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Formation of crystalline complexes between amylomaize dextrin and ceramide

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ABSTRACT

Complexes between amylomaize dextrin (average DP 311) and ceramide were prepared by using two different blending systems: an aqueous batch system containing ethanol and a two-phase system of isopropyl ether and water. The organic solvents and complex formation temperature (50–90 °C) were important in determining the level of complex formation and its crystalline structure. Under X-ray diffraction analysis, the solvents as well as ceramide could form complexes with dextrin as weak V_{6I} type crystals. However, the crystallinity of complexes was much higher in the presence of ceramide, which would enhance complex formation by forming ternary co-inclusion complexes of dextrin–solvent–ceramide. Compared to the two-phase system, the batch system yielded much higher crystallinity of complexes. With a minor use of ethanol (0.5 mL) in the batch system, aqueous blending of dextrin and ceramide at 50 °C for 2 days followed by a storage at 25 °C for 1 day produced well-defined V_{6I} crystal particles as precipitates. The isolated particles had rectangular shapes with a size of 1 μm or less, and contained about half of the ceramide initially added. The ceramide–dextrin complex exhibited enhanced water dispersibility, up to 45% based on the ceramide content in complex.

1. Introduction

Various bioactive compounds having health-promoting benefits have been attracting industrial interest as functional ingredients in foods, cosmetics and nutraceuticals. Ceramide is one of the natural bioactive components for its skin barrier function and widely used as an additive in cosmetic and pharmaceutical products (Dickson & Lester, 1999; Raith & Neubert, 2000). Structurally, it is a long-chain amino alcohol, covalently linked via an amide linkage to a fatty acyl chain (Cremesti & Fischl, 2000). Several reports have described the benefits of the oral intake of glucosyl ceramide contained in cereal and legumes such as rice, wheat and soybeans as well as sphingomyelin contained in milk. The major benefits of ceramide include improvement of dry skin and alleviation of atopic dermatitis (Asai & Miyachi, 2007; Tsuji et al., 2006).

However, the industrial utilization of ceramide is limited because it is insoluble in water. The hydrophobicity may lead to a poor absorption in the gastrointestinal tract when ceramide is orally administrated (Senkal et al., 2006). Therefore, new formulation or structural modification with ceramide has been required and widely studied to enhance its water solubility and bioavailability. One of potential approaches to increase its water solubility might be the formation of complex with hydrophilic carriers such as cyclodextrin and starch. Recently, genistein–amylose complex was introduced with improved aqueous solubility and bioavailability of genistein (Cohen, Schwartz, Peri, & Shimoni, 2011).

Amylose, a linear starch molecule, tends to form a single helix by accommodating hydrophobic guest molecules inside the helices, which are laterally stacked and form a crystal, the so-called V-amylose. The V-amylose crystals are classified into several families depending on their morphology and electron diffraction diagram (Cardoso et al., 2007). The best documented V-amylose is the V_6 complex, which contains left-handed helices consisting of six D-glycosyl units per turn with a pitch of 0.805 nm (Rappenecker & Zugenmaier, 1981). The structure of this complex is usually induced by the presence of linear molecules such as linear alcohols and lipids (Whittam et al., 1989). With bulky molecules (e.g., naphthol), amylose could form a larger helices consisting of eight D-glycosyl units per turn (Bail, Rondeau, & Buléon, 2005).

The degree of polymerization (DP) of amylose affects the properties of complexes. If the amylose chains are too long, a conformational disorder may be induced, resulting in an imperfection in the crystal structure (Gelders, Vanderstukken, Goesaert, & Delcour, 2004). In contrast, if the amylose chains are too short, crystallization of the complexes can be disturbed. Kim and Lim

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(2009) used dextrins with different degree of polymerizations (DP) prepared by an acidic hydrolysis of a high amylose maize starch in alcohol for the complex formation with *n*-butanol. They found that the complex formation with butanol required only fragment of starch chains (DP_n 311), and thus starch dextrins could form complexes with butanol more efficiently than did intact starch. In addition to the chain length of amylose, the morphology and hydrophobicity of guest molecules also affect the complex formation characteristics with starch or dextrin.

Complex formation with amylose or starch dextrins for various bioactive components (i.e., bioactive long chain FAs such as conjugated linoleic acid and docosahexadecanoic acid) has been extensively studied (Gelders, Goesaert, & Delcour, 2006; Lalush, Bar, Zakaria, Eichler, & Shimoni, 2005). However, research data on the complex formation with ceramide is scarce. Therefore, in this study, the complex formation between a dextrin of amylomaize starch (70% amylose) and ceramide by using batch and two-phase systems was characterized, and the effects of physicochemical conditions on the crystalline structure of complexes were examined.

2. Materials and methods

2.1. Materials

Amylomaize starch (Hylon VII, 70% amylose) was purchased from the National Starch & Chemical Company (Bridgewater, NJ, USA). Ceramide that had been chemically synthesized (>95% purity) was supplied by Doosan Corporation (Kyunggi-do, Korea).

