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Preparation and characterization of aqueous dispersions of dextrin and policosanol composites

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ABSTRACT

Policosanol (50–100 mg) was dispersed in an aqueous solution (0.5–2.0% solids, w/v, 50 mL) of an amylomaize starch dextrin at 90 °C under different conditions. The dispersion of the mixture was opaque but remained homogenous for up to 7 days at an ambient temperature. The precipitates obtained by centrifuging the dispersion (5000 × *g*, 30 min) contained crystalline V-amylose complex of policosanol. The supernatant also contained policosanol but not in the complex form. Stepwise addition of policosanol and longer time complex formation increased the dispersible policosanol: about 95% by 30 min interval, 1.0% dextrin solution and 12 h complex formation time. Among the dispersible policosanol (95%), 70% policosanol resided in the precipitates and 25% was in the supernatant, indicating the dextrin behaved as a stabilizer for the dispersed policosanol as well as a complex forming agent with policosanol. The policosanol dispersion was sonicated up to 30 s to evaluate physical stability. Around 70% policosanol in the dispersion remained stable against the sonication treatment.

1. Introduction

Policosanol, which may be isolated from various plant materials including sugar cane, bees wax, rice bran and wheat germ, is a mixture of aliphatic primary long-chain alcohols with chain lengths varying from C_{20} to C_{36} (Cherif, Ben Messaouda, Kaabi, Boukhchina, Pepe & Kallel, 2010; Irmak, Dunford & Milligan, 2006). The main aliphatic alcohols in policosanol are docosanol (C_{22}), tetracosanol (C_{24}), hexacosanol (C_{26}), octacosanol (C_{28}), and triacontanol (C_{30}) (Irmak, Dunford & Milligan, 2006). It has been reported that the aliphatic primary alcohols provide various physiological effects relating to cardiovascular diseases and dermatological diseases (Arruzazabala, Carbajal, Mas, Garcia & Fraga, 1993; Carbajal, Arruzazabala, Valdés & Más, 1998; Varady, Wang & Jones, 2003). Policosanol may also act as a cholesterol-lowering agent by inhibiting cholesterol biosynthesis and enhancing LDL decatabolism (Gouni-Berthold & Berthold, 2002; Menendez et al., 1994). It also enhances the stability of lipoproteins against oxidation (Menéndez, Fraga, Amor, González & Más, 1999). Currently, policosanol is commercially utilized in dietary supplements in many countries (Irmak, Dunford & Milligan, 2006). However, policosanol is hydrophobic and insoluble in aqueous media, which limits its application in a variety of industrial products.

Delivery systems that make policosanol readily dispersible in aqueous media are thus very useful for its application not only in foods but in cosmetics and pharmaceuticals. Simple oil-in-water emulsions are widely used in the preparation of aqueous dispersions of lipophilic components; however, the emulsions are susceptible to breakdown when exposed to various environmental stresses (Maswal & Dar, 2014; Rousseau, 2000). Multilayer emulsions are more stable and effective delivery systems for hydrophobic compounds than monolayer emulsions; however, they require complicated fabrication techniques with a large quantity of surfactant (Maswal & Dar, 2014). Another approach for the dispersion of hydrophobic compounds is molecular encapsulation in polymeric matrices. Cyclodextrin is one of the common encapsulating agents because it has a unique structure with hydrophobic interior cavity but hydrophilic exterior allowing the guest components to be soluble or dispersible in water (Vos, Faas, Spasojevic & Sikkema, 2010). Cyclodextrin is produced industrially from starch using an enzyme called cycloamylose glucanotransferase (Yamamoto, Zhang & Kobayashi, 2000).

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Starch, which is a raw material for cyclodextrin production, offers a similar function of incorporating various hydrophobic compounds. Moreover, it has a distinct advantage over cyclodextrin in industrial utilization because of its renewability and low price (Wu, Wang, Zhi, Jiang, Zhang & Wang, 2011). Encapsulation of hydrophobic components such as policosanol within starch chains can be achieved by forming random and/or ordered structures similar to that of cyclodextrin. Amylose, a relatively linear glucan with mainly α-(1,4) linkages possibly plays a major role in encapsulation, because it can form inclusion complexes with hydrophobic compounds which is called V-amylose. The central cavity of V-amylose inclusion complex can host hydrophobic compounds whereas the hydrophilic outer surface enhances the dispersibility of host compounds in aqueous media (Eliasson, 2004; Tomasik & Schilling, 1998a,b). In this way, starch may be applied as a carrier for various bioactive compounds such as CoQ10 and beta-carotene (Heinemann, Zinsli, Renggli, Escher & Conde-Petit, 2005; Jouquand, Ducruet & Le Bail, 2006; Kim, Kim, Chung & Lim, 2012; Kim, Seo & Lim, 2013; Lalush, Bar, Zakaria, Eichler & Shimoni, 2004; Wulff, Avgenaki & Guzmann, 2005). Although policosanol has been widely used as a potent nutraceutical, no study on the preparation of its aqueous dispersion by forming the complex with starch has not been performed yet.

