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Enhancing dispersion stability of alpha-tocopherol in aqueous media using maize starch and ultrasonication

Chuqiao Ren [a], Eun Young Park [a], Jong-Yea Kim [b, **], Seung-Taik Lim [a, *]

[a] School of Life Sciences and Biotechnology, Korea University, 5-1, Anam-dong, Sungbuk-ku, Seoul 136-701, South Korea
[b] Department of Food Science and Biotechnology, Kangwon National University, Chuncheon 200-701, South Korea

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ABSTRACT

Alpha-tocopherol (30–120 mg) was dispersed in an aqueous maize starch solution (0–240 mg starch solids in 30 mL) at various temperatures (60–80 °C) for 3 h, followed by cooling to 30 °C for different periods (0–12 h). The dispersion prepared under an optimized condition was subjected to ultrasonic treatment (up to 5 min in ice bath) to enhance the dispersion stability. When an aqueous α-tocopherol (60 mg) dispersion in a starch solution (120 mg in 30 mL) was prepared at 70 °C with cooling for 3 h and ultrasonic treatment for 3 min, a homogenous and opaque dispersion was obtained with 74% (w/w) of the α-tocopherol added was stably dispersed. The ultrasonic treatment decreased hydrodynamic diameter (from 1253 to 416 nm) and zeta-potential (from −6.28 to −22.40 mV) of the dispersed α-tocopherol particles, improving the dispersion stability. During a storage for 28 days at room temperature, the dispersion remained stable without producing any precipitates or aggregates. When the α-tocopherol dispersion in starch solution was subjected to autoclave treatment (121 °C for 20 min), 25% of the α-tocopherol in the dispersion was transformed to immiscible phase and phase-separated.

1. Introduction

Vitamin E, a well-known lipophilic natural antioxidant, is a widely used as an additive in foods, cosmetics and pharmaceuticals (Constantinides, Han, & Davis, 2006). It has physiological benefits, e.g., preventing oxidative damage and lipid peroxidation in the central and peripheral nervous systems (Scholz et al., 1997; Teranishi, Nakashima, & Wakabayashi, 2001; Terrasa, Guajardo, Marra, & Zapata, 2009). Tocols and tocotrienol derivatives exhibit an activity of vitamin E (Traber & Sies, 1996), but vitamin E is often referred to as α-tocopherol given which is predominant in nature with the highest biological activity (Brigelius-Flohé & Traber, 1999; Cheong, Tan, Man, & Misran, 2008).

Intestinal absorption of α-tocopherol requires the formation of micelles that contain dietary lipids and emulsification in the presence of bile slats, so the bioavailability of α-tocopherol is affected by food consumption, lipid digestion, and the formation of micelles (Hatanaka et al., 2010; Lodge, Hall, Jeanes, & Proteggente, 2004). The water-immiscibility of α-tocopherol, however, results in low bioavailability and limits its use in beverage products (Chen & Wagner, 2004). Recently, great attention has been drawn to functional beverages, and thus a demand for the use of vitamin E in beverages has increased. Emulsification is a common approach to overcome this problem associated with α-tocopherol use in food industry (Chen & Wagner, 2004; Cheong et al., 2008; Hatanaka et al., 2010). Thermodynamically metastable emulsion systems require emulsifying agents such as surfactant molecules (e.g., polysorbates) (Rousseau, 2000). Emulsifying agents for food and cosmetic applications are considered safe, but developing alternative approaches has been carried out to meet the consumer's demand for “clean label” products (Patel & Velikov, 2011; Wilcock, Pun, Khanona, & Aung, 2004).

Hydrocolloids and proteins may stabilize emulsion systems and are commonly used to control their stability (Dickinson, 2009). Under favorable conditions, proteins tend to be more efficient than hydrocolloids in the utilization as emulsifying agents because proteins generally have some affinity to hydrophobic compounds with surface activity (Dickinson, 2009). Protein-based emulsions, however, are susceptible to the destabilization under unfavorable

* Corresponding author.
** Corresponding author.
E-mail addresses: jongkim@kangwon.ac.kr (J.-Y. Kim), limst@korea.ac.kr (S.-T. Lim).