2.2. Preparation of dextrin

A dextrin was prepared following the method of Kim, Yoon, and Lim (2009). Amylomaize starch was hydrolyzed in an acidic alcohol solution (HCl and ethanol) at 20 °C for 72 h. Hydrolyzed dextrins (1 g, dry basis) were purified by dispersing the dextrin in 1 M NaOH solution (10 mL) by vortexing, and the solutions were diluted with distilled water (40 mL). After neutralizing with 1 M HCl (10 mL), the solutions were autoclaved (121 °C, 20 min) before precipitating with 95% ethanol, washing twice with 95% ethanol and once with acetone, and drying at room temperature overnight. The DP_n value of resulting dextrin, calculated as the ratio between total carbohydrate content and the reducing value (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956; Somogyi, 1952), was 311.

2.3. Preparation of complexes

An aqueous solution containing dextrin (250 mg, dry basis) was prepared by dissolving the dextrin in 1 M NaOH solution (2 mL). Distilled water (21 mL) and 1 M HCl solution (2 mL) were then added for neutralization. The resulting solution was purged with nitrogen gas for 3 min to prevent oxidation and autoclaved (121 °C, 20 min). Ceramide (30 mg, dry basis), the guest compound, was dissolved in ethanol (0.5–2.0 mL) under mild stirring at 50 °C and the solution was slowly added to the dextrin solution. The mixture was then stirred vigorously (550 rpm) at 50 °C for 2 days for complex formation.

To compare the complex forming ability with the above-mentioned batch system, another method referred to as two-phase system was investigated. In this system, ceramide (30 mg, dry basis) was dissolved in isopropyl ether (25 and 35 mL, organic phase) under mild stirring at 50 °C and slowly added to the dextrin solution (25 mL, aqueous phase). The two-phases were mixed vigorously (550 rpm) at 50 °C, and the isopropyl ether continually evaporated from the open system during 2 days. After complex formation, the aqueous solution was cooled down to ambient temperature slowly and stored at 25 °C for 1 day. Complexes obtained as precipitates were recovered by centrifuging (5000 × *g*, 15 min) and freeze-drying the solution overnight.

To obtain an in-depth understanding of complex formation behaviors, the crystalline structures and thermal transitions of complexes formed in the batch system were investigated further using the following complex formation conditions: reaction temperature of 50, 70 or 90 °C for 2 days followed by storage at 25 °C for 1 day or 3 days.

2.4. X-ray diffraction pattern

The crystalline structures of the dextrin–ceramide complexes were determined with an X-ray diffractometer (XPERT MPD, Philips Analytical, Almelo, the Netherlands) at a target voltage and current of 40 kV and 30 mA, respectively. The scanning range and rate were 3–30° (2θ) and 1.0°/min, respectively.

2.5. Thermal analysis

The thermal transition of the dextrin–ceramide complexes was determined by differential scanning calorimeter (DSC 6100, Seiko Instruments, Chiba, Japan). The DSC instrument was calibrated with indium, and an empty pan was used as a reference. Freeze-dried complex and water were weighed into an aluminum pan in a 1:2 ratio. The sealed pan was equilibrated in a cold chamber (4 °C, 2 h) before the analysis. The sample was scanned from 40 to 120 °C at a rate of 5 °C/min. The sample was then quickly cooled and reheated under the same conditions for second scanning.

2.6. Quantification of dextrin and ceramide

The re-dispersion of freeze-dried complex (1 mg/10 mL) was autoclaved (121 °C, 20 min) to disrupt any linkages between ceramide and dextrin. The amount of dextrin was quantified using the phenol-sulfuric acid method (Dubois et al., 1956). For the analysis of ceramide, freeze-dried complex (20 mg) were dissolved in an isopropanol/water mixture (7:3, v/v, 10 mL) under a mild stirring at 50 °C for 1 h. The ceramide extracted from the complexes was quantified using a high-performance liquid chromatography (HPLC; Varian Prostar 210, Varian, Palo Alto, CA, USA) with a refractive index detector (Shodex RI-71, Tokyo, Japan). The HPLC-RI system consisted of a pump (P2000, Spectra System, San Jose, CA), an injector valve with a 0.1 mL loop (Rheodyne 7072, Cotati, CA, USA), and a column (TSKgel silica-60, 4.6 mm × 250 mm, Tosoh Bioscience, Montgomeryville, PA, USA). The eluent was an isopropanol/water mixture (7:3, v/v) that had been filtered through a 0.1 μm pore PTFE filter (Whatman, Kent, UK) and the flow-rate of eluent was 0.4 mL/min. Samples were filtered through a membrane filter (5.0 μm pore size, Advantec Inc., Japan) before HPLC analysis.

2.7. Water dispersibility

Water dispersibility of ceramide in the complexes prepared in batch or two-phase system was determined. The freeze-dried complex samples (dry basis, 80 mg) were dispersed in 40 mL of distilled water and the dispersion was magnetically stirred for 3 h at 25 or 50 °C. The dispersion was stored at 25 °C for 24 h and then centrifuged at 5000 × *g* for 15 min to remove the precipitates. The ceramide dispersed in the supernatant was quantified using HPLC as previously described.

2.8. Transmission electron microscopy (TEM)

The morphology of the dextrin–ceramide complexes was observed using a transmission electron microscopy (FEI Technai

G2 F30, Eindhoven, the Netherlands). The re-dispersion of the centrifuged residue (0.3 mg/1.0 mL) was deposited on a carbon-coated microscopy grid. It was negatively stained with a drop of 2% (w/v) uranyl acetate and dried at room temperature. The dried sample was imaged by TEM at an accelerating voltage of 200 kV.

