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β-Carotene nanodispersions: preparation, characterization and stability evaluation

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Abstract

The aim of the present study was to investigate the preparation of β-carotene nanodispersions as potential active ingredients for food formulations. Nanodispersions containing β-carotene were obtained by a process based on an emulsification–evaporation technique. The preparation method consisted of emulsifying an organic solution of β-carotene in an aqueous solution containing emulsifier using two different homogenizers (a conventional homogenizer and a microfluidizer), followed by direct solvent evaporation under reduced pressure. The influence of different homogenizing conditions (pressure and cycle) and two organic/aqueous phase ratios on particle size parameters and content of β-carotene was investigated. In addition, the stability of β-carotene nanodispersions was carried out at a storage temperature of 4 °C. The particle size distribution of β-carotene in nanodispersions was demonstrated with a laser diffraction particle size analyzer and the retention of β-carotene in the prepared nanodispersions was studied by high-pressure liquid chromatography. In general, homogenization pressure and cycle had significant ($P < 0.05$) effects on various particle size parameters. A volume-weighted mean diameter ($D_{4,3}$) of β-carotene nanoparticles, ranging from 60 to 140 nm, was observed in this study.

Keywords: Emulsification-evaporation; β-carotene; Nanodispersion; High-pressure homogenization; Particle size analysis

1. Introduction

Functional lipids, such as carotenoids, phytosterols, ω-3 fatty acids, natural antioxidants, and numerous other compounds, are widely used as active ingredients in various food products. However, the poor water solubility of functional lipids has made their use problematic for food formulations. Most of the functional lipids are almost insoluble in water or show a very low water solubility. The solubility of functional lipids in food formulations is a major consideration for the food industry. Moreover, functional lipids with a low water solubility may be prone to reduced bioavailability. For these reasons, it is important to find solutions to this problem. Nanotechnology provides a good opportunity to improve the solubility of such active ingredients and to increase bioavailability. Today, nanotechnology is one of the new frontiers in the field of food science and it offers one of the most exciting prospects for technological innovation (Moraru et al., 2003).

Nanotechnology has been explored in the past decade, primarily as the result of the development of new tools that have made the characterization of nano-size materials practical, and also as a result of various new methods for preparation of these materials. In the field of pharmaceuticals and medicine, drug particles in the nanometer range show a substantial increase of solubility in water, which should lead to improved bioavailability (Grau,

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Kayser, & Muller, 2000; Muller, Beker, Kruss, & Peters, 1999; Trotta, Gallarate, Pattarino, & Morel, 2001). Therefore, considerable work has been devoted, in the past decade, to preparing drug nanoparticles of narrow size distribution. Emulsification-evaporation, a process of emulsification followed by solvent evaporation, is the most widely used techniques for preparing nanoparticles containing drugs (Kwon, Lee, Choi, Jang, & Kim, 2001). High-pressure homogenization is extensively used in the food, pharmaceutical and biotechnology industries to emulsify, disperse, mix and process various products (Floury, Desrumaux, & Lardières, 2000; Floury, Desrumaux, Axelos, & Legrand, 2002). Microfluidization technology, a type of high-pressure homogenization, is currently used by the pharmaceutical and cosmetic industries to produce fine emulsions. O'Donnell & McGinity (1997) and Couvreur, Blanco-Prieto, Puisieux, Roques, & Fattal (1997) reviewed the preparation and characterization of many of the different types of emulsification–evaporation microspheres based on biodegradable polymers and copolymers of hydroxy acids containing small peptides.

Recently, Moraru et al. (2003) reported that the development of new functional materials is one of the major areas in food industry that will probably be significantly enhanced by the development of nanotechnology. Carotenoids are one of the most important group of natural pigments, because of their wide distribution in plant tissues, structural diversity and numerous functions. In addition to the provitamin A activity of some carotenoids, these pigments have recently been implicated in the prevention of, or protection against, serious human health disorders such as cancer, heart disease, macular degeneration and cataracts (Che Man & Tan, 2003). Among carotenoid pigments, β-carotene provides the highest vitamin A activity.

Today, scientists are just beginning to try to understand the physicochemical properties of small-size materials (a leap from micro to nano). For example, variations of the particle size within the colloidal range may affect some of the physicochemical properties of nanoparticles. It has been shown earlier that the behaviour of colloidally dispersed triacylglycerols differs significantly from that of their bulk phases, e.g., in terms of melting and recystallization temperatures and time course of polymorphic transitions (Bunjes, Koch, & Westesen, 2000; Bunjes, Westesen, & Koch, 1996; Higami, Ueno, Segawa, Iwanami, & Sato, 2003; Siekmann & Westesen, 1994; Westesen, Siekmann, & Koch, 1993).

Hitherto, however, no information is available concerning the physicochemical properties of β-carotene nanodispersions prepared by the emulsification-evaporation technique. This point is of particular interest, since changes in physicochemical properties of β-carotene nanodispersions may affect the use of this active ingredient in food formulations. Moreover, the physicochemical state of β-carotene is crucial for the in vivo and in vitro performance of the preparation. Therefore, the purpose of this study was to apply the emulsification-evaporation technique for preparing β-carotene nanodispersions, as well as to study the physicochemical properties and storage stability of the prepared β-carotene nanodispersions. We were interested in potential changes of the nanodispersion properties, e.g., regarding particle size and distribution as well as the chemical stability of prepared dispersions during storage, due to the various processing parameters. The effect of processing conditions on the mean particle size of nanodispersions was determined, to design suitable process conditions for preparing β-carotene nanodispersions of less than 100 nm in size.

