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Stability and Bioaccessibility of β-Carotene in Nanoemulsions Stabilized by Modified Starches

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ABSTRACT: Oil-in-water nanoemulsions stabilized by food-grade biopolymer emulsifiers (modified starches) were fabricated using high-pressure homogenization in an effort to improve the stability and bioaccessibility of β-carotene. Physicochemical and biological properties of β-carotene nanoemulsions were investigated considering the particle size, β-carotene retention, and in vitro digestion. During 30 days of storage at different conditions, the mean diameters of the emulsion systems were increased by 30−85%. The retention of β-carotene in nanoemulsions was significantly higher compared to that of the β-carotene dispersed in bulk oil. After in vitro digestion, the bioaccessibility of β-carotene was increased from 3.1% to 35.6% through nanoencapsulation. The results also indicated that modified starch with high dispersed molecular density led to a higher retention but lower bioaccessibility of β-carotene in nanoemulsions. This could be due to the thick and dense interfacial layer around the oil droplets. This result provides useful information for developing protection and delivery systems for carotenoids.

KEYWORDS: *β-carotene, nanoemulsion, modified starch, stability, bioaccessibility*

INTRODUCTION

Carotenoids represent a large group of tetraterpenoid organic pigments, which are widespread among various fruits and vegetables, as well as some animal products.[1,2] β-Carotene, a common carotenoid, is a well-known active phytochemical with many health-promoting properties. Biologically, it is a precursor of vitamin A which possesses the greatest "provitamin A" activity.[2,3] It also has a strong antioxidant activity, thus protecting cells from the damaging effects of free radicals.[4−7] It may help prevent some chronic diseases caused by free radicals such as cancer, heart disease, and aging.[8,9] β-Carotene can also enhance the immune system response to infections and help the reproductive systems function properly.[10]

However, the pure hydrogen−carbon skeleton structure and high unsaturation degree of β-carotene greatly restrict its use in foods and beverages. First, β-carotene is insoluble in water and only slightly soluble in oil at room temperature.[11] Second, as a bioactive labile lipophilic compound, β-carotene is sensitive to oxygen, light, and temperature. This leads to degradation reactions such as oxidation, isomerization, and photosensitization during processing and storage.[12−14] Lastly, β-carotene exists in a crystalline form which has been reported to have poor uptake and low oral bioavailability.[15,16]

To overcome these limitations, a useful method is to entrap the compound into appropriate delivery vehicles. One such vehicle is an oil-in-water (O/W) nanoemulsion-based delivery system which is considered an efficient way to increase the dispersibility, stability, and bioavailability of nutraceuticals.[17] Nanoemulsions are colloidal systems of small droplets ($r < 200$ nm). Compared with conventional emulsions, the small size is useful for increasing kinetic stability and improving the oral bioavailability of many functional food ingredients.[18−20] Numerous reports regarding the incorporation of β-carotene in various nanoemulsion systems have been recently published. For example, Tan et al. first prepared β-carotene nanodispersions through the emulsification−evaporation technique by using Tween 20 as an emulsifier and hexane as the solvent.[21] Yuan et al. also used Tween 20 as an emulsifier to prepare β-carotene nanoemulsions by high-pressure homogenization.[1,15] Despite successful formation of nanodelivery systems using Tween 20 as the emulsifier, such systems may not be safely used in foods and beverages when consumed at a high level.[22] With a growing concern about the safety and potential toxicity of nanoparticles, the utilization of biopolymeric emulsifiers was perceived to be more "label friendly" and preferred than the synthetic and semisynthetic surfactants.[23] Materials "generally recognized as safe" (GRAS) that can fulfill these requirements mainly include food proteins and polysaccharides of plants or microbial origin.[17]

Previous researchers have tried to use food-grade materials to prepare β-carotene delivery systems. Chu et al. chose five different proteins as emulsifiers using the same emulsification−evaporation method to prepare β-carotene nanodispersions. The results showed that sodium caseinate was the most effective emulsifier to prepare a nearly monodispersed β-carotene nanodispersion with a mean particle size of 17 nm.[24] Mao et al. investigated the effect of Tween 20, decaglycerol monolaurate, octenylsuccinate starch, and whey protein isolate on the characteristics of β-carotene nanoemulsions prepared by high-pressure homogenization.[5] From their reports, nanoemulsions stabilized by proteins were most susceptible to environmental stresses (e.g., pH, salt, heating, and freezing). Similar results were

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also reported by Qian et al.[25] and Charoen et al.[26,27] Relatively speaking, the other main food-grade biopolymeric emulsifiers, modified starches (MSs), could provide high stability against oil droplet coalescence caused by the change of pH, ionic strength, and temperature. These differences could be attributed to the characteristics of MSs. Due to their branched structures, MSs are prone to stabilize emulsions mainly through steric repulsion, therefore making them less affected by pH and ionic strength.[28] In addition, MSs tend to be less sensitive to the heating process.[29] With these advantages, MSs, as abundant and low-cost food ingredients, were widely utilized to prepare emulsions in foods and beverages.[30−32]

Among these hydrophobically modified starches, octenylsuccinic anhydride (OSA)-modified starch is the main and the most important one which has a strong surface activity.[33] Numerous reports have indicated that OSA-modified starch is especially suitable for the encapsulation of flavors, clouds, vitamins, and spices.[34−36] However, little research has been reported on preparing β-carotene emulsions using OSA-modified starches. In this research, the main objective was to select OSA-modified starches as the emulsifiers to prepare β-carotene nanoemulsions and investigate the effect of these emulsifiers on the stability of β-carotene O/W nanoemulsions. The in vitro digestion experiment was set up to mimic the digestion process in gastric fluid and intestinal fluid to determine the bioaccesibility of β-carotene after nanoencapsulation.