3. Results and discussion

3.1. Formation of dextrin–ceramide complexes

Complex formation between dextrin and ceramide was accomplished using two different systems and they were referred to as "batch system" and "two-phase system", respectively. Preliminary studies on the complex formation were carried out by dissolving dextrin and mixing it with the powdered ceramide. The results revealed that complex formation without any solvents is difficult because of low solubility of ceramide. Thus, a suitable solvent for dissolution solid ceramide is needed to help the increase of the reactivity with dextrin. In this study, two different solvents (aqueous or organic) were chosen to dissolve ceramide prior to the complexing process and investigated how V-amylose structures are affected by the solubility of ceramide.

In the batch system, ceramide (30 mg) was dissolved in small amounts of ethanol (0.5–2.0 mL) under mild heat (50 °C) and then this solution was directly added to a dextrin solution (250 mg/25 mL). Two-phase system was conducted in the same way as the batch system, but in this case, ceramide was dissolved in isopropyl ether (25–35 mL). In two-phase system, ceramide and dextrin solution remained in separated phases, unlike the batch system forming only one-phase. This is because isopropyl ether is slightly soluble in water but miscible with most organic solvents. By the extensive blending, however, ceramide molecules in the organic solution were diffused into the dextrin solution, as a result, such physical contact led the complex formation.

Separation of the dextrin–ceramide complexes from the suspensions prepared by both systems was done by centrifugation ($5000 \times g$, 10 min). These complexes that could be recovered as precipitates after an ambient storage were also found by many literatures (Biliaderis, Page, Slade, & Sirett, 1985; Galloway, Biliaderis, & Stanley, 1989; Karkalas & Raphaelides, 1986; Raphaelides & Karkalas, 1988). It should be noted that liquefied ceramide could be dispersible in aqueous media when dextrin co-exist, as a result of structural associations between ceramide and dextrin. However, it was assumed that the characteristics of the obtained dextrin–ceramide complexes would differ between two different systems. More study should be done to understand the formation of the complexes.

3.2. X-ray diffraction (XRD) patterns

The complex samples yielded a typical V_{6I} type X-ray diffraction pattern with two main reflections corresponding to the Bragg angles [2θ] of 13.03 and 20.35° independent of the complex formation methods (Fig. 1). A minor reflection at 7.0° [2θ] was also observed, which overlapped with a peak for free ceramide that could not participate in the complex formation. V_{6I} complex crystal structures have been proposed for a variety of linear guest molecules such as fatty acids (Buléon, Duprat, Booy, & Chanzy, 1984), emulsifiers (Brisson, Chanzy, & Winter, 1991), and alcohols (Whittam et al., 1989). Ceramide used in this study is composed of a long-chain amino alcohol that is covalently linked via an amide linkage to a fatty acyl chain (Cremesti & Fischl, 2000). Therefore, it was possible that ceramide having a linear molecular structure induced another type of V-amylose complex consisting of six D-glucosyl residues per turn.

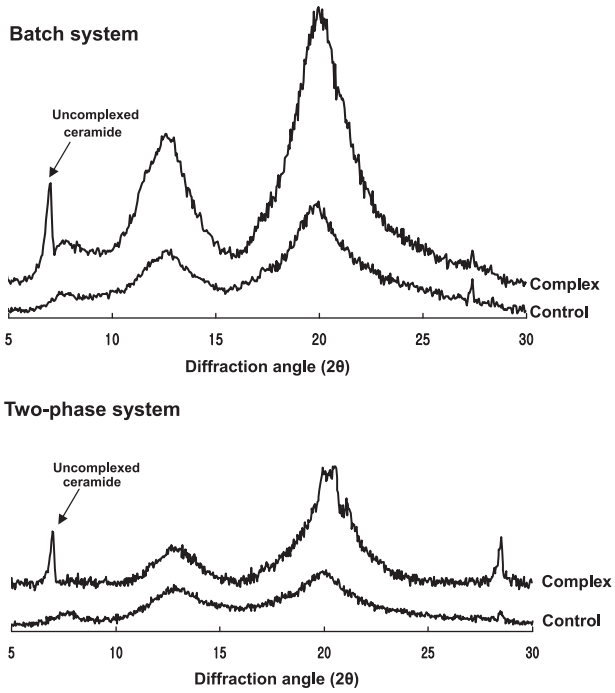

Fig. 1. X-ray diffraction (XRD) patterns of complex samples prepared in the batch and two-phase systems. The complex formation was conducted at 50 °C for 2 days followed by a storage at 25 °C for 1 day. Ceramide (30 mg) was dissolved in 0.5 mL of ethanol for the batch system or 25 mL of isopropyl ether for the two-phase system. "Control" was the sample prepared without ceramide.