2. Materials and methods

2.1. Materials

β-Carotene, polyoxyethylene sorbitan mono-laurate (Tween 20), HPLC grade acetonitrile and ethanol, together with analytical grade hexane, were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Standard β-carotene (>97% purity) was supplied by EMD Biosciences, Inc. (San Diego, USA). Distilled water was of Milli-Q quality (Milipore, Bedford, USA). All other chemicals used were of analytical grade. For HPLC analysis, an independent β-carotene standard stock solution, containing β-carotene at 1 g/l in cyclohexane, was prepared and stored at 4 °C in an amber glass bottle. Intermediate and standard solutions were prepared by appropriate dilutions of the existing stock solution.

2.2. Preparation of β-carotene nanodispersions

Hexane was used as the organic phase to prepare oil-in-water emulsions. β-Carotene (0.3% w/w) was dissolved and dispersed in hexane (40 °C). The organic solution was then poured into the aqueous phase containing 0.5% (w/w) Tween 20 in Milli-Q water. The pre-mix was homogenized using a conventional homogenizer (Polytron® PT3000, Kinematica AG, Lucerne, Switzerland) at 5000 rpm for 5 min to produce coarse oil-in-water emulsion, followed by a high-pressure homogenization (Model M-110EHi Microfluidizer Processor, Microfluidics™ Corporation Newton, USA). To improve the efficiency of fine emulsification, the coarse emulsions were immediately passed through the microfluidizer. Hexane was then removed from the fine emulsion by rotary evaporation (Eyela rotary evaporator NE-1101, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) under reduced pressure (0.25 bar and 40 °C). Several batches were prepared, using the above-mentioned tech-

nique, by varying the homogenization pressure (60–140 MPa) and cycle (one to three cycles) and organic/aqueous phase volume ratio (1:9 and 2:8). Each dispersion sample was carefully sampled and immediately analyzed for particle size distributions and β-carotene concentrations, and then stored in screw-capped amber bottles at 4 °C for microscopic observations (within 24 h) and for further storage evaluations (sampling at 4 week intervals to a period of 12 weeks).

2.3. Characterization of β-carotene nanodispersions

2.3.1. Particle size analysis

The mean particle diameter and size distribution of β-carotene nanodispersions were measured, using a laser diffraction particle size analyzer (LS 13320, Beckman Coulter Ltd., Florida, US). The experiments were carried out on the undiluted suspensions after preparation. All measurements were repeated twice for all batches prepared. The particle size of the prepared β-carotene nanodispersions was described by the volume-weighted mean diameter ($D_{4,3}$) and surface-weighted mean diameter ($D_{3,2}$). The polydispersity of the particles was expressed by the span. Span = $[d_{v,90} - d_{v,10}]/d_{v,50}$, where $d_{v,90}$, $d_{v,10}$ and $d_{v,50}$ are the equivalent volumes diameters at 90%, 10% and 50% cumulative volume, respectively. Span is one of many possible measures of relative width for a unimodal distribution.

2.3.2. Scanning electron microscopy analysis

The nanodispersions were also determined by scanning electron microscopy (SEM). An aqueous dispersion of nanoparticles was finely spread over a slab and dried under a nitrogen stream. The sample was then coated in a quick auto coater (JFC-1500, JEOL, Tokyo, Japan) with a gold layer in vacuum condition. The electron microscopic photographs were taken using a JSM-5600LV scanning electron microscope (JEOL, Tokyo, Japan).

2.3.3. Sample preparation for β-carotene determination

The sample preparation procedures were modified from Iwase's work (2002). The reverse phase solid-phase extraction C18 cartridges (Alltech Associates, Inc., Deerfield, US) were conditioned by washing with 3 ml of methanol and then with 5 ml of Milli Q water prior to use. In a brown volumetric flask, 2.5 g of sample was dissolved in 5% (w/v) aqueous sodium sulfate solution containing 1 mM ethylenediaminetetraacetic acid disodium dihydrate (EDTA). This solution (1 ml) was applied to the conditioned C18 cartridge. The cartridge was washed with deionized water (10 ml) and then 10% aqueous ethanol (5 ml), followed by elution with acetonitrile–ethanol (7:3, v/v; 10 ml). This final elution fraction was collected and used for the determination of β-carotene.

2.3.4. Determination of β-carotene content

β-carotene was assayed by HPLC, using a UV–Vis detector. HPLC separation was performed with a JASCO liquid chromatograph system (JASCO International Co., Ltd., Tokyo, Japan), equipped with a UV-1575 UV–Vis spectrophotometric detector, a PU-1580 pump system, a CO-1560 column thermostat, and a AS-950 autosampler. Quantitative measurement of β-carotene was done at 450 nm. The β-carotene was separated on a 4.6×250 mm 5 μm silica gel column (Daisopak SP-120-5-SIL-P; Daiso Co., Ltd., Osaka, Japan) with a mobile phase of aceotonitrile–ethanol (7:3, v/v) at 1.6 ml/min according to an established method (Iwase, 2002). A 30-μl aliquot of sample was injected. The calibration of peak area versus β-carotene concentration was linear in the concentration range of 0.10–8.00 mg/l ($R^2 = 0.9982$, $n = 8$). Injections, in duplicate, were done at each concentration for standards and samples. All results were expressed in mg/l.

2.3.5. Statistical analysis

All experiments and/or measurements were duplicated. Data were statistically analyzed by a one-way analysis of variance procedure, using SAS (SAS, 1989) software package. Significant differences ($P < 0.05$) between means were further determined by Duncan's multiple range test.