In this study, commercial policosanol was dispersed in aqueous media containing a dextrin of amylomaize starch, and physical characteristics of the dispersion were examined. Various parameters in relations to the complex formation and dispersibility of policosanol, such as complex formation time, solute concentrations, and stepwise addition of policosanol were examined.

2. Materials and methods

2.1. Materials

Amylomaize starch (amylose content 70%) was obtained from Ingredion Incorporated (Bridgewater, NJ). Policosanol extracted from sugarcane wax (octacosanol > 65%, 1-triacontanol 20%, and 1-hexacosanol 9.9%) was purchased from Active Ingredient Group Inc. (Changsha, China). All other chemicals used were of analytical grade.

2.2. Dextrin preparation

Starch was subjected to an acid hydrolysis according to the method of Kim, Yoon, and Lim (2009). Amylomaize starch (25 g) was suspended in anhydrous ethanol with 0.36% hydrochloric acid (w/v) and then incubated at 20 °C for 72 h. The resulted mixture was collected, washed with 70% ethanol, and then dried at 40 °C for 24 h. The resulted dextrin was purified using dimethylsulfoxide solution (90%) and absolute ethanol (Lee, Han & Lim, 2009). The number-averaged degree of polymerization of the dextrin was approximately 300 as calculated from the ratio of reducing value and total carbohydrate content (DuBois, Gilles, Hamilton, Rebers & Smith, 1956; Jane & Robyt, 1984; Somogyi, 1952).

2.3. Preparation of aqueous dispersions

The dextrin (500 mg dry solids) was dissolved in an aqueous NaOH solution (1 M, 5 mL) and then the solution was diluted by adding deionized water (40 mL). After neutralizing with 1 M HCl, the solution was adjusted to 50 mL by adding distilled water, and then autoclaved at 121 °C for 20 min under N_2 gas to ensure the complete dissolution of dextrin. The solution was cooled to 90 °C, and then aliquots of policosanol (20 mg) were stepwise added to the dextrin solution (1.0% solids, 50 mL) for five times (total 100 mg) at different intervals (5–60 min) while magnetically stirring (550 rpm) at 90 °C. The mixture was additionally stirred for 2.5 h, and then gradually cooled to an ambient temperature for 12 h. Then the dispersion was centrifuged (5000 × *g*, 30 min, room temperature), and then the precipitates and supernatant were fractionated for further analyses.

2.4. Effects of complex formation parameters

To examine the effect of time, the dispersion was continually stirred after the stepwise addition for additional periods up to 24 h at 90 °C. The addition levels of dextrin and policosanol were also varied from 0.5% to 2.0%, and from 50 to 100 mg, respectively, to examine their effects on the dispersion properties.

2.5. Stability of policosanol dispersion against sonication

The dispersion was sonicated for 10 and 30 s to evaluate physical stability of the dispersion (200 W/cm^2, JAC-2010, Jinwoo Engineering, Seoul, Korea).

2.6. X-ray diffraction (XRD) analysis

The precipitates and supernatant obtained from the aqueous dispersion of dextrin and policosanol were freeze-dried, and then crystalline properties of those fractions were measured using an X-ray diffractometer (MAC Science Co. Ltd., Tokyo, Japan) 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. XRD spectra of pure dextrin and pure policosanol powders were also analyzed for comparison.

2.7. Differential scanning calorimetry (DSC)

The thermal transition properties of dextrin, policosanol, the precipitates, and the supernatant were assessed via differential scanning calorimetry (Seiko DSC 6100, Chiba, Japan). The samples (3.0 mg, dry basis) were mixed with water (7.0 mg) in an aluminum DSC pan. The mixture was equilibrated at 4 °C over 2 h, and scanned from 25 to 150 °C at a heating rate of 5 °C/min against an empty reference pan.