Dextrin dispersion, processed in a similar way but without ceramide, was used as control group (Fig. 1). They also had typical V_{6I} patterns, probably due to the complexation of solvents with some part of the dextrin (Whittam et al., 1989). However, the intensity peaks at Bragg angles [2θ] ~13° and ~20° was much weaker than those observed for the complexes. It indicated that ceramide played an important role in the induction of the structural changes, which led to more tight V_{6I} structure. Moreover, it was found that the dissolution of ceramide was critical for the complex formation with dextrin. The samples without solvents did not show any crystalline XRD pattern, indicating that no complex had been formed between the dextrin and ceramide (data not shown). Solid ceramide has limited physical contact to dextrin, and thus the reactivity with dextrin becomes very low. Similar result was reported by Tapanapunnitikul, Chaiseri, Peterson, and Thompson (2008). They claimed that the ligand needs to be in solution to interact with amylose. The presence of flavor compounds with a relatively high solubility may sufficient to induce complex formation, resulting in V_7-amylose. For flavor molecules of low solubility, however, a stable complexation only takes place in the presence of lipids, including a V_6-conformation for the ternary complex.

The extent of complexation varied depending on the type and amount of solvent used (Fig. 2). Both ethanol and isopropyl ether serving as solvent led to the formation of V_{6I} crystal structure, but the extent of complex formation appeared different. Probably, superior dissolution ability of ethanol compared to isopropyl ether could be a reason for the higher crystallinity of the complexes in the batch system. In fact, only 0.5 mL of ethanol was sufficient for dissolving ceramide 30 mg. It was also found that the amount of solvent did not positively relate to the crystallinity. Relatively small amount of solvents (0.5 mL of ethanol or 25 mL of isopropyl ether, respectively) were best for complex formation in the both systems. The presence of the desired amount of solvent would lead to the positive cooperativity with ceramide whereas excess use of solvent might limit the function of ceramide, resulting in the formation

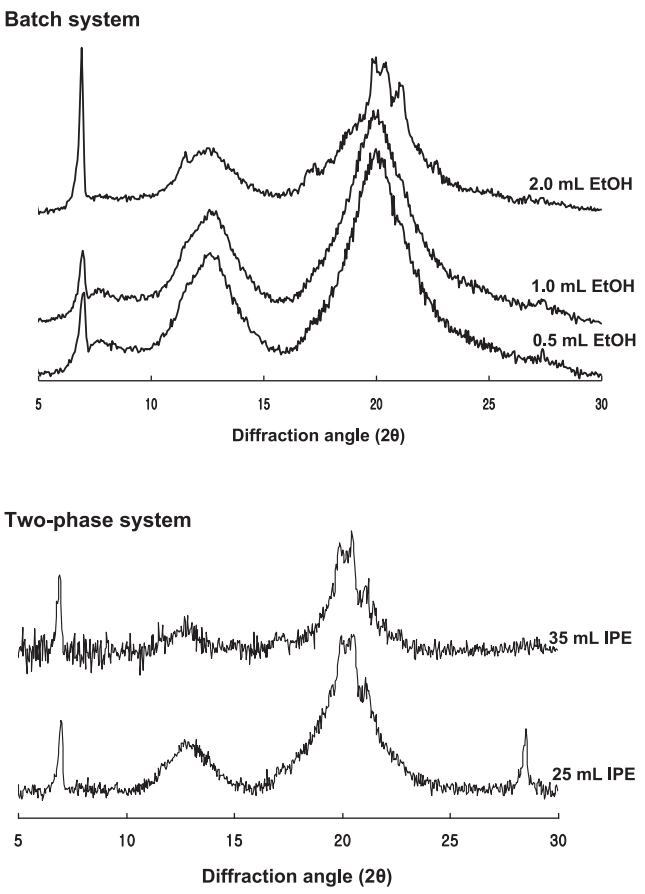

Fig. 2. Effect of solvents on the XRD patterns of complex samples formed in the batch and two-phase systems. Ceramide (30 mg) was dissolved in different amounts of ethanol (0.5–2.0 mL) for the batch system or isopropyl ether (25–35 mL) for the two-phase system.

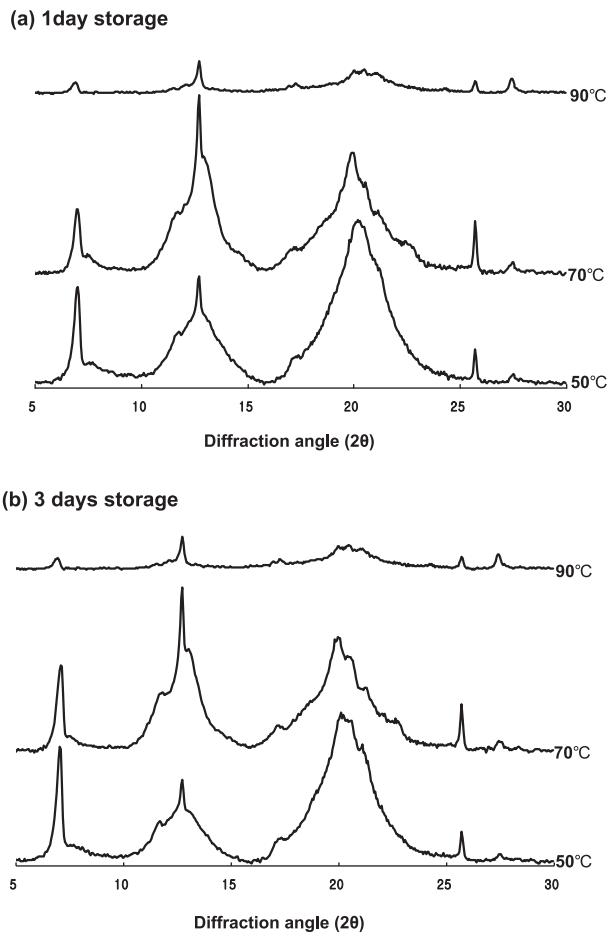

Fig. 3. Effect of reaction temperature on the XRD patterns of complex samples formed in the batch system. The complex formation was performed at 50, 70 or 90 °C for 2 days followed by a storage at 25 °C for 1 day (a) or 3 days (b). Ceramide (30 mg) was dissolved in 0.5 mL of ethanol to be used in the complex formation.