3. Results and discussion

3.1. General

In this study, aqueous dispersions of β-carotene nanoparticles were prepared by an emulsification-evaporation technique, which has been widely utilized for the production of drug particles. Owing to the insolubility of β-carotene in water, the disperse phase, consisting of β-carotene, was first dissolved in hexane, while the continuous phase was emulsifier in Milli-Q water. In order to reach submicron size, we used a microfluidizer as a homogenization device, with a view to obtaining nanodispersions with a very high grade of monodispersity. It is well known that high-pressure homogenization allows reduction of the droplet size of emulsions produced and improves shelf life of the products by reducing creaming rate (Paquin, 1999). In addition to the reduction of the mean droplet diameter of emulsions, high-pressure homogenization can also deflocculate clusters of primary fat globules and disperse agglomerates uniformly. The hexane is then removed under reduced pressure. The formation of β-carotene particles happens as hexane diffuses into the aqueous phase and evaporates at the water/air interface. Due to the high interfacial tension between the organic and aqueous phases, an emulsifier, such as Tween 20, is indispensable for preventing

coalescence of the formed β-carotene particles. A number of variables affect the solvent droplet size of the emulsions and probably the properties of the resulting β-carotene particles; these include phase ratio, type and concentrations of emulsifiers, mixing technique, and other technological conditions of manufacturing. In the present study, we fixed the type and concentration of emulsifier, while the influence of phase ratios and homogenization conditions were investigated.

3.2. Effect of homogenization parameters and organic/aqueous phase ratio on the physicochemical properties of β-carotene nanodispersions

Table 1 shows the particle size parameters of β-carotene nanodispersions determined by the laser diffraction technique for two different phase ratios (organic:aqueous), namely 1:9 and 2:8 ratios. The particle size distributions were unimodal and typically extended from 40 to 260 nm. The volume-weighted mean diameters ($D_{4,3}$) of the dispersions were between 60 and 135 nm, with span values being approximately in the range of 0.4–0.7 (Table 1). For both organic/aqueous ratios, mean particle diameters ($D_{4,3}$ and $D_{3,2}$) significantly ($P < 0.05$) decreased when the homogenizing pressure increased. These results show that,under the conditions of the present study, the intensity of the high shear forces, and the turbulence and/or cavitation produced during the homogenization process determine particle size. As the pressure of the homogenizer is increased, the size of the dispersed droplets decreases as a result of the various forces induced in the microfluidizer. At the same homogenization pressure, the mean particle diameter increased with increase in the organic phase ratio (Table 1). Part of the effect may be due to the limitation on emulsifier in the prepared organic-in-water emulsions due to the strong increase of the interfacial area created by the homogenizing process. As the organic volume increases, the emulsifier available decreases, limiting the stabilizing benefits of the formed droplets, which favours organic droplet coalescence and thus increases the mean droplet diameters. In addition, this observation might be also attributed to a reduction of the shear stress during the homogenization process, resulting from higher viscosity of the organic phase and consequently a less favourable mixing efficiency and larger emulsion droplets. The $d_{v,10}$, $d_{v,50}$ and $d_{v,90}$ values indicate that 10%, 50% and 90% of the particles measured were less than or equal to the size stated. Increasing the homogenization pressure caused a decrease in these three parameters, whilst increasing the ratio of organic to aqueous phase tended to increase these values.

The span index measures the width of the particle size distribution, as described in the British Standard (1993). Hence, a small span value indicates a narrow particle size distribution. Both ratios show a narrow particle size distribution (span index <0.7). For a 1:9 ratio, increasing the homogenization pressure also caused a significant ($P < 0.05$) decrease in span value. For example, the sample prepared from 60 MPa was the broadest; the sample from 140 MPa was the narrowest. However, this phenomenon was not observed in samples prepared with a 2:8 ratio. The cause of this observation could not be exactly clarified. Tentatively, the differences might be due to the organic/aqueous phase variations.

In general, a high homogenization pressure ensures a good emulsification process and therefore leads to smaller particles with a satisfactory span value (lower than 0.6). For example, by using the organic/aqueous ratio of 1:9, the 90th percentile diameter by volume ($d_{v,90}$) for samples prepared a with homogenization pressure equal to or higher than 100 MPa indicated that 90% of all the particles had a diameter of less than 100 nm.

The influence of homogenization cycle on the particle size of nanodispersions was also studied. The results are shown in Table 2. As expected, the mean particle diameters decreased with increase of the number of homogenization cycles applied at 140 MPa. Results accord with previous findings with several high-pressure homogeniz-

Table 1
Characteristics of particle size of β-carotene nanodispersions prepared with different homogenizing pressures and two different ratios of mixtures[A]

