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Factors affecting droplet size of sodium caseinate-stabilized O/W emulsions containing β-carotene

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Abstract

It has been reported that solubilization, and therefore, bioavailability of water insoluble bioactive compounds can be improved by incorporating the compounds in emulsions. This work was initiated to prepare an oil-in-water (O/W) emulsion containing β-carotene by microfluidization. The β-carotene was dissolved in triolein and microfluidized with an aqueous phase containing sodium caseinate (SC) as the emulsifier. Microfluization at 140MPa resulted in O/W emulsions with a mean droplet diameter of *ca.*120nm which was further confirmed with a transmission electron microscopy (TEM) analysis. The influences of SC concentration and microfluidization parameters on the droplet size of the emulsions were studied. The results showed that mean droplet diameter decreased significantly ($P<0.05$) from 310nm to 93nm with the increase in SC concentration from 0.1%wt to 2%wt. However, further increase in SC concentration did not change the droplet diameter much, although the polydispersity of the emulsions was slightly improved. The droplet diameter of the emulsions was found to decrease from 200nm to 120nm with increasing microfluidization pressure, with narrower droplet size distribution. Storage study showed that the emulsions were physically stable for about two weeks at 4^{o}C in dark. The results provide a better understanding on the performance of SC in stabilizing the O/W emulsions.

Keywords: β-carotene, emulsion, high-pressure homogenization, microfluidization

Introduction

Bioactive compounds such as carotenoids, phytosterols, natural antioxidants and many others have been receiving much attention from pharmaceuticals and food industries for many years. Although the physiological functions of many bioactive compounds have not yet fully understood, it is well recognized that these compounds contribute to the improvement of public health. The effectiveness of bioactive compounds in preventing diseases depends on preserving the bioavailability of the bioactive ingredients. However, many of the bioactive compounds are almost water-insoluble and thus have a low uptake in the body. A strategy to improve the bioavailability of these bioactive compounds involves the preparation of O/W emulsions with droplet size in sub-micrometer range.

Preparation of an emulsion generally requires energy input *via* a homogenization process. The energy must overcome Laplace pressure, which increases with decreasing emulsion droplet size (McClements, 2004). High-pressure homogenization is extensively used in the food, pharmaceuticals and biotechnology industries to mix, disperse, emulsify and process many products (Floury *et al.* 2002). Microfluidization is a high-pressure homogenization technique that is efficient for preparing O/W emulsions with fine droplet size. The interaction chamber of the microfluidizer has a fixed geometry and is present in the form of a confined capillary tube where disruption of the droplets occurs due to liquid-liquid, and above all, liquid-solid shear forces (Perrier-Cornet *et al.*, 2005).

Milk proteins are good emulsifiers and hence are used as ingredients in a wide range of formulated food emulsions. Caseinates are important milk proteins and have a number of advantages over the whey proteins. SC has a better solubility in water and more thermally stable compared to whey proteins, presumably because the relatively flexible casein molecules do not undergo appreciable heat-induced conformational changes like the globular

whey proteins (Srinivasan *et al.*, 2002). SC contains a soluble mixture of surface-active caseins that absorb rapidly at the oil-water interface during emulsification and stabilize the dispersions by a combination of electrostatic repulsion and steric stabilization (Dickinson *et al.*, 1998). SC is also highly effective at protecting emulsified oils from oxidation, owing to their unique iron chelating property and the ability to produce thick interfacial layers around the droplets (Hu *et al.*, 2003).

In this work, O/W emulsions containing β-carotene as a model of water-insoluble bioactive compounds were prepared using a microfluidizer. The β-carotene was dissolved in triolein and emulsified with the aqueous phase containing SC as the emulsifier. The main objectives of this work were to study the performance of SC in stabilizing the emulsions and the effect of microfluidization in the emulsions droplet size.

Materials and methods

Materials

SC, β-carotene and triolein were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Deionized water purified by a Milli-Q Organex system (Millipore, Bedford, Connecticut, USA) was used for preparing the aqueous phase. All other chemicals used were of analytical grade.