MATERIALS AND METHODS

Materials. β-Carotene (≥97.0%, UV) was purchased from Fluka (St. Louis, MO). Medium-chain triacylglycerol (MCT; Neobee 1053) with 44% C-10 and 56% C-8 was donated by Stepan Co. (Maywood, NJ). OSA-modified starches HI-CAP 100 (A), CAPSUL (B), and CAPSUL TA (C) were obtained from National Starch (Bridgewater, NJ). According to the technical service bulletin from the company and the research from Soottitantawat et al., these three OSA-modified starches are suitable to encapsulate compounds with high oil loadings.[34] All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. Deionized water obtained from a Milli-Q water purification system (Millipore Co., Bedford, MA) was used in all experiments.

β-Carotene Anaylsis by High-Performance Liquid Chromatography (HPLC). Quantification of β-carotene was carried out following the method reported by Arias and Lee with slight modifications.[37] The HPLC system (Dionex Ultimate 3000) consisted of an autosampler and a Dionex Bio LC AD25 UV/vis detector (Dionex Corp., Sunnyvale, CA). The β-carotene was separated on a polymeric carotene C_{30} column (250 × 4.6 mm i.d., 5 μm, YMC, Inc., Wilmington, NC) at room temperature. The injection volume for all samples was 20 μL, and quantitative measurements were carried out at 450 nm. The mobile phase consisted of methanol (A) and methyl *tert*-butyl ether (B) at a flow rate of 1.0 mL/min. The solvent gradient was programmed linearly from 90% A/10% B at 0 min to 70% A/30% B at 4 min and to 15% A/85% B at 7 min, followed by a flat profile for the next 8 min and then a linear change back to 90% A/10% B at 17 min. The data were quantified with Dionex Chromeleon chromatography management system software. The measurement of each stock or working solution was made in triplicate. The standard curve of the absorption peak area versus β-carotene concentration was plotted and fitted with a linear function.

Determination of Molecular Weight Distributions of Modified Starches. *Sample Preparation.* Three modified starch samples were first dissolved in 0.1 M $NaNO_3$ at a concentration of 1% (w/w) and then filtered through 1.2 μm nylon syringe filters. The filtrates were collected in 2 mL HPLC amber vials and then injected into the high-performance size-exclusion chromatography−multiangle laser light scattering−refractive index (HPSEC−MALLS−RI) system. Each sample was replicated three times for analysis.

HPSEC−MALLS−RI System. The HPSEC system consisted of an HP 1050 series pump and an autoinjector valve (Hewlett-Packard, Valley Forge, PA) with a 100 μL injection loop. The detection part of the system was composed of an MALLS detector (Dawn DSP-F, Wyatt Technology, Santa Barbara, CA) with a He−Ne laser source (k = 632.8 nm), a K-5 flow cell, and an RI detector (model ERC-7512, ERMA Inc., Tokyo, Japan). Two aqueous SEC columns (Ultrahydrogel 250 and 1000, Millipore Co.) were connected in series to determine the molecular weight distributions of modified starches. The temperature of the column was maintained at 40 °C. $NaNO_3$ (0.1 M) with 0.02% NaN_3 was selected as the mobile phase with a flow rate of 0.6 mL/min.

Data Analysis. The data from the system were analyzed by Astra software (version 5.3.4, Wyatt Technology), which had been described by Li et al.[38] A refractive index of 1.336 and a dn/dc value of 0.160 were used in the second-order Berry method to calculate the weight-average molecular weight (M_w) and the z-average radius of gyration (R_z) of starch in $H_2O/NaNO_3$.

Preparation of β-Carotene Nanoemulsions. Modified starch powder (30%, w/w) was first dispersed in deionized water at room temperature and stirred overnight to enhance the hydration of the starch. The oil phase was prepared by dispersing β-carotene (0.3%, w/w) in MCT and mixing until a homogeneous dispersion was produced. Then the oil dispersion was emulsified into the hydrated water phase in a mass to modified starch solid ratio of 3:7. A coarse emulsion was produced using a high-speed homogenizer (Ultra-Turrax T25, IKA Works Inc., Willington, NC) equipped with an S25 N18 G rotor working at 24 000 rpm for 1 min at room temperature. Next, high-pressure homogenization (EmulsiFlex-C3, Avestin, Inc., Ottawa, Ontario, Canada) was conducted for 10 cycles at a pressure of 150 MPa to produce a fine emulsion by reducing the particle size. A heating exchange unit was used to cool the inlet reservoir and homogenization valve and maintain a controlled temperature of 15 °C. This helped inhibit the degradation of β-carotene. Light exposure of the samples was also avoided during the process by covering the whole device and flask with aluminum foil paper and preparing the samples under dim light.

