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Spray-Drying Microencapsulation of β-Carotene by Soy Protein Isolate and/or OSA-Modified Starch

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ABSTRACT: Microencapsulation is an effective strategy to improve the storage stability of β-carotene. This article investigated the potential and effectiveness of soy protein isolate (SPI) and octenylsuccinic anhydride-modified starch (MS), alone or in combination (1:1, w/w), to encapsulate β-carotene by spray drying. The results indicated that the microcapsule with MS exhibited much lower encapsulation efficiency (NE) and poorer dissolution behavior, but much better redispersion behavior, than that with SPI or its blends with MS. The NE was basically unaffected by total solid content (TC) or core/wall ratio; increasing the TC impaired the dissolution and/or redispersion behavior. The dispersion behavior was closely associated with the morphology of the microcapsules. The encapsulated β-carotene suffered a progressive loss upon storage under high humid or temperature environment, but it exhibited extraordinary stability at low temperatures (e.g., 4°C). The β-carotene degradation was independent of sunlight. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40399.

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INTRODUCTION

β-Carotene is an important member of the carotenoids family found in many fruits and vegetables. Besides its high provitamin A activity and antioxidant capacity, β-carotene possesses many health-benefiting effects, including protection against a number of serious health disorders, e.g. cancer, cardiovascular disease, macular degeneration.[1,2] For these reasons, there is an increasing interest in incorporating β-carotene as functional ingredients in food formulations. However, β-carotene is insoluble in water, and only marginally soluble in oil at room temperature because of its crystalline form. This greatly limits the development of β-carotene -richened food products, and even decreases its bioavailability. Another limitation for β-carotene to be applied in food industry is that β-carotene is sensitive to light, oxygen, and even heat.[3]

One effective strategy to protect and deliver the sensitive ingredients including β-carotene is to microencapsulate them within a coating or wall material. Spray-drying is one of the most common used microencapsulation techniques in the food industry. In this process, the sensitive ingredient solubilized or dispersed in an organic or oil phase is homogenized in an aqueous phase containing wall materials to form a kinetically stable emulsion. The emulsion is further fed into a spray drying device, and subsequently, transformed into a dried product consisting of particles. The choice of effective wall materials is a vital step for the microencapsulation by spray drying. Encapsulation of carotenoids or their oleoresins makes them easier to handle and improves their stability to oxidation.[4] The main wall materials for the spray-drying encapsulation of carotenoids or their oleoresins include gum Arabic,[5] modified starch,[6–8] gelatin,[9,10] sodium caseinate,[11] and soy protein isolate (SPI).[5] In some cases of proteins, low molecular weight carbohydrates, e.g. lactose and maltodextrins, are usually co-applied for the improvement of encapsulation efficiency.[12] Although gum Arabic is a well-known effective wall material for many years, recent works suggest that other wall materials, e.g., modified starch and SPI, might be a better choice as the wall materials due to their low cost, vast supply, and even excellent emulsifying properties. Rascón et al.[5] pointed out that the microcapsules prepared with SPI exhibited much higher ability to retain their structural integrity at high water activities (e.g. above 0.743) than those using gum Arabic as the wall material.

A modified starch, by the modification with a lipophilic component, octenylsuccinate (OSA-starch), has been widely applied as the encapsulation material in the food industry, with approval of the FDA as food additive when its content does not exceed 30 g kg^{-1}.[13] Compared with native starch, this modified starch has been confirmed to exhibit excellent capacity to retain

volatiles during atomization in a spray dryer.[8] In a recent work, OSA-starch has been applied to prepare spray-dried β-carotene nanoemulsions with a good dissolution and/or redispersion in water.[6] On the other hand, SPI has been also confirmed to exhibit good microencapsulation properties when oil is used as the core material.[12,14] However, the effectiveness of these wall materials to encapsulate β-carotene still needs to be well characterized and confirmed. Furthermore, to the best of the authors' knowledge, no work is available to compare the microencapsulation properties of these two wall materials, and the storage stability of β-carotene encapsulated in the correspondingly spray-dried products.

Thus, the main objective of the present work was to compare the potential of OSA-starch, SPI and their 1:1 mixture to be applied as the wall materials for the preparation of spray-dried β-carotene microcapsules. Some characteristics including encapsulation efficiency (of β-carotene), dissolution and/or redispersion behavior and microstructure of microcapsules, as well as storage stability of β-carotene in the microcapsules, were characterized. Furthermore, the influence of wall/core ratio and total solid content on the properties of the microcapsules using the SPI/OSA-starch 1:1 mixtures as the wall materials was also investigated. For storage stability experiments, β-carotene degradation upon storage in the microcapsules at various storage temperatures, with or without light, was monitored.

EXPERIMENTAL

Materials

The concentrated β-carotene dispersion (300 g kg^{-1} in vegetable oil) was purchased from BASF Co., Ltd. (England). Standard β-carotene (> 96%, UV) was purchased from Aladdin Jingchun Reagent Co., Ltd. (Shanghai, China). Commercial soy protein isolate (SPI), containing 6.2% moisture and 910 g kg^{-1} protein (on dry basis), was purchased from Fuji Oil Technology Ltd. (Beijing, China). Soy oil was purchased from a local supermarket in Guangzhou (China). OSA-modified starch (MS) was prepared according to the process described in our previous literature.[15] All other chemicals used were of analytical grade.

Emulsion and Powder Preparation

The SPI and/or MS dispersions (with a total concentration of 5 g L^{-1}), alone or in combination (1:1, w/w), were prepared in de-ionized water containing 0.1 g L^{-1} sodium azide, under stirred conditions at room temperature for 2 h. If necessary, the pH of the dispersions was adjusted to pH 7.0 with 0.2*M* NaOH, and the dispersions were then kept overnight at 4°C, as the stock dispersions. Before the emulsion preparation, all the dispersions were heated up to 60°C in a water bath. The concentrated β-carotene dispersion was diluted 30 times by soy oil preheated to approximately 60°C (in a water bath), and the diluted dispersion was further stirred using a magnetic stirrer for 1 h. Then, the diluted β-carotene dispersion was little by little added into the corresponding aqueous phase, at different core/wall ratios of 1:1, 1:2, or 2:1, and prehomogenized using a high-speed dispersing unit (Ultra-Turrax T25, IKA-Labortechnik, Staufen, Germany) at 7000 rpm for 3 min to produce coarse emulsions. The coarse emulsions were further passed at 40 MPa using a microfluidiser (M110EH model, Microfluidics Co., Newton, MA) two times, to produce the final emulsions (denoted as the original emulsions for spray-drying).