of amorphous aggregates of dextrin. This result was supported by the peaks around 7.0° [2θ] characteristic of uncomplexed ceramide, which increased as the amount of solvent increased. Therefore, the type or amount of solvent should be controlled to induce complex formation properly.

The structural characteristics of the complexes also strongly influenced their complexation temperature (Fig. 3). Complexes formed using batch system at 50 °C had diffraction pattern similar to those obtained at 70 °C, but the position of major peak differed from each other. Whereas XRD peak obtained after blending at 90 °C was much less pronounced than those at 50 and 70 °C, which may indicate very poor complexation ability. The result of this study was incompatible with previous reports (Buléon, Colonna, Planchot, & Ball, 1998; Godet, Bizot, & Buléon, 1995). These authors proposed that complexes formed at a higher temperature (at least 90 °C) exhibited more crystalline V-type XRD pattern, whereas those formed at a temperature of maximally 60 °C showed an amorphous XRD pattern. Pure ceramide melts at ~90 °C (López-Montero, Monroy, Vélez, & Devaux, 2010) so the ceramide may remain as crystalline solids at 50 and 70 °C tested in this study, which make it difficult for ceramide to physical contact with dextrin. Therefore, it is worth noting that sharp V-form peaks appeared after blending at 50 or 70 °C. This is attributed to the solvent used for dissolving ceramide. With the help of ethanol, the interaction between dextrin and ceramide and subsequent complex formation would be feasible even temperature below ceramide melting. While, ethanol at 90 °C was consumed rapidly due to the temperature above boiling point, as a result, it could not the role as an assistant for complex formation.

When the temperature of complex dispersions decreased from 50, 70, 90 °C to 4 or 25 °C, dispersions contained crystalline precipitates (Fig. 3). Precipitates in these dispersions were classified into two forms, that is, either B or V-type crystalline form depending on the storage temperature. In general, low storage temperature (at 4 °C) has been shown retrogradation easily (Zhang, Huang, Luo, & Fu, 2012). The refrigerated solution may allow the dextrin chains to crystallize, thus it contained mainly B-type crystals (data not shown). Solution containing the V-type crystals could be prepared at ambient temperature. Unlike our result, Cohen, Orlova, Kovalev, Ungar, and Shimoni (2008) claimed that the ratio between the B form and the V form can be determined by the amount of guest compound added to the complexation solution. They observed that as genistein–amylose ratio increase, the complexes contained mostly V form.

Based on the X-ray diffraction results, dextrin–ceramide complexes were obtained in the V_{6I} type crystal form in which ceramide was entrapped only within the helices. And it is possible by using solvent as a vehicle for ceramide dissolution. The degree of crystallinity of these complexes varied depending on the complex formation method including type or content of solvent, complexation temperature, etc.

3.3. Thermal transition of complexes

Thermal melting property of the precipitated complexes recovered by centrifuging the dispersions was determined by using a

