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Reversed Phase HPLC Analysis of Stability and Microstructural Effects on Degradation Kinetics of β-Carotene Encapsulated in Freeze-Dried Maltodextrin−Emulsion Systems

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ABSTRACT: Degradation of dispersed lipophilic compounds in hydrophilic solids depends upon matrix stability and lipid physicochemical properties. This study investigated effects of solid microstructure and size of lipid droplets on the stability of dispersed β-carotene in freeze-dried systems. Emulsions of β-carotene in sunflower oil were dispersed in maltodextrin systems (M040/DE6, M100/DE11, and M250/DE25.5) (8% w/w oil) and prefrozen at various freezing conditions prior to freeze-drying to control nucleation and subsequent pore size and structural collapse of freeze-dried solids. The particle size, physical state, and β-carotene contents of freeze-dried emulsions were measured during storage at various water activity (a_w) using a laser particle size analyzer, differential scanning calorimeter, and high performance liquid chromatography (HPLC), respectively. The results showed that M040 stabilized emulsions in low temperature freezing exhibited lipid crystallization. Collapse of solids in storage at a_w which plasticized systems to the rubbery state led to flow and increased the size of oil droplets. Degradation of β-carotene analyzed using a reversed-phase C_{30} column followed first-order kinetics. Porosity of solids had a major effect on β-carotene stability; however, the highest stability was found in fully plasticized and collapsed solids.

KEYWORDS: *freeze-drying, emulsion, β-carotene, structure, particle size*

INTRODUCTION

Encapsulation of functional lipid ingredients, including β-carotene, in hydrophilic solids is often achieved by freeze-drying. β-Carotene is a health promoting substance that naturally occurs in plants either in the crystalline, e.g., carrots and tomatoes, or in the noncrystalline form, e.g., mangoes.[1,2] The provitamin A and antioxidant activity of β-carotene makes it an important bioactive nutrient for human consumption. It is common in freeze-dried and spray-dried functional food and pharmaceutical products. A high number of conjugated double bonds make β-carotene susceptible to oxidation as well as isomerization, causing losses of bioactive properties. Previous studies have shown factors which accelerate degradation of β-carotene in food systems, e.g., exposures to oxygen, heat, acid, light, metal ions, etc.[3−5] β-Carotene losses occur in food processing as well as storage, and first order loss kinetics in dehydrated foods stored over 20−60 °C and 0−0.8 water activity (a_w) have been reported.[6−11]

β-Carotene has poor water solubility, and a hydrophobic lipid is often required to dissolve it. In a food or a delivery system, β-carotene may be dispersed in a water phase as part of an emulsion system. Several studies have demonstrated factors affecting the stability of dispersed β-carotene in emulsion systems at high water contents.[5,12−15] For instance, crystallization of the lipid carrier in dispersed lipid particles can expel β-carotene to the droplet surface where it is in closer proximity to prooxidants in the aqueous phase.[13] The droplet size and subsequent interface area of the emulsion also play an important role in the degradation of β-carotene, i.e., the contact surface of β-carotene and the surrounding aqueous phase.[12,15] In addition, manipulation of interfacial properties using emulsifiers contributed to the stabilization of β-carotene as a result of the modification of physical barriers (thickness and multilayer interface), radical scavenging, and control the size and surface area of the droplets.[14,15]

Emulsions are susceptible to structural changes resulting from freezing and dehydration processes. Hydrophilic components are often the main determinants of the physicochemical stability of dehydrated systems. For example, sugar crystallization in a dried emulsion was reported to result in the release of dispersed compounds with subsequent exposure to oxygen and degradation of bioactive components.[16] Collapse of dried solids may also enhance the stability of dispersed compounds by the inhibition of oxygen diffusion.[6,17] Studies of β-carotene stability in dehydrated systems have included storage of solids under various a_w conditions and monitoring the amount of β-carotene at intervals at a single temperature. Prado et al.[6] found increased stability of β-carotene at increasing a_w that was coincident with structural collapse of solids and in agreement with others.[18,19] These authors reported decreased degradation rates for beetroot and water-soluble carotenoid compounds encapsulated in polymeric matrices during storage at high a_w. The solids were fully plasticized showing viscous flow and structural collapse. Ramoneda et al.,[11] however, reported increased rates of β-carotene losses in freeze-dried systems during storage at high a_w and presumably fully collapsed structures. Such controversial

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results suggest additional factors affecting the stability of β-carotene when dispersed in freeze-dried systems and stored at various a_w. It should also be noted that other influences, such as the oil particle size as well as the effect of other solutes on degradation reactions, have rarely been established.

Our previous studies have demonstrated that the stability of bioactive components in freeze-dried systems may be controlled by the manipulation of the freeze-dried solid's porosity and structure, which result from freezing conditions and solute properties.[20] Our earlier study showed that the stability of dispersed crystalline β-carotene in freeze-dried, glassy solids was strongly dependent on porosity. At conditions resulting in glass transition, β-carotene degradation rate increased with increasing water plasticization.[21] In the present study, β-carotene was fully dissolved in a lipid phase that was emulsified in glass-forming carbohydrate solutions prior to freeze-drying.

The objectives of the present study were to investigate the effects of glass formation in freezing and freeze-drying on the stability of dispersed lipid particles with dissolved, noncrystalline β-carotene and to determine the extents of degradation of the β-carotene at various levels of water plasticization of the encapsulant solids. The β-carotene in sunflower oil was dispersed in solutions of maltodextrins of various dextrose equivalents (DE) prior to prefreezing at various freezing temperatures to control the solids' porosity and collapse. The freeze-dried solids were stored at various a_w and analyzed for the stability of β-carotene and emulsion particle size during storage.