Droplet Size Measurements. The particle sizes and size distributions of the nanoemulsions were measured by dynamic light scattering using a BIC 90 plus particle size analyzer equipped with a Brookhaven BI-9000AT digital correlator (Brookhaven Instrument Corp., New York, NY). To avoid multiple scattering effects, emulsions were first diluted 100 times using deionized water and stirred continuously before a measurement to ensure the samples were well dispersed. All measurements were made at a fixed scattering angle of 90° at 25.0 °C. The light source of the particle size analyzer was a solid-state laser operating at 658 nm with 30 mW power. The mean diameters of the emulsions were determined by cumulant analyses of the intensity−intensity autocorrelation functions, $G(q,t)$.

Storage Stability of β-Carotene Nanoemulsions. The nanoemulsion samples were transferred into screw-capped glass vials immediately after homogenization. The samples were divided into four groups which were stored at (1) 25 °C under light, (2) 25 °C in the dark, (3) 4 °C in the dark and (4) flushed with nitrogen and then stored at 4 °C in the dark. The particle sizes and β-carotene concentrations of the nanoemulsions were measured during a 30 day storage period.

In Vitro Digestion. To determine the bioaccessibility of β-carotene in nanoemulsions, a dynamic in vitro digestion procedure with simulated gastric fluid (SGF) and intestinal fluid (STF) was conducted with minor modifications of a previously described method.[39,40] During the digestion process the samples were incubated at 37 °C under magnetic stirring. First, 1.5 mL of the nanoemulsion was mixed with 13.5 mL of basal saline (140 mM NaCl, 5 mM KCl, and 150 μM BHT) for 10 min. To initiate the gastric digestion, the pH of the mixture was adjusted to 2.0 by adding 0.1 M/1.0 M HCl, and then the gastric juice (40 mg/mL procine pepsin in 0.1 M HCl) was added. After 1 h, the pH of the sample was adjusted to 7.5 with 0.2 M/1.0 M NaOH. Then the small intestinal digestion was started by adding 4.5 mL of duodenal juice (containing 2 mg/mL pancreatin and 12 mg/mL porcine bile extract in saline buffer). During 2 h of the intestinal digestion process, the pH of the solution was maintained at 7.5 by adding 0.2 M NaOH manually. The amount of NaOH added over time was recorded throughout the digestion.

According to the research by Netzel et al., 1.5 mL of bulk MCT with dispersed β-carotene (0.3%, w/w) was treated with the same process as a control.[41]

During the digestion process it was assumed that one molecule of MCT liberated two fatty acid molecules by consuming two molecules of NaOH. Therefore, the percentage of free fatty acids (FFAs) released from the system was calculated using the following equation:

$$\text{percentage of FFAs released} = \frac{V_{NaOH}(t)C_{NaOH}M_{w,lipid}}{2m_{lipid}} \times 100 \quad (1)$$

where m_{lipid} is the total mass of oil present in the sample during digestion (g), $M_{w,lipid}$ is the average molecular weight of the lipid (g/mol), C_{NaOH} is the concentration of NaOH in the titration buret (mol/1000 cm^3), and $V_{NaOH}(t)$ is the volume of NaOH titrated into the reaction vessel at the digestion time (t) to neutralize the FFAs released.

Bioaccessibility Determination. After in vitro digestion, the dispersion was separated into an opaque sediment phase and an aqueous fraction containing formulated β-carotene micelles by using an ultracentrifuge at 4 °C and 40 000 rpm (113613g) for 40 min (Beckman Coutler, Inc., Brea, CA) with a 60 Ti rotor. The amount of β-carotene in the micelles was determined by HPLC. The bioaccessibility (%) of β-carotene was calculated using following equation:

$$\text{bioaccessibility (\%)} = \frac{\text{amount of solubilized β-carotene in micelle}}{\text{amount of β-carotene in the formulations}} \quad (2)$$

Statistical Analysis. The whole experiment was conducted in duplicate, and all analyses were carried out at least in triplicate and expressed as the mean ± standard deviation (SD). The one-way analysis of variance (ANOVA) test was analyzed using the SPSS 17.0 package. Duncan's multiple range test was used to determine the significant differences of the mean values ($P < 0.05$).

RESULTS AND DISCUSSION

Development of the HPLC Method To Quantify β-Carotene. β-Carotene naturally exists in the *all-trans* form, which has shown better antioxidant properties and higher bioavailability than the *cis* form.[42,43] However, due to the high degree of unsaturation, the *trans* form would easily undergo isomerization and oxidation during processing and storage.[44] Therefore, a sensitive HPLC method was set up to detect the *all-trans* form before the experiments with the β-carotene nanoemulsions.

The HPLC retention time for *all-trans*-β-carotene was approximately 9.80 min (Figure 1). Since the β-carotene concentrations in emulsions varied due to the different formulations and storage conditions, a broad concentration range (0.005−50 μg/mL) of β-carotene was used for HPLC analysis to establish the calibration curves. The large range of concentrations was divided into four regions (0.005−0.05, 0.05−0.5, 0.5−5, and 5−50 μg/mL), and separate calibration curves were determined for each. As shown in Table 1, each region had a correlation index of $R^2 > 0.999$ for *all-trans*-β-carotene.