Preparation of the β-carotene O/W emulsions

Unless otherwise specified, β-carotene O/W emulsion was prepared by the following method. SC (1%wt) was dissolved in 0.05M phosphate buffer pH7 (at 20^{o}C), containing 0.02%wt sodium azide. The aqueous solution was magnetically stirred for 1h before the organic phase (0.1%wt β-carotene in triolein) was added. The ratio of organic phase to aqueous phase was 1:9 by weight. The pre-mix was homogenized using a conventional homogenizer (Polytron®

PT300, Kinematica AG, Lucerne, Switzerland) at 5000rpm for 5min to produce a coarse O/W emulsion, immediately followed by microfluidization (Model M-110EHi Microfluidizer Processor, Microfluidic™ Corporation, Newton, Maine, USA) for a single pass at 140MPa (Tan and Nakajima, 2005).

The O/W emulsions containing β-carotene were prepared under various operating conditions to study the effect of the experimental parameters on the droplet size of the emulsions. The concentration of SC was varied from 0.1 to 10%wt to study the effect of the protein concentration. Several other batches of emulsions were prepared under different microfluidization pressures (20 to 160MPa). For storage stability study, emulsions prepared with 1%wt SC and microfluidized at 140MPa were kept at 4^{o}C in dark. Samples were withdrawn for analysis at weekly interval. Each sample was analyzed for particle-size distribution.

Particle-size analysis

A laser-diffraction particle-size analyzer (LS 13320, Beckman Coulter, Inc., Florida, USA) was used to measure the droplet size distribution of the emulsions. A refractive index of 1.47 for β-carotene in polar solution (water) and that of 1.33 for water were used to calculate the droplet size. The reported droplet diameter was calculated from the average of at least three measurements.

TEM analysis

O/W emulsion prepared with 1%wt SC and 0.1%wt β-carotene, and microfluidized at 140MPa was selected for TEM analysis to observe the microstructure and droplet size distribution of the emulsion. The sample was prepared using the freeze-fracture replica method. The surface of the fractured sample was coated with a platinum layer followed by a

carbon layer in vacuum. The metal atoms were applied at 40^{o} to the fractured surface to produce a shadow effect. TEM images were then obtained using a JEOL-JEM 200CX TEM (JEOL, Tokyo, Japan) working at an accelerating voltage of 80kV.

Statistical analysis

Statistical analysis was performed on the data by a one-way analysis of variance using SAS (SAS, 1989) software package release 6.1. The significant differences (P<0.05) between means were further determined by Duncan's multiple-range test.

Results and discussion

Fig.1 shows the typical changes in droplet size of the O/W emulsion after the coarse homogenization and microfluidization at 140MPa. Coarse homogenization by a conventional rotor-stator homogenizer resulted in large droplets with a mean diameter of 1.7μm with CV 61%. The coarse homogenization involved the breakup and intermingling of bulk oil phase and aqueous phase. Fairly large droplets were formed due to the low homogenization pressure. The following microfluidization reduced the droplet size distribution into the range of 40 to 700nm with a mean value of 120nm (CV45%), owing to the fact that microfluidization applied higher disruptive energy than the conventional rotor-stator homogenizer. The breakup of the large droplets to smaller ones in the microfluidizer was initiated by a combination of turbulence and laminar-shear stress, which increased the droplet-specific surface area up to disruption. The protein rapidly adsorbed at the surface of the newly formed smaller droplets.

Fig.2 depicts the effect of SC concentration on the droplet size distribution of the emulsions after microfluidization. At low SC concentration (0.1%wt), the emulsion exhibited a bimodal droplet distribution, with a mean droplet size of 310nm and CV104%. Even the microfluidizer was capable of producing smaller droplets, there were insufficient protein molecules to adsorb

onto the newly formed surface and formed a protective interfacial layer around the droplets that prevented them from coalescing with their neighbors. Increasing SC concentration to 2%wt significantly (P<0.05) decreased the mean droplet diameter to 93nm, as well as the polydispersity of the emulsion (CV35%). Higher protein concentration in the aqueous phase provided better availability of the emulsifier to stabilize the droplets before they re-aggregated and therefore narrowed the range of droplet size. However, there was no significant difference (P>0.05) in the mean droplet size with further increase in SC concentration to 10%wt. There were more SC molecules present than were required to cover the droplet surface formed during microfluidization and the droplet size was independent of the protein concentration. The results were in accordance with previous findings that there was a critical concentration of SC at which the emulsifying capacity was the maximum (Carrera Sánchez and Rodríguez Patino, 2005). Nevertheless, the polydispersity of the emulsions was improved slightly in system with excess SC. The CV decreased from 35% to 27% with increasing SC concentration from 2%wt to 10%wt.