Table I. Formulations and Process Parameters for the Preparation of Original Emulsions

Nos.	Core (g kg^{-1})	Wall materials (g kg^{-1})		Total solid content (TC; g kg^{-1})	Core/wall ratio
		SPI	MS		
1	50	50	0	100	1 : 1
2	50	0	50	100	1 : 1
3	50	25	25	100	1 : 1
4	75	37.5	37.5	150	1 : 1
5	50	50	50	150	1 : 2
6	100	25	25	150	2 : 1
7	100	50	50	200	1 : 1

The original emulsions were spray-dried in a laboratory drier (Lapland, SD-06, England) fitted with 1.5 mm nozzle atomizer. Each emulsion was pumped to the spray-drier at room temperature at a flow rate of 10 mL/min, and dried with an inlet temperature of 160°C and outlet temperature of ~85°C. The obtained powder samples were stored in brown bottles (60 mL) sealed with screw glass plug in a desiccator at 4°C before use. The formulation or processing parameters for various original emulsions or spray-dried samples (denoted as Nos. 1–7) are shown in Table I.

Droplet size distribution and Average Droplet Size ($d_{4,3}$)

The size distribution of droplets in original emulsions, as well as reconstituted emulsions from the spray-dried powders, was evaluated using a Malvern MasterSizer 2000 (Malvern Instruments Ltd, Malvern, Worcestershire, UK). Distilled water was used as the dispersant. Calculation of the droplet size distribution was based on a relative refractive index of 1.095 [the ratio of the refractive index of soy oil (1.456) or β-carotene (1.47) to that of the continuous phase (1.33)] and absorption of 0.001. Droplet size data are reported as the volume-average diameter, $d_{4,3}$ ($=\sum n_i d_i^4 / \sum n_i d_i^3$, where ni is the number of droplets with diameter di). For each emulsion, three replicates were applied, and each replication was the mean of duplicate measurements.

Total and Surface β-Carotene, and Encapsulation Efficiency (NE)

The total and surface β-carotene contents of the microencapsulated powders were determined as the process described by Loksuwan,[7] with a few modifications. For total β-carotene content determination, 50 mg of each powder was weighed into the 125 mL-flask, dispersed in 10 mL deionized water under stirred conditions for 30 min. An aliquot (1 mL) of the dispersion was mixed and extracted with 5 mL of acetone/petroleum ether (1:4, v/v), and the extraction was repeated until the bottom aqueous phase was clear and colorless. The organic phase was pooled and dehydrated using anhydrous sodium sulfate. The adsorption of the resultant organic was spectrophotometrically measured at a wavelength of 450 nm on a UV spectrophotometer (UV 2300, Techcomp, Shanghai, China), with petroleum ether as blank

Table II. Droplet Size of Original and Reconstituted Emulsions Formed with Various Core/Wall Material Formulations, as well as Encapsulation Efficiency (NE), Surface Content, and Storage Stability of β-Carotene in the Resultant Microcapsules (mean ± SD, $n = 3$)

	Emulsion size, $d_{4,3}$(μm)				β-Carotene retention (%)[b]	
Sample nos.	Original	Reconstituted	NE (%)	Surface β-carotene (%)[a]	After wet storage at 75% RH for 7 days	After dry storage at 4°C for 35 days
1	0.64^{c}	16.4^{a}	35.4 ± 6.5^{a}	8.4 ± 0.6^{a}	38.7 ± 2.6^{a}	92.1 ± 8.1^{a}
2	0.28^{f}	0.75^{e}	20.9 ± 4.9^{b}	7.6 ± 0.5^{b}	31.2 ± 7.5^{a}	97.9 ± 2.9^{a}
3	0.32^{e}	9.8^{c}	38.8 ± 5.3^{a}	7.5 ± 1.0^{b}	15.9 ± 2.7^{c}	87.0 ± 3.2^{b}
4	0.65^{c}	9.1^{c}	34.7 ± 7.4^{a}	7.7 ± 0.3^{b}	19.1 ± 4.3^{b}	102.1 ± 2.8^{a}
5	0.78^{a}	8.0^{d}	24.0 ± 1.5^{b}	3.2 ± 0.4^{c}	20.4 ± 1.4^{b}	102.4 ± 2.0^{a}
6	0.60^{d}	13.4^{b}	24.6 ± 5.8^{b}	8.1 ± 0.4^{a}	37.0 ± 8.4^{a}	102.3 ± 1.7^{a}
7	0.69^{b}	12.6^{bc}	29.0 ± 2.1^{ab}	3.6 ± 0.1^{c}	16.4 ± 1.0^{c}	104.3 ± 6.2^{c}

[a] Surface β-carotene (%) was calculated by the surface β-carotene content (in the powders) compared to the β-carotene initially applied to produce them.
[b] β-carotene retention (%) was calculated by the total β-carotene content in the stored powders compared to that in the fresh powders.
Different superscripts (a–f) represent significant difference at $P < 0.05$ level among the same column.

control. For surface β-carotene content determination, 50 mg of each powder was extracted with 25 mL of petroleum ether. After shaking at 100 rpm for 15 s, the dispersion was centrifuged at 1000 × *g* for 1 min. The adsorption of the resultant supernatant was determined at 450 nm. The concentration of β-carotene in the organic solutions was calculated using a previously established standard curve of standard purified β-carotene in the same solvent. Encapsulation efficiency (NE) was calculated as the total content of β-carotene present in the powders compared to the β-carotene initially applied to produce them.

Dissolution and Redispersion Behavior

The dissolution behavior was spectrophotometrically measured on a UV spectrophotometer (UV 2300, Techcomp, Shanghai, China), according to the method of Millqvist-Fureby et al.,[16] with a few modifications. A powder sample (5 mg) was layered on top of 3.0 mL water in a cuvette (1 × 1 cm cross-section), and the increase in absorbance at 450 nm (A_{450}) with time was recorded. The rate of increase in absorbance (during initial dissolution; k_0) and maximal A_{450} (approximately constant after dissolution) were applied as the indicators to evaluate the rate and potential of dissolution.

Redispersion behavior of the powders was evaluated according to the method of Hogan et al.[17] with minor modifications. The powders (0.1 g) were dispersed in 100 mL deionized water. The dispersions were gently stirred at room temperature for 30 min and the particle size distribution or $d_{4,3}$ was determined using laser diffraction as described above.

Microstructure of Powder Particles

Scanning electron microscopy (SEM) was used to observe the morphology of particles for the spray-dried powders, without or with storage at 75% RH for 7 days. Each sample was attached to SEM stubs using double-side adhesive tape. The specimens were coated with gold and examined using EVO18 scanning electron microscope (Carl Zeiss AG, Oberkochen, Germany) at an accelerating voltage of 10 kV.