Ratio (organic:aqueous)	Pressure (MPa)	Volume-weighted mean diameter, $D_{4,3}$ (nm)	Surface-weighted mean diameter, $D_{3,2}$ (nm)	Diameter at 10%, $d_{v,10}$ (nm)	Diameter at 50%, $d_{v,50}$ (nm)	Diameter at 90%, $d_{v,90}$ (nm)	Span
1:9	60	89.3 ± 0.6^{a}	83.5 ± 0.3^{a}	60.6 ± 0.5^{a}	86.7 ± 0.6^{a}	121.5 ± 0.6^{a}	0.702^{a}
	80	82.7 ± 0.6^{b}	77.7 ± 0.7^{b}	57.5 ± 0.4^{b}	80.2 ± 0.5^{b}	111.5 ± 0.6^{b}	0.673^{b}
	100	75.7 ± 0.3^{c}	72.0 ± 0.2^{c}	54.6 ± 0.2^{c}	74.2 ± 0.2^{c}	99.5 ± 0.2^{c}	0.605^{c}
	120	70.9 ± 0.2^{d}	67.8 ± 0.3^{d}	52.8 ± 0.3^{d}	69.7 ± 0.3^{d}	90.5 ± 0.3^{d}	0.541^{d}
	140	62.7 ± 0.2^{e}	60.7 ± 0.2^{e}	48.6 ± 0.1^{e}	62.1 ± 0.2^{e}	77.1 ± 0.5^{e}	0.459^{e}
2:8	60	134.0 ± 2.3^{a}	130.0 ± 2.3^{a}	106.0 ± 2.0^{a}	132.0 ± 1.9^{a}	163.5 ± 1.7^{a}	0.436^{d}
	80	118.0 ± 1.2^{b}	113.0 ± 1.2^{b}	88.4 ± 0.9^{b}	116.0 ± 1.2^{b}	149.0 ± 1.0^{b}	0.522^{b}
	100	109.5 ± 0.6^{c}	105.5 ± 0.6^{c}	82.9 ± 0.4^{c}	107.5 ± 0.5^{c}	138.5 ± 0.6^{c}	0.517^{b}
	120	102.0 ± 1.2^{d}	98.6 ± 0.9^{d}	78.1 ± 0.6^{d}	101.5 ± 0.6^{d}	129.0 ± 1.2^{d}	0.501^{c}
	140	92.7 ± 0.3^{e}	88.6 ± 0.5^{e}	68.7 ± 0.5^{e}	91.4 ± 0.5^{e}	118.0 ± 1.2^{e}	0.539^{a}

[A] Each value in the table represents the mean ± SD of four measurements from two replications. For each ratio, means within each column with different superscripts are significantly different ($P < 0.05$). Span = $[d_{v,90} - d_{v,10}]/d_{v,50}$.

Table 2
Characteristics of particle size of β-carotene nanodispersions prepared with different homogenizing cycles and two different ratios of mixtures (at 140 MPa)[A]

Ratio (organic:aqueous)	Number of cycle	Volume-weighted mean diameter, $D_{4,3}$ (nm)	Surface-weighted mean diameter, $D_{3,2}$ (nm)	Diameter at 10%, d_{10} (nm)	Diameter at 50%, d_{50} (nm)	Diameter at 90%, d_{90} (nm)	Span
1:9	1	62.7 ± 0.2^{a}	60.7 ± 0.2^{a}	48.6 ± 0.1^{a}	62.1 ± 0.2^{a}	77.1 ± 0.5^{a}	0.459^{a}
	2	58.8 ± 1.0^{b}	57.3 ± 0.9^{b}	46.3 ± 0.6^{b}	58.3 ± 0.9^{b}	71.8 ± 1.8^{b}	0.437^{b}
	3	55.7 ± 0.1^{c}	54.4 ± 0.1^{c}	44.6 ± 0.1^{c}	55.2 ± 0.1^{c}	67.6 ± 0.1^{c}	0.417^{c}
2:8	1	92.7 ± 0.3^{a}	88.6 ± 0.5^{a}	68.7 ± 0.5^{a}	91.4 ± 0.5^{a}	118.0 ± 1.2^{a}	0.539^{b}
	2	82.8 ± 0.5^{b}	78.6 ± 0.4^{b}	56.0 ± 0.6^{b}	81.6 ± 0.6^{b}	109.0 ± 1.2^{b}	0.650^{a}
	3	77.7 ± 0.2^{c}	73.5 ± 0.2^{c}	55.2 ± 0.2^{c}	75.8 ± 0.2^{c}	102.5 ± 0.6^{c}	0.624^{a}

[A] Each value in the table represents the mean ± SD of four measurements from two replications. For each ratio, means within each column with different superscripts are significantly different ($P < 0.05$). Span = $[d_{v,90} - d_{v,10}]/d_{v,50}$.

ers (Trotta et al., 2001). It was also observed that the span values were obviously reduced with the increased number of cycles, as demonstrated by the laser diffraction particle size analyzer, for samples prepared from a 1:9 ratio (organic/aqueous phase). However, the recycling process of homogenzation did not affect the span values of 2:8 ratio samples in the same way. The span values, for both two and three homogenization cycles were significantly higher than those with only one cycle (for 2:8 ratio).

Figs. 1 and 2 indicate the size distribution of the β-carotene nanodispersions after the emulsification-evaporation process for 1:9 and 2:8 ratios, respectively. It has been shown that the emulsification-evaporation technique can be used to produce β-carotene dispersions with particles in the nanometer range (40–260 nm). These results indicate that, by varying the production parameters, a desired particle size distribution can be produced in a controlled way. As the homogenization pressure increased, the particle size distribution shifted indeed to smaller particles (Figs. 1 and 2) and distribution pattern narrowed (Fig. 1). Fig. 3 shows the relationship between homogenization pressure and $D_{4,3}$ for the β-carotene nanodispersions prepared at two organic/aqueous phase ratios. The fitting by simple regression of homogenization pressure against $D_{4,3}$ showed a good correlation coefficient ($R^2 > 0.97$) for both organic/aqueous ratios (Fig. 3).

SEM images of a coarse β-carotene crystalline and a dried suspension of the β-carotene nanodispersion are presented in Fig. 4. The results of the microscopic analysis supported the particle sizing results. A representative picture of the β-carotene particles prepared from the 1:9 ratio (organic/aqueous phase) is presented in Fig. 4(b). The dried dispersions, contained particles of a much smaller size than the coarse β-carotene crystalline sample (Fig. 4(a)). The absence of larger particles means the homogenization and evaporation processes gave significantly smaller mean particle sizes.

