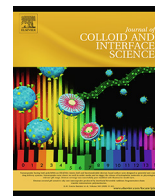




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Novel strategy for enhancing the color intensity of β -Carotene: Enriching onto the oil-water interface



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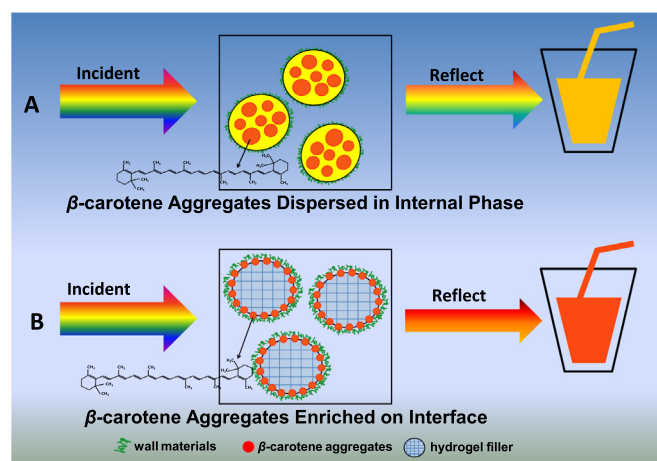
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GRAPHICAL ABSTRACT



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ABSTRACT

A novel strategy to enhance the color intensity of β -carotene (BC), namely, “interfacial enriching”, was developed in this work. As the sole emulsifier in W/O emulsion, BC particles were enriched onto the droplet surface through emulsifying process. By increasing the concentration of BC in oil phase from 1 mg/g to 5 mg/g, the average droplet size of the emulsion decreased from $92.2 \pm 5.1 \mu\text{m}$ to $34.0 \pm 5.4 \mu\text{m}$. Too low (e.g. $\leq 1 \text{ mg/g}$) or too high (e.g. $\geq 25 \text{ mg/g}$) concentration of BC in the oil phase yielded an insufficient coverage or flocculation of the droplets. By enriching onto the interface, the color intensity of BC were enhanced apparently at the reflectance wavelength ranging from 500 nm to 700 nm, compared with that of the BC encapsulated within the emulsion droplets. This enhancement was due to the higher availability of incident light for the BC particles on the interface than that of the BC particles buried inside the droplets.

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1. Introduction

Most of the colorations in food matrix use non-self-luminous colors. The optical properties of the colorants are entirely induced by energetic interactions of electromagnetic waves with the bound electrons of atoms, molecules, particles, or crystallites of the color-producing material [1]. Such kinds of light interactions are called selective if they are effective in a narrow range of wavelengths in the visible spectrum. Carotenoids, for example, with their different supramolecular short-range order structures, can be obtained in a targeted manner by variation in the precipitation conditions, which at strong intermolecular dipole interaction lead to marked changes in the absorption spectrum and thus the optical properties [2].

Numerous synthetic food colorants have been applied in food products to improve their quality and organoleptic characteristics. However, most of them were banned due to the clearly evident side effects, toxicity at short or long terms, and possible carcinogenic effects [3,4]. The use of more natural ingredients in the food formulation is one of the new trends in the food industry, and replacing the synthetic food colorants by natural ones has attracted a growing interest from both food manufacturers and consumers. In addition, natural food colorants revealed to be as much effective as those derived from chemical synthesis, with the advantages of being more safe, providing health benefits besides conferring organoleptic features, acting as multifunctional food ingredients (in fact several food additives acting not only as colorant but also as antioxidants and even preservatives), and conferring functional properties to food products [4–6]. However, replacement of synthetic colorants by natural ones is challenging, because natural colorants are more costly, usually less stable [7], and require more material to achieve equivalent color strength and limited range of hues.