MATERIALS AND METHODS

Pre-Emulsion with β-Carotene. Emulsions with β-carotene were prepared according to Cornacchia and Roos.[22] β-Carotene (crystalline Type II, synthetic, 95% HPLC, Sigma-Aldrich Ireland Ltd., Dublin, Ireland) was dissolved in sunflower oil (Pan Euro Foods, EU) at a ratio of 0.05% (w/w) at 50 °C under continuous stirring with a magnetic rod in the dark for at least 2 h to completely dissolve it. A solution of polysorbate 20 (2.4%w/w) (Tween 20, Merck, Germany) in deionized water was prepared and heated to 50 °C. Sunflower oil−β-carotene solution was pre-emulsified to this water−polysorbate solution at a 40:60 ratio using a high speed homogenizer (T 25 Digital Ultra-Turrax, Germany) for 30 s. The pre-emulsions (40% w/w oil) were subsequently homogenized at 50 °C using a two-stage valve homogenizer (APV-1000 high-pressure homogenizer, Denmark) with 3 cycles at 250 bar (approximately 10% of the total pressure was applied for the second stage). The emulsions with β-carotene were cooled to room temperature and mixed with solutions of respective maltodextrins and maltodextrin−agar systems.

Maltodextrin and Maltodextrin−Agar Gel Systems. The gel and liquid systems containing β-carotene were prepared by dispersing the β-carotene emulsion into the solution of maltodextrin with and without agar mixtures. Agar (microbiology grade, Fluka, Switzerland, Sigma-Aldrich Ireland Ltd., Dublin, Ireland) and various maltodextrins (M040, DE6; M100, DE11; M250, DE25.5; Grain Processing Corporation, Iowa, USA) were premixed, stirred, and hydrated in deionized water (KB Scientific, Ireland) using a magnetic stirrer at room temperature (24 °C) for at least 20 min until further heated to 90 °C while stirring and held for 5 min to ensure complete melting of the agar. The β-carotene emulsions (20%w/w of 40:60 oil/water containing 0.02% w/w β-carotene) were poured into the carbohydrate solutions (80% w/w) during cooling at 60 °C. Consequently, the gel solutions contained 2% (w/w) agar, 18% (w/w) maltodextrins, and 8% (w/w) oil. After stirring for further 5 min, a homogeneous turbid yellow color was obtained. The gelling solutions were poured into containers and left for gelation at 24 °C for at least 1 h. The emulsion systems containing no agar were prepared with the same maltodextrin ratio (18%w/w) in the final liquid systems and procedures for the measurement of the droplet particle size with laser diffraction particle analyzer were followed. The final β-carotene contents of the systems were 0.01%, i.e., 0.05% (w/w) in the oil phase.

Freezing and Freeze-Drying. Samples of maltodextrin systems were prepared by cutting gelled slabs into cubes (10 × 10 × 10 mm) with a razor blade. The cubic samples were placed on aluminum trays and frozen in still air at −20 °C, −40 °C, or −80 °C for 20 h. All samples were subsequently subjected to freezing at −80 °C for 3 h prior to rapid loading onto a laboratory freeze-dryer for freeze-drying (Steris Lyovac GT2, Germany with Leybold Trivac, Germany vacuum pump) for 48 h. This eliminated possible ice melting during loading on freeze-drying shelves. The chamber pressure was decreased to <0.1 mbar corresponding to ice sublimation at less than −40 °C. The freeze-dryer was operated at ambient room temperature, and at the end of freeze-drying, the vacuum was broken using ambient air. The freeze-dried materials were stored in evacuated desiccators containing P_2O_5 for at least 4 days in the dark prior to further analysis.

Emulsion Particle Size Measurement. The emulsion systems were characterized for particle size distribution, and the average particle diameter was measured by using a laser diffraction particle size analyzer (Malvern Master Sizer MSS, Malvern Instruments Ltd., U.K.) as described by Cornacchia and Roos.[22] The refractive index of the emulsion droplets was 1.456, while the refractive index of the dispersion medium was 1.33. The absorbance value of the emulsion droplets was 0.1. Droplet size measurements are reported as the surface-weighted or Sauter, mean diameter, d_{32}. In the storage stability study, the freeze-dried emulsion solids were stored in evacuated desiccators containing P_2O_5 as well as saturated salt solutions of $MgCl_2$ and NaCl (Sigma-Aldrich, St. Louis, MO, U.S.A.) with respective a_w values of 0, 0.33, and 0.75. The solids were reconstituted with 3 mL of deionized water at 24 °C for 30 min and immediately measured for the droplet size of dispersed lipid particles.

Differential Scanning Calorimetry. As the glass transition is a likely event of the hydrophilic phase components only, freeze-dried maltodextrin solids without dispersed oil were crushed into powder, and the glass transition temperatures (T_g) were measured using a differential scanning calorimeter (DSC, Mettler Toledo 821e with liquid N_2 cooling, Schwerzenbach, Switzerland). The DSC was calibrated for temperature and heat flow using *n*-hexane, mercury, water, gallium, and indium, as reported by Haque and Roos.[23] An empty aluminum pan was used as a reference. Samples (7−15 mg) were transferred into 40 μL aluminum pans (Mettler Toledo, Schwerzenbach, Switzerland) and equilibrated over saturated salt solutions of $MgCl_2$ and NaCl (Sigma-Aldrich, St. Louis, MO, U.S.A.) with respective a_w values of 0.33 and 0.75. The samples in open pans were stored in evacuated desiccators as in the water sorption study for 4−6 days. DSC pans were immediately sealed after removal from the desiccators. Triplicate samples were scanned at 5 °C/min from at least 40 °C below to well above the glass transition. All samples were rescanned over the same temperature range to confirm the location of the glass transition in heating and cooling, and to detect possible enthalpic relaxations around the glass transition. The T_g values were confirmed from heating and cooling scans and recorded from the second heating scan onset temperatures for the endothermic change in heat flow over the glass transition using STAR Software, version 8.10 (Mettler Toledo, Switzerland).

β-Carotene Extraction and HPLC Analysis. Freeze-dried cubic gels (10 × 10 × 10 mm) containing dispersed emulsion of β-carotene were placed on an open and transparent Petri dish and stored in desiccators over P_2O_5 and saturated salt solutions of $MgCl_2$ and NaCl to obtain the respective 0, 0.33, and 0.75 a_w of the samples. Vacuum was applied to the desiccators for 30 s for rapid establishment of the desired equilibration conditions. The samples were exposed to normal light from the laboratory at the same environment at room temperature (24 °C).