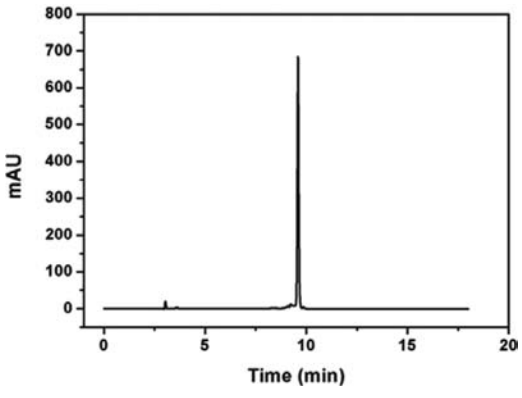

Figure 1. Representative chromatogram (at 450 nm) of β-carotene by HPLC analysis. A 20 μL injection of 5 μg/mL β-carotene is shown.

Table 1. Summary of the Equations of the Calibration Curves for β-Carotene from HPLC

	x range (concn, μg/mL)	y range (peak area, mAU·min)	equation	R^2
all-trans-β-carotene	0.005−0.05	0.0054−0.0592	$y = 1.1956x - 0.0004$	0.9992
	0.05−0.5	0.0592−0.592	$y = 1.1822x + 0.001$	0.9998
	0.5−5	0.592−5.90	$y = 1.1727x + 0.0448$	0.9998
	5−50	5.90−59.4	$y = 1.1515x + 0.1732$	0.9996

Molecular Weights and Radii of Modified Starches. Molecular characteristics based on absolute M_w values and R_z values of three different OSA-modified starches were analyzed using HPSEC combined with MALLS and RI detectors. The M_w versus elution volume curves were measured with superimposed concentration curves of MSs A, B, and C and are shown in Figure 2. The summarized M_w and R_z results are given in Table 2. According to the concentration curve in Figure 2a, MS A displayed two M_w populations, populations 1 and 2, with M_w values of 2.1×10^6 and 7.9×10^4 g/mol, respectively. The average of the whole sample of MS A (population 1 + population 2) was 9.4×10^5 g/mol. As shown in Table 2, MSs B and C had lower M_w values of 8.3×10^4 and 4.0×10^4 g/mol, respectively. From the M_w results, all the modified starches showed much lower M_w than the original starches (waxy maize and tapioca), which agreed with the results of Qi et al.[45] and Scheffler et al.[46] The processes to prepare OSA-modified starches normally include depolymerization first by alkali hydrolysis, pyrodextrinization, or enzyme hydrolysis to decrease the viscosity. Further starch hydrolysis may also occur during the reaction of OSA with starches.

R_z represents the volume occupied by molecules in a solution, which is related to the branch chain length and the pattern of molecules.[47] The R_z values for MSs A, B, and C were 32.3, 18.7, and 12.6 nm, respectively (Table 2). The results were lower than those of the original starches, which was consistent with the M_w values. This means that both M_w and R_z decreased after OSA substitution.

The dispersed molecular densities (ρ, g/mol·nm^3), defined as $\rho = M_w/R_z^3$, are listed in Table 2 for MSs A, B, and C.[48] From the results, ρ values increased in the following order: B < C < A. According to the research by Yoo et al., the starches with the larger ρ values have more branched chains, resulting in more densely packed molecules.[47] Therefore, since MS A has the largest ρ value, this suggests that molecules of MS A had greater branched and dense structures than MSs B and C. The different structure properties of MSs would affect the properties of β-carotene emulsions stabilized by different MSs (e.g., particle size distribution, bioaccessibility, and storage stability).

Physical Stability of β-Carotene Nanoemulsions. The mean diameters of β-carotene nanoemulsions are shown in Figure 3. The initial mean diameters were 157, 142, and 148 nm for freshly made β-carotene emulsions stabilized by MSs A, B,