Beta-carotene (BC) is one of naturally-derived food colorants, and it has been widely applied in food products. BC is known to be bioactive, possessing many health benefits [8]. Their antioxidative potential is widely known and scientifically recognized. BC is also used in large scale by the food industry as natural preservatives [5,9]. Due to its hydrophobicity, BC is usually encapsulated in an O/W emulsion [10], and then spray-dried to form a dispersible powder for using in hydrophilic food environments. In fact, the dispersibility of BC in edible oils, such as soy bean oil and sesame oil, is unsatisfactory. Therefore, BC existing as aggregated granules in the core of spray-dried microcapsule. This means that the chromogenic efficiency of BC in food is inhibited seriously. Apart from microencapsulation, various carrier systems also have been developed to improve the stability and dispersibility of BC, which include protein nanoparticles, starch-based micelles, and solid lipid particles etc [11–13]. However, the loading efficiency of BC in these carriers were too low to achieve an acceptable color intensity. So far as we know, there are limited reports investigating how to enhance the color intensity of BC.

BC has been shown to be surface active [14–16], and the morphology of BC adsorbed on the interface has been reported [16,17]. We speculate that the BC adsorbed at the surface of microcapsule might have a higher availability to the incident light, and thus an enhanced coloration efficiency, compared with those encapsulated inside the spray-dried microcapsules. The aim of this work is to investigate how the BC adsorbs on the interface and how this localization at the interface affects their color intensity.

To enrich the BC on the surface of the microcapsule, a processing method was developed in this work and it is presented in Fig. 1. BC was firstly dispersed in soy oil to form a BC/oil dispersion, and κ -carrageenan and KCl were dispersed in deionized water to form the aqueous phase. Both of the oil and water phases were pre-

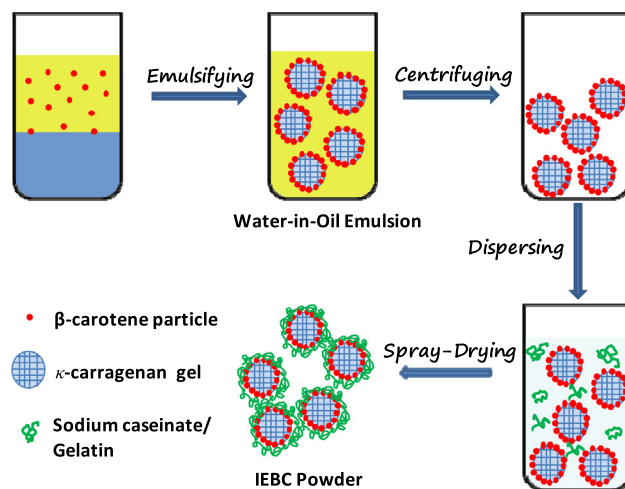


Fig. 1. Schematic diagram of a general processing for preparing the IEBC Powder.

heated to 85 °C before emulsifying. After emulsifying with a high-speed blender, the formed W/O emulsion was cooled down and centrifuged to collect the BC coated droplets. The obtained droplets were then mixed with sodium caseinate/gelatin dispersions to allow the adsorption of sodium caseinate/gelatin at the surface of the droplets. After that, the dispersion was spray dried to obtain the desired interfacial enriched BC (IEBC) powder.

2. Methods and materials

2.1. Materials

BC (purity $\geq 99\%$) was provided by Zhejiang Medicine Co., Ltd, soybean oil was purchased from the local supermarket. Sodium caseinate and gelatin were purchased from Sigma-Aldrich. Commercial BC powder (has a BC content of 1%) was purchased from Wuhan Stars Modern Bio-engineering Co., Ltd. Papain (P3375) with an activity of 1.5–10 units/mg was purchased from Sigma-Aldrich. Other chemical reagents were of analytical grade.

2.2. Preparation of BC/oil dispersion for interfacial measurements

Soybean oil was used as dispersant, and BC powder was added into the soybean oil at different concentrations. The dispersion was stirred at 400 rpm and 25 °C for 2 h. During the stirring, the container was packaged with tinfoil to protect BC from degradation. Then, the dispersion was submitted to a water bath sonication (25 °C, 30 min) to improve its dispersion. Before use, the soy oil was purified by the method of Gaonkar [18], and surface tension was checked to make sure the absence of surface-active contaminants.