β-Carotene was extracted from freeze-dried gels at intervals during storage using a solvent extraction method. Duplicate samples were removed from the desiccators at intervals and transferred to 13 mL screw-capped polypropylene test tubes. Sample cubes (approximately

0.2 g each) were then rehydrated with 3 mL of deionized water at room temperature for at least 30 min to ensure complete rehydration. The rehydrated samples were crushed with a spatula in the tubes. Methanol and hexane (HPLC grade, Sigma-Aldrich Ireland Ltd., Dublin, Ireland) containing 0.1% of butylated hydroxytoluene (≥99%, SAFC, USA) were added to the mixture at a ratio of 1:1 (6 mL) prior to mixing with a vortex for 2 min. The residues were added with 3 mL of hexane and re-extracted with the same procedure. Approximately 2 mL of methanol was further added after mixing to enhance separation of the hexane layer. The clear yellow supernatant of β-carotene in hexane and the corresponding colorless precipitate were obtained. β-Carotene in hexane solutions were transferred to HPLC vials, and 10 μL of each samples was injected into HPLC systems.

β-Carotene contents were quantitated using HPLC (Dionex ICS3000, Sunnyvale, CA, U.S.A.) with a dual pump (DP-1, Dionex, Sunnyvale, CA, U.S.A.), autosampler (AS-1, Dionex, Sunnyvale, CA, U.S.A.), and photodiode-array detector (PDA ICS Series, Dionex, Sunnyvale, CA, U.S.A.). The analytical condition was modified from Liu et al.[24] The HPLC column used was a 250 mm × 4.6 mm i.d., 5 μm, reversed-phase Acclaim C30 analytical column with a 4 mm × 4 mm i.d. guard column of the same material (Dionex, Sunnyvale, CA, U.S.A.). A gradient solvent system of MeOH/MeCN/H_2O (84/14/2, v/v/v) (solvent A) and CH_2Cl_2 (solvent B) was used: a mixture of 80% A and 20% B was used initially, and then the mixing was programmed linearly to 55% B within 5 min and retained from 5 to 12 min. The flow rate was 1.0 mL/min, and detection was performed at 450 nm. The amounts of β-carotene were calculated from the standard curve of all-*trans* β-carotene.

β-Carotene degradation data were fitted to first-order kinetics: $-kt = \ln A/A_0$, and the rate constants (k) were derived from the slopes of linear regressions. In accordance with the first-order equation, the amounts of β-carotene at storage intervals divided by the initial contents (A/A_0) were converted to a natural logarithmic form (ln), and linear regression (R^2 ranged from 0.73 to 0.99) was used to obtain the rate constants.

Statistical Analysis. The analysis of β-carotene stability was conducted in two separate experiments with duplicate samples for each. The first-order rate constants were calculated at 5 intervals during 37 days of storage for each experiment and were shown with standard deviation (SD) bars from two separate experiments. A one-way analysis of variance (ANOVA) and Duncan's multiple range tests were used to analyze the differences among experimental data at 95% confidence level using SPSS 12.0 software for Windows.

RESULTS AND DISCUSSION

Freezing and Emulsion Particle Size. The freezing profiles of frozen agar gels containing emulsion and maltodextrin (M040, M100, and M250) with corresponding onset of ice melting temperature (T_m') are shown in Figure 1. The presence of dispersed oil (8%w/w) in maltodextrin-agar gave a freezing profile similar to that of nondispersed oil systems (data not shown), which indicated the key role of hydrophilic phase components on the freezing properties of bulk systems. The initial slope indicated the cooling rates affecting the rate of nucleation of water molecules, which were insignificantly different for the systems at each freezing temperature. As expected, faster cooling rates and shorter freezing times were found at the lower freezing temperatures in agreement with our previous study.[20] Moreover, the lower temperature freezing gave faster cooling rates, which contributed to faster water nucleation as discussed by Harnkarnsujarit et al.[20]

Samples of the frozen emulsions were thawed and immediately measured for particle size distributions and compared with those of fresh emulsions. The results showed that freezing significantly increased the size of oil particles in maltodextrin−emulsion systems (Figure 2). Low temperature

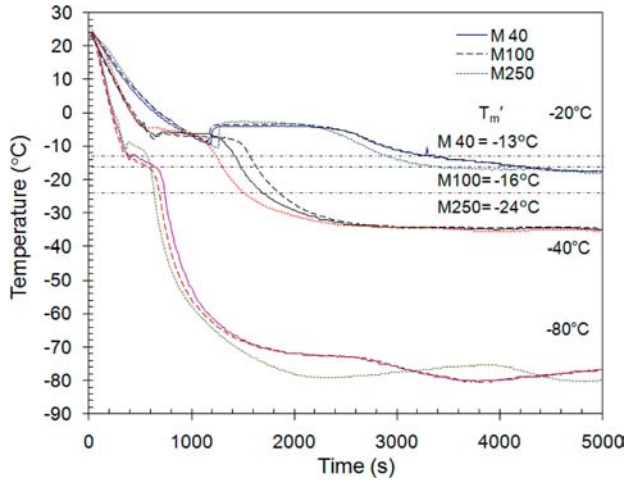

Figure 1. Freezing profile of maltodextrin-agar gels containing dispersed emulsion of β-carotene at freezing temperatures of −20 °C, −40 °C, and −80 °C with the corresponding onset of ice melting temperature (T_m') of maltodextrin (M040/DE6, M100/DE11, and M250/DE25.5) systems (dotted lines) reported previously by others.[20]

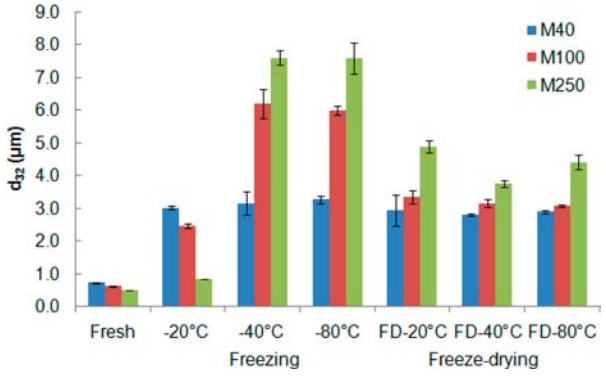

Figure 2. Influence of freezing at −20 °C, −40 °C, and −80 °C, and freeze-drying (FD) on average particle size d_{32} of β-carotene emulsion in various maltodextrin solids (M040/DE6, M100/DE11, and M250/DE25.5).