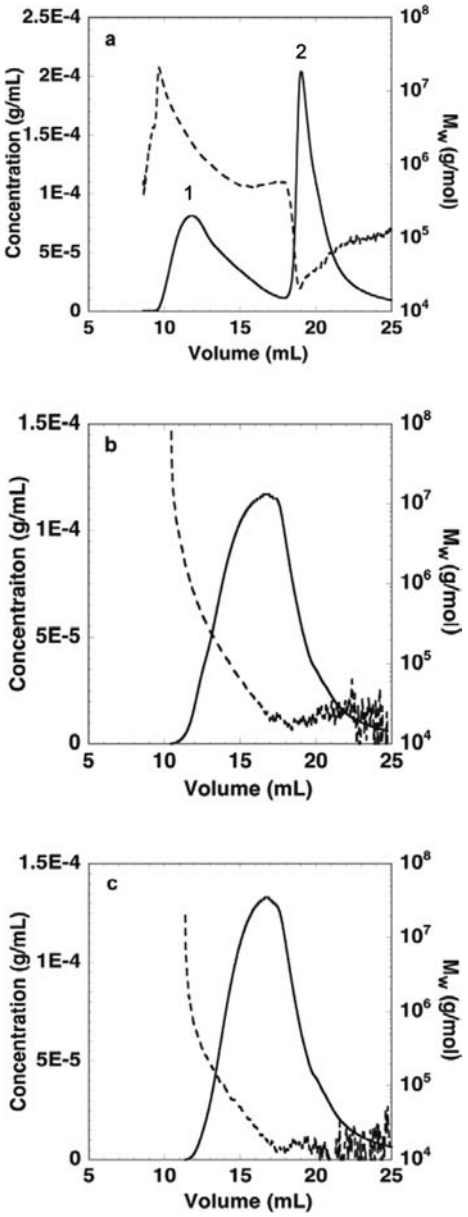

Figure 2. Weight-average molecular weight (M_w) and concentration signals vs elution volume profiles of MSs A (a), B (b), and C (c) determined by an HPSEC−MALLS−RI system: a solid line denotes the concentration signal, and a dashed line denotes the M_w distribution.

and C, respectively. These results were consistent with the results of the ρ values of modified starches. The report by Bi et al. indicated that a high density of molecule tends to produce a thick and dense interfacial layer over oil droplets in emulsions,[49] and according to the results of Varona et al., the surface loading of OSA starch is very high and likely to be determined by the amount of surface area and the concentration of OSA starch available in the system.[31] It is reasonable to assume that all the MS molecules would be adsorbed at the interfacial layers around the oil droplets. Therefore, MS with higher ρ would give a thicker and denser interfacial layer. Furthermore, the mean diameter of nanoemulsions should also increase in the following order: B < C < A.

To obtain more information about the physical stability of these β-carotene nanoemulsions, extended storage tests were conducted under the following four different conditions: 25 °C under light, 25 °C in the dark, 4 °C in the dark, and 4 °C in the dark and flushed by nitrogen. The mean diameters of nanoemulsions were measured during the 30 day storage period.

As shown in Figure 3, the mean diameters of nanoemulsions at all the storage conditions increased with the storage time. Increased diameters of about 59, 81, and 127 nm, which were around 41%, 51%, and 85% increased compared to the initial mean diameters, were observed for the nanoemulsions stabilized by MSs B, A, and C, respectively, at 25 °C under light after 30 days (Figure 3a). The same trend for the mean diameter increase was observed for the nanoemulsions stored at 25 °C in the dark (Figure 3b). However, no significant differences were observed for the mean diameters of nanoemulsions stabilized by the same MS between these two groups. When the storage temperature was 4 °C, the nanoemulsions were more stable with smaller increases of the mean diameters. According to the results from Figure 3c, increases of about 43, 55, and 100 nm, which respectively correspond to 30%, 35%, and 67% increases compared to the initial mean diameters, were found for the nanoemulsions stabilized by MSs B, A and C. Also, the mean diameters at day 30 for the two groups stored at 4 °C showed the same level for nanoemulsions with the same formulation. From the results, it can be concluded that the storage temperature is the most important factor for the physical stability of nanoemulsions. These results were consistent with the work of Qian et al., which indicated that β-carotene emulsions exhibited better stability when stored at 5 °C than at 20 °C.[25] According to the research by Tse et al., the greater instability of emulsions with increased temperature was attributed to the loss of viscosity and the increased mobility of the system.[50] Overall, there was no obvious phase separation or creaming observed for any of the samples during the 30 day storage period.

Chemical Stability of β-Carotene Nanoemulsions. Since β-carotene has a high sensitivity to environmental conditions, 30 day storage was carried out with four different conditions to investigate the effect of temperature, light, and oxygen on the chemical stability of β-carotene emulsions. For each group, samples of β-carotene dissolved in bulk MCT oil were used as the control. The chemical stability was represented by the amount of β-carotene retention measured by HPLC analysis. As shown in Figure 4, the retention of β-carotene in samples at all the storage conditions decreased during the storage period. For the control samples, 42.0%, 48.7%, 88.0%, and 90.1% of β-carotene remained

Table 2. Weight-Average Molecular Weight (M_w), *z*-Average Radius of Gyration (R_z), and Dispersed Molecular Density (ρ) of OSA-Modified Starches A, B, and C[a]

modified starch	peak 1 M_w (10^4g/mol)	peak 2 M_w (10^4g/mol)	av M_w (10^4g/mol)	R_z (nm)	ρ (g/mol·nm^3)
A	206.6 ± 0.02	7.85 ± 0.01	94.2 ± 0.03	32.3 ± 0.1	28.0 ± 0.3 c
B			8.29 ± 0.02	18.7 ± 0.5	12.7 ± 1.4 a
C			4.02 ± 0.01	12.6 ± 0.2	20.1 ± 1.3 b

[a]Data expressed as the mean ± SD (n = 3). For ρ values, data followed by different online letters (i.e., a−c) are significantly different (P < 0.05).