2.8. Analysis of policosanol content

The dry solids of the precipitates and supernatant were re-dispersed in deionized water (48 mL), and an aliquot of the dispersion (5 mL) was mixed with chloroform (5 mL) in a screw cap tube. The mixture was vigorously vortexed for 30 s and then stirred at 60 °C for 15 min to extract the policosanol. An aliquot of the chloroform solution (0.5 mL) was taken, and mixed with a 250 silylation reagent (N-Methyl-N-[trimethylsilyl] trifluoroacetamide, 250 μL). The mixture was stirred at 60 °C for 15 min for derivatization according to the method of Dunford, Irmak, and Jonnala (2010). The policosanol content of the derivatized sample was analyzed by gas chromatography (GC) with a flame ionization detector (Hewlett Packard 5890 Series II, Agilent Technologies, Palo Alto, CA, USA). An HP-5 MS column (30 m × 0.25 mm × 0.25 μm film thickness, Agilent Technologies, Palo Alto, CA, USA) was used, and the GC oven temperature was programmed as follows: initial temperature 250 °C for 1 min, heat to 318 °C with 8 °C/min ramp, hold for 4.5 min. Nitrogen gas was used as a carrier at a flow rate of 20 mL/min, and the inlet and detector temperature were 310 and 320 °C, respectively.

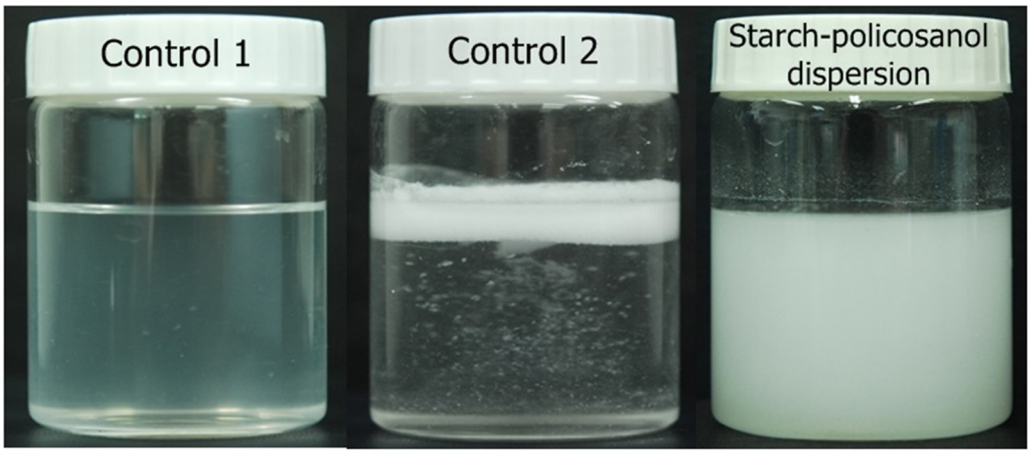

Fig. 1. Photograph of aqueous dextrin–policosanol dispersions (100 mg) after stirring policosanol (100 mg) in dextrin solution (1.0% solids) for 2.5 h at 90 °C followed by cooling for 12 h. Control 1 and 2 were dispersions of pure policosanol and pure dextrin, respectively, which subjected to same condition.

3. Results and discussion

3.1. Aqueous dispersion formation

Gas chromatography analysis revealed that the policosanol used in this study consisted of 1-octacosanol (62.52%), 1-triacontanol (20.05%), 1-hexacosanol (9.88%), and other minor variations of policosanol (4.55%), which was similar to the composition reported by the manufacturer (data not shown). Complete dissolution of amylomaize in aqueous solution requires severe thermal treatment (over 150 °C), which possibly results in chain degradation of starch. The dextrin prepared from the acid hydrolysis of amylomaize starch and tested in this study was completely dissolved in an aqueous solution by autoclaving. The dextrin itself (1.0%, Control 1) afforded a transparent solution, which implied that the dextrin was completely dissolved and did not form the precipitates during cooling, (Fig. 1). Without dextrin, policosanol (100 mg, Control 2) was immiscible and readily separated from water. However, the dispersion containing both dextrin and policosanol together was homogeneous with opacity, and the dispersion remained homogeneous without forming any precipitates for up to 7 days at an ambient temperature, suggesting that starch could stabilize policosanol in the aqueous dispersion (Fig. 1). There might be physical interaction between dextrin chains and policosanol resulting in the formation of homogenous dispersion with improved stability.