freezing at −40 °C and −80 °C gave similar particle size distributions, and the particles were larger than those when freezing at −20 °C for M100 and M250 systems. M040 systems showed a significant difference in freezing behavior and provided similar particle sizes at various freezing temperatures. This emphasized the effect of maltodextrin DE on the freezing point depression and the onset of ice melting at the maximally freeze-concentrated state, T_m'. M040 systems had the highest T_m' and could become frozen and maximally freeze-concentrated at a higher temperature. Maximum freezing at a higher temperature supported the liquid state of the sunflower oil which was found to reduce destabilization in freeze−thaw cycling of emulsions.[14] Freezing of M100 and M250 systems at −40 °C and −80 °C produced the maximally freeze-concentrated state at a lower temperature concomitant to possible crystallization of the lipid phase, which could promote emulsion destabilization in the freeze−thaw processes. These results suggested that freezing conditions of delivery systems with sensitive bioactive components need to be optimized using data on component effects on the freezing of water and lipid crystallization. In agreement with this conclusion, the higher

DE maltodextrins maintained smaller particle sizes at −20 °C freezing. Mun et al.[25] also found less stable emulsion droplets in systems containing lower DE maltodextrins. Depending on the freezing conditions, droplet aggregation by the depletion attraction caused by the nonadsorbed free molecules in the aqueous phase may also increase emulsion destabilization. Attractive forces have been reported to be proportionate to the molecular weight of the free molecules.[25−27] Consequently, the highest DE of M250 system could show least depletion flocculation at −20 °C, where it was also the least freeze-concentrated system. The ice formation during freezing concentrated the oil particles and forced them into closed proximity with one another in the freeze-concentrated solids phase.[28] The M250 system with the lowest average molecular weight and T_m' (Figure 1) had the highest unfrozen water content and most reduced tendency for emulsion droplets adhesion. Upon freezing at −20 °C, the temperature of M040 was lower than its T_m', which could result in maximum ice formation and increased aggregation of oil droplets as compared to the M100 and M250 systems.

Although the small ice crystals formed by rapid freezing could reduce emulsion destabilization, the results showed an increased size of emulsion droplets at low temperature freezing (Figure 2). The onset glass transition and ice melting temperatures of maximally freeze-concentrated solids, T_g' and T_m' as previously reported[20,29] for freezing at −40 °C and −80 °C, were higher than the freezing temperature and could support a high level of freeze-concentration. This was the primary cause for the increased size of oil particles, and it also coincided with crystallization of the surfactant and lipid particles that could rupture interphases and cause emulsion destabilization. Cornacchia and Roos[14] reported the major crystallization of sunflower oil to occur around −50 °C. In M100 and M250 systems, freezing at −40 °C and −80 °C could cause lipid crystallization and destabilization by the disruption of the interfacial membranes leading to the formation of lipid bridges and aggregation of particles particularly as the fat melted upon thawing.[14,28,30−32]

Freeze-Dried Emulsion. The hydrophilic components of maltodextrins formed solid matrices in freezing and contained dispersed emulsion particles after freeze-drying. Figure 3 shows the appearance of freeze-dried emulsions containing maltodextrin solids during storage at 0.75 a_w at room temperature. No changes in visual appearance were found during storage at 0 and 0.33 a_w as the glassy state of the maltodextrins was maintained. Water plasticization depressed the T_g by increasing the free volume, which led to structural changes as a result of viscous flow above the T_g. Structural collapse was found in M100 and M250 during storage at 0.75 a_w. The results were in agreement with the previous findings, which indicated the higher rate of the structural collapse with increasing $T-T_g$.[6,33]

M100 and M250 stored at 0.75 a_w had the largest particle sizes significantly exceeding those of solids stored at anhydrous and 0.33 a_w conditions (Figure 4). The increased particle sizes of droplets were due to structural collapse and appearance of liquid properties allowing separation of the hydrophilic and hydrophobic phases. M250 systems showed the largest lipid particle size, which was coincident to the lowest T_g of the systems and hence the highest $T-T_g$ during storage. Conversely, emulsion destabilization was not present in systems stored in the glassy state.

***β*-Carotene in Freeze-Dried Solids.** The stability of noncrystalline *β*-carotene in freeze-dried maltodextrin-agar

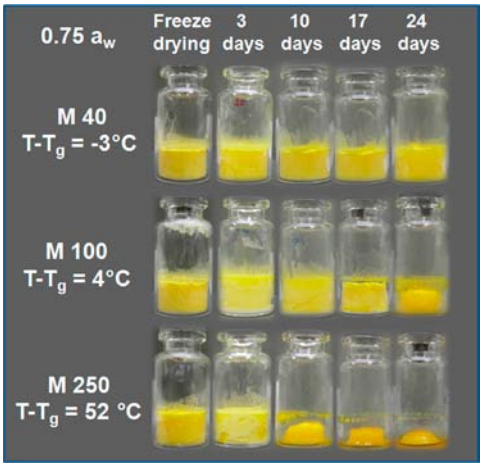

Figure 3. Appearance of freeze-dried emulsions (without agar) after freeze-drying and subsequent storage for 24 days at 0.75 a_w at room temperature (24 °C) with the corresponding $T-T_g$ values referring to the difference between storage temperature and glass transition temperature. All systems showed no structural changes during storage at anhydrous and 0.33 a_w (data not shown).