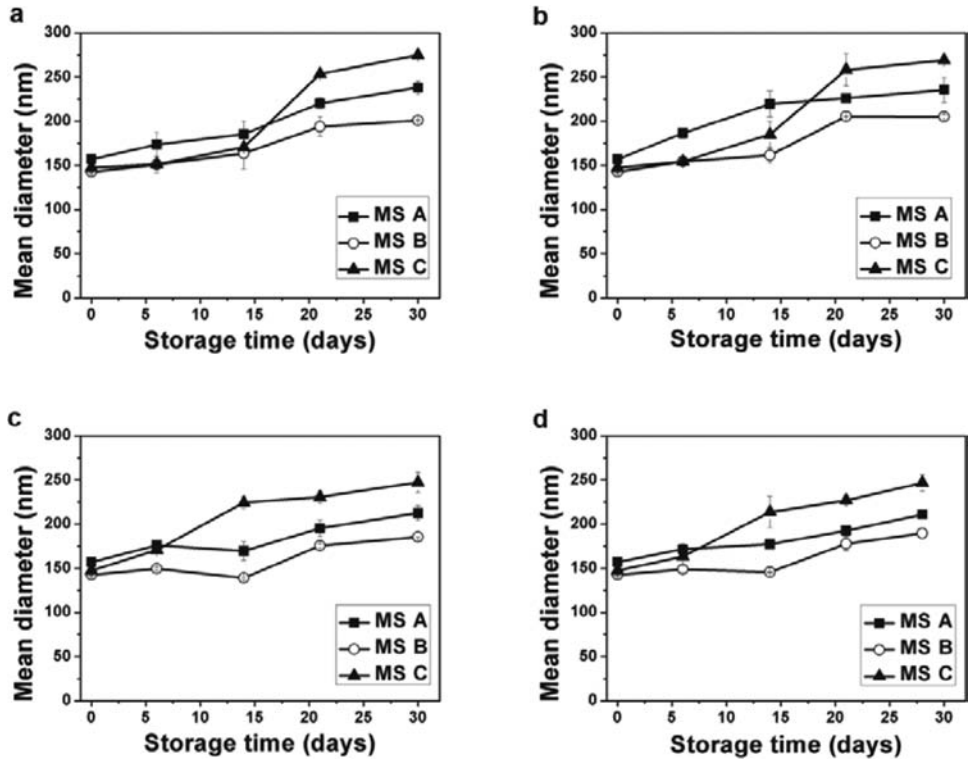

Figure 3. Evolution of mean diameters of β-carotene nanoemulsions over time under different storage conditions: (a) 25 °C under light; (b) 25 °C in the dark; (c) 4 °C in the dark; (d) 4 °C in the dark and flushed by nitrogen. Data are represented as the mean ± standard deviation (n = 3).

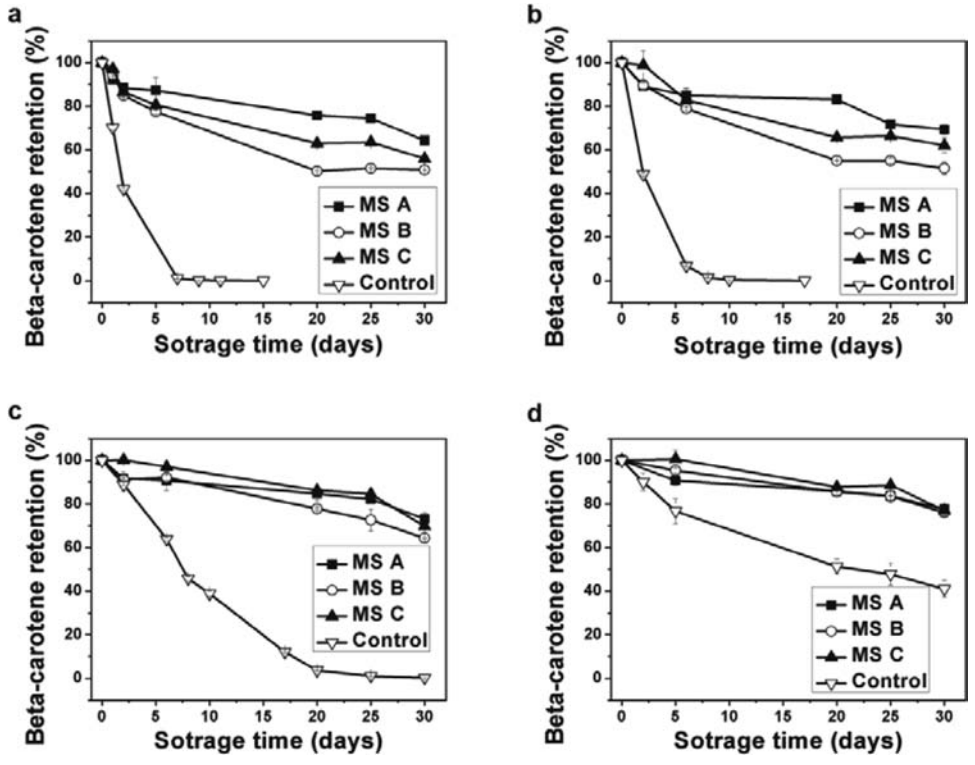

Figure 4. Retention of β-carotene in nanoemulsions over time with different storage conditions: (a) 25 °C under light; (b) 25 °C in the dark; (c) 4 °C in the dark; (d) 4 °C in the dark and flushed by nitrogen. Data are represented as the mean ± standard deviation (n = 3).

