwat et al., 2008). However, the crystallinity which indicates the degree of complex formation was decreased as the reaction pH increased from 7 to 8. It may indicate that the alkaline condition was not favorable in complex formation in this experiment possibly due to the disruption of complexes. Overall, the data revealed that the reaction at pH 7 and 90 °C for 24 h

was optimum for the formation of crystalline starch-CLA complexes. The X-ray diffraction results confirmed that the resultant complexes had V6I-type crystals in which CLA was entrapped within single amylose helices. However, the crystallinity of the complexes did not necessarily represent the recovery yield of CLA in the precipitates isolated from the reaction mixture.

3.4. Thermal transition of starch-CLA complexes

The wet precipitates obtained by centrifuging the reaction dispersions were promptly used for DSC analysis. As shown in Fig. 2, broad endotherms showing two transition peaks were observed for the precipitate samples from the reaction dispersions prepared at 70 °C and 90 °C, whereas the sample obtained from the dispersion prepared at 50 °C displayed a single transition peak on DSC thermogram. As reaction temperature increased, the melting of starch-CLA complex crystals appeared at a higher temperature. Peak temperature for the first melting gradually increased from 99.7 °C to 112.8 °C as the reaction temperature increased from 50 °C to 90 °C. The second melting peak also moved to higher temperature range with the increase of reaction temperature from 70 °C and 90 °C (113.2 °C to 119.1 °C). Generally, type I crystals begin melting at around 100 °C, whereas, type II crystals require higher thermal energy to melt with an approximate onset of 115 °C (Biliaderis & Galloway, 1989). Thus, starch-CLA complex formed at 50 °C likely possessed type I crystalline structure, but the complex formed at 70 °C might be a mixture of type I and II crystals. The weight ratio of both crystals was estimated to be 56:44 based on the melting enthalpy of the two peaks. Further increase of reaction temperature to 90 °C resulted in the formation of starch-CLA complex having mainly type II crystals (Fig. 2). Tufvesson, Wahlgren, and Eliasson (2003) have also reported similar results in which the increases in endothermic peak temperature and enthalpy were observed with increasing reaction temperature. It was reported that the crystallinity of type II complex is generally greater than that of type I complex (Biliaderis & Seneviratne, 1990).

The DSC thermograms agreed with the XRD patterns: the crystallinity increase with temperature increase observed on XRD pattern was in agreement with enthalpy increased in DSC