3.2. Crystalline structure and thermal transition

To evaluate driving forces for the improved stability of policosanol in aqueous dispersion, the homogenous dispersion was fractionated by centrifugation (5000 × *g*, 30 min, room temperature); oil layer of policosanol on the upper surface, clear solution of supernatant, and precipitates. The crystalline structure and thermal transition properties of the solids isolated from the supernatant and precipitates of the dextrin–policosanol dispersion were analyzed. Pure dextrin, as a control, exhibited an amorphous X-ray diffraction pattern whereas pure policosanol showed sharp diffraction peaks as a proof of its crystallinity (Fig. 2a). The precipitates exhibited diffraction peaks consistent with those of pure policosanol. In addition, two broad peaks at 13° and 20° corresponding with the amylose-inclusion complex (Lesmes, Cohen, Shener & Shimoni, 2009) were also observed for the precipitates. This result suggested that the policosanol in the precipitates existed either in pure crystalline form or in the V-amylose complex. However, the freeze-dried supernatant exhibited diffraction peaks only for pure crystalline policosanol with relatively low intensity (Fig. 2b),

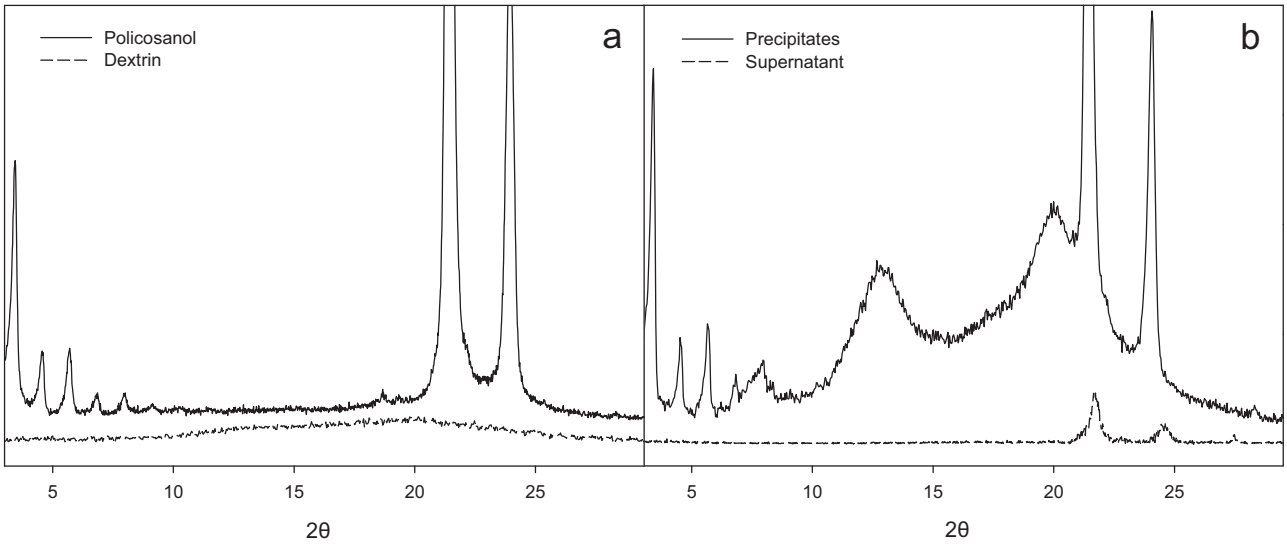

Fig. 2. Comparison of X-ray diffraction patterns of pure starch and pure policosanol (a), and those of dried precipitates and supernatant from a policosanol dispersion (100 mg) in dextrin solution (1.0% solids) at 90 °C for 2.5 h (b).