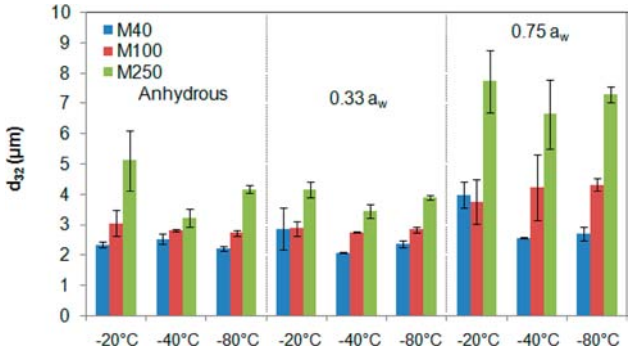

Figure 4. Average particle size d_{32} of the dispersed phase in reconstituted freeze-dried maltodextrin solids (M040/DE6, M100/DE11, and M250/DE25.5) prefrozen at −20 °C, −40 °C, and −80 °C prior to freeze-drying and storage under anhydrous, 0.33, and 0.75 a_w at room temperature (24 °C) for 24 days.

systems prefrozen at −20 °C, −40 °C, and −80 °C prior to freeze-drying was monitored during storage at anhydrous, 0.33, and 0.75 a_w conditions for 37 days using HPLC. The HPLC conditions developed in the present study revealed a significantly faster detection of all-*trans*-*β*-carotene with a C_{30} reversed-phase column.[24] A sharp and symmetric eluent peak was observed at a retention time of 9.4 ± 0.2 min with relative standard deviation, RSD < 1.67%, suggesting good reproducibility. The mobile phase and stationary phase of the C_{30} column used in our study were reported to effectively separate the isomers of *β*-carotene.[24] However, only a significant peak of all-*trans*-*β*-carotene was found in the HPLC chromatograms, which suggested that the isomerization of *β*-carotene was negligible during storage at various a_w (Figure 5). The mechanism of *β*-carotene degradation has been extensively reviewed by previous researchers.[3−5] Generally, various factors during food processing and storage, e.g., heat, acid, light, oxygen, metal ions, accelerate oxidation, and isomerization of carotenoids, lead to the degradation and loss of bioavailability.[3,4] Autoxidation is known as the major cause of carotenoid loss in dehydrated foods.

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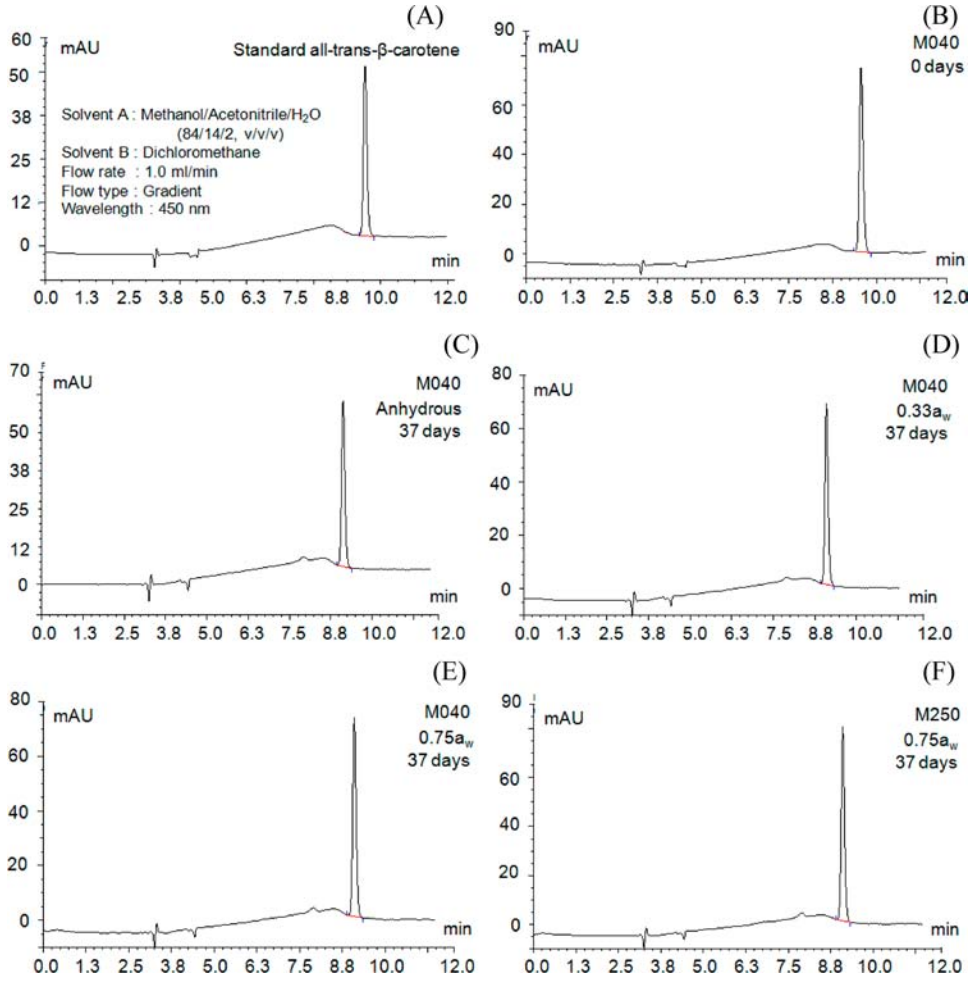

Figure 5. HPLC chromatogram of β-carotene from (A) standard all-*trans*-β-carotene, (B) M040 after freeze-drying, (C) M040 after storage at anhydrous, (D) M040 after storage at 0.33 a_w, (E) M040 after storage at 0.75 a_w, and (F) M250 after storage at 0.75 a_w for 37 days.