The effect of microfluidization pressure on the droplet size distribution is shown in Fig.3. The mean droplet size of the emulsions decreased significantly (P<0.05) from 200 to 120nm with the increase in microfluidization pressure from 20 to 100MPa. Increasing the microfluidization pressure or energy input caused the generation of more intense disruptive forces when the fluid inside the homogenisation chamber collided with each other, resulting in emulsion with smaller droplet size. The results agreed well with other works with several high-pressure homogenizers (Trotta *et al.*, 2001; Tan and Nakajima, 2005). However, there was a maximum limit of homogenization efficiency for the microfluidizer at which a minimum emulsion droplet size could be achieved under a given condition. In this work, there was not much change in mean droplet size when the microfluidization pressure was further increased to 140MPa, although the droplet diameter showed was some tendency to increase at

160MPa.

Fig.4 shows the changes in droplet size distribution during storage at 4^{o}C in dark. There was no significant (P>0.05) change in mean droplet diameter after one week of storage, but the mean droplet size increased gradually from 120nm in the first week to 201nm in the fourth week with increasing polydispersity from CV 45% to 53%. Phase separation was observed after two weeks of storage but the creaming layer disappeared after gentle shaking. Flocculation, which occurred due to the inability of SC to prevent close approach of the droplets, partly caused the phase separation (Dalgleish, 1997). Other reason for the creaming included the gravitational separation of the oil phase from the aqueous phase owing to the large density difference between the two phases (McClements, 2004).

A representative TEM image of O/W emulsion containing β-carotene prepared with 1%wt SC and homogenized at 140MPa is illustrated in Fig.5. The droplet size of the emulsion in the image agreed well with the results from particle size analyser, although some smaller droplets were also observed. The droplets had a well-defined boundary and fairly smooth surface which was of characteristic of oil droplets.

One of the difficulties in application of emulsions as bioactive compound delivery systems is the limitation in the choice of emulsifier. This work demonstrated the potential of SC as an emulsifier for preparing the emulsions. The droplet size of the emulsions depended on the SC concentration and microfluidization pressure. Due to the high solubility of β-carotene in triolein, the use of triolein in preparation of the emulsions would allow more β-carotene to be loaded in the system. Future works include the addition of a co-emulsifier into the system to improve the storage stability of the emulsions, as well as the bioavailability study with *in vivo* animal trials.

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List of figures

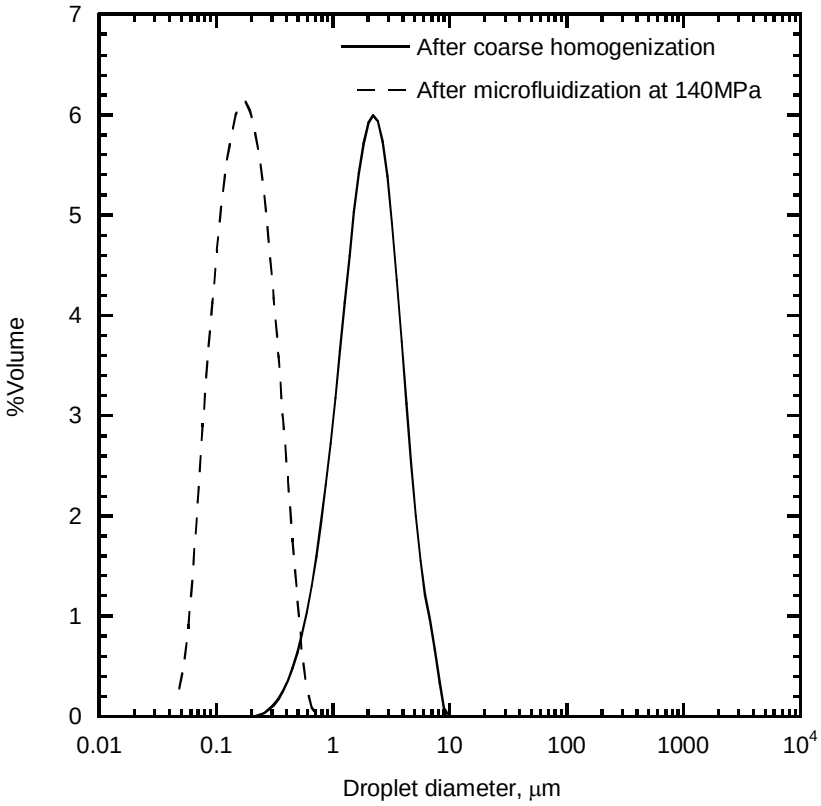
After coarse homogenization
After microfluidization at 140MPa
%Volume
Droplet diameter, μm
0
1
2
3
4
5
6
7
0.01
0.1
1
10
100
1000
10^4