2.3. Interfacial adsorption kinetics

The interfacial adsorption kinetics of BC at the oil-water interface were observed by a drop profile tensiometer (Teclis Tracker, France). The experiments were carried out at 25 °C. Five mL BC/-soybean oil dispersions with different BC contents were loaded into the sample cell. A drop (10 μ L) of deionized water was delivered from syringe and allowed to stand at least for 3 h to achieve the equilibrium adsorption of BC at the oil–water interface. The surface tension (σ) was calculated according to the fundamental Laplace equation. The surface pressure is $\pi = \sigma_0 - \sigma$, where σ_0 is the surface tension of the oil without BC.

2.4. Interfacial microstructure observation

The microstructure of BC adsorbed onto the air-water interface was observed by the combination of Langmuir-Blodgett trough (KSV-Nima, Biolin Scientific) and Brewster angle microscopy (EP3-BAM, Nanofilm, Germany). The trough, with symmetric Delrin twin barriers, was equipped with a surface pressure sensor that uses a Wilhelmy plate technique to determine the change of surface pressure of the air-water interface.

Deionized water was loaded in the trough as the sub-phase, and then the dust at the surface was sucked off to avoid its effect on the surface pressure. BC was dissolved in ethyl acetate with a concentration of 0.1% (w/v), and a volume of 50 μL was dispersed on the air-water interface. Then, ethyl acetate was allowed to volatilize for at least 5 min before measurements. The isotherm was measured by recording the compress surface pressure as a function of surface area, at a barrier compression speed of 5 cm min^{-1} . Measurements were repeated at least 3 times until good reproducibility was achieved.

All BAM micrographs were taken on the EP3-BAM with a lateral resolution of approx. 1 mm. The size of the micrographs was 600 $\times$ 500 μm and images were not processed in any way except for background correction carried out with the provided software. BAM micrographs were recorded at selected surface pressures.

2.5. Preparation of BC stabilized W/O emulsion

BC/soybean oil dispersion was used as the continuous phase, with BC concentration ranging from 1 to 25 mg/g . κ -carrageenan was dispersed in deionized water (10 mg/mL) followed by addition of 0.1 M KCl. The dispersions were then stirred at 85°C for 2 h before emulsifying. The W/O emulsion was obtained by subjecting the mixture of water and oil phases (at a ratio of 2:8) to high speed mixing using a PT-MR2100 blender (Kinematica, Swiss) at 20000 rpm for 2 min.

2.6. Optical light microscopy observation

The microstructure of the emulsions was observed using a conventional optical light microscope (Nikon YS100), equipped with a CCD camera (HV2001UC).

2.7. Droplet size analysis of W/O emulsion

The droplet size of the W/O emulsion was analyzed using a software of NIKON NIS-Elements AR. At least 10 micrographs of the samples were analyzed and the average droplet size was used.

2.8. Surface loading measurement

The amount of BC adsorbed at the oil-water interface was the difference between the amount of BC in the oil phase before and after emulsification according to the method described by Roos et al., [19] with a slight modification. A certain volume of freshly prepared emulsions (described as above mentioned) was stored at 25 °C overnight to let the water droplets precipitate at the bottom. The serum (soybean oil) was then collected for analysis of BC concentration in the oil phase.