To describe the degradation rate of β-carotene during storage, the first-order kinetics was fitted to the experimental data as previously reported for dehydrated fruits and model systems stored at various temperatures (20−60 °C) and humidities (0−0.8 a_w).[6−11] The degradation rate of β-carotene in M040 and M100 systems was found to be dependent on the freezing temperature with a clear difference between −20 °C and −80 °C (Figure 6). Our previous study showed that lower temperature freezing gave a faster cooling rate and water nucleation, which led to smaller pores and thinner wall membranes of freeze-dried maltodextrin-agar solids.[20] The lower temperature freezing gave a higher β-carotene degradation rate in M040 and M100 solids (Figure 6) and the result agreed with the loss of entrapped crystalline β-carotene. The high amount of small pores with corresponding thinner spaces between voids locating solids and entrapped lipid particles gave higher surface area and oxygen exposure of β-carotene. This presumably increased access of oxygen and accelerated degradation of β-carotene.[21] However, the stability of β-carotene in M250 systems was independent of prefreezing conditions. The lowest rate constants for β-carotene loss were found in M250 systems at all freezing and storage conditions. This accounted for the partial flow or minor structural collapse during freeze-drying of M250 solids irrespective of freezing conditions (Figure 7). Our previous study showed a non-collapsed structure of M250 solids at identical prefreezing and

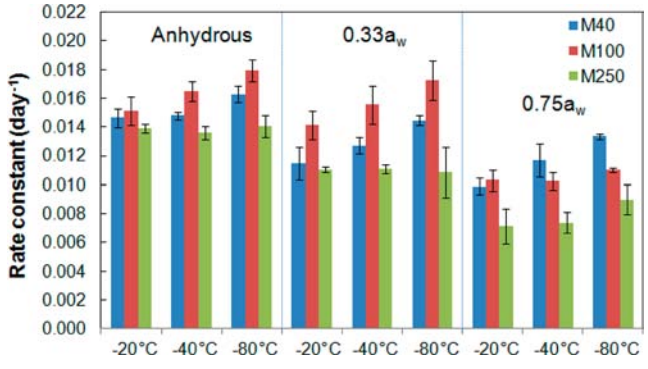

Figure 6. First-order rate constants for β-carotene degradation in freeze-dried maltodextrin systems stored at anhydrous, 0.33, and 0.75 a_w conditions for 37 days. Systems were prefrozen at −20 °C, −40 °C, and −80 °C prior to freeze-drying and stored at room temperature (24 °C).

freeze-drying conditions;[20] however, the present study indicated structural collapse with lipid components. The hydrophobic lipid particles possibly hindered diffusion through the unfrozen matrix during sublimation, which could accelerate collapse. The structural collapse of glassy solids has been reported to effectively enhance the stability of dispersed crystalline β-carotene as well as oil-solubilized β-carotene in freeze-dried mangoes by prevention of oxygen permeation

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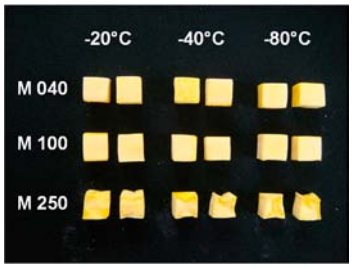

Figure 7. Appearance of freeze-dried agar-maltodextrin (M040, M100, and M250) solids containing dispersed oil-dissolved β-carotene particles prefrozen at −20 °C, −40 °C, and −80 °C prior to freeze-drying.

through the collapsed solids.[10,21] This stabilization by the collapse of solids reduced loss of oil-dissolved β-carotene in the present study. It should also be noted that increasing destabilization of lipid droplets also reduced β-carotene loss rates. Such lower losses could result from the smaller surface area and reduced direct exposure of the β-carotene to the surrounding atmosphere and the presence of natural antioxidants in the sunflower oil.

M100 and M250 systems stored at 0.75 a_w also showed the flow of solids plasticized to above T_g, and the structural collapse was apparent with increased emulsion destabilization and size of oil particles during storage (Figures 3 and 4), which led to a lower β-carotene degradation rate. Our results agreed with Prado et al.,[6] who reported a lower degradation rate of oil-solubilized β-carotene in polyvinylpyrrolidone (PVP-40) matrices as humidity increased and the highest retention was found in systems within a fully collapsed matrix. Similarly, Selim et al.[18] and Serris and Biliaderis[19] also measured the highest retention of saffron and beetroot pigments in systems stored at high a_w at which full collapse of the structures of matrices occurred. As water plasticized the encapsulant, matrices showed viscous flow and structural collapse, the reduction of matrix micropores and dense structure effectively controlling the amount and rate of oxygen transport through solids giving a higher retention of β-carotene.[6,18,19] Conversely, Ramoneda et al.[11] demonstrated increased degradation rate of β-carotene emulsion encapsulated in maltodextrin solids with increasing a_w, and the fully plasticized and collapsed matrices gave the highest degradation. The emulsifier effects on β-carotene retention in maltodextrin solids were also reported. Gelatin gave a faster degradation rate than the nonemulsifier and gum arabic systems respectively.[11] Various solid components, however, gave diverse results and could possibly be explained by the oxygen water solubility,[34] as well as the emulsion stability, i.e., the size of oil droplets during storage at high humidities.

However, the relationship between the stability of β-carotene and size of emulsion droplets dispersed in dehydrated solids stored at various a_w has not yet been established. The present study showed that the lowest β-carotene degradation rate of M250 systems at 0.75 a_w was coincident to the largest size of lipid particles (Figure 4). Several studies indicated the effect of oil droplet size on the stability of dispersed β-carotene emulsions.[12,15] Qian et al.[15] reported higher stability for a β-carotene nanoemulsion with β-lactoglobulin than that for a Tween 20 stabilized system, which was possibly due to the larger size of oil particles (80 and 60 nm, respectively, after 15 days of storage at 37 °C). This confirmed the previous finding by Tan and Nakajima,[12] who observed the increased stability of β-carotene nanoemulsion in systems with larger particle sizes (d_{43} ranging from 60 to 140 nm) prepared in various homogenizing conditions (pressure and cycle) after storage at 4 °C for 12 weeks. Soottitantawat et al.[35] also demonstrated higher retention as the particle size of D-limonene increased in spray-dried powders, which could be explained by the surface area to expose and react with oxygen. The higher β-carotene degradation in small dispersed lipid particle systems accounted for the higher effective surface area of β-carotene particles exposed to the environment. The results indicated that coalescence of lipid particles during storage above T_g increased the size of emulsion droplets, which decreased the surface area of oxygen exposure and subsequently increased β-carotene stability. M100 systems showed similarities in rate constants for storage at anhydrous and 0.33 a_w conditions, whereas lower degradation rates were found at 0.75 a_w, which was coincident to the flow of solids and subsequent increase of droplet size.