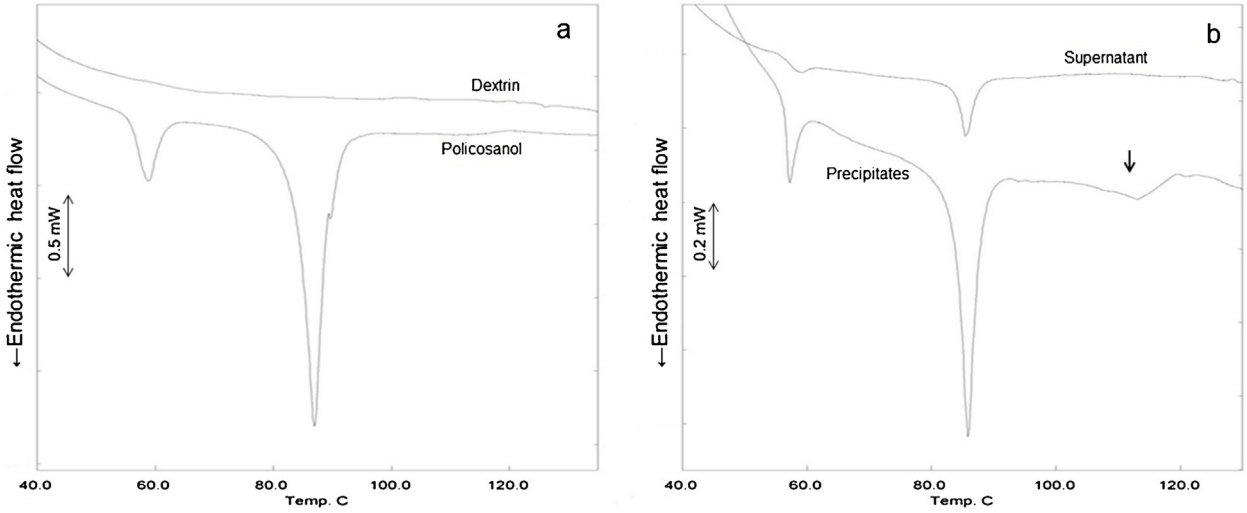

Fig. 3. Comparison of DSC thermograms of pure starch, pure policosanol (a), and precipitates and supernatant from a policosanol dispersion (100 mg) in dextrin solution (1.0% solids) at 90 °C for 2.5 h (b). The DSC samples contained 70% water.

indicating that the policosanol in the supernatant was not in the form of V-amylose complex. It might exist as amorphous state as added to the dispersion, but the crystalline structure was formed during freeze-drying.

Differential scanning calorimetry for pure dextrin exhibited no thermal transition suggesting that the dextrin was amorphous, which agreed with the XRD result. Pure policosanol exhibited two endothermic peaks (Fig. 3a), representing its crystalline structure as reported in previous publications (Madhavi & Kagan, 2013; Martínez, Uribarri & Laguna, 1999). The melting temperatures of the consisting policosanols (1-octacosanol, 1-hexacosanol, and 1-triacontanol) are similar (81.3, 83.0, and 85.7 °C, respectively), which possibly caused the eutectic thermal transition of policosanol in the major melting endotherm starting at around 80 °C (Martínez, Uribarri & Laguna, 1999). The precipitates from the dextrin–policosanol dispersion exhibited similar endotherms, but they showed an additional transition at around 110 °C (marked by arrow, Fig. 3b), which confirmed the formation of an inclusion complex between dextrin and policosanol (Gelders, Vanderstukken, Goesaert & Delcour, 2004; Jovanovich & Añón, 1999; Tufvesson & Eliasson, 2000). The supernatant displayed double endothermic peaks just as shown by pure policosanol, but no peak for the complex. These DSC results agreed with the XRD data, indicating that a portion of the policosanol added could be well dispersed in the supernatant without forming the complex with dextrin. However, both DSC and XRD analyses revealed that a portion of the policosanol added could form the V-amylose inclusion complex with dextrin, which was exclusively recovered in the precipitates. It was hypothesized that the complex formation made the policosanol more miscible in aqueous solution, because the hydrophilic outer surface of the single helices of dextrin enabled it to be dispersed in an aqueous solution (Eliasson, 2004). Although there was no proof of V-amylose complex in the supernatant under XRD and DSC analyses (Figs. 2 and 3), a portion of policosanol could remain in the supernatant with a great stability against the centrifugal force. In this case, the dextrin in the supernatant might act as a stabilizer for the dispersed policosanol which was assumed to be insoluble solids. A variety of hydrocolloids which have thickening ability in aqueous media may provide stabilizing effects to suspended solids (Whistler & BeMiller, 1993). It was hypothesized that there was some interactions between dextrin chains and policosanol molecules for the stabilization.