Storage Stability

Three kinds of experiments were performed for evaluation of storage stability of β-carotene in spray-dried powders, with or without sunlight. For the first experiments, all the spray-dried powders (freshly prepared) were placed in a desiccator containing saturated NaCl slurry (relative humidity, RH = 75%), at ambient temperature in the dark, for a period up to 7 days. Another set of experiments to evaluate the influence of sunlight on the degradation of β-carotene, the powders were placed in transparent plastic bottles with sealed lids. In this case, all the samples were divided into two parts, with one part of the samples further tightly packed with a tinfoil material. All the samples were stored in a glass desiccator with dry silica, at ambient temperature up to 25 days (the desiccator was placed in the lab without direct contact with sunlight). At fixed time intervals, the changes in total β-carotene content for these powders were determined as mentioned above. At last, the thermal degradation of β-carotene in the spray-dried powders, at a high (60°C) or low (4°C) temperature was also evaluated in the dark. In this case, each powder sample was put into an aluminum pan (covered with a loose lid) that was placed in an incubator with a constant temperature of 60°C, or in a refrigerator at 4°C. At specific storage intervals (1 or 2 weeks for 60°C and 35 days for 4°C, respectively), the total β-carotene content of the stored powders was determined as mentioned above.

Statistical Analysis

A one way analysis of variance (ANOVA) of the data was performed to determine the significant difference at $P < 0.05$ level.

RESULTS AND DISCUSSION

Characterization of Original Emulsions

The oil phase containing 10 g L^{-1} β-carotene was prepared by diluting the β-carotene oleoresin with soy oil, while stirring at 60°C for 1 h. The resultant β-carotene dispersion was transparent with no crystallization of β-carotene occurring. To avoid the β-carotene crystallization occurring during the emulsification, due to the difference in temperature, the aqueous dispersions containing SPI or MS were also heated in a water bath up to around 60°C, and then subjected to the emulsification. Table II

summarizes the volume-weighted mean diameter ($d_{4,3}$) values of various original emulsions, formed according to the formulations in Table I. The $d_{4,3}$ values of these emulsions ranged from 0.28 to 0.78 μm. The $d_{4,3}$ (0.644 μm) of the original emulsion stabilized by SPI alone is distinctly higher than that (0.265 μm) of the native SPI emulsion, formed at comparable oil fraction and protein concentration.[12,14] The difference is clearly due to the difference in emulsifying properties between the two SPI samples.

At $\varnothing = 0.05$ and TC = 50 g kg^{-1}, the emulsion stabilized by MS alone had lowest $d_{4,3}$ value (0.28 μm), followed by the emulsions by the 1:1 mixture (0.32 μm) and SPI alone (0.64 μm), respectively (Table II, Nos. 1–3). The observation indicated that under the conditions, MS seems to exhibit better emulsifying properties than the SPI. At the same core/wall ratio (1:1), increasing the TC (in the emulsions stabilized by the 1:1 mixture) from 100 to 200 g kg^{-1} led to a gradual increase in emulsion size, e.g. with $d_{4,3}$ increasing from 0.32 to 0.69 μm (Table II, Nos. 3,4, and 7), suggesting enhanced inter-droplet interactions upon increasing the TC. On the other hand, we can unexpectedly see that at a constant TC (150 g kg^{-1}), increasing the core/wall ratio on the contrary led to a reduction in $d_{4,3}$ (Table II, Nos. 4–6). A similar reduction in $d_{4,3}$ has been observed for the emulsions stabilized by native SPI with core/wall ratios increasing from 1:2 to 3:1,[14] where it has been attributed to decreased interdroplet interactions, as a result of decreased amount of effective emulsifying agents to stabilize the emulsions. For SPI-stabilized orange oil emulsions, Kim et al.[18] also observed that increasing oil content from 100 to 300 g L^{-1} resulted in a decrease in droplet size from 2.804 to 1.687 μm. Whereas in the work of Nesterenko et al.,[19] a contrasting result was reported that varying the core/wall ratio from 1:2 to 2:1 did not significantly change the $d_{4,3}$ of SPI stabilized α-tocopherol emulsions.

Characteristic of Microcapsules

Encapsulation Efficiency (NE). The NE of β-carotene for various spray-dried microcapsules is also shown in Table II. As expected, the NE considerably varied with the composition of encapsulating materials, and processing parameters, e.g., core/wall ratio and TC. At the core/wall ratio of 1:1 and TC = 100 g kg^{-1}, the NE (35.4%) of the microencapsulated powder with SPI alone was significantly higher than that (20.9%) with the MS alone (Table II, Nos. 1 and 2), indicating better microencapsulating property of the protein. The NE (38.8%) for the microcapsule with the SPI/MS mixture as the wall material was slightly but insignificantly higher than that for the SPI counterpart (Table II, Nos. 1 and 3). Similar improvements by the blending with carbohydrates (e.g., lactose) have been observed for the microencapsulation of oils with sodium caseinate, whey proteins and SPI as emulsifying and encapsulating materials.[14,17,20] The NE of the microcapsule with MS is similar to that (21–30%) reported for the spray-dried lycopene microcapsules with a similar MS as the wall material,[8] but considerably lower than that (54–71%) of carotenoids in spray-dried encapsulated *rosa mosqueta* oleoresin with potato starch.[9] The NE value for the microcapsule with SPI alone is considerably lower than that (~83%) of carotenoids in spray-dried encapsulated paprika oleoresin using SPI as the wall material (at a comparable inlet air temperature of 160°C).[5] The differences in NE for carotenoids in spray-dried microcapsulated products might be associated with the differences in the core/wall ratio, composition of wall materials, homogenization conditions, as well as conditions of drying.[10]

At a same core/wall ratio (1:1), increasing the TC from 100 to 200 g kg^{-1} resulted in a significant reduction in NE (from 38.8 to 29.0%), for the encapsulated powders using the 1:1 mixture as the wall material (Table II, Nos. 3, 4 and 7). In the spray-dried microencapsulated lycopene with MS as the wall material, Rocha et al.[8] similarly observed that at a constant TC (of 300 g kg^{-1}), increasing the core concentration (in regard to TC) from 50 to 150 g kg^{-1} resulted in a progressive decrease of NE from 30 to 21%. In the microencapsulated lycopene using gelatin and sucrose blends as the wall material, Shu et al.[10] also verified that increasing the core/wall ratio led to a lower NE. On the other hand, we can observe that for the microcapsules with the 1:1 mixture as wall materials, the NE was slightly changed by the variation in core/wall ratio from 2:1 to 1:2 (Table II, Nos. 4–6). This observation seems to be a bit contrasting from the previous findings that the core/wall ratio was the most pronounced parameter affecting the microencapsulation efficiency of carotenoids or oil retention efficiency.[10,12]

Besides the NE, we also evaluated the surface β-carotene content of all the spray-dried powders, and the results indicated that (1) there was no significant difference in surface β-carotene content between the microcapsules obtained with SPI, MS or their 1:1 mixture as the wall materials, at the same core/wall ratio and TC (Table II; Nos. 1–3); (2) significant decreases in surface β-carotene content were observed at highest TC (200 g kg^{-1}; compared to that at 100 or 150 g kg^{-1}, at a same core/wall ratio), or lowest core/wall ratio (1:2; compared with that at 2:1 or 1:1; at TC = 15%). These observations suggest that although the NE of β-carotene in the microcapsules was insignificantly affected by changing the TC or core/wall ratio, the surface β-carotene content was highly dependent on these parameters.