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environmental conditions. For example, casein-based emulsions are readily destabilized by acidification and calcium addition, and whey protein-based emulsions are unstable under thermal treatment (Dickinson, 2006, 2009; Dickinson & Parkinson, 2004). On the other hand, hydrocolloid-based emulsions containing gum arabic or modified starch are mostly stable over a wide range of physical conditions, such as thermal shock treatment and the addition of calcium salts (Chanamai & McClements, 2002). Considering the environmental conditions associated with typical beverage production (i.e., generally acidic conditions and thermal treatment for pasteurization), hydrocolloids are more suitable as the primary emulsion stabilizer for functional beverages (Dickinson, 2009). As structuring, thickening, and/or gelling agents in aqueous media or oil-in-water emulsions, many hydrocolloids may modify the rheology of the media or emulsions in which biopolymer network contributes in immobilizing the dispersed particles or droplets (Dickinson, 2009).

Starch is a low-cost, renewable, non-toxic hydrocolloid with wide uses in the food industry as a thickening and/or gelling agent in its native and modified forms. Aside from its major applications, starch may also be utilized as a stabilizer in dispersions and emulsions. As an example, octenylsuccinic starch could be used to stabilize vitamin E in aqueous dispersions (Chen & Wagner, 2004; Qiu, Yang, & Shi, 2015). However, no study has been carried out in the stabilization of vitamin E in aqueous media using native starch. In this study, the effects of starch addition and post-ultrasonication on the stability of α-tocopherol dispersed in aqueous solution were investigated.

2. Materials and methods

2.1. Materials

Normal maize starch was provided by Samyang Genex Company (Seoul, Korea) and α-tocopherol (purity > 95.5%) was purchased from Sigma Aldrich Company (St. Louis, MO, USA). All other chemicals used were of analytical grade.

2.2. Preparation of α-tocopherol dispersions

The aqueous α-tocopeherol dispersions (30 g total) were prepared under different conditions: 30–120 mg α-tocopherol, 60–240 mg starch (dry solids), reaction temperatures between 60 and 80 °C, dispersing times of 0, 1, 2, or 3 h, and cooling periods during 0, 3, 7 or 12 h. The α-tocopherol solids were pre-dissolved in absolute EtOH (10 mL). Normal maize starch dispersions were prepared by autoclaving at 121 °C for 20 min with purged N_2 gas for complete gelatinization of starch granules. The pre-dissolved α-tocopherol solutions was quickly poured into the starch solutions while vigorously stirring at 70 °C. The dispersions were then immediately evaporated (N-1100 rotary evaporator, EYELA, Tokyo, Japan) at 60 °C until the final volume reached approximately 30 mL to remove most of the ethanol. The treated dispersions were stirred in a water bath (SWB-10L-2, Major Science, Saratoga, CA, USA) at different temperatures (60–80 °C) and times (0–3 h) at a constant speed (1500 rpm). After the stirring, the dispersions were subsequently cooled to 30 °C in the water bath while stirring for different periods (0–12 h). To improve the stability of α-tocopherol dispersed in aqueous starch solutions, ultrasonic treatment (frequency 20 kHz, amplitude 20%, output power 350 W) in an ice bath for different periods (0–5 min) using an ultrasonic processor (Branson Sonifier-450, Emerson Industrial Automation, St. Louis, MO, USA). The dispersions were centrifuged (10,000 × *g* for 30 min at 30 °C), and then any precipitates and flocculants were removed. The homogeneous supernatant was tightly sealed and kept in a dark place before analyses. Control dispersions without tocopherol or starch were prepared according to the same procedure.