after 2 days when stored at four different conditions, 25 °C under light, 25 °C in the dark, 4 °C in the dark, and 4 °C in the dark and flushed by nitrogen, respectively. After day 7 and day 10, the β-carotene was totally lost in the control samples stored at 25 °C under light and at 25 °C in the dark, respectively. For control samples stored at 4 °C in the dark, only 0.2% of β-carotene was left at the end of the measurement (Table 3), but the β-carotene

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Table 3. β-Carotene Retention Levels in Nanoemulsions Stabilized by Modified Starches after 30 Days of Storage[a]

	β-carotene retention (%)			
sample	25 °C, L	25 °C, D	4 °C, D	4 °C, D, N_2
MS A	$64.29 \pm 0.39^{d,AB}$	$69.29 \pm 2.3^{f,BC}$	$73.09 \pm 2.83^{j,CD}$	$77.49 \pm 1.56^{l,D}$
MS B	$50.82 \pm 0.04^{b,E}$	$51.52 \pm 2.92^{e,E}$	$64.36 \pm 0.4^{hi,F}$	$76.02 \pm 1.72^{l,G}$
MS C	$56.01 \pm 1.69^{c,H}$	$62.01 \pm 3.36^{f,H}$	$69.73 \pm 1.41^{ij,I}$	$77.01 \pm 2.31^{l,J}$
control	0	0	0.20 ± 0.04^{g}	41.02 ± 3.88^{k}

[a]Storage tests for nanoemulsions were carried out at four different conditions: 25 °C under light (L), 25 °C in the dark (D), 4 °C in the dark, and 4 °C in the dark and flushed with nitrogen (N_2). Data are expressed as the mean ± standard deviation ($n = 3$). Values of means followed by different lowercase letters (b–l) in the same column are significantly different ($P < 0.05$). Values of means followed by different uppercase letters (A–J) in the same rows are significantly different ($P < 0.05$).

retention (41.0%) was extremely higher for the control samples stored at 4 °C in the dark with the nitrogen flush.

Compared to the control samples, β-carotene nanoemulsions exhibited improved stability and higher retentions at all storage conditions. As shown in Table 3, when stored at 25 °C under light 64.3%, 50.8%, and 56.0% of β-carotene was retained in the emulsions at the end of 30 days when stabilized by MSs A, B, and C, respectively. A significant decrease was observed for β-carotene retention in the order A > C > B, which was consistent with the decrease of ρ values in the same order, A > C > B. The molecules with a larger ρ value would benefit the emulsions by forming a dense and thick film around the oil droplets and further protect the β-carotene dissolved in the oil phase. Research by Bi et al. also produced similar results.[49] At 25 °C in the dark, no significant improvement was achieved for β-carotene retentions. This was consistent with the results of Wagner et al.[51] and Boon et al.,[52] which indicated exposure to light did not accelerate degradation of the carotenes. The differences in retentions between MSs A and C were not significant. This may be due to the change of mean diameters. According to the research by Tan et al., the small mean particle diameter with a big interfacial surface would increase the degradation of β-carotene.[21] Therefore, the increase of mean diameters for emulsions stabilized by MS C could be a benefit to the storage stability of β-carotene. When the storage temperature was lowered to 4 °C, the retentions of β-carotene for all emulsions increased significantly; at the same time the effect of the MS on the retentions became not significant as shown in Table 3. Even at 4 °C in the dark with a nitrogen flush, the β-carotene retentions for emulsions stabilized by MSs A, B, and C stayed at a similar level. These results were consistent with the β-carotene retentions for control samples and in agreement with previous research by Goldman et al., who reported the oxygen concentration in the medium was the most important factor for the degradation of β-carotene.[53] Therefore, the nitrogen-flushed samples may benefit the stability of β-carotene. In summary, the molecular properties of the MS affect the storage stability of β-carotene emulsions at normal conditions. However, when the storage temperature and oxygen in the samples are low enough, the effect of the molecular properties becomes negligible. Therefore, appropriate storage conditions should be taken into account to improve the stability of β-carotene emulsions.

Bioaccessibility of β-Carotene during in Vitro Digestion. The in vitro digestion was examined for β-carotene nanoemulsions to study their potential value as delivery systems for nutraceuticals. In this study, two steps were conducted to mimic the in vitro digestion process which included treatments with SGF and STF. Rapid lipolysis normally happens in the STF process by converting triacylglycerols and diacylglycerols to monoacylglycerols and free fatty acids,[54] so the percentage of FFAs released versus the digestion time was determined by the pH-stat method for different formulations. As shown in Figure 5,