Dissolution and Redispersion Behavior. The dissolution behavior of the powder products in water is one of the important quality parameters affecting the preference of the consumer. We evaluated the dissolution behavior of various β-carotene microencapsulates in water, by detecting the changes in absorbance at 620 nm (of the dispersions) over time. In this case, the increasing rate (or slope) of absorbance during the initial incubation with water (e.g., less than 10 min) can reflect the ability of the microcapsule products to be dispersed, while the magnitude of maximal absorbance (at the end of dissolution; $A_{120\ min}$) is an indication for the capacity of the powder products to be dissolved. From Figure 1(A), we can observe that at comparable core/wall ratio and TC (1:1 and 100 g kg^{-1}), all the microcapsule powders using SPI, MS or the 1:1 mixture thereof, exhibited a similar increasing rate of absorbance, during the initial dissolution period, but the $A_{120\ min}$ progressively decreased with the order: SPI/MS mixture > SPI > MS. The observation indicated that the blending of SPI and MS remarkably improved the dissolution behavior of the microcapsule products, especially

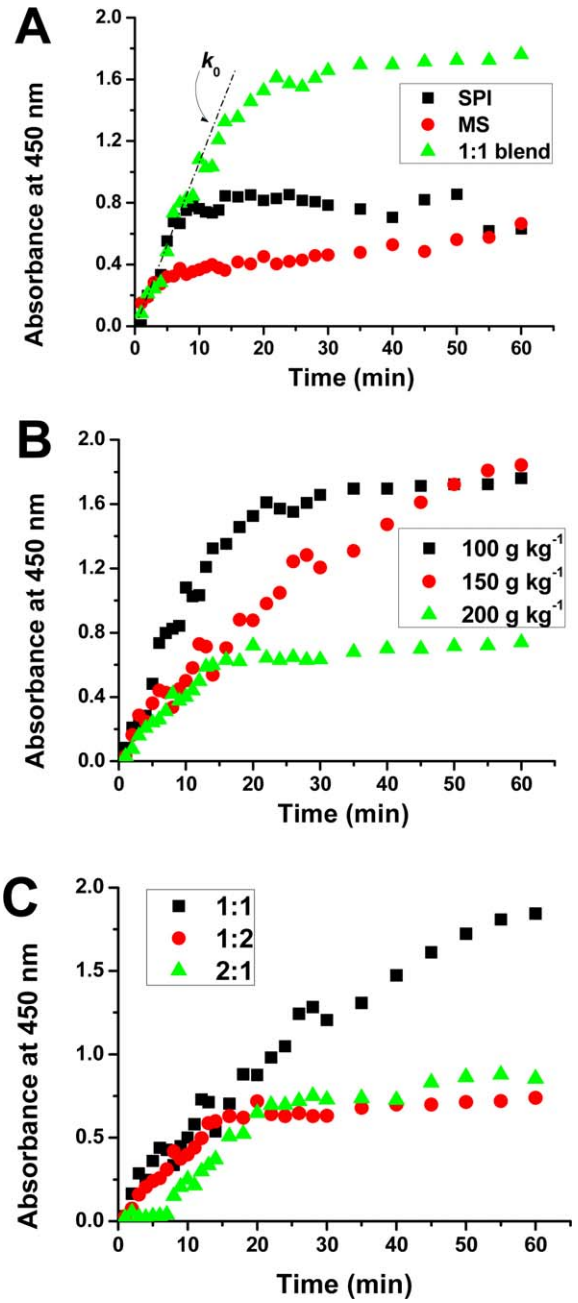

Figure 1. Typical dissolution profiles of various spray-dried β-carotene microcapsules using SPI, MS or their 1:1 mixture as the wall materials, as affected by composition of wall materials (A), TC (B), and core/wall ratio (C). Conditions: A) core/wall ratio = 1:1, TC = 100 g kg^{-1}; B) core/wall ratio = 1:1, the SPI/MS mixture as wall material; C) TC = 150 g kg^{-1}, SPI/MS blend as the wall material. k_0: the slope of absorbance against time during the initial incubation period. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

at prolonged periods of dissolution. The order for the $A_{120\ min}$ is well consistent with the order for the NE of the microcapsule products (Table II, Nos. 1–3), possibly suggesting a close correlation between the dissolution capability and the surface oil coverage of the powders. The importance of surface oil coverage to the dissolution behavior has also been confirmed in the spray-dried milk protein- or SPI-stabilized emulsions.[12,16]

At a given core/wall ratio (1 : 1), increasing the TC from 100 to 200 g kg^{-1} progressively impaired the dissolution behavior of the microcapsule products using the 1 : 1 mixture as the wall materials, including reduction in increasing rate of absorption, or decreased $A_{120\ min}$ [Figure 1(B)]. The observation further confirmed the relationship between the dissolution behavior and the NE. On the other hand, variation in core/wall ratio did not distinctly affect the dissolution behavior during initial incubation, while highest capacity to be dispersed was observed at the core/wall ratio of 1:1 [Figure 1(C)]. This seems to be not well in agreement with the NE data (Table II), reflecting that the dissolution of the microcapsule products was a complex process involving many parameters, including surface oil coverage, nature and interactions of the wall materials in the matrix, and core/wall ratio.

The redispersion behavior of the spray-dried microcapsules was characterized by evaluating the droplet size of their reconstituted emulsions, which can provide information about the stability of emulsion droplets during the drying process. The $d_{4,3}$ data of the reconstituted emulsions (diluted in water) are also summarized and included in Table II. In general, all of these reconstituted emulsions exhibited considerably higher $d_{4,3}$ than their original counterparts, with an exception for the microencapsulate using MS alone as the wall material, where it was observed that although the $d_{4,3}$ of the re-constituted emulsion was significantly higher than that of the original counterpart, but the difference in magnitude (from 0.28 to 0.75 μm) was marginal (Table II). The considerably higher $d_{4,3}$ data clearly reflected destabilization of oil droplets during the drying process, including droplet flocculation and/or coalescence. Thus, the observations indicated that the microcapsule product using MS alone exhibited an excellent dispersion behavior, and the presence of SPI greatly impaired the dispersion behavior of the spray-dried products. This can be well corroborated by the observation that at given core/wall ratio and TC (1:1 and 100 g kg^{-1}), the $d_{4,3}$ of the reconstituted emulsions considerably decreased in the order: SPI (16.4 μm) > 1:1 mixture (9.8 μm) > MS (0.75 μm), while the $d_{4,3}$ of their original counterparts slightly varied (Table II). The excellent redispersion behavior of the β-carotene microcapsules using MS as the wall material has also been previously reported by Liang et al.[6] The underlying reason causing the differences in redispersion behavior between MS and SPI encapsulated powders might be that the magnitude of inter-droplet attractive interactions in the SPI microcapsule case was considerably stronger than that in the MS case. Our previous work[12] confirmed that the blending with lactose could remarkably improve the redispersion behavior of spray-dried SPI-stabilized emulsions, through decreasing the inter-droplet interactions in the powders.