In addition to particle size analysis, the concentration of prepared nanodispersions of the β-carotene was investigated. Tables 3 and 4 present the data on the loss

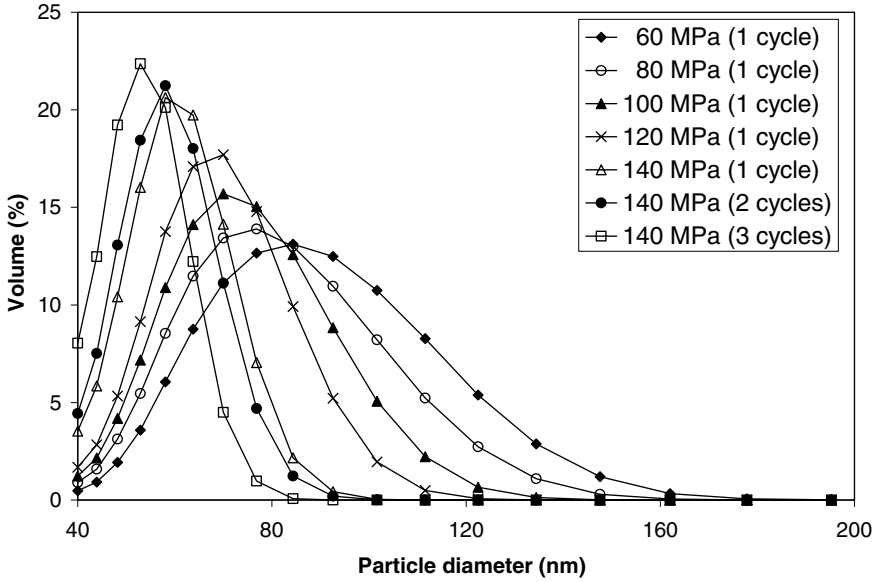

Fig. 1. Particle size distribution of β-carotene nanodispersions prepared with different homogenization pressures and cycles (for organic/aqueous phase ratio of 1:9).

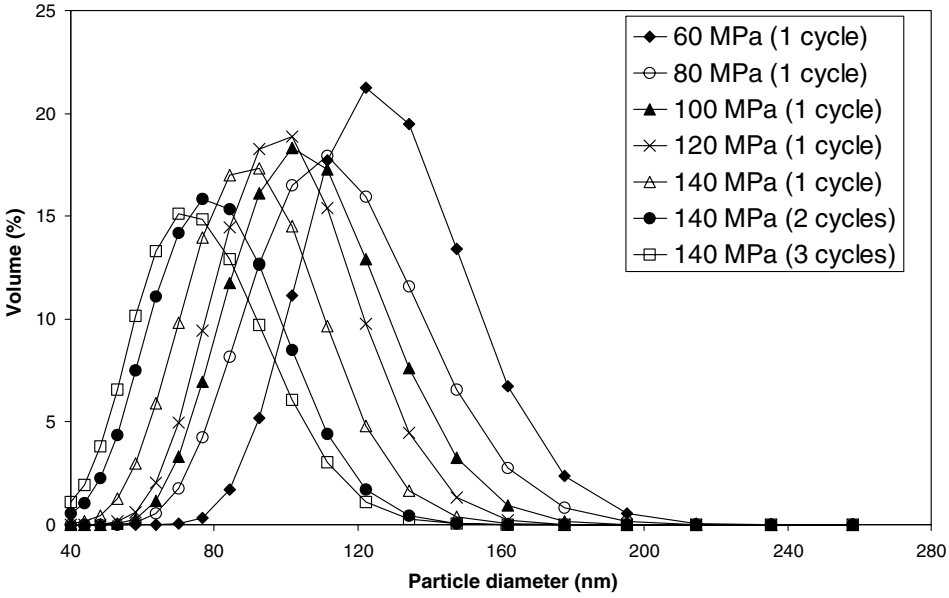

Fig. 2. Particle size distribution of β-carotene nanodispersions prepared with different homogenization pressures and cycles (for organic/aqueous phase ratio of 2:8).

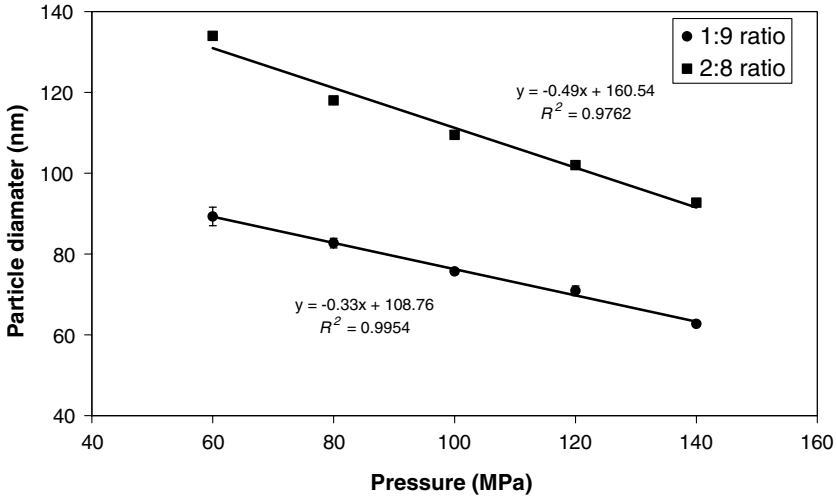

Fig. 3. Linear correlations between the homogenization pressure and volume-weighted mean diameter, determined by laser diffraction particle size analyzer, for two different organic/aqueous phase ratios. Regression analysis was carried out considering all conditions. The data ($n = 5$). Error bar express SD ($n = 4$).