Fig.1

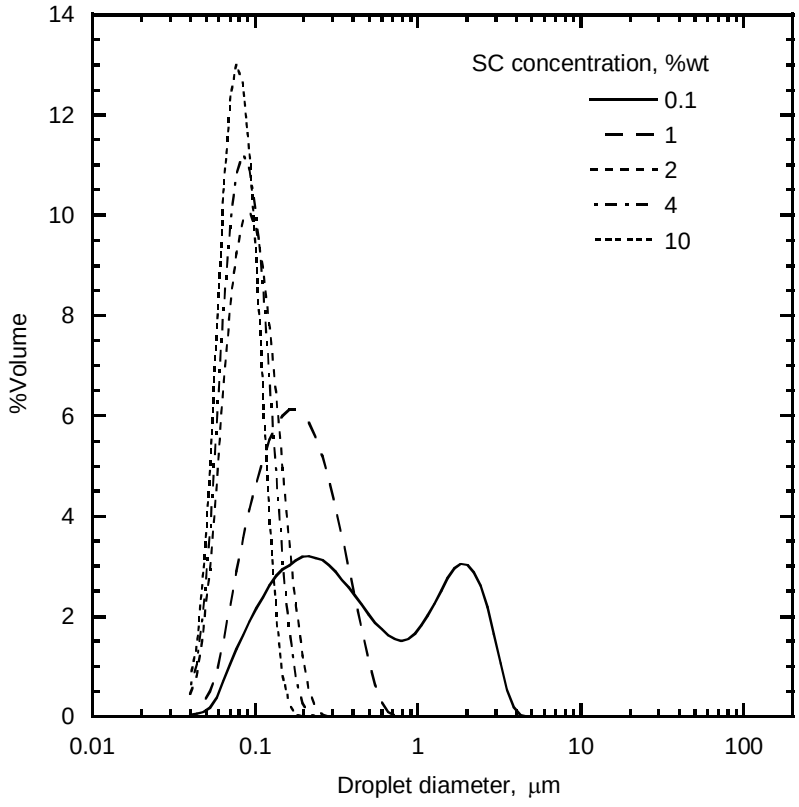
SC concentration, %wt
0.1
1
2
4
10
%Volume
Droplet diameter, μm
0
2
4
6
8
10
12
14
0.01
0.1
1
10
100

Fig.2

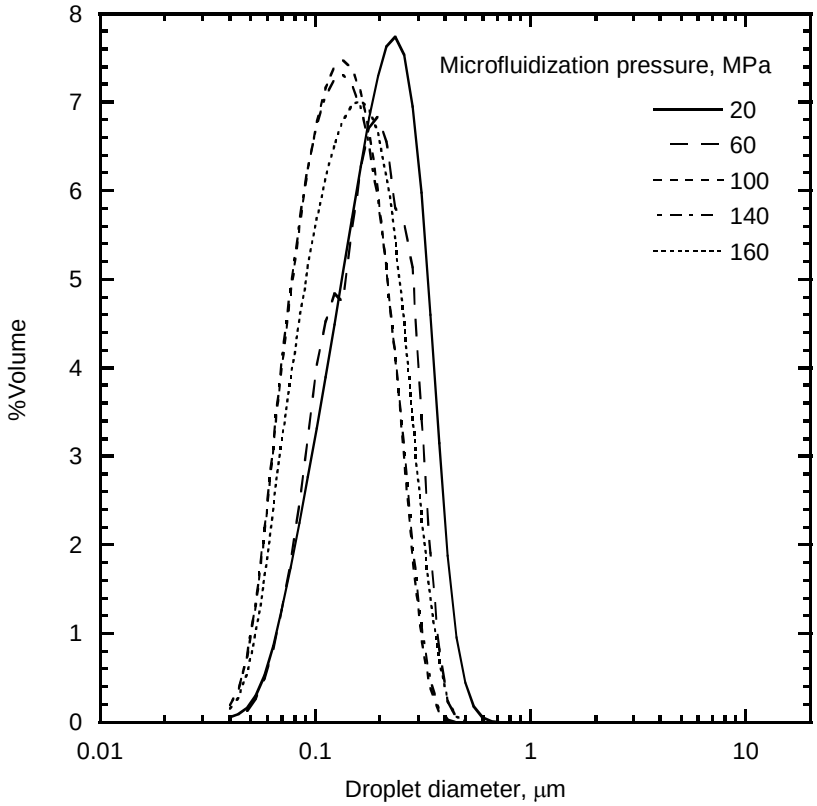
Microfluidization pressure, MPa
20
60
100
140
160
%Volume
Droplet diameter, μm
0
1
2
3
4
5
6
7
8
0.01
0.1
1
10