2.3. Tocopherol content

The α-tocopherol content in the dispersions was assessed using a high performance liquid chromatography (HPLC; Prostar 240, Varian medical systems, Palo Alto, CA) with a C-18 column (Shiseido, 4.6 × 150 mm, 5 μm, 120 Å) and monitored with a UV detector at 295 nm (Prostar 320 UV–VIS detector, Varian medical systems, Palo Alto, CA). Methanol was used as the mobile phase with flow rate at 1.2 mL/min, and injection volume was 50 μL. An aliquot of α-tocopherol dispersion (1 mL) was poured into an absolute EtOH (20 mL), and the mixture was vigorously stirred for 10 min to extract α-tocopherol and precipitate starch. After centrifugation (2280 × *g* for 15 min at 4 °C), the starch precipitate was removed and then the remained supernatant was filtered through a 0.50 μm hydrophobic syringe filter (Advantec MFS, Inc., Dublin, CA) prior to injection to the HPLC system. α-Tocopherol was also dissolved in EtOH (0.02–0.1 mg/mL) to prepare standard solutions. Standard curve (concentration verses peak area) was calculated by linear regression analysis to determine concentration of α-tocopherol in the samples. Recovery of α-tocopherol was calculated according to the equation below:

$$\text{Recovery of } \alpha-\text{tocopherol} = \frac{\text{Weight of } \alpha-\text{tocopherol in the dispersion}}{\text{Weight of added } \alpha-\text{tocopherol into reaction system}} \times 100$$

2.4. Particle size analysis

The hydrodynamic mean diameter of the particles in the dispersions was determined with a dynamic light scattering detector (Dynapro Titan, Wyatt Technology, Santa Barbara, CA) using a Dynamics program (Version 6.9.2.9, Wyatt Technology, Santa Barbara, CA). The viscosity and refractive index of the water at 20 °C, determined using calculation software, were 1.00 cP and 1.333, respectively.

2.5. Zeta potential analysis

The zeta potential value of the starch-tocopherol dispersions was measured using a Zeta-sizer (3000HS Advance Malvern Instruments Ltd., Worcestershire, UK). The measurements were carried out at an ambient temperature and pH 5.5.

2.6. Storage stability of dispersions

During the storage of the starch-tocopherol dispersions at an ambient temperature and in a dark place, an aliquot of the dispersion (1 mL) was taken. The hydrodynamic diameter and

remaining α-tocopherol content of the samples were analyzed according to the methods described previously.

2.7. Thermal stability of dispersions

A freshly prepared starch-tocopherol dispersions was purged with N_2 gas to minimize the oxidation of the sample and then autoclaved at 121 °C for 20 min. The thermal stability of the dispersions was characterized by comparing the tocopherol content in the dispersion before and after autoclaving.

2.8. Statistical analyses

Statistical analysis consisted of an analysis of variance (ANOVA) using SAS 9.2 software (SAS Institute, Cary, NC, USA). Duncan's multiple range test ($p < 0.05$) was used to identify statistical differences between mean values.

3. Results and discussion

3.1. Preparation of aqueous tocopherol dispersions

The α-tocopherol could be dispersed in homogeneous status regardless of the presence of starch, but the dispersion appeared more opaque when starch was present (Fig. 1). The residual amount of ethanol in the dispersions was negligible because of the evaporation process (<0.01%, w/v, method and data not shown). The α-tocopherol could be dispersed in water even without the presence of starch by the simple blending used for the preparation of dispersions. The physical force applied might offer enough energy to overcome the interfacial tension between α-tocopherol and water, possibly inducing the formation of a crude emulsion. The greater opacity of the starch-tocopherol dispersion, as shown in Fig. 1, however, suggested that the starch in the reaction mixture might influence the α-tocopherol dispersal in aqueous medium. After an ambient storage for 4 weeks, the starch-tocopherol dispersion remained almost unchanged, whereas the tocopherol dispersion without starch became heterogeneous showing transparent phase separated from the immiscible tocopherol. It indicates that a portion of the α-tocopherol dispersed underwent coalescence and phase separation. This result proved that the starch in aqueous tocopherol dispersion inhibited the coalescence and phase separation of α-tocopherol which might occur during storage. The control sample of aqueous starch dispersion without tocopherol showed an increase of opaqueness which indicates that chain association of starch occurred during the storage.

Amylose in starch may form inclusion complexes with various lipophilic compounds, which assists the dispersal of the compounds in aqueous media because the hydrophilic outer surface of the complexes enhances the dispersibility of the guest compounds (Eliasson, 2004; Tomasik & Schilling, 1998a, 1998b). It was expected that the controlled heating followed by cooling might induce the formation of inclusion complex between starch chains and α-tocopherol. According to the results from differential scanning calorimetry and X-ray diffractometry of the freeze-dried starch-tocopherol dispersions, however, no significant evidence was observed for the presence of inclusion complexes (data not shown).