of β-carotene during preparation steps for 1:9 and 2:8 ratios, respectively. The content of β-carotene of a freshly prepared coarse emulsion (by Polytron® PT3000) was approximately 278 mg/l. Since β-carotene loss during the preparation should be kept to a minimum during emulsification steps, the stability of the emulsion is crucial. In general, a loss of 1–8% of β-carotene from the initial added amount was observed in all samples after both the emulsification and evaporation processes (Tables 3 and 4). For both organic/aqueous phase ratios, the losses were significantly ($P < 0.05$) higher with an increase in the number of homogenizing cycles. It is well known that β-carotene is sensitive to light, oxygen and heat (Che Man & Tan, 2003). In a dynamic high-pressure system, such as in the microfluidizer, temperature rise in the reaction chamber is expected. Therefore, a loss in the content of β-carotene in the sample, after high-pressure homogenization, is predictable. However, the role of heating on the samples produced by high-pressure homogenization is uncertain, although the heating is of short duration in the homogenizing chamber. This phenomenon is different from the deliberate application of heat in the food industry to heat food samples over longer times. The presence of

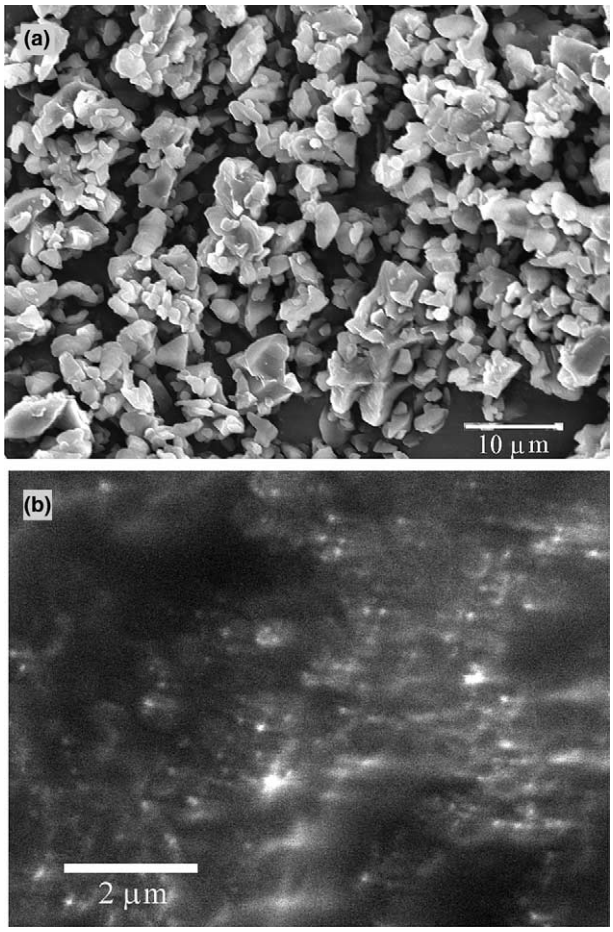

Fig. 4. Scanning electron micrograph of: (a) coarse crystalline β-carotene and (b) dried β-carotene nanodispersion sample prepared by the emulsification–evaporation technique (140 MPa, one cycle) using 1:9 ratio (organic/aqueous phase).

heat, light and oxygen also contributed to the losses of β-carotene during the evaporation process.

3.3. Stability evaluation of prepared β-carotene nanodispersions during storage

A higher specific surface area of an active ingredient generally leads to a higher dissolution rate and, as a consequence, to higher bioavailability. However, particles in the micrometer and nanometer range need, in many cases, to be chemically or physically stabilized. For this reason, it is important to consider stability problems. In the present study, the influence of particle size on particle distribution is far less pronounced than that on chemical stability of β-carotene. In general, micro- or nanoemulsions are thermodynamically stable (Ruckenstein, 1999). Mei, Che, Weng, Yang, & Yang (2003) noted that microemulsion with a droplet size <100 nm does not have a tendency to coalesce. Figs. 5 and 6 show the influence of storage on the particle size distribution of β-carotene nanodispersions for 1:9 (80 MPa, one cycle) and 2:8 (60 MPa, one cycle) ratios, respectively. In general, the analysis of the nanodispersion stability, in terms of size distribution by particle size analyzer for up to 12 weeks at 4 °C showed no distinct changes in particle size distribution for the two ratios with any of the preparation parameters.

However, the content of β-carotene was significantly reduced ($P < 0.05$) during the storage study. Figs. 7 and 8 show the degradation profile of β-carotene as a function of storage time at 4 °C. The results show that the mean particle diameter of β-carotene has a significant ($P < 0.05$) influence on the stability of β-carotene in nanodispersions. The degree of degradation of β-carotene in the nanodispersions was found to increase with an decrease in mean particle diameter. After 12 weeks of storage, a range of 25–44% and 32–56% of residual intact β-carotene was detected for samples prepared from 1:9 and 2:8 ratios, respectively (Table 5). In another study, Yoshida, Sekine, Matsuzaki, Yanaki, & Yamaguchi (1999) reported the stability of retinol (vitamin A alcohol) in multiple emulsions. The authors found that the remaining percentage of vitamin A in an oil-in-water emulsion, after 4 weeks of storage at 50 °C, was 32.3%.

There are several possible explanations for the degradation of β-carotene in the nanodispersion samples during storage. Among them, the influence of surface area is relevant to the present study. As compared to bulk crystalline β-carotene, the surface area of β-carotene

Table 3
Changes in β-carotene concentration during preparation steps (for organic/aqueous phase ratio of 1:9)[A]

Homogenization pressure (MPa)	Number of cycles	After microfluidizer (mg/l)		After evaporation (mg/l)	
		Concentration[B]	Loss (%)	Concentration[B]	Loss (%)
60	1	269 ± 0.5[a]	3.15	260 ± 5.6[a,b]	3.14
80	1	269 ± 7.9[a]	2.90	257 ± 6.1[a,b]	4.77
100	1	271 ± 3.0[a]	2.54	262 ± 7.7[a]	3.21
120	1	274 ± 5.7[a]	1.28	262 ± 0.1[a]	4.51
140	1	273 ± 3.3[a]	1.54	264 ± 6.9[a]	3.34
140	2	266 ± 6.9[a,b]	4.19	254 ± 7.1[a,b]	4.38
140	3	261 ± 4.9[b]	6.10	251 ± 8.3[b]	3.83

[A] Concentration of β-carotene after 1st stage homogenization with Polytron® PT3000 was 278 ± 28.0 mg/l.