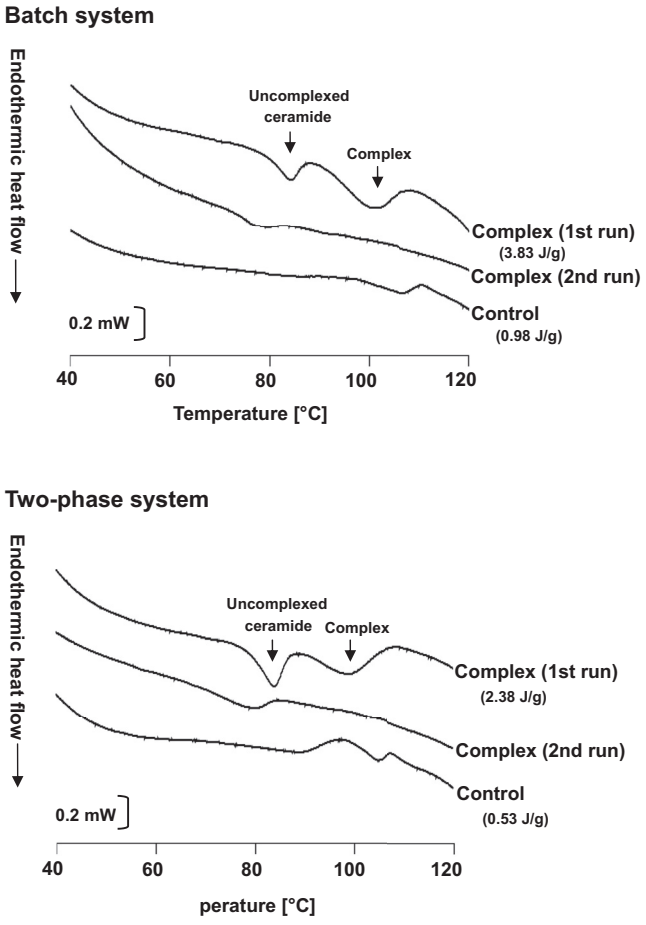

Fig. 4. DSC thermal transitions of the complex samples formed in the batch and two-phase systems. Complex formation was conducted at 50 °C for 2 days followed by a storage at 25 °C for 1 day. Ceramide (30 mg) was dissolved in 0.5 mL of ethanol for the batch system or 25 mL of isopropyl ether for the two-phase system. "Control" was the sample prepared without ceramide.

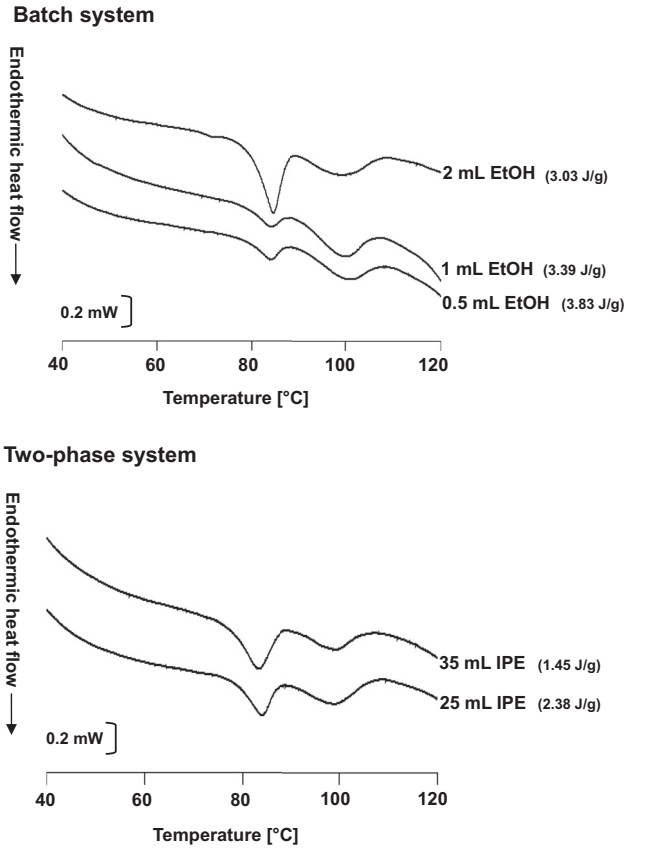

Fig. 5. Effect of solvents on the DSC thermal transitions of complex samples formed in the batch and two-phase systems. Ceramide (30 mg) was dissolved in different amounts of ethanol (0.5–2.0 mL) for the batch system or isopropyl ether (25–35 mL) for the two-phase system.

differential scanning calorimeter. The simple control group showed only a single endotherm at around 105 °C (Fig. 4), indicating that some portion of dextrin formed complexes with solvent. For the dextrin–ceramide complexes, however, broad endotherms with two peaks were observed in the both systems: one at around 84 °C for the melting of free ceramide and the other at around 105 °C for the melting of V-type crystals of dextrin–ceramide complex. The melting temperature for the second peaks was considered as the melting of type I complex, based on the report by Karkalas, Ma, Morrison, and Pethrick (1995). However, since type I complex is usually associated with an amorphous XRD pattern, this result seemed to conflict with the previous XRD results showing crystalline V-type diffraction pattern. Biliaderis and Seneviratne (1990) explained this phenomenon to be the result of the drying procedure used to prepare the complexes. Freeze-drying modified the structure of type I complexes by formation of chain aggregates of a much higher order. The evidence of type II became more clearly by rescanning complex samples immediately after the first scan. Upon cooling and reheating (second run in Fig. 4), the melting endotherm of the complexes disappeared, leaving the peak of only uncomplexed ceramide. This peak became smaller and shifted to lower temperature (~78 °C) compared to the first scan. If complexes are type I form, they would reform at a temperature ca. 10–20 °C below the T_p after rescan (Biliaderis et al., 1985; Karkalas et al., 1995; Rappenecker & Zugenmaier, 1981).

The melting enthalpy (ΔH) that reflects the degree and perfection of the crystallinity (Durrani & Donald, 1995) also analyzed. The melting enthalpies of complexes (3.83 and 2.38 J/g in the batch and two-phase system, respectively) were higher than those of control group (0.98 and 0.53 J/g in the batch and two-phase system, respectively), probably due to the presence of ceramide. The ceramide would enhance complexation by forming ternary coinclusion complexes of dextrin–solvent–ceramide, which resulted in more perfection of the crystallinity. The enthalpy values of the complexes produced under the specific conditions are in agreement with the crystallinity shown by the XRD peak intensity. The enthalpy values showed the tendency to increase when solvent content or complexation temperature was decreased. As shown in Fig. 5, it represented 3.03 J/g for 2 mL, 3.39 J/g for 1 mL, and 3.83 J/g for 0.5 mL ethanol in the batch system, 1.45 J/g for 35 mL and 2.38 J/g for 25 mL in the two-phase system, respectively. Along with the increase of complexation temperature, the enthalpy values of complexes increased, ranging from 0.32 J/g at 70 °C, and to 3.83 J/g at 50 °C. In the case of 90 °C, however, no endotherm for complex was observed (Fig. 6). When the storage of complex dispersions continued up to 3 days under the same condition, the enthalpy values for the complexes decreased. And such trend was predominant in dispersion stored after blending at 50 °C. In contrast, the enthalpy values for free ceramide increased with increasing storage time up to 3 days. The reduced ΔH values for the complexes may result from the increased amount of precipitates, probably because free ceramide molecules were associated for 3 days of storage.