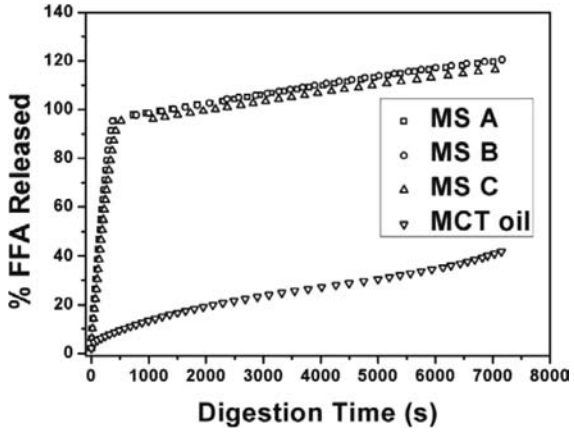

Figure 5. FFA release profiles from β-carotene nanoemulsions stabilized by different MSs as a function of small intestine digestion time.

a rapid increase of FFAs was exhibited in the first 500 s followed by a more gradual increase of digestion time. The final percentages of FFAs produced were around 120% for MS A, 120% for MS B, and 117% for MS C. No significant difference was observed for different nanoemulsions stabilized by MS. Similar results were also obtained by Sandra et al.[55] McClements et al. indicated that the type of initial emulsifier did not have a large effect on the rate and extent of FFAs released from the emulsion.[56] Furthermore, the type and concentration of the oil phase were both the same for all emulsion systems, which may be more helpful to eliminate the difference in FFAs released during digestion. Compared to β-carotene emulsions, the sample of β-carotene dissolved in bulk MCT oil showed a much lower percentage of FFAs released during digestion (42%). These results suggested that lipid digestion was an interfacial phenomenon, and the oils in colloidal particles had a larger surface area, which benefited the interaction with lipase,[57] whereas for the bulk lipids insoluble in the water phase, the reaction between the enzyme and the oil phase would be delayed, which further decreased the FFA release from lipids.

The emulsion system showed a greater bioaccessibility than the bulk lipid sample. As shown in Figure 6, the bioaccessibility of β-carotene dispersed in bulk MCT oil was only 3.1 ± 0.6%. These values increased to 19.4 ± 2.2%, 35.3 ± 0.8%, and 24.9 ± 0.4% for emulsions stabilized by MSs A, B, and C respectively. These results were consistent with the report by Yu et al. that the extent of lipolysis was proved to be positively correlated with the bioaccessibility (%) and the increase of the percentage of FFAs released could improve the bioaccessibility.[58] In addition, the

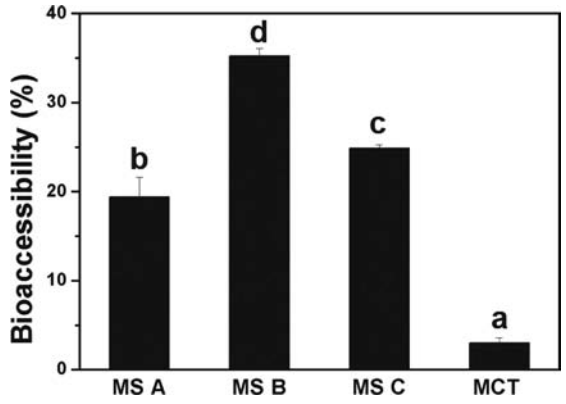

Figure 6. Bioaccessibility (%) of β-carotene after in vitro digestion of nanoemulsions stabilized by different MSs. Different letters (i.e., a–d) indicate a significant difference ($P < 0.05$).

bioaccessibility was higher than that of the nanodispersion system stabilized by decaglycerol monolaurate[8] and a little bit lower than that of the nanoemulsion system stabilized by protein,[59] which indicated that the biological fate of the emulsion system varied with the different digestion methods and physicochemical properties of the emulsions, e.g., particle size, emulsifier, and oil phase.[20] The bioaccessibility values among the three emulsions were significantly different and decreased in the order MS B > MS C > MS A. According to the paper published by Golding et al., this phenomenon was related to the micellization process during digestion when the bile salts and phospholipids were adsorbed to the droplet surfaces and displaced any existing emulsifier molecules to form a micelle with the products of digestion and nutraceuticals, such as β-carotene.[60] As suggested by the results above, MS A with a high ρ value showed higher branched and dense structures around the oil phase, which would make it hard to be displaced and further decreased the micellization of β-carotene. The trend of a decrease of bioaccessibility (B > C > A) was in agreement with the increase of the ρ value (B < C < A). Overall, the emulsion systems showed improvement of the β-carotene bioaccessibility over the bulk oil phase. The structure and properties of the emulsifiers influence the bioaccessibility of β-carotene by affecting the micellization process.

In summary, stable nanoemulsions of β-carotene were produced using MSs as the emulsifiers. From storage tests, temperature and nitrogen were shown to be the main parameters which affect the mean diameters and β-carotene retentions. Through in vitro digestion, the bioaccessibility of β-carotene was found to improve significantly after encapsulation in nanoemulsions. Our results indicated that the dispersed molecular density of MS could affect the droplet diameters, chemical stability, and in vitro digestion of nanoemulsions. Typically, MS with higher dispersed molecular density would give a thicker and denser layer over the oil droplets, which would further increase the mean diameter, improve the chemical stability, and lower the bioaccessibility during in vitro digestion. Further work is still under way to establish the correlation between in vitro bioaccessibility and in vivo bioavailability for nutraceuticals. The knowledge obtained will be important in the design and development of new strategies to improve the oral bioavailability of active ingredients.

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Notes

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