The redispersion behavior of the microcapsule products was nearly unaffected by increasing the TC from 100 to 200 g kg^{-1}, as evidenced by insignificant changes in $d_{4,3}$ of the reconstituted emulsions (Table II, Nos. 3, 4, and 7). On the other hand, we can see that increasing the core/wall ratio led to a gradual impairment of the redispersion behavior, though to a certain extent (Table II, Nos. 4–6). A similar effect of the influence of core/wall ratio on the redispersion behavior of powder products

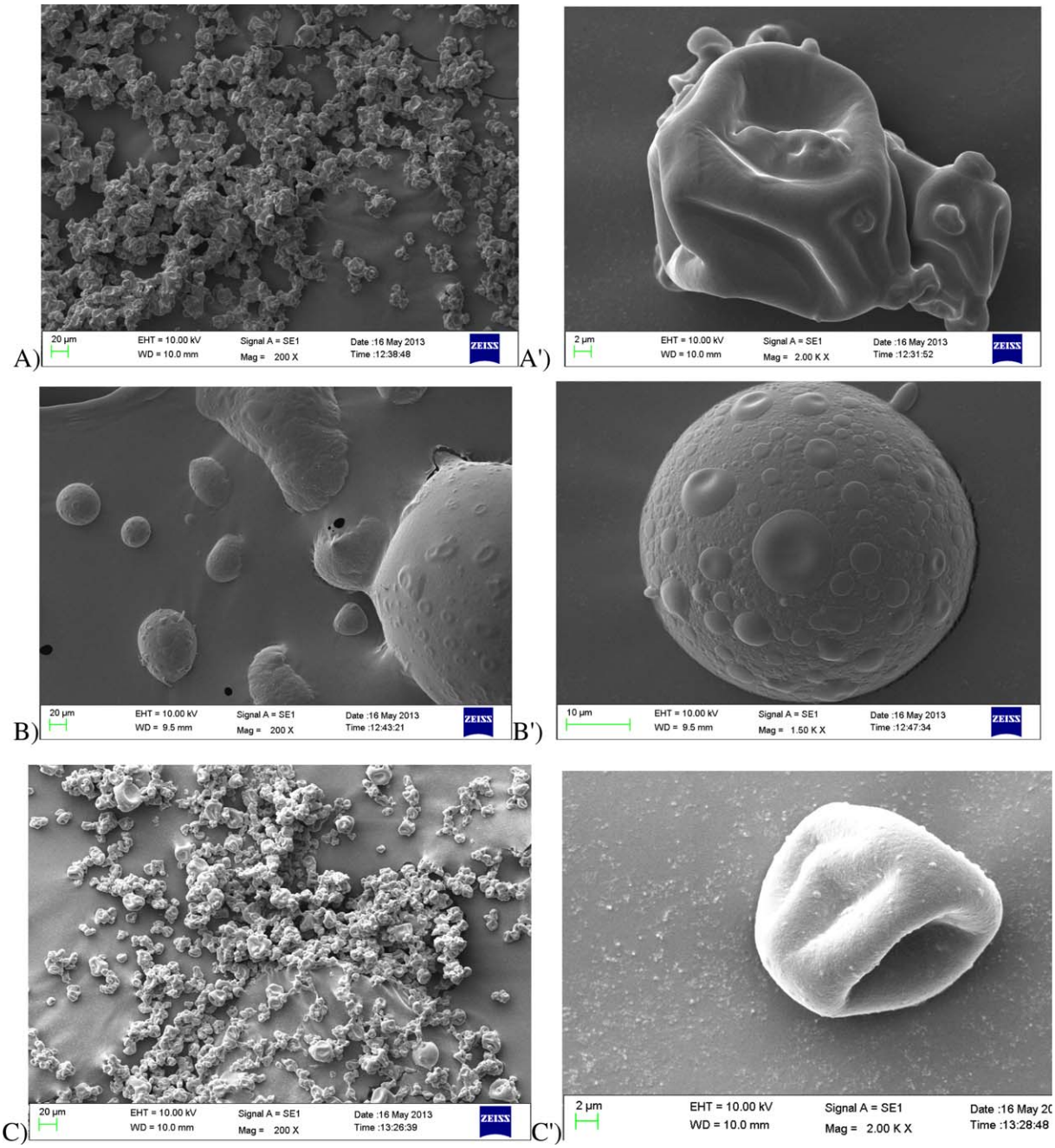

Figure 2. Typical SEM micrographs of β-carotene microcapsules with SPI (A, A′), MS (B, B′) and their 1 : 1 mixture (C, C′). The microcapsule sample with 1:1 mixture was obtained at the core/wall ratio of 1 : 2 and TC of 150 g kg^{-1} (corresponding to No. 5 in Table I). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

has been widely observed for many spray-dried emulsions stabilized by SPI, sodium caseinate and even gum Arabic.[12,17,21]

Microstructure of Microencapsulates. The microstructure of spray-dried β-carotene microcapsules was evaluated using SEM, with some representative micrographs displayed in Figure 2. As expected, different microcapsules with different composition of wall materials exhibited remarkable differences in morphology. For the microcapsule using SPI alone as the wall material, most of the particles had indented and wrinkled surface morphology [Figure 2(A,A′)]. This is consistent with the general viewpoint that the presence of dents on the surface is common for powders with high protein content, which is largely associated with uneven shrinkage during drying and/or cooling.[12,22] In this case, most of the particles were to a great extent adjoined one another [Figure 2(A)], indicating high stickiness between the particles. In contrast, the particles in the microcapsule with MS alone as the wall material were much larger in size, with round and smooth surface in morphology observed [Figure 2(B,B′)]. A similar powder surface