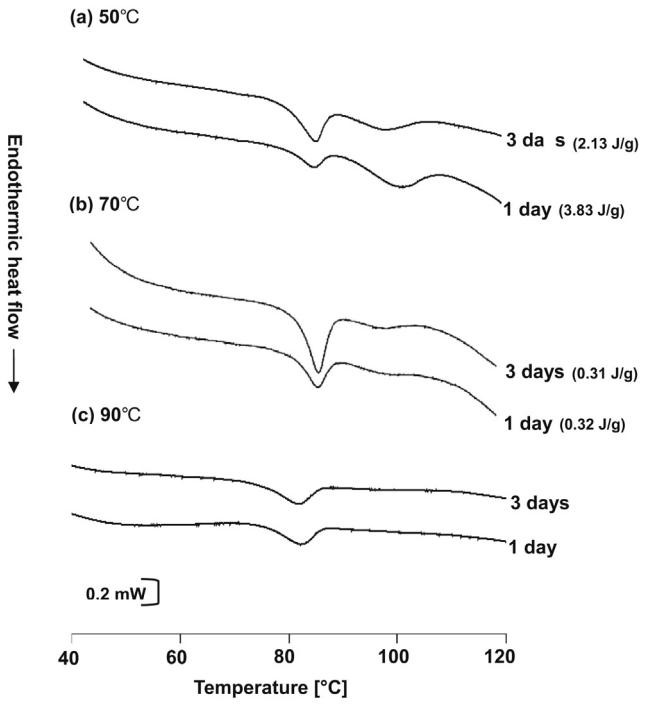

Fig. 6. Effect of reaction temperature on the DSC thermal transitions of complexes formed in the batch system. The complex formation process was performed at 50 (a), 70 (b) or 90 °C (c) for 2 days followed by storage at 25 °C for 1 day or 3 days. Ceramide (30 mg) was dissolved in 0.5 mL of ethanol to be used in the complex formation.

3.4. Yields and water dispersibility

The yields of dextrin and ceramide were calculated based on the precipitates recovered by centrifuging the dispersions after complex formation under the optimal conditions (Fig. 7a). There was no apparent discrepancy in dextrin yield even though two different systems were employed. From 250 mg of initially dextrin addition more than 110 mg of dextrin was recovered as co-precipitates with ceramide, showing the dextrin yields of 51.2% and 47.8% similarly in the batch and two-phase system, respectively. However, it was found that the amount of ceramide in the batch system (46.7%) was much higher than that in the two-phase system, nearly 2-fold of that in the two-phase system. This result indicates that the complex formation in batch system was more favorable for ceramide incorporation in dextrin complex, which was consistent with the results observed under X-ray diffractions and DSC.

One of the major attributes in complex formation with dextrin is enhancing water solubility of the incorporated compounds. The ceramide–dextrin complexes prepared in both batch and two-phase systems were obtained not fully soluble, but in the form of dispersed particles. The complex particles obtained under the optimal condition were chosen to measure the water dispersibility at two different temperatures: 25 and 50 °C. As shown in Fig. 7b, the complex particles displayed a good water dispersibility showing nearly more than one third of the ceramide in the complexes being homogeneously dispersed in water. The amounts of ceramide dispersed in water at 25 °C were 45.5% and 35.8%, in the batch and two-phase systems, respectively. It indicates that the complex formation in the batch system was more effective in increasing the water dispersibility of ceramide. The effect of temperature increase (50 °C) on the water dispersibility appeared not significant: 45.4% and 36.7% water dispersibilities for the complexes prepared in the batch and two-phase systems, respectively. The aqueous dispersion of ceramide complexes (45.4% ceramide) remained homogeneous and stable without forming precipitates while storing at