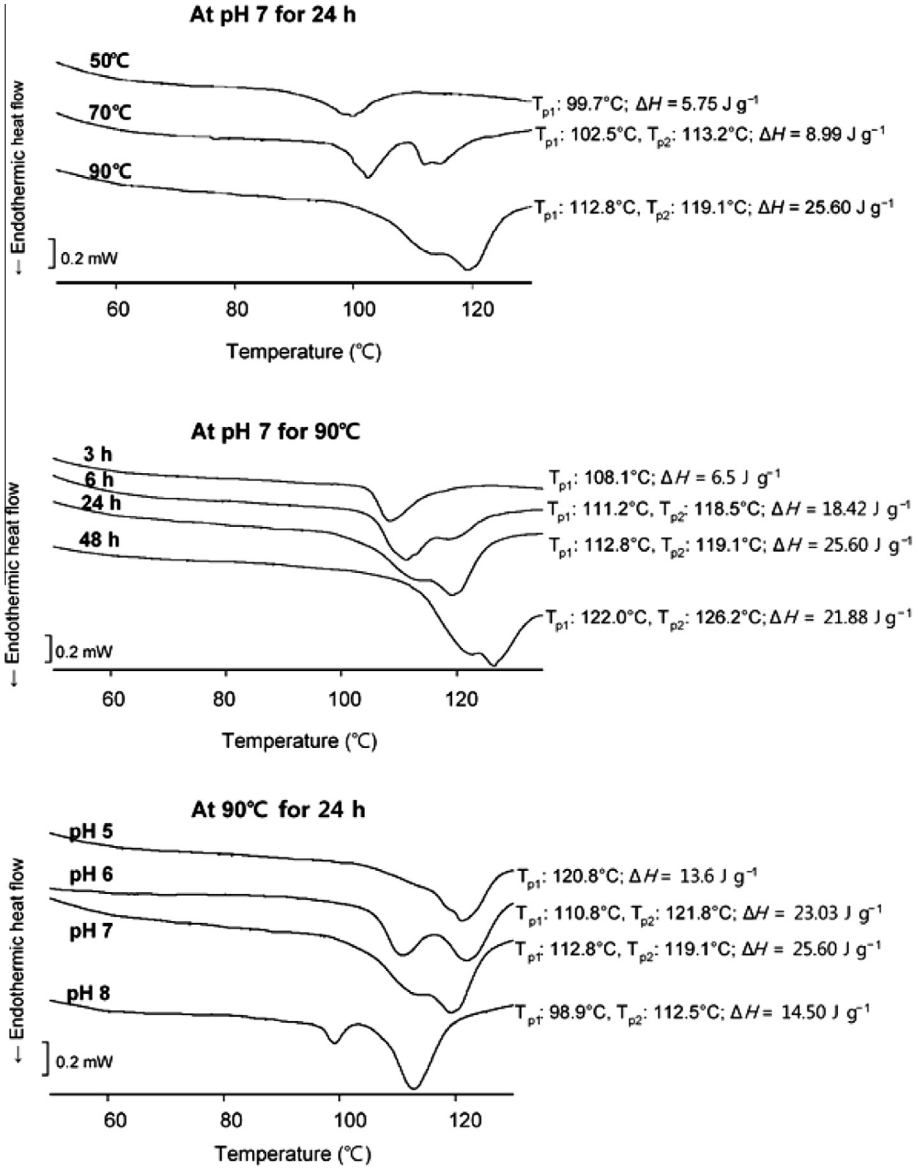

Fig. 2. DSC thermograms of the precipitates obtained for the reaction dispersions prepared under different conditions.

thermograms (Fig. 1 and 2). Crystallization occurs in three consecutive steps – nucleation, propagation, and maturation – which are temperature-dependent and kinetically controlled (Silverio, Fredriksson, Andersson, Eliasson, & Åman, 2000). At a relatively low temperatures (e.g., 50 °C), nucleation proceeds rapidly, and the formation of type I polymorph is generally favored. Yet at a high temperatures (e.g., 90 °C), the nucleation density becomes low, and the crystallization leads to more perfection which appears in type II polymorph for amylose complexes (Biliaderis & Galloway, 1989).

Reaction time and pH also affected the DSC thermograms of the starch-CLA complexes (Fig. 2). As the reaction time increased, endothermic peaks of the complexes were shifted to higher temperatures. However, pH increase shifted these peaks toward lower temperatures. The increase in reaction time also showed an increase in melting enthalpy, which indicated that progressive crystal formation as the reaction advanced. The melting enthalpy (ΔH) of the complexes was 6.5 J g^{-1} at the reaction time of 3 h but continually increased up to 25.6 J g^{-1} when the reaction time was extended to 24 h. Further extension of reaction time to 48 h, however, resulted in a decrease of melting enthalpy to 21.8 J g^{-1}. A similar trend was observed with the effect of reaction pH. The melting enthalpy (ΔH) of the complexes increased as the reaction pH increased from 5 to 7 (13.6 J g^{-1} for pH 5 and 25.6 J g^{-1} for pH 7), but decreased with a pH increase to 8.0 (14.5 J g^{-1} for pH 8). These results indicate that some crystals formed by complex formation may have experienced disruption due to severe physical and alkaline conditions (48 h, pH 8). Additionally, excessive heating (90 °C, 48 h) under alkaline condition (pH 8) could have induced oxidative degradation of CLA molecules as shown in the data of recovery yield (Table 1).

Immediate rescanning following the initial scanning showed a disappearance of the melting endotherms observed in the first thermograms of the complexes (data not shown). This irreversibility of the crystal melting was also observed in a study on the complexes between starch dextrin and β-carotene (Kim, Seo, & Lim, 2013b), which might be related to reaction time for the complex formation. As shown in the DSC result of the complexes prepared at different reaction time, the formation of crystalline structure in the V-amylose complex might be kinetically controlled. Thus, the immediate rescanning for the test of reversibility of crystal melting might not provide sufficient time for the recrystallization of V-amylose crystals. Overall, the melting enthalpy measured by DSC analysis showed a relatively good concordance with the CLA recovery yield (Table 1): higher values of CLA recovery yield corresponded with higher melting enthalpy of complex crystals.