The BC concentration in the oil phase could be analyzed as follows: weighed aliquots of 1 g of the oil phase were poured into a 13 mL screw-capped polypropylene test tube at 25 °C. One mL of saturated potassium hydroxide in methanol was added in order to separate the lipid carrier (saponized fraction) from BC (unsaponized). The mixture was placed in a thermostatted water bath and heated at 50 °C for 30 min. After cooling to 22 $\pm$ 2 °C, the unpolar BC was extracted three times with 2 mL of *n*-hexane. The

organic solvent was added to the oil- KOH/methanol mix and stirred (Vortex for 30 s) and then left to stand for 10 min to obtain the separation of an organic layer containing BC on the top. The top layer was accurately removed with a glass Pasteur pipette and transferred to a glass vial containing a small amount ($\sim$ 0.5 g) of anhydrous sodium sulfate powder which absorbed possible residual water. After 1:4 dilution with *n*-hexane, extracts were analyzed by a UV-2500 spectrometer (Shimadzu, Japan) at the maximum absorbance wavelength for BC, $\lambda_{\text{max}} = 450 \text{ nm}$.

The mass of BC adsorbing on the interface (M_a) is expressed as $M_a = M_i - M_d$, where M_i is the mass of BC initially added into the emulsion and M_d is the mass of BC existing in the oil phase of the emulsion. The surface loading of BC Γ is determined by the following equation:

$$\text{Surface loading}(\text{mg/m}^2) = (M_a \times d_{3,2}) / (6 \times V \times \Phi_{\text{oil}}) \times 10^{-2}$$

where V (mL) is the volume of the emulsion, and Φ_{oil} was the volume fraction of oil phase.

2.9. Preparation of the spray-dried BC powder

To apply the above interfacial enriched BC in hydrophilic food environment, it should be encapsulated by a hydrophilic wall. The BC stabilized W/O emulsion (BC concentration in oil phase was 5 mg/g) was isolated through centrifuge at 1000g for 15 min. The sediment (BC particles stabilized droplets) was collected and dispersed in a wall materials dispersion (composed of 5% sodium caseinate and 5% gelatin) with a weight ratio of 1:9. The mixing was reinforced by a PT-MR2100 blender (Kinematica, Swiss) at 10,000 rpm for 2 min. IEBC powder was obtained by spray-drying of the formed W/O/W emulsion. Spray-drying was conducted on a Mini Spray Dryer (B-290, BÜCHI, Switzerland). Temperature at the inlet and the outlet were 120°C and 90°C, respectively, and the rate of the airflow was 35 $\text{m}^3 \text{h}^{-1}$. The fluid speed rate was 5 mL min^{-1} . The obtained spray-dried powder contained 0.39 $\pm$ 0.03% BC (the method for measurement will be introduced later).

2.10. Particle size measurement of IEBC powder

The particle size of the dispersed IEBC powder was measured using laser diffraction on Master Sizer 2000 (Malvern Instruments Ltd., UK). The powders were diluted approximately 200-fold using deionized water. The samples were stirred continuously throughout the measurements to ensure their homogeneity. The refractive index ratio used to calculate the particle size distribution was 1.09. Droplet size was reported as average diameters $d_{4,3}$, which was defined as $\sum n_i d_i^4 / \sum n_i d_i^3$, where n_i is the number of particles with diameter d_i . Mean particle diameters were calculated as the average of duplicate measurements.

2.11. Determination of BC content in IEBC powder

Weighed aliquots of 1 g of IEBC powder, 1 g of ascorbic acid, 0.5 g of papain and 50 mL deionized water were mixed in a 300 mL beaker. The mixture was stirred at 55°C for 30 min to hydrolyze the protein. Then, 75 mL saturated potassium hydroxide in methanol was added in order to separate the lipids (saponized fraction) from the BC (unsaponized). The following process for isolation and determination of BC content was carried out as in the section "Surface loading measurement".

2.12. Scanning electronic microscopy

IEBC powders were attached to a SEM aluminum plate through double sided conductive carbon tabs. After coating with gold in an E-1010 ion sputter (Hitachi, Tokyo, Japan), IEBC was observed using a JSM-6390LV SEM (JEOL, Japan) at an accelerating voltage of 15 kV.