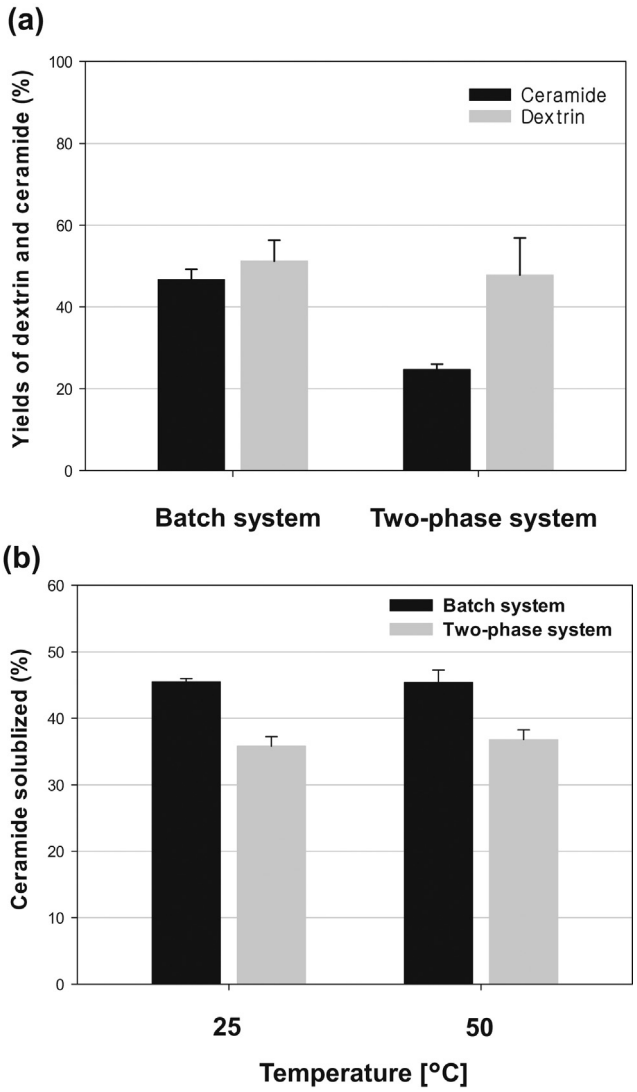

Fig. 7. (a) Yields of dextrin and ceramide in the isolated complexes from the batch (0.5 mL ethanol, 50 °C for 2 days) and two-phase systems (25 mL isopropyl ether, 50 °C for 2 days), respectively. (b) Percent ceramide in the complexes dispersed in water at 25 or 50 °C.

ambient temperature for a week (data not shown). Considering that pure ceramide is hydrophobic and not dispersible in water, the water dispersibility of ceramide endowed by the complex formation with dextrin may provide extended possibility for the ceramide to be utilized in various commodities of aqueous basis. This result was in agreement with the findings in several literatures showing that some of the hydrophobic compounds could have increased water solubility by complex formation with cyclodextrin or starch (Samperio et al., 2010; Stancanelli et al., 2007).

3.5. Morphology of complexes

Fig. 8 depicts the transmission electron microscopy (TEM) images of the dextrin–ceramide complexes formed under the optimal conditions. The TEM images revealed that complexes formed in the batch system yielded apparent aggregates with morphologies distinctly different from those of complexes formed in the two-phase system. The complexes formed in the batch system were uniformly distributed, with a length ranged in about 1 μm. In contrast, complexes formed in the two-phase system did not form regular rectangular shapes but rather a mixture of various

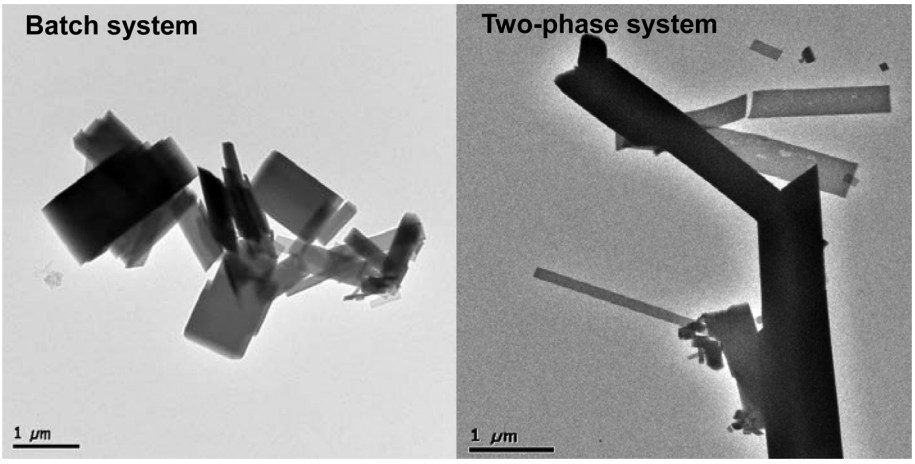

Fig. 8. TEM images of a negatively stained complexes from the batch (0.5 mL EtOH, 50 °C for 2 days) and two-phase systems (25 mL isopropyl ether, 50 °C for 2 days), respectively.

platelets with a broader range of sizes of 1–3 μm. This may be a result of different complexation kinetics or different complexation mechanisms, or both. According to the report by Lesmes, Cohen, Shener, and Shimoni (2009), V-amylose crystals produced by complex formation with guest compounds (three different fatty acids varying only in their degree of unsaturation) can be obtained as micrometer-sized particles. And the morphology of these particles was highly dependent on the structure of guest molecules and the complex formation method. Storage process after the complex formation may cause the increase of the particle size up to micro-size. Moreover, the dehydration of the isolated crystal particles for the TEM observation may induce the aggregation, affecting the shape and size of the particles.

4. Conclusions

Crystalline complexes between amylomaize dextrin and ceramide could be formed by blending both components in an aqueous batch system or a two-phase system. In the batch system, better defined V_{6I} crystalline complexes were formed compared to the two-phase system. The complex formation enhanced water dispersibility of ceramide, which may extend the utilization of ceramide in various aqueous commodities.

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