3.5. Ultrasonication effect

The precipitates isolated from the starch-CLA complex dispersion were re-dispersed in hot water (80 °C, 50 mg/50 mL) for the evaluation of particle size and dispersion stability. Changes in the hydrodynamic mean diameter and zeta potential of starch-CLA complex particles before and after ultrasonication treatment were presented in Fig. 3. The mean diameter of the complex particles was within a relatively large-size range from approximately 650 nm to 900 nm before ultrasonication. However, the particle size rapidly decreased after an ultrasonication treatment was applied for 3 min (307 nm) possibly demonstrating that the complex particles could effectively be fragmented into small-sized particles. Extended treatment for 10 min resulted in further decrease in particle size to 201.5 nm. Similar results were found in previous studies with nano-dispersions of starch complexes with coenzyme Q_{10} and beta-carotene (Kim et al., 2012; Kim, Seo, et al., 2013b), which demonstrated that ultrasonication treatment should be controlled for effective reduction of complex particle size to the nanoscale.

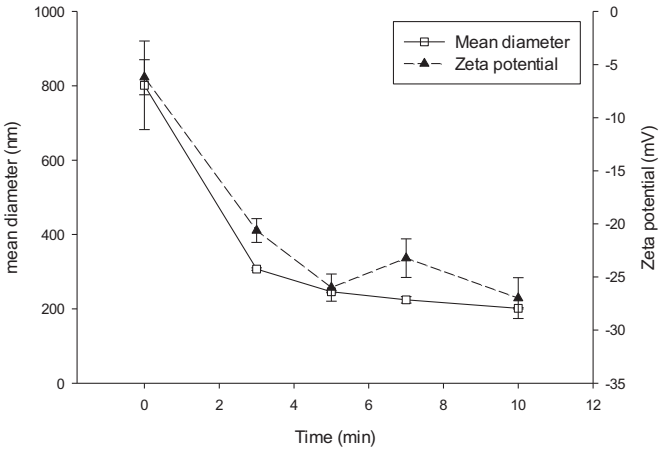

Fig. 3. Effect of ultrasonication for up to 10 min on particle size and zeta potential of starch-CLA complex dispersion.

To verify the effect of ultrasonication on dispersion stability, zeta potential was measured using a zeta potential analyzer. As shown in Fig. 3, the zeta potential of the starch-CLA dispersion prior to treatment was around −10 mV, which represented that the particles in the dispersion were unstable because a zeta potential above ±30 mV signifies a stable dispersion without forming precipitates (Kim et al., 2012). By applying ultrasonic treatment for up to 10 min, the absolute value of zeta potential for the complex dispersion gradually increased to −27 mV. In this research, ultrasonic treatment effectively fragmented the complex particles which might have been obtained as aggregates, and thus increased the surface area of the particles resulting in the enhancement of dispersion stability for storage.

The ultrasonication effect was visually observed with the starch-CLA complex dispersions stored for 4 weeks under an ambient condition (data not shown). The dispersion freshly prepared appeared homogeneous with milky-white opacity. The dispersion treated by ultrasonication (3 or 5 min) remained homogeneous with the absence of any precipitate formation for a month, whereas the untreated dispersion showed the precipitate formation within a week under ambient conditions.

Subsequently, the effect of ultrasonication on the crystalline structure of starch-CLA complexes was investigated. As shown in Fig. 4. XRD analysis revealed that the crystallinity of the complex gradually decreased as the treatment time increased. This result agreed with previous research on acid-hydrolyzed starch and nano-particles reporting that long-ranged arrangement of starch chains in nanocrystals could be collapsed by ultrasonic treatment (Kim, Han, Kweon, Park, & Lim, 2013a). Also, the minor peaks (about 17, 18, and 23°) were completely disappeared by ultrasonication. It was considered that the other type crystals present in small quantity were more susceptible to the physical treatment than V-type crystals. In the DSC thermograms, the melting enthalpy (ΔH) of the complex did not significantly change with the ultrasonication treatment (actual values not shown). However, the Type IIb crystal portion which appeared in relatively high temperature was substantially transformed to Type IIa crystals showing low melting temperature, possibly due to the physical force which induced the crystalline rearrangement.