M040 showed lower β-carotene degradation than M100 systems stored at anhydrous and 0.33 a_w. The stabilizing effect of M040 was also found in the frozen emulsions that underwent freezing at various temperatures (Figure 2). Nonpolar groups of emulsifiers on droplet interphase can be incorporated into the helical coils formed by maltodextrin in the emulsion systems.[25] Therefore, it could stabilize emulsion droplets upon freeze-thawing. Upon water removal, the structure formed might physically protect β-carotene against reactive oxygen species during storage. In addition, the higher average molecular weight of M040 also gave a larger pore size and membrane thickness, which led to a lower oxygen exposure to dispersed β-carotene particles as described by Harnkarnsujarit et al.[21]

In the glassy solids, the gaseous oxygen diffused through solids and to β-carotene particles located at the pore interfaces. Therefore, a smaller pore size and hence a larger surface area of β-carotene exposed to oxygen leads to an increased degradation, whereas β-carotene particles dispersed in the solids membrane were more effectively protected. The present study also showed a higher stability of oil-dissolved β-carotene than the crystalline β-carotene particles reported by Harnkarnsujarit et al.[21] The results indicated that the oil droplets also protected β-carotene. The oxygen needs to diffuse through the oil particles to interact with β-carotene located at the core of the lipid droplets and, therefore, retards degradation. In addition, the sunflower oil dissolving β-carotene contained antioxidant components, such as tocopherols, which potentially interact and terminate radicals formed in the lipid droplets increasing β-carotene stability. The present study showed a decrease of β-carotene degradation as a_w increased. Oxidation is the main factor that accelerates β-carotene loss in freeze-dried solids. The results agreed well with the classical food stability map showing the oxidative reaction of food established by Labuza et al.[36] They demonstrated that the oxidation rate decreased toward the monolayer value, which was caused by various factors. The first factor is the hydration of transition metals, particularly cations, rendering them less active promoters of autoxidation. Second, lipid hydroperoxides move to oil−water boundaries and are taken out of the propagation reactions due to hydrogen bonding with water. Third, the free radical termination reactions increase as polar radicals congregate at the oil−water interfaces.[36] As a result, intermediate moisture foods exhibit higher stability against oxidation. They also suggested that from a_w 0.4−0.7, the lipid oxidation rate increased again owing to the increased mobility

of transition metal cations and the reduction of viscosity.[36] However, the further increase of a_w to above 0.7 contributed to reduced degradation because of the dilution of reacting species.[17,36] In addition, the greatest stability to oxidation has been extensively observed at a high a_w at which solids were in the rubbery state, and structural collapse or flow occurred, which prevented oxygen diffusion through the matrix.[17] Therefore, β-carotene degradation could be interpreted as a result of oxidative reactions with a relatively high rate within the glassy state particularly in anhydrous conditions. The results indicated that not only the structural collapse of the rubbery systems but also the increased size of lipid particles as well as thicker encapsulant matrix membranes could enhance the stability of dispersed β-carotene in freeze-dried solids.

In conclusion, the freezing temperature below T_m' resulted in an increase in the size of emulsion particles. In particular, the solidification of lipid particles at low temperature freezing caused the destabilization of emulsions. However, M040 gave a significant stabilizing effect on emulsions in freezing and freeze-drying. Freeze-dried emulsion solids stored at a_w above T_g caused the flow and structural collapse as well as the increased size of emulsion particles. As oxygen freely diffused through the glassy solids, the stability of β-carotene was dependent on matrix porosity, which was controlled by the prefreezing conditions prior to freeze-drying. The structural collapse during freeze-drying was confirmed to effectively stabilize β-carotene during storage at various a_w. The highest stability of β-carotene at high a_w of 0.75 was coincident with the increased size of lipid particles during storage above T_g. The larger lipid particles resulted in higher stability because of the lower effective surface area for oxygen exposure. The results indicated that the stability of dispersed β-carotene emulsion in freeze-dried solids was strongly dependent on matrix porosity and components, and the increased size of lipid particles during storage enhanced the stability of dispersed β-carotene. Moreover, the present study developed HPLC conditions for the rapid detection of all-*trans*-β-carotene using a reverse-phased C_{30} column. The present study indicated that the microstructures of solids as well as lipid particle size are effective parameters to improve the stabilization of lipophilic components dispersed in hydrophilic solids for delivery systems.

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Notes

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REFERENCES

(1) Harris, W. M.; Spurr, A. R. Chromoplasts of tomato fruits. II. The red tomato. *Am. J. Bot.* **1969**, *56*, 380−389.

(2) Vásquez-Caicedo, A. L.; Heller, A.; Neidhart, S.; Carle, R. Chromoplast morphology and β-carotene accumulation during postharvest ripening of mango Cv. 'Tommy Atkins. *J. Agric. Food Chem.* **2006**, *54*, 5769−5776.

(3) Britton, G. Structure and properties of carotenoids in relation to function. *FASEB J.* **1995**, *9*, 1551−1558.

(4) Gregory, J. F., III Minerals. In *Food Chemistry*, 4th ed.; Damodaran, S., Parkin, K. L., Fennema, O. R., Eds.; CRC Press: Boca Raton, FL, 2007.

(5) Boon, C. S.; McClements, D. J.; Weiss, J.; Decker, E. A. Factors influencing the chemical stability of carotenoids in foods. *Crit. Rev. Food Sci.* **2010**, *50*, 515−532.

(6) Prado, S. M.; Buera, M. P.; Elizalde, B. E. Structural collapse prevents β-carotene loss in a super-cooled polymeric matrix. *J. Agric. Food Chem.* **2006**, *54*, 79−85.

(7) Koca, N.; Burdurlu, H. S.; Karadeniz, F. Kinetics of colour changes in dehydrated carrots. *J. Food Eng.* **2007**, *78*, 449−455.

(8) Lavelli, V.; Zanoni, B.; Zaniboni, A. Effect of water activity on carotenoid degradation in dehydrated carrots. *Food Chem.* **2007**, *104*, 1705−1711.

(9) Ferreira, J. E. M.; Rodriguez-Amaya, D. B. Degradation of lycopene and β-carotene in model systems and in lyophilized guava during ambient storage: Kinetics, structure, and matrix effects. *J. Food Sci.* **2008**, *73*, 589−594.