Starch and hydrocolloids have thickening ability when dispersed in aqueous media, providing a stabilizing effect for suspended solids. The polymers thus may be used in food emulsions and particle suspensions to reduce the separation of the oil phase and solids during storage (Brennan, 2011; Whistler & BeMiller, 1993). Some of hydrophilic hydrocolloids are able to absorb oil droplets and sterically stabilize emulsions to prevent or minimize coalescence (Huang, Kakuda, & Cui, 2001). Therefore, the starch added in the tocopherol dispersions possibly played the same role in stabilizing the α-tocopherol dispersed in aqueous media.

3.2. Effects of addition levels of starch and α-tocopherol

Fig. 2 illustrates the effect of starch and α-tocopherol levels on the α-tocopherol content in the resulting aqueous dispersion. Given an equal α-tocopherol addition level (60 mg), about 40% of the α-tocopherol remained in the aqueous dispersion in the absence of starch, whereas this value continuously increased up to about 70% as starch addition increased to 120 mg (Fig. 2A). The higher levels of starch (180 and 240 mg), however, did not produce any further improvement. This result suggests that the starch contributed to the enhanced stabilization of α-tocopherol, even though native starch had no emulsification capacity due to the absence of surface activity at the oil-water interface. As a thickening agent, starch may stabilize solid suspensions by modifying the rheology of the continuous phase, with solid particles becoming immobilized in a network of starch. Therefore, the buoyancy force on individual particles insufficient to overcome the yield stress of the surrounding hydrocolloid network (Dickinson, 2009).

Given an equal starch addition level (120 mg), the percent recovery of α-tocopherol could be slightly increased as the addition of α-tocopherol increased from 30 to 60 mg. The higher addition level of α-tocopherol (90 and 120 mg), however, resulted in the significant reduction of α-tocopherol recovery (Fig. 2B). However, the higher addition level of α-tocopherol generally resulted in the

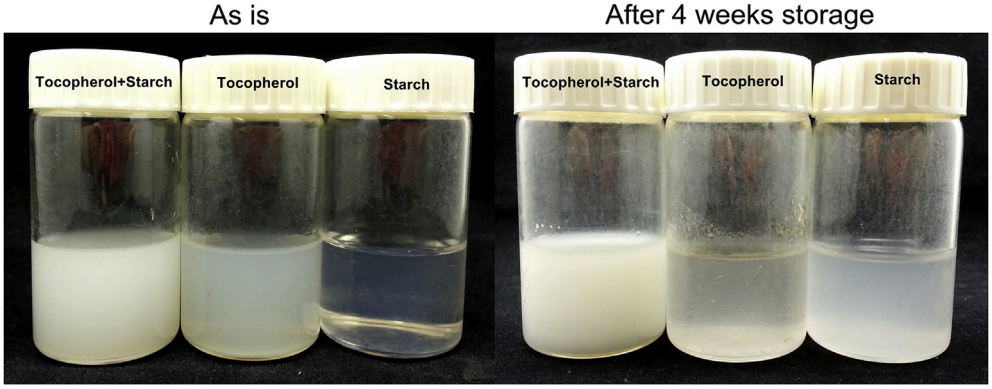

Fig. 1. Photographs of starch-tocopherol dispersion (60 mg α-tocopherol, 120 mg starch in 30 mL dispersion) prepared by stirring for 1 h at 70 °C and ultrasonic treatment for 3 min, in comparison with control samples containing each of tocopherol and starch. The dispersions were also stored for 4 weeks under an ambient condition.