2.13. XRD patterns analysis of the powder

XRD patterns were obtained using a D/Max-3B diffractometer with Cu K α radiation (40 kV, 100 mA), at a scanning rate of 5°·min⁻¹. The IEBC powder was mounted on a vitreous sample holder and scanned with a step size of 2 θ = 0.02° from 2 θ = 5° to 80°.

2.14. Color analysis

IEBC powders were firstly dispersed in deionized water at a BC concentration of 0.1 mg/mL. The spectral reflectance of the IEBC dispersions were measured using a spectrophotometer with an integrated sphere setup (CM-5; Konica Minolta Co., Japan). Spectra were obtained over the wavelength range from 350 to 750 nm at 10-nm intervals relative to a barium sulphate (BaSO₄) standard white plate.

The color of dispersions was measured in the L*, a*, b* system using the Konica Minolta CM-5 spectrophotometer in the reflectance mode, with illuminant D65 and the 10° observer. In the color space, L* represents the lightness, which ranges from 0 to 100; the parameters a* (from green to red) and b* (from blue to yellow) are the two chromatic components.

2.15. Statistical analysis

Unless otherwise specified, three independent trials were carried out for each experiment. All measurements were repeated in triplicate and the results were given as mean $\pm$ standard deviation.

3. Results and analysis

3.1. Interfacial behavior of BC particles

BC was regarded as surface active according to their spreading behavior in Langmuir-Blodgett trough [14–16]. Their adsorbing behavior on the interface, however, has never been presented so far as we know. The variation of interfacial tension as function of time was shown in Fig. 2A. Since a too higher concentration of BC leads to the instability of the results (data not shown), we just analyzed the interfacial behavior of BC at the concentration range from 1 to 2 mg/g. It could be observed that BC can adsorb onto the oil-water interface and produce a surface pressure. All the three samples showed a two-stage adsorption behavior in which a rapid increase of surface pressure was observed during the first 1000 s (first stage), and followed by a slow increase until reaching an equilibrium state (second stage). A higher concentration (such as 2 mg/g) resulted in a more rapid adsorption at the first stage and slower adsorption at the second stage. While a lower concentration (such as 1 mg/g) showed a slower adsorption at the first stage, and needed longer time to reach the equilibrium state. It could also be observed that their final surface pressures were almost the same. This indicated that through free adsorption, BC could reach a saturated adsorption on oil–water interface at a concentration of 1 mg/g, and higher concentration did not contribute to the coverage of the interface. BC particle size was also considered to have an effect on the interfacial adsorption rate of the first stage. For the concentration of 1 mg/g or 2 mg/g, the BC particle size was 698 ± 7.1 nm