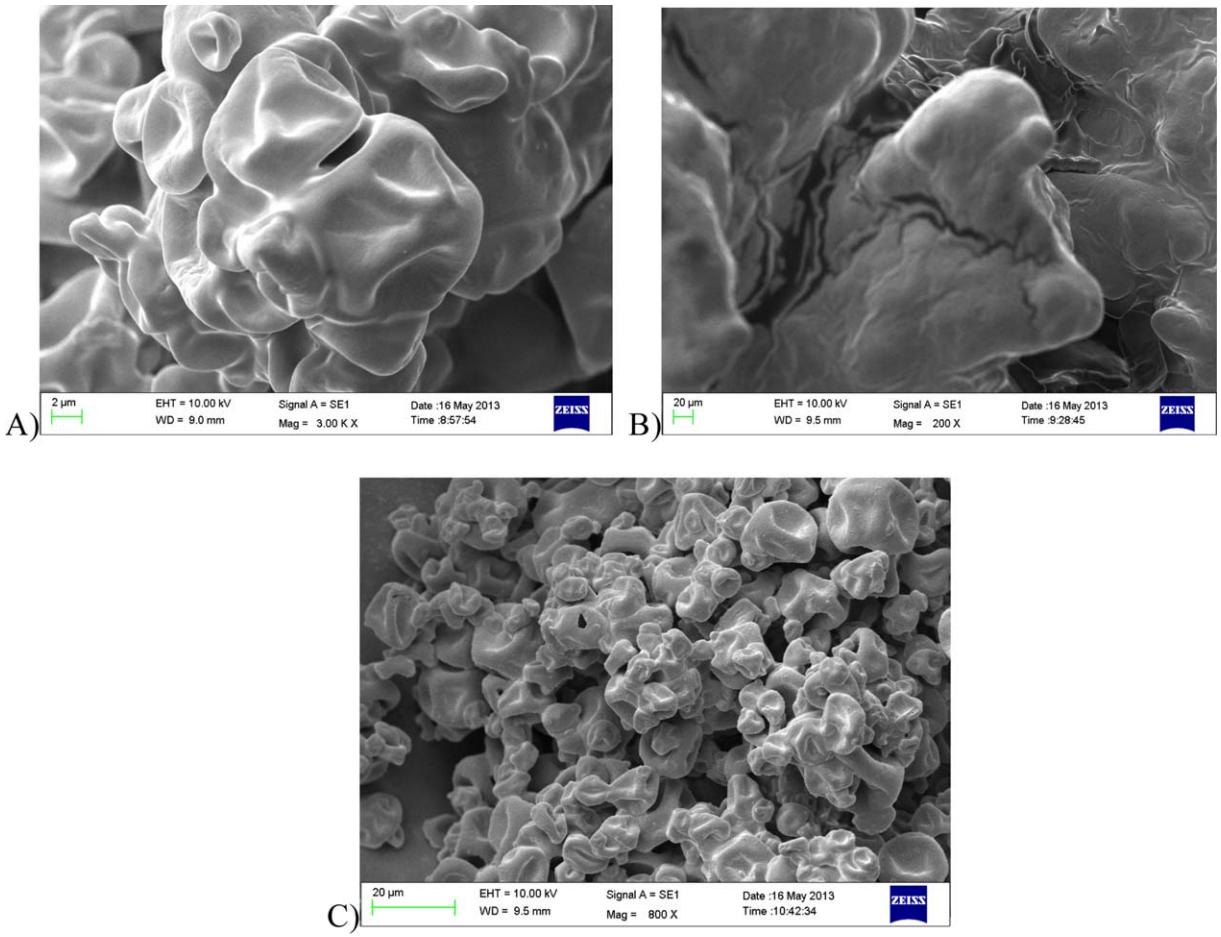

Figure 3. Typical SEM micrographs of β-carotene microcapsules with SPI (A), MS (B), and their 1 : 1 mixture (C), after a storage at 75% RH for 7 days. The microcapsule sample with 1 : 1 mixture was obtained at the core/wall ratio of 1:2 and TC of 150 g kg^{-1} (corresponding to No. 5 in Table I). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

morphology has been previously observed for spray-dried β-carotene microcapsules using HI-CAP (OSA 1).[6] However, a distinctly different surface morphology for the spray-dried microcapsules with the same MS as the wall material has also been previously observed,[6,8] where the wrinkled surface or concavities on the surface were observed. Interestingly, free oil spots could be distinctly observed on the particle surface of the microcapsule with MS alone [Figure 2(B′)], which is well consistent with the lowest NE value of this microcapsule (Table II). In contrast, the surface morphology of the microcapsule with 1:1 mixture was in the intermediate between the microcapsules obtained with SPI and MS alone [Figure 2(A–C)]. The observations confirmed that the differences in dissolution or redispersion behavior between the different microcapsule samples were largely attributed to the differences in microstructure of the microcapsules.

Storage Stability of β-Carotene Microcapsules

Storage at 75% RH for 7 Days. The moisture adsorption behavior is an important parameter reflecting the storage stability of the powders. We evaluated the influence of storage at 75% RH for a 7-day period on the β-carotene loss in the spray-dried microcapsules (Nos. 1–7). The β-carotene retention of the microcapsules after the storage is also included in Table II. The moisture contents of all the spray-dried powders ranged from 15 to 30 g kg^{-1}, depending on the composition of wall material, TC and core/wall ratio (data not shown). This is consistent with the results of many previous works on spray-dried protein-stabilized emulsions,[12,14,17,22] or starch-stabilized emulsions.[7] Upon storage at 75% RH, all the spray-dried powders absorbed moisture fast during initial storage period up to 20 h, and after that, the rate of moisture adsorption progressively decreased (data not shown). After 7-day storage, the moisture content for the No. 5 microcapsule was greatest (approximately 4.1%), while that for the No. 6 microcapsule was least (~18 g kg^{-1}; data not shown), confirming that the moisture adsorption behavior of the spray-dried microcapsules was mainly determined by the core/wall ratio.[12]

We also evaluated the changes in surface morphology of the spray-dried microcapsules after the storage, and some representative SEM micrographs of the stored microcapsules are presented in Figure 3. We can see that the microcapsules using SPI alone or its blends with MS as wall materials basically retained their surface morphology after the storage, while the surface morphology of the microcapsule with MS alone changed

considerably (Figure 3). In the stored No. 2 microcapsule, all the particles appeared to adhere to one another, and as a result, the structure of globule-like microcapsules was completely collapsed [Figure 3(B)]. A similar phenomenon has been reported for spray-dried β-carotene microcapsules with MS as the wall materials.[6] The considerable change in surface morphology in the No. 2 microcapsule might be largely attributed to the transformation of an amorphous to rubber state occurring for the powders containing high content of starch.