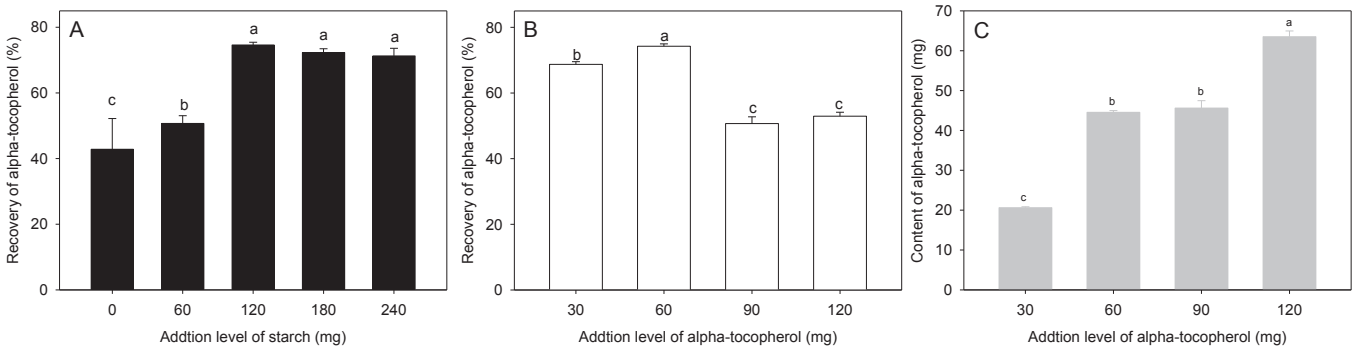

Fig. 2. Recovery of α-tocopherol in dispersion (percent ratio of tocopherol in dispersion against that initially added) at different levels of starch and tocopherol addition in dispersion (bars along the x-axis denoted a common letter are not significantly different, $p < 0.05$).

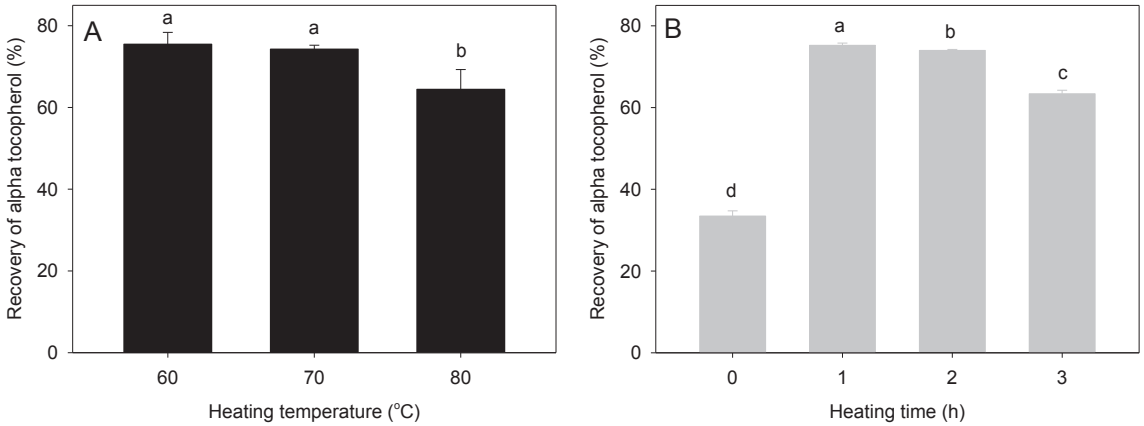

Fig. 3. Recovery of α-tocopherol in the dispersion under different physical conditions for dispersion preparation (bars along the x-axis denoted a common letter are not significantly different, $p < 0.05$).

higher content of the residual α-tocopherol in the dispersion (Fig. 2C).

3.3. Effect of physical parameters

Fig. 3 depicts the effect of temperature and mixing time during the preparation process on the percent recovery of α-tocopherol. When a dispersion containing 60 mg α-tocopherol and 120 mg starch was blended for 1 h at different temperatures with 3 h for cooling, the temperature of 60 or 70 °C resulted the relatively high recovery of around 75% of α-tocopherol whereas the higher heating temperature tested (80 °C) led to significantly lower recovery (Fig. 3A). When α-tocopherol (60 mg) was mixed with the starch solution (120 mg) at 70 °C followed by a cooling for 3 h, an additional heating time up to 3 h led to the considerable decrease in the recovery of α-tocopherol (Fig. 3B). Thus, relatively short time for mixing (1 h) was effective in dispersing the tocopherol, possibly indicating that the tocopherol in dispersion might be decomposed as the reaction time increased. On the other hand, cooling time tested up to 12 h did not show any influence on the tocopherol recovery (data not shown).