[B] Each value in the column represents the mean ± SD of four analyses from two replications. Means within the column with different superscripts are significantly ($P < 0.05$) different.

Table 4
Changes in β-carotene concentration during preparation steps (for organic/aqueous phase ratio of 2:8)[A]

Homogenization pressure (MPa)	Number of cycles	After microfluidizer (mg/l)		After evaporation (mg/l)	
		Concentration[B]	Loss (%)	Concentration[B]	Loss (%)
60	1	563 ± 5.5[a]	3.20	537 ± 4.1[a,b]	4.64
80	1	565 ± 3.2[a]	2.74	544 ± 7.7[a]	3.69
100	1	565 ± 3.8[a]	2.85	545 ± 11.0[a]	3.43
120	1	566 ± 1.7[a]	2.64	545 ± 10.5[a]	3.63
140	1	568 ± 2.9[a]	2.24	542 ± 10.5[a]	4.49
140	2	548 ± 4.7[b]	5.77	526 ± 3.3[b,c]	4.00
140	3	538 ± 4.9[c]	7.36	520 ± 8.4[c]	3.48

[A] Concentration of β-carotene after 1st stage homogenization with Polytron® PT3000 was 581 ± 63.3 mg/l.
[B] Each value in the column represents the mean ± SD of four analyses from two replications. Means within the column with different superscripts are significantly ($P < 0.05$) different.

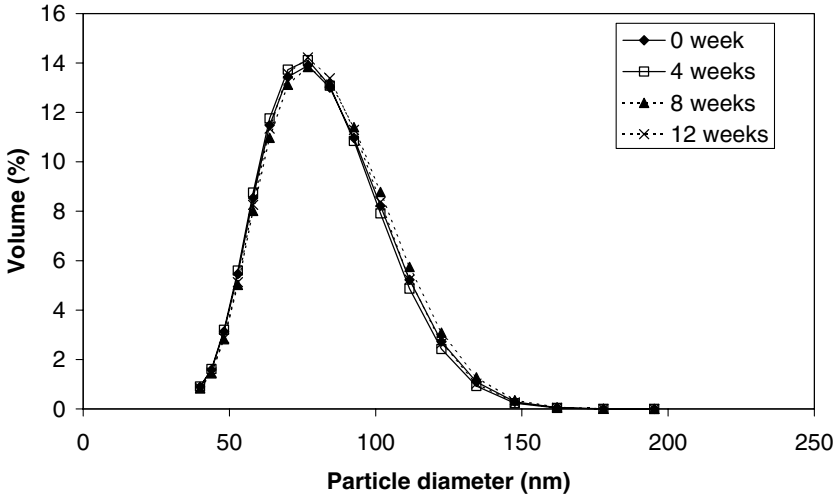

Fig. 5. Changes in particle size distribution for β-carotene nanodispersions prepared using a homogenization pressure of 80 MPa for one cycle during storage at 4 °C (for organic/aqueous phase ratio of 1:9).

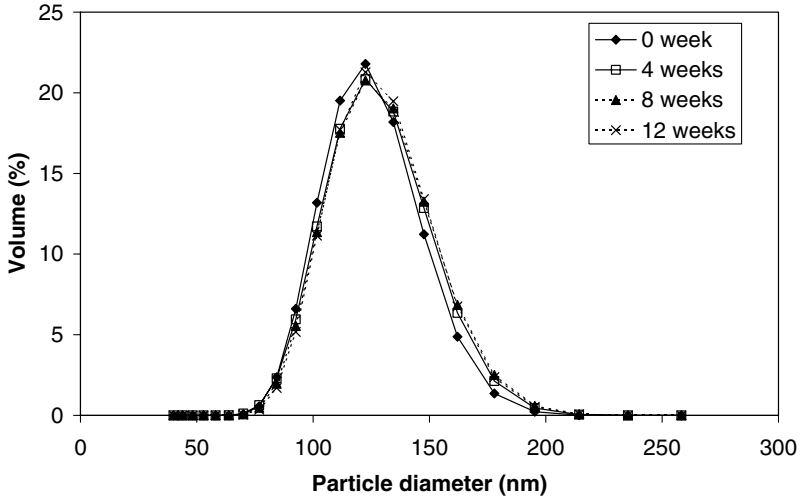

Fig. 6. Changes in particle size distribution for β-carotene nanodispersions prepared using a homogenization pressure of 60 MPa for one cycle during storage at 4 °C (for organic/aqueous phase ratio of 2:8).

in the nanometer range is significantly larger. This may significantly reduce the stability β-carotene nanodispersions by providing more contact surface between β-carotene particles and the aqueous environment. In addition, the occurrence of cavitation within the homogenizer has been demonstrated by the detection