(10) Harnkarnsujarit, N.; Charoenrein, S. Influence of collapsed structure on stability of β-carotene in freeze-dried mangoes. *Food Res. Int.* **2011**, *44*, 3188−3194.

(11) Ramoneda, X. A.; Ponce-Cevallos, P. A.; Buera, M. D. P.; Elizalde, B. E. Degradation of β-carotene in amorphous polymer matrices: Effect of water sorption properties and physical state. *J. Sci. Food Agric.* **2011**, *91*, 2587−2593.

(12) Tan, C. P.; Nakajima, M. β-carotene nanodispersions: Preparation, characterization and stability evaluation. *Food Chem.* **2005**, *92*, 661−671.

(13) Helgason, T.; Awad, T. S.; Kristbergsson, K.; Decker, E. A.; McClements, D. J.; Weiss, J. Impact of surfactant properties on oxidative stability of β-carotene encapsulated within solid lipid nanoparticles. *J. Agric. Food Chem.* **2009**, *57*, 8033−8040.

(14) Cornacchia, L.; Roos, Y. H. Lipid and water crystallization in protein-stabilised oil-in-water emulsions. *Food Hydrocolloids* **2011**, *25*, 1726−1736.

(15) Qian, C.; Decker, E. A.; Xiao, H.; McClements, D. J. Physical and chemical stability of β-carotene-enriched nanoemulsions: Influence of pH, ionic strength, temperature, and emulsifier type. *Food Chem.* **2012**, *132*, 1221−1229.

(16) Buera, P.; Schebor, C.; Elizalde, B. Effects of carbohydrate crystallization on stability of dehydrated foods and ingredient formulations. *J. Food Eng.* **2005**, *67*, 157−165.

(17) Nelson, K. A.; Labuza, T. P. Water activity and food polymer science: Implications of state on Arrhenius and WLF models in predicting shelf life. *J. Food Eng.* **1994**, *22*, 271−289.

(18) Selim, K.; Tsimidou, M.; Biliaderis, C. G. Kinetic studies of saffron carotenoids encapsulated in amorphous polymer matrices. *Food Chem.* **2000**, *71*, 199−206.

(19) Serris, G. S.; Biliaderis, C. G. Degradation of beetroot pigment encapsulated in polymeric matrices. *J. Sci. Food Agric.* **2001**, *81*, 691−700.

(20) Harnkarnsujarit, N.; Charoenrein, S.; Roos, Y. H. Microstructure formation of maltodextrin and sugar matrices in freeze-dried systems. *Carbohyd. Polym.* **2012**, *88*, 734−742.

(21) Harnkarnsujarit, N.; Charoenrein, S.; Roos, Y. H. Porosity and water activity effects on stability of β-carotene crystals in freeze-dried solids. *J. Food Sci.* **2012**, doi: 10.1111/j.1750-3841.2012.02935.x.

(22) Cornacchia, L.; Roos, Y. H. Stability of β-carotene in protein-stabilized oil-in-water delivery systems. *J. Agric. Food Chem.* **2011**, *59*, 7013−7020.

(23) Haque, Md.K.; Roos, Y. H. Water plasticization and crystallization of lactose in spray-dried lactose/protein mixtures. *J. Food Sci.* **2004**, *69*, 23−29.
(24) Liu, S.; Lin, J.; Yang, D. Determination of cis- and *trans-α-* and *β*-carotenoids in Taiwanese sweet potatoes (*Ipomoea batatas* (L.) Lam.) harvested at various times. *Food Chem.* **2009**, *116*, 605−610.
(25) Mun, S.; McClements, D. J.; Surh, J. Influence of maltodextrin addition on the freeze-dry stability of *β*-lactoglobulin-based emulsions with controlled electrostatic and/or steric interactions. *Food Sci. Biotech.* **2011**, *20*, 1143−1150.
(26) Klinkesorn, U.; Sophanodora, P.; Chinachot, P.; McClements, D. J. Stability and rheology of corn oil-in-water emulsions containing maltodextrin. *Food Res Int.* **2004**, *37*, 851−859.
(27) McClements, D. J. Protein-stabilized emulsions. *Curr. Opin. Colloid Interface Sci.* **2004**, *9*, 305−313.
(28) Ghosh, S.; Coupland, J. N. Factors affecting the freeze-thaw stability of emulsions. *Food Hydrocolloids* **2008**, *22*, 105−111.
(29) Roos, Y.; Karel, M. Water and molecular weight effects on glass transitions in amorphous carbohydrates and carbohydrate solutions. *J. Food Sci.* **1991**, *56*, 1676−1681.
(30) Vanapalli, S. A.; Palanuwech, J.; Coupland, J. N. Stability of emulsions to dispersed phase crystallization: Effect of oil type, dispersed phase volume fraction, and cooling rate. *Colloid. Surf., A* **2002**, *204*, 227−237.
(31) Cramp, G. L.; Docking, A. M.; Ghosh, S.; Coupland, J. N. On the stability of oil-in-water emulsions to freezing. *Food Hydrocolloids* **2004**, *18*, 899−905.
(32) Thanasukarn, P.; Pongsawatmanit, R.; McClements, D. J. Impact of fat and water crystallization on the stability of hydrogenated palm oil-in-water emulsions stabilized by whey protein isolate. *Colloid. Surf., A* **2004**, *246*, 49−59.
(33) Levi, G.; Karel, M. Volumetric shrinkage (collapse) in freeze-dried carbohydrates above their glass transition temperature. *Food Res. Int.* **1995**, *28*, 145−151.
(34) Whitcombe, M. J.; Parker, R.; Ring, S. G. Oxygen solubility and permeability of carbohydrates. *Carbohydr. Res.* **2005**, *340*, 1523−1527.
(35) Soottitantawat, A.; Bigeard, F.; Yoshii, H.; Furuta, T.; Ohkawara, M.; Linko, P. Influence of emulsion and powder size on the stability of encapsulated D-limonene by spray drying. *Innov. Food Sci. Emerg. Technol.* **2005**, *6*, 107−114.
(36) Labuza, T. P.; McNally, L.; Gallagher, D.; Hawkes, J. Stability of intermediate moisture foods. I. Lipid oxidation. *J. Food Sci.* **1972**, *37*, 154−159.