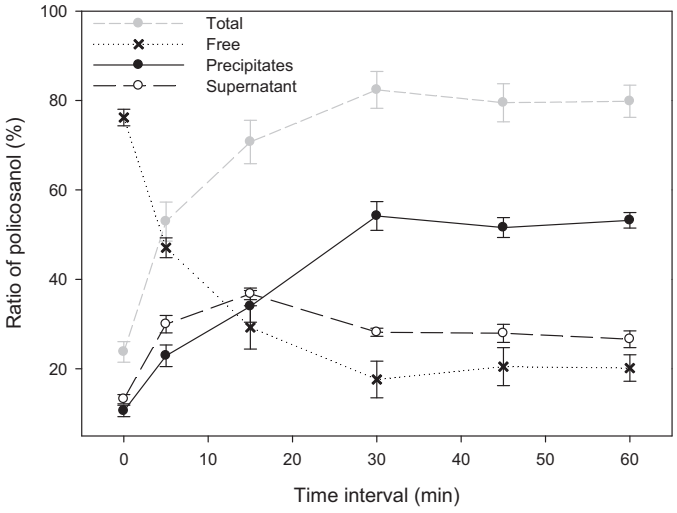

Fig. 4. Ratio of dispersible policosanol (total), either in supernatant and precipitates, and free policosanol (not dispersed) in dextrin solution (1.0% solids) prepared by stepwise additions (20 mg × 5 times) at different time intervals. The temperature and time were 90 °C and 2.5 h (after stepwise addition of policosanol), respectively.

3.3. Optimized conditions for dispersing policosanol

3.3.1. Effect of stepwise addition of policosanol

Fig. 4 illustrates the effect of stepwise addition of policosanol (20 mg × 5 times) in the dextrin solution (1.0% solids, 50 mL) on the policosanol contents in the different fractions (supernatant, precipitates, and free form). The interval for the stepwise addition was varied for 0–60 min. Total complex formation time for the stepwise addition was five times of each interval plus additional 2.5 h. For example, the total complex formation time was 7.5 h when the interval was 60 min (1 × 5 h + 2.5 h). The amount of free policosanol which was not dispersed in dextrin solution and remained in a separated phase became decreased as the interval increased up to 30 min. However, no change was found after 30 min (about 20% free policosanol). This result implied that a sufficient time was required for policosanol to become dispersible in the dextrin solution. The interaction between policosanol and dextrin chains for the dispersion of policosanol, possibly including the V-amylose complex formation, was thus kinetically controlled.

The hydrophobic policosanol might tend to reassociate quickly upon the addition in the aqueous solution, resulting in the separation of undissolved policosanol (free policosanol in Fig. 4). The policosanol reassocation might be hindered when the addition of policosanol was done stepwise with sufficient time interval. By the optimized stepwise addition (30 min, five times), approximately 80% of the policosanol added, which was equivalent to about 80 mg policosanol in this case, could be homogeneously dispersed in the dextrin solution (1% solids in 50 mL). This dispersion may be used as a commercial nutraceutical beverage because dosages of policosanol of 5–40 mg/day were suggested for a lipoprotein-lowering effect among the patients with hypercholesterolemia or combined hyperlipidemia (Berthold, Unverdorben, Degenhardt, Bulitta & Gouni-Berthold, 2006).

The policosanol incorporated in dextrin solution could be fractionated into precipitates and supernatant by a centrifugation (Fig. 4). The residual content in the precipitates continuously increased when the time interval increased up to 30 min, and then remained unchanged afterward. However, that in the supernatant reached to the highest at the interval of 15 min and continually decreased. These trends suggest that the policosanol in the supernatant partially transferred to the precipitates when the interval increased from 15 to 30 min. During this period, it was expected that the policosanol dispersed in the supernatant interacted with dextrin chains possibly forming the V-amylose complex. Overall data revealed that 50–55% of the policosanol could be maximally recovered in the precipitates under the optimal conditions (30 min interval), whereas 25–30% of the policosanol remained in the supernatant with a stability against the centrifugation.

3.3.2. Effect of extended complex formation time

The policosanol dispersion which had been prepared by a stepwise additions for 5 times at 30 min interval was continually stirred with additional complex formation time up to 24 h, and the changes in the policosanol contents in the precipitates and supernatant were examined (Fig. 5). As the complex formation was continued by stirring the dispersion up to 12 h, the total amount of dispersible policosanol was gradually increased up to about 95%. Additional stirring after 12 h appeared no more effective in dispersing policosanol. During the extended complex formation time by stirring, opposite trends were observed for the policosanol contents in the precipitates and the supernatant. The policosanol level in the precipitates increased, whereas that in the supernatant continuously decreased. It proved that the extended stirring for the complex