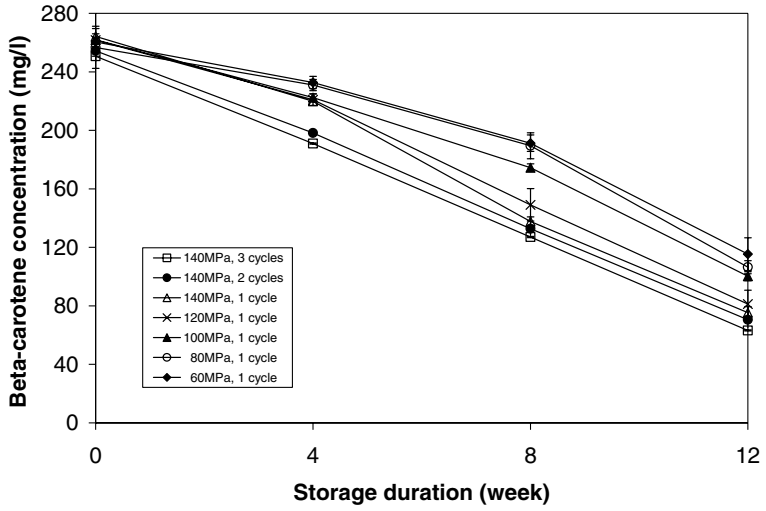

Fig. 7. Changes in β-carotene content for β-carotene nanodispersions prepared using various homogenization conditions during storage at 4 °C (for organic/aqueous phase ratio of 1:9).

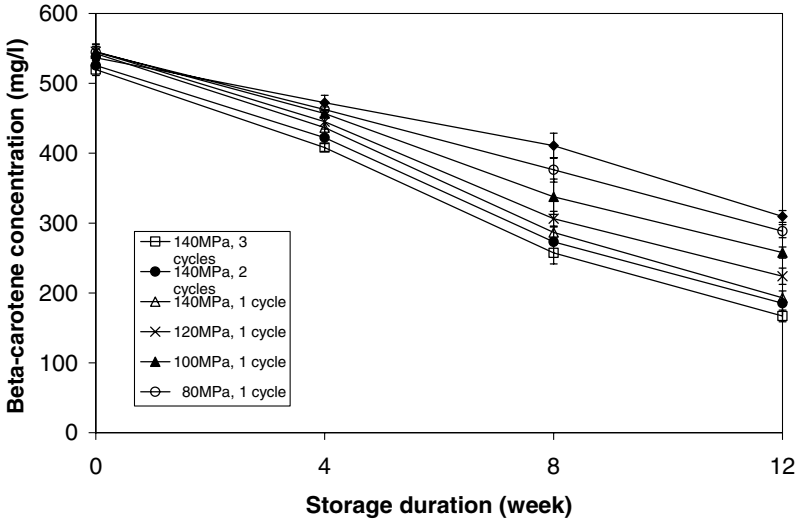

Fig. 8. Changes in β-carotene content for β-carotene nanodispersions prepared using various homogenization conditions during storage at 4 °C (for organic/aqueous phase ratio of 2:8).

Table 5
Retention of β-carotene after 12 weeks of storage at 4 °C

Homogenization pressure (MPa)	Number of cycles	1:9 ratio		2:8 Ratio	
		Concentration (mg/l)[A]	Retention (%)	Concentration (mg/l)[A]	Retention (%)
60	1	115 ± 11.2[a]	44.3	310 ± 8.5[a]	56.0
80	1	106 ± 4.5[a,b]	41.5	288 ± 9.4[b]	53.0
100	1	100 ± 3.1[b]	38.3	258 ± 8.0[c]	47.3
120	1	81.2 ± 9.5[c]	31.0	224 ± 11.7[d]	41.1
140	1	75.4 ± 4.1[c,d]	28.5	193 ± 9.9[e]	35.6
140	2	70.5 ± 5.4[d,e]	27.7	185 ± 16.6[e]	35.2
140	3	63.2 ± 0.4[e]	25.2	167 ± 8.3[f]	32.1

[A] Each value in the column represents the mean ± SD of four analyses from two replications. Means within the column with different superscripts are significantly ($P < 0.05$) different.

of free radicals (Lander, Manger, Scouloudis, Ku, & Lee (2000)). Therefore, it is also possible that the free radical generated during the homogenization process could trigger a loss of β-carotene in the prepared nanodispersions. High shear stress has been assumed to be the major cause, and evidence exits of free radical

formation in a polysaccharide medium (Lander et al., 2000). The free radical formation begins in a pressure range of 11.03–34.47 MPa (1600–5000 psi). Moreover, it was also found that high-pressure homogenization causes degradation of DNA and albumin (Mehnert & Mäder, 2001). However, according to Mehnert & Mäder (2001), high-pressure homogenization-induced degradation is not a serious problem for the majority of active ingredients.

4. Conclusion

In general, we have demonstrated that it is possible to prepare β-carotene nanodisperisons by using the emulsification–evaporation technique. Significant ($P < 0.05$) difference was observed between particle size parameters and homogenization pressure/cycles, suggesting that the particle formation is affected by homogenization conditions. An increase of the homogenization pressure/cycles may give finer and more homogeneous emulsions after the evaporation process. Particle size analysis also clearly illustrated the differences between the two different ratios used for the preparation of β-carotene nanodispersions. An increase in the level of organic phase in the final volume led to a shift in the size distribution to larger diameter range.

It was demonstrated that physical stability of β-carotene nanodispersions was superior during storage. However, β-carotene nanodispersions are chemically unstable during storage. This study revealed that β-carotene degradation is very dependent on the mean particle diameter. These results suggest that a special emulsifier system could be utilized to stabilize chemically unstable β-carotene as well as to protect poorly soluble β-carotene particles against coalescence. The following study, will focus on the effects of various emulsifiers on the physicochemical properties of β-carotene nanodispersions, as they have an important role in the stabilization of emulsions. Further investigations are also underway to increase the content of β-carotene in the final dispersions. However, the preliminary results, presented herein, indicate that emulsification–evaporation is capable of producing β-carotene nanodispersions with particle sizes below 100 nm.

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