Fig.3

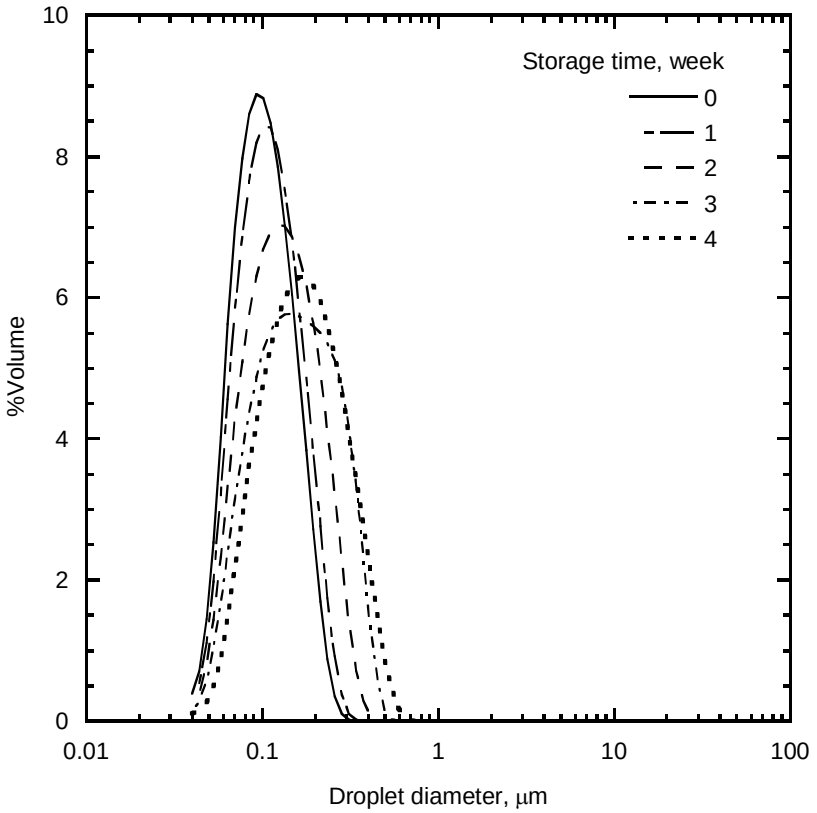
Storage time, week
0
1
2
3
4
%Volume
Droplet diameter, μm
0
2
4
6
8
10
0.01
0.1
1
10
100

Fig.4

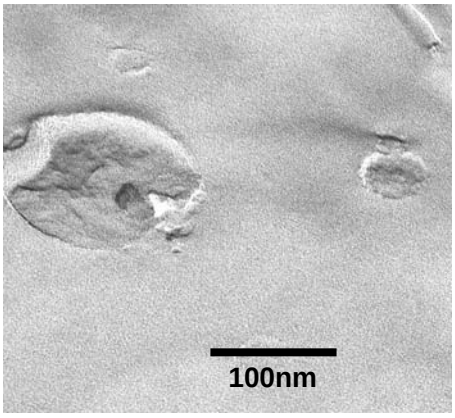

Fig.5