As expected, the storage resulted in a considerable loss of β-carotene in the spray-dried microcapsules, but the magnitude in loss was closely dependent on the type of the microcapsules (Table II). At the core/wall ratio of 1:1 and TC = 100 g kg^{-1}, the β-carotene retention (38.7%) of the stored No. 1 microcapsule was similar to that of the No. 2 counterpart, much higher than that (15.9%) of the No. 3 microcapsule (Table II), indicating that the blending considerably decreased the storage stability of β-carotene at high RH. The β-carotene stability at high RH seems to be not related to the encapsulation efficiency of the microcapsule products, since in the case, highest NE was observed for the No. 3 microcapsule (Table II). The differences in β-carotene stability at high RH might be associated with the structure stability of the microcapsules. In the SEM micrograph of the microencapsulated product with the SPI/MS mixture as the wall material, we can observe that although the morphology of the microencapsulated particles did not distinctly change upon the storage, there were some fractures or holes occurring in the particles [Figures 2(C) and 3(C)]. Thus, the much lower β-carotene retention for the No. 3 microcapsule (relative to the Nos. 1 and 2) can be to a certain extent attributed to the disruption of microcapsule structure after the storage.

For the microcapsules using the SPI/MS mixture as the wall materials, the β-carotene retention (15.9–19.1%) was not significantly affected by increasing the TC from 100 to 200 g kg^{-1} (at the same core/wall ratio of 1:1) (Table II, Nos. 3, 4, and 7). At a given TC (150 g kg^{-1}), we observe that the β-carotene retention was significantly increased by increasing the core/wall ratio to 2:1 as compared with that at core/wall ratios of 1:1 or 1:2 (Table II, Nos. 4–6), though the NE of the former microcapsule was much lower than the latter two. This is contrasting from the observation of Rocha et al.,[8] who reported that increasing the core concentration resulted in a reduction in lycopene retention for the spray-dried microcapsules using MS as the wall material. The higher stability of β-carotene at higher core/wall ratio (2:1; as compared with the 1:1 or 1:2 ratios) could be also attributed to the much less moisture contents of the microcapsules at higher core/wall ratios. At 2:1 core/wall ratio, much less amount of moisture was adsorbed and retained after the storage at 75% RH (18 vs. 27–41 g kg^{-1}; relative to the 1:1 and 1:2 ratios). Liang et al.[6] observed that at prolonged periods of storage (e.g., 30 days), the β-carotene retention progressively decreased with the RH (at which the spray-dried microcapsules were stored) increasing from 11 to 52%. This observation to a great extent supported the above explanation, confirming that the moisture content might produce a vital influence on the β-carotene stability.

Degradation Kinetics of β-carotene at Various Temperatures

It is well recognized that carotenoids are unstable against light, oxygen, moisture, and even temperature. The kinetics of degradation of β-carotene in all the microcapsules (placed in a desiccator) at ambient temperature (around 25°C), in the dark or in the presence of sunlight, were monitored by the change in β-carotene retention upon storage up to 25 days, as shown in Figure 4. As expected, increasing storage period resulted in a progressive loss of β-carotene in all the microcapsules, confirming instability of β-carotene. On the first hand, we can observe that for each test microcapsule, the pattern of β-carotene degradation in the dark was basically the same as that in the presence of sunlight (Figure 4), indicating high light stability of β-carotene in the microcapsules. On the other hand, it can be seen that the degradation pattern of β-carotene was largely related to the composition of wall materials, and variations in TC or core/wall ratio did not produce a remarkable influence on the β-carotene degradation (Figure 4).

Different fitting models have been applied to describe the degradation kinetics of carotenoids in spray-dried powder products encapsulated using various types of carbohydrates or proteins as the wall materials, including first-order kinetics of degradation,[5,8,11,23] first-order kinetics depending on two separate storage periods,[24] and a pseudo-first order kinetic.[6,9,25] In the present work, we see that the degradation of β-carotene in the microcapsules with SPI or MS alone as the wall materials basically followed the first-order kinetics, while that in the microcapsules with the SPI/MS mixtures as the wall materials could be more preferably described by the pseudo-first order kinetic model (Figure 4). The reason causing the differences in β-carotene degradation pattern is still unknown yet.

The No. 1 microcapsule with SPI alone as the wall material almost followed the same degradation of β-carotene as that of the No. 2 counterpart with MS alone [Figure 4(A,B), Nos. 1 and 2]. At the same TC and core/wall ratio, the 1:1 blending of SPI and MS greatly accelerated the β-carotene degradation, upon storage up to 15 days. For example, at the 15-day storage period, almost 90% of β-carotene in the No. 3 microcapsule was degraded, while it was only 65 to 70% for the other two counterparts [Figure 4(A,B), No. 3]. When the storage was prolonged above 15 days, the rate of β-carotene degradation remarkably decreased [Figure 4(A,B), No. 3]. A similar observation has been reported by Desobry et al.,[24] who attributed the first period rapid loss of β-carotene to the oxidation of surface carotenoids. If this explanation does work, the relative content of surface carotenoids should be higher in the No. 3 microcapsule than in the other two counterparts. In fact, the No. 3 microcapsule on the contrary showed higher NE (Table II), implying lower surface oil content. One of the reasonable explanations for this inconsistency is that the 1:1 blending of SPI and MS might decrease the matrix homogeneity of the microcapsules, thus causing enhanced susceptibility of encapsulated β-carotene to oxidation.

Among the microcapsules with the SPI/MS mixture as the wall materials, we can observe that increasing TC from 100 to 200 g kg^{-1} did not distinctly affect the β-carotene degradation,