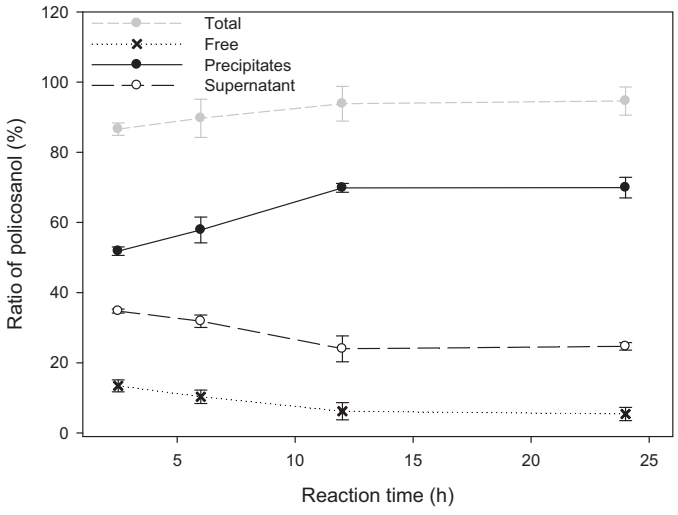

Fig. 5. Effect of complexation time on dispersible policosanol (total), either in supernatant and precipitates, and free policosanol (not dispersed) in dextrin solution (1.0% solids) after a stepwise addition (20 mg × 5 times) at an interval of 30 min.

formation increased the interactions between policosanol and dextrin chains, triggering the formation of precipitates and causing the transfer of the policosanol in the supernatant to the precipitates.

3.3.3. Effects of levels of dextrin and policosanol

Fig. 6 illustrates the effects of the levels of dextrin and policosanol added in the dispersion on the distribution of policosanol in different fractions of the dispersion. When the policosanol addition was as low as 50 mg under the optimal stepwise addition with 30 min interval and 12 h at 90 °C, the majority of policosanol could be isolated in the precipitates. However, as the policosanol level increased above 50 mg, the yield of precipitated policosanol was decreased whereas the amount of policosanol in the supernatant was increased (Fig. 6a). Overall, the total yield of incorporated policosanol, both in the supernatant and the precipitates, decreased slightly as the addition level of policosanol increased. These results indicate that excessive addition of policosanol might result in decreased yield of policosanol (amount of dispersible policosanol). For the maximum dispersion of the policosanol, stepwise addition of small quantity of policosanol may be suggested. It is possibly because the interaction between dextrin and policosanol for

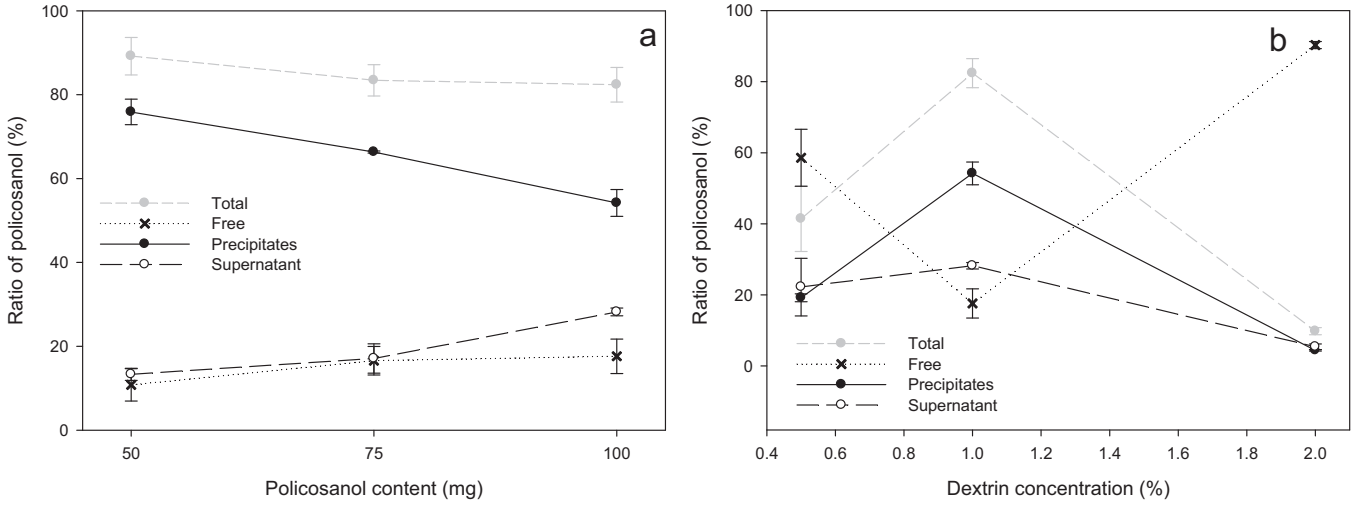

Fig. 6. Effect of addition levels of dextrin (0.5–2.0%) and policosanol (50–100 mg, stepwise addition for 5 times at interval of 30 min) on dispersible policosanol (total), either in supernatant and precipitates, and free policosanol (not dispersed) in dextrin solution (1.0% solids). The temperature and time were 90 °C and 12 h, respectively.