As the starch in dispersion had been fully gelatinized when mixed with tocopherol, the heating of dispersions was solely for the possible interactions between starch chains and dispersed tocopherol. The data in this study revealed that a short time for blending with mild heating (60–70 °C for 1 h), α-tocopherol could be effectively dispersed in aqueous media containing starch at a recovery yield higher than 70% (1:2 weight ratio of tocopherol and starch).

3.4. Effect of ultrasonic treatment

When an optimally prepared aqueous dispersion of α-tocopherol (60 mg) with starch (120 mg) at 70 °C, the mean hydrodynamic diameter of the droplets or particles in the dispersion was 1253 nm and the absolute zeta potential of the dispersion was −6.28 mV (Fig. 4). In a colloidal system, the surface charge of the colloids, as

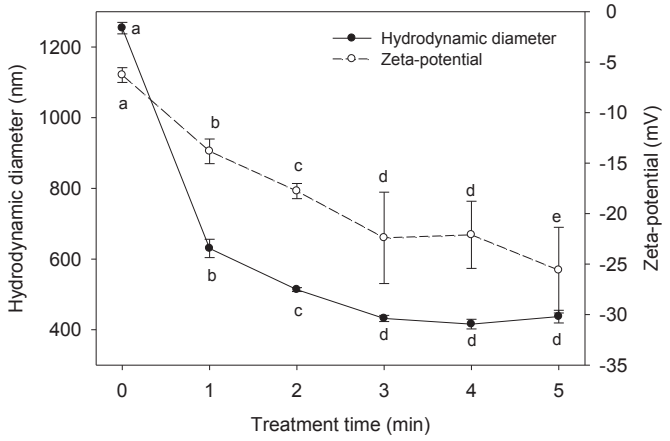

Fig. 4. Changes in hydrodynamic diameter and zeta-potential of a starch-tocopherol dispersion by ultrasonic treatment (α-tocopherol 60 mg and starch 120 mg/30 mL dispersion; a common letter above a set of data points denotes are not significantly different, $p < 0.05$).

measured by zeta potential, can generally enhance the stability of the dispersion during storage because mutual electrostatic repulsion in the dispersion (Liu, Wu, Chen, & Chang, 2009). Reducing particle size in colloids possibly increase the absolute zeta potential of a dispersion because greater surface area per mass unit of the smaller particles renders it more electrostatic state compared with the larger particles. The starch-tocopherol dispersions were subjected to varying lengths of ultrasonic treatment in order to enhance the stability by reducing the particle size of tocopherol droplets. Fig. 4 illustrates the changes in the hydrodynamic diameter and zeta potential of the dispersion according to ultrasonic treatment time. As ultrasonic treatment time increased, the hydrodynamic diameter of the particles in the dispersion decreased, reaching around 400 nm with an absolute zeta potential of higher than 22 mV after the treatments over 3 min, which suggests that the ultrasonic treatment effectively enhanced the dispersal of α-tocopherol droplets. These significant changes in particle size and zeta potential may be attributed to additional mechanical energy via ultrasonic treatment, an action which possibly broke down the individual droplets in the starch-tocopherol dispersions. Moreover, ultrasonic treatment (for longer than 2 min) increased the recovery of α-tocopherol in the dispersion from 70 to 74%.

According to simple calculations, the optimal configuration of the process presented in the present study may produce an aqueous dispersion containing 1.47 g of α-tocopherol per 1 L dispersion, a value similar to the α-tocopherol content of nanodispersions using a commercial emulsifier (Tween 20) (Cheong et al., 2008). In addition, around 10 mL of the optimal starch-tocopherol dispersion had an α-tocopherol level similar to the recommended dietary allowance of α-tocopherol (15 mg/d) reported by the Food and Nutrition Board at the National Academy of Science (Maras et al., 2004). As a result, it can be concluded that this dispersion shows a potential to be used as commercial nutraceutical beverages.