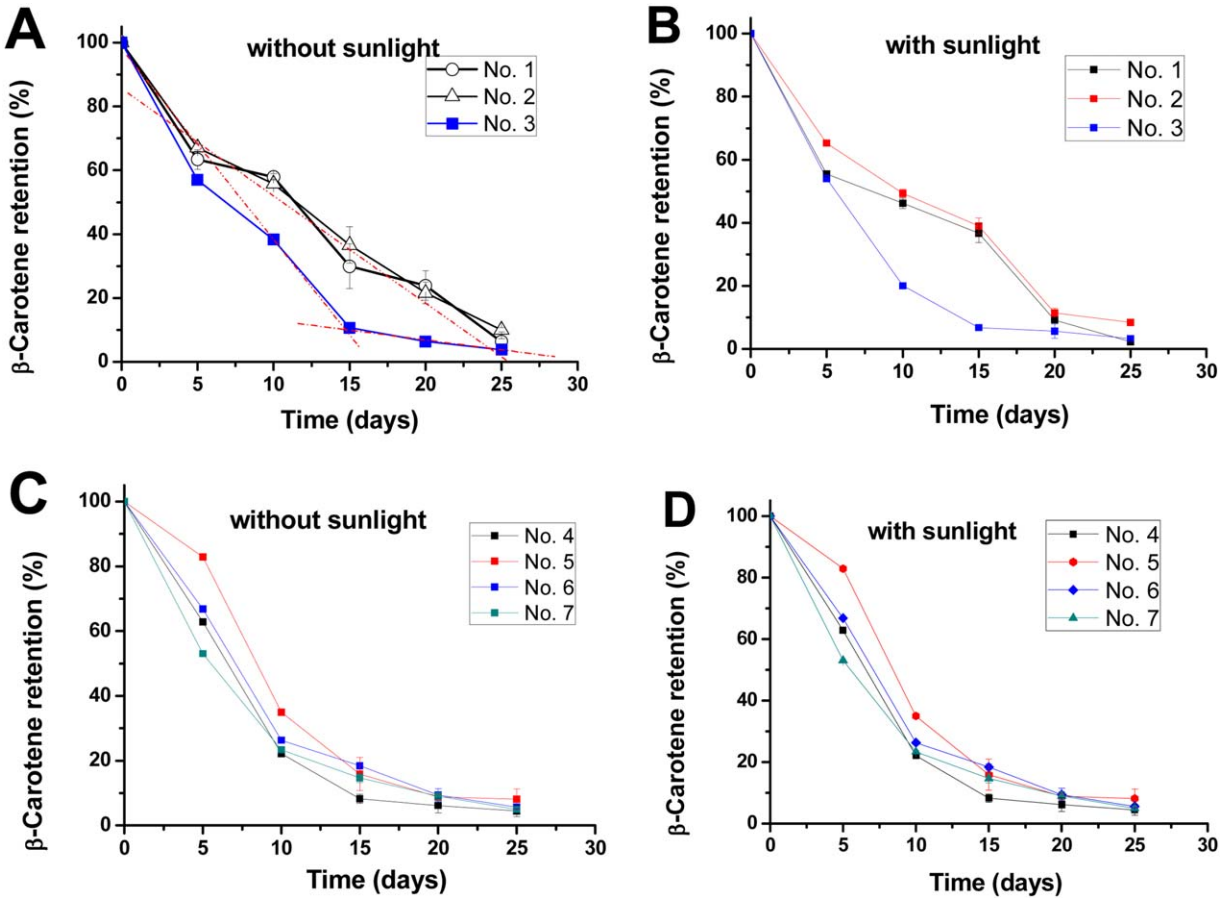

Figure 4. Changes in β-carotene retention for the microcapsules, without (A, C) or with (B, D) sunlight, upon storage at ambient temperature (~25°C) up to 25 days. The symbols (Nos. 1–7) for the samples are the same as in the Table II. Each datum is expressed as mean and standard deviation ($n = 3$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

but lowering the core/wall ratio from 2:1 to 1:2 considerably delayed the degradation, during the initial storage period up to 15 days [Figure 4(C,D)]. The slower β-carotene degradation at lower core/wall ratios for the No. 5 microcapsule (at the core/wall ratio of 1:2) might be largely attributed to its much lower surface β-carotene content (3.2%; relative to 8.1% at the 2:1 core/wall ratio). The importance of encapsulating materials or microcapsule matrix to the storage stability of carotenoids has been confirmed in many previous works.[9,23]

Many previous works[9,11,24,26] had well indicated that the rate of carotenoid degradation in microencapsulated powders could be greatly enhanced by increasing the storage temperature. Figure 5 shows the influence of storage at 60°C for a period of 1 or 2 weeks on the β-carotene retention of various microcapsules (Nos. 1–7). In general, we can see that the β-carotene degradation considerably varied with the type of test microcapsules, and even the time period of storage. At given core/wall ratio (1:1) and TC (100 g kg^{-1}), about 22% β-carotene was retained for the No. 2 microcapsule with MS alone as the wall material, after one-week storage at 60°C, followed by the No. 1 and No. 3 microcapsules (about 12.5% and 2%), respectively (Figure 5). When the storage period was prolonged up to 2 weeks, the retaining β-carotene for the No. 2 microcapsule was reduced to 3.0%, while in the other two microcapsules, almost all of β-carotene was degraded (Figure 5). Among the microcapsules with the MS/SPI mixture as the wall materials, we can observe that

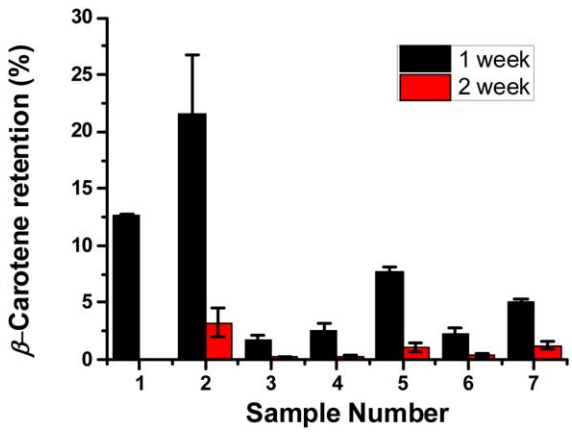

Figure 5. Retention of β-carotene in various spray-dried microcapsules, after storage at 60°C for 1 or 2 weeks. Each datum is the mean and standard deviation ($n = 3$). The sample Nos. 1–7 are the same as in table. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

increasing the TC (from 10 to 20%) or the ratio of wall materials could progressively improve the thermal stability of β-carotene, especially upon storage up to one week (Figure 5). The core/wall ratio seems to be a more important parameter affecting the thermal stability of β-carotene than the TC, since the β-carotene retention (7.8%) of the No. 5 microcapsule after 1-week storage was significantly higher than that of the No. 7 microcapsule (5.2%). The observations are basically consistent with the β-carotene degradation at ambient temperature, with or without sunlight [Figure 4(A,B)], furthering indicating that the β-carotene encapsulated with MS alone exhibited highest thermal stability, followed by that with SPI alone, while the thermal stability was least for the microcapsule with the MS/SPI mixture.

We also evaluated the β-carotene degradation in the microcapsules in a desiccator at 4°C, and found that more than 90% of β-carotene could be retained after a storage up to 35 days (Table II), suggesting that despite of the test core/wall ratio and TC, these microcapsules with SPI and/or MS alone or in combination as the wall materials exhibited extraordinary stability of their encapsulated β-carotene at low temperatures.

CONCLUSIONS

The encapsulation efficiency, dissolution and redispersion behavior, and storage stability of β-carotene in spray-dried microcapsules using SPI and/or MS as the wall materials were affected by the composition of wall materials, as well as many processing parameters, including TC and core/wall ratio. At comparable conditions, highest NE was observed for the microcapsule using the SPI and MS blend (1 : 1) as the wall material, while that with MS was least. The blending could improve the dissolution behavior, but best redispersion behavior was observed for the microcapsule with MS alone as the wall material. On the other hand, the storage stability of β-carotene encapsulated in these materials, at high RH or at ambient or higher temperatures was basically the reversal. Among the microcapsules with the blend as the wall materials, lowering the core/wall ratio improved the storage stability of encapsulated β-carotene (in dry state). Furthermore, the encapsulated β-carotene was not sensible to sunlight, and extraordinarily stable upon storage at low temperatures (e.g. at 4°C). These results are of vital importance for the development of β-carotene or other lipid-soluble bioactive containing microcapsules with SPI or MS as the wall materials.

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