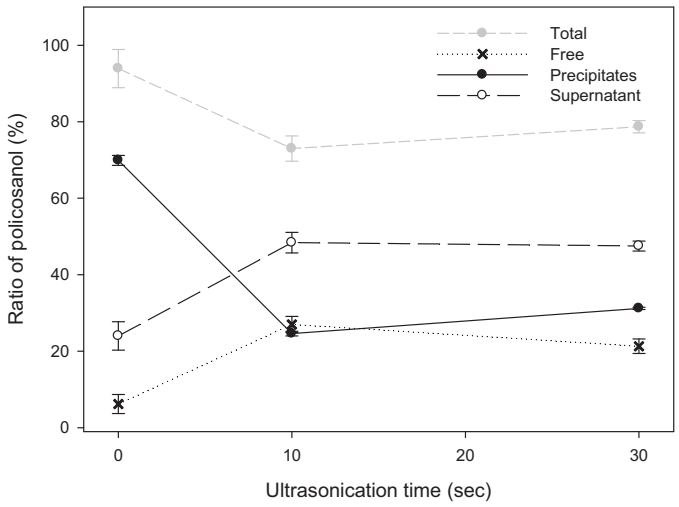

Fig. 7. Effect of ultrasonication on dispersible policosanol (total), either in supernatant and precipitates, and free policosanol (not dispersed) in dextrin solution (1.0% solids) after a stepwise addition (10 mg × 5 times) at an interval of 30 min. The temperature and time were 90 °C and 12 h, respectively.

the formation of a stable dispersion of policosanol is kinetically dependent requiring sufficient time, as shown in the previous results.

Fig. 6b shows the ratio of free policosanol and dispersible policosanol (total, precipitates and supernatant) plotted as a function of dextrin content in the dispersion prepared under an optimal condition (12 h at 90 °C with an addition interval of 30 min). The dextrin content significantly affected the yield of policosanol in the aqueous dispersion. Increasing the dextrin concentration from 0.5% to 1.0% almost doubled the ratio of total dispersible policosanol content. However, the ratio was decreased to less than 10% by increasing the dextrin concentration from 1.0% to 2.0%. The dextrin in the dispersion might function as a stabilizer for the suspending solids, either by thickening the dispersion or by forming the complex. The stabilization function of the dextrin reached the maximum when its concentration became 1.0%.

3.4. Effect of ultrasonication

It was expected that the dextrin–policosanol dispersion could be applied as a functional beverage. In the preparation of beverage products, intense shear is often subjected (e.g. sonication) for blending various compounds. Fig. 7 illustrates the effect of ultrasonication on the free policosanol and dispersible policosanol in precipitates and supernatant of the dispersion prepared from 50 mg policosanol in 1.0% dextrin. The ultrasonication for 10 s decreased the ratio of total dispersible policosanol and thus increased the free form, but the extended sonication (30 s) did not cause further decrease. It suggests that the treatment could break the interaction between dextrin and policosanol and thus decreased the dispersible amount of policosanol in the dextrin solution (around 15–20%). However, the treatment induced the change in the ratio between the policosanol content in the precipitates and that in the supernatant. A portion of policosanol in the precipitates was transferred to the supernatant by the ultrasonication for 10 s. It proved that the ultrasonication disrupted the matrices of dextrin and policosanol in the precipitates, transferring those to the supernatant.

4. Conclusions

Hydrophobic policosanol could be homogeneously dispersed in aqueous medium containing a dextrin produced after a mild hydrolysis of amylomaize starch. The dextrin could stabilize the dispersed policosanol in the form of V-amylose complex as well as a thickening agent. The yield of policosanol (percentage of dispersible policosanol) could be improved by a stepwise addition, and extended complex formation time, reaching around 90%. The amylomaize dextrin could be used as an effective stabilizer for the preparation of aqueous dispersions of various hydrophobic ingredients. However, additional research should be followed to ensure the dispersion stability against thermal and physical processes.

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