3.5. Storage stability of starch-tocopherol dispersion

Fig. 5 illustrates the change in hydrodynamic diameter of dispersed tocopherol droplets and α-tocopherol content in a dispersion treated by ultrasonication during a storage for up to 28 days at an ambient temperature. The hydrodynamic diameter of the dispersion remained constant to be around 400 nm over the storage period tested (30 days). During the storage, re-association of starch chains in the dispersion could result in the increase of particle size as the control starch dispersion became opaque during the storage (Fig. 1) (Gidley & Bulpin, 1989; Putaux, Buléon, & Chanzy, 2000). The particle size of the starch-tocopherol dispersion, however, did not exhibit a continuous increase over the storage period, indicating that the meaningful aggregation or coalescence of α-tocopherol droplets and/or starch chains might not have occurred. The starch-tocopherol dispersion prepared without ultrasonication showed a continuous increase of particle size (data not shown) indicating that the positive effect of the physical treatment on stabilization of the dispersion.

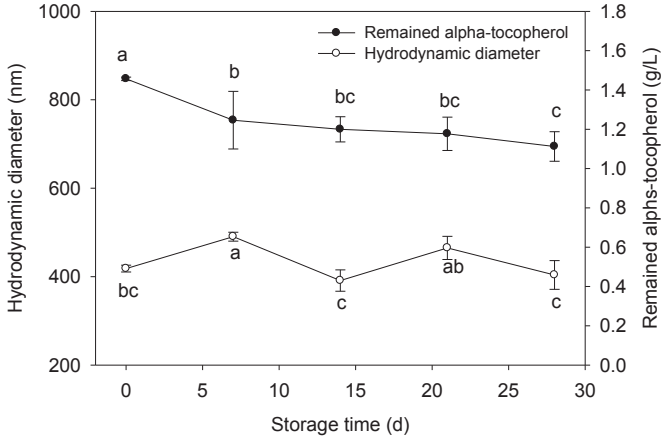

Fig. 5. Changes in hydrodynamic diameter and α-tocopherol content in a starch-tocopherol dispersion optimally prepared with ultrasonic treatment during an ambient storage for 28 days (a common letter above a set of data points denotes are not significantly different, $p < 0.05$).

The α-tocopherol content in dispersion, however, continually decreased. During the first 7 days of storage, the α-tocopherol concentration in the dispersion decreased from 1.45 to 1.24 g/L. The decrease afterward was relatively less, reaching 1.11 g/L after 28 days. Because no change in diameter was observed, this decrease was possibly from the oxidative degradation during the storage as reported by other researchers (Cheong et al., 2008).

For the starch-tocopherol dispersion to be used in the production of functional beverages, thermal stability is often required because heating is a common method for beverage sterilization. It has been reported that thermal treatment may readily result in flocculation of the oil droplets in the emulsion and phase separation (Kulmyrzaev, Bryant, & McClements, 2000; Srinivasan, Singh, & Munro, 2002), especially emulsion stabilized globular proteins readily destabilize when subjected to heat treatment above the denaturation temperature of the protein (Demetriades, Coupland, & McClements, 1997; Hunt & Dalgleish, 1995; Kim, Decker, & McClements, 2005). When the starch-tocopherol dispersion produced under the optimal conditions and ultrasonic treatment was subjected to an autoclave treatment (121 °C for 20 min), approximately 25% of the α-tocopherol in the dispersion was lost by phase separation (data not shown). The hydrothermal treatment may increase the mobility of starch chains in a dispersion which result in reducing the stabilization effect of starch chains for tocopherol droplets. It is thought that the starch-tocopherol dispersion may be relatively stable against thermal treatment compared to the emulsions stabilized globular protein, however, additional study is required to improve the thermal stability of starch-tocopherol dispersion.

4. Conclusion

Using a simple blending with maize starch and post ultrasonication, α-tocopherol was successfully dispersed in aqueous media. More than 70% of the α-tocopherol added could be stably and homogeneously dispersed in aqueous starch solution at 1:2 ratio of both solids, producing a dispersion at α-tocopherol concentration of approximately 1.4 g/L. However, no helical complex between amylose and tocopherol was formed under the conditions for dispersion preparation. A dispersion prepared at 70 °C with ultrasonic treatment for 3 min remained stable under ambient condition for a month without showing any notable changes in particle size, coalescence, and phase separation.

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