Effect of ultrasonication on the recovery yields of starch and CLA in a re-dispersion of the complex precipitates prepared from a reaction at pH 7.0, 90 °C for 24 h is shown in Table 2. It was found that the ultrasonication decreased the recovery yields of both CLA

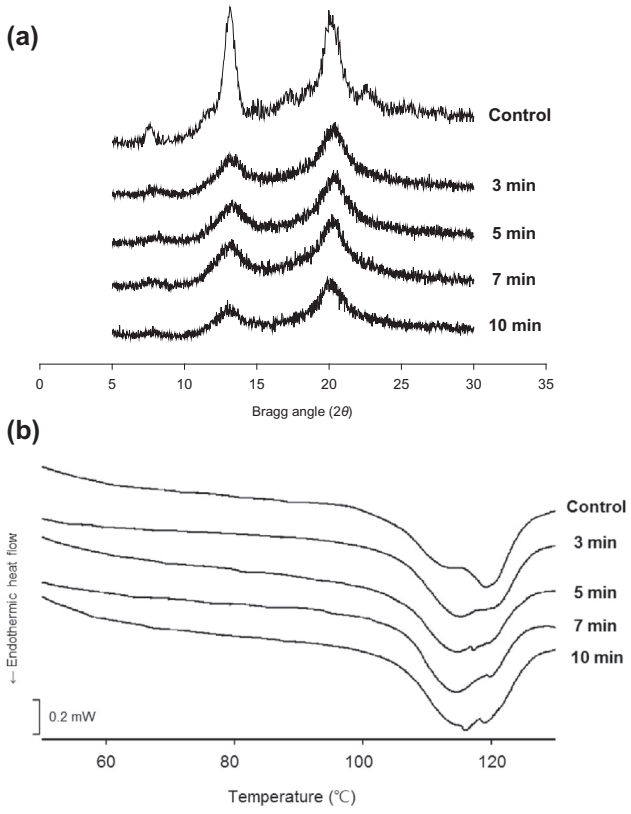

Fig. 4. (a) X-ray diffraction patterns and (b) Thermal transitions of starch-CLA complexes after ultrasonication at different periods (3, 5, 7, or 10 min).

Table 2
Recovery yields (%) of CLA and starch in a re-dispersion of the starch-CLA complex after ultrasonication for different periods.

Ultrasonication time	Yields of CLA (%)	Yields of starch (%)
0 min	63.5 ± 4.2^{a}	33.4 ± 4.4^{a}
3 min	53.0 ± 5.7^{b}	31.3 ± 2.3^{ab}
5 min	48.4 ± 4.6^{bce}	28.3 ± 2.6^{ab}
7 min	47.6 ± 2.2^{bce}	27.9 ± 1.2^{ab}
10 min	43.8 ± 4.4^{c}	25.7 ± 4.5^{b}

All data are presented as mean ± SD of triplicate determinations. Values with different alphabets in the same column are significantly different ($p < 0.05$).

and starch. The yield decrease was highly proportional to the treatment period. A similar result was found for the complex of starch dextrin and beta-carotene (Kim, Seo, et al., 2013b) showing that increasing ultrasonic treatment time promoted disruption of complexes. The authors suggested that the severe physical treatment caused the breaking of hydrophobic interactions between starch dextrin and β-carotene. The data in this study revealed that the treatment up to 10 min resulted in approximately 20% and 8% loss of CLA and starch, respectively. The greater loss of CLA than starch suggested that CLA in the complex released more freely than that of starch from the complex crystals. It could be suggested that a short-term ultrasonic treatment, 3 min in this case, was found to be effective in both increasing storage stability and minimizing loss of recovery yield for the starch-CLA dispersion.

4. Conclusions

V-type amylose complexes could be formed by blending CLA in an aqueous dispersion of high amylose maize starch. Mild, not excessive, heating was required for the maximum complex formation into the crystalline structure, and neutral pH appeared favorable. The weight ratio between starch and CLA in the precipitated complex from a dispersion reacted at 90 °C for 6 h was about 10:1. Post-treatment with mild ultrasonication demonstrated its applicability in effectively decreasing particle size and improving the storage stability of the dispersion of starch-CLA complex. An optimally prepared dispersion of starch-CLA complex remained homogeneous for a month without any phase separation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2016.05.078.

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