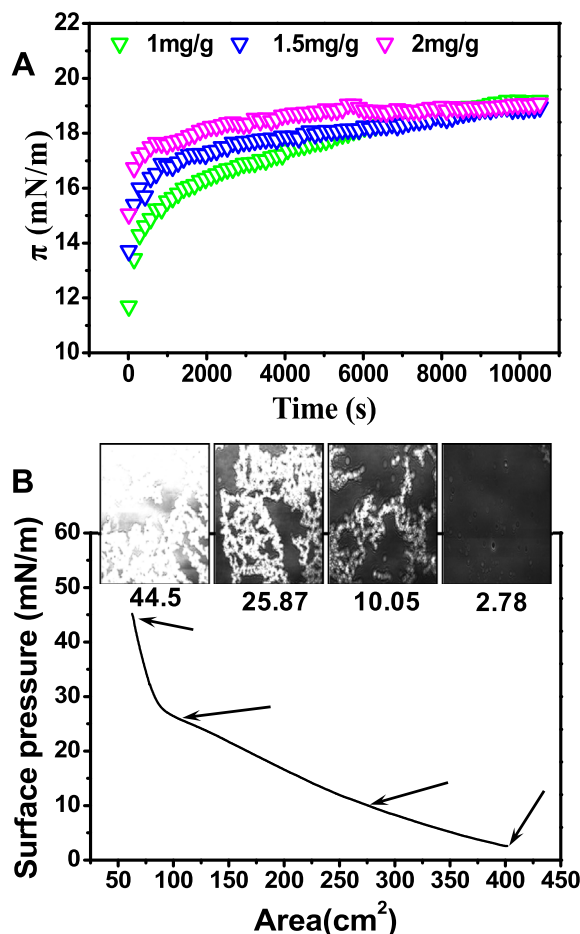


Fig. 2. Interfacial behavior of BC during the adsorption processing and compressing. A is the changes of the surface pressure of BC on oil-water interface during its adsorption. B shows the compressing isotherm of BC on the air-water interface. Inset displays the Brewster Angle Micrograph of the BC on the air-water interface.

or 825 ± 11.6 nm, respectively (data not shown). This indicated that the main factor affecting the interfacial adsorption behavior of the BC particles was their concentration but not their size.

Fig. 2B showed the π -A isothermal curve of BC on air-water interface. Upon compression, the surface pressure increased monotonically until reaching the smallest trough area, and it increased even more steeply after reaching 25 mN/m. During the whole compressing process, no indication of collapse had been observed under our experiment conditions, such as abrupt dropping of the surface pressure. The morphology of the BC adsorbed at the air-water interface was monitored by Brewster Angle Microscopy (BAM) during the compressing (Fig. 2B inset). At low surface pressure (e.g. 2.78 mN/m), most region of the BAM image was dim, which might be occupied by the monomolecular layer, and the floated BC particles could scarcely be observed. At higher surface pressure (e.g. 10.05 mN/m), reticulate fragments of the aggregated BC emerged, and the background was shown to be brighter. On continuing compression of the film, the surface pressure increased to 25.87 mN/m, the fragments changed to be more crowded and showed to be thicker since the image became brighter. With further compression, when the surface pressure reached 44.50 mN/m, the film became very thick and it seems to be an integral network. It could be observed clearly that the spread BC on air-water interface tended to form a multilayer film or aggregated network at such surface pressure. Unlike other monolayer films formed by low molecular weight surfactant which had a collapse point when

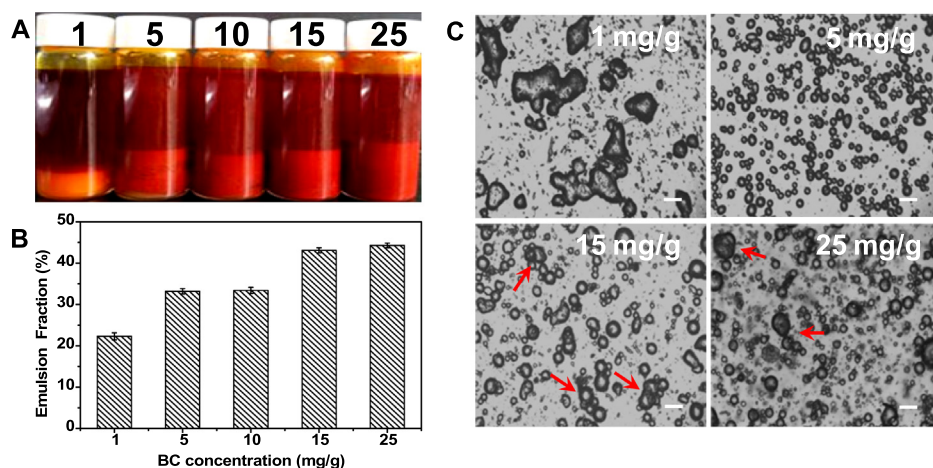


Fig. 3. Appearance (A), emulsion fractions (B) and optical micrographs (C) of the BC particle stabilized W/O emulsion, with BC concentration varied from 1 mg/g to 25 mg/g. The red arrows marked in C show the flocculation and coalescence between the droplets. Scale bar in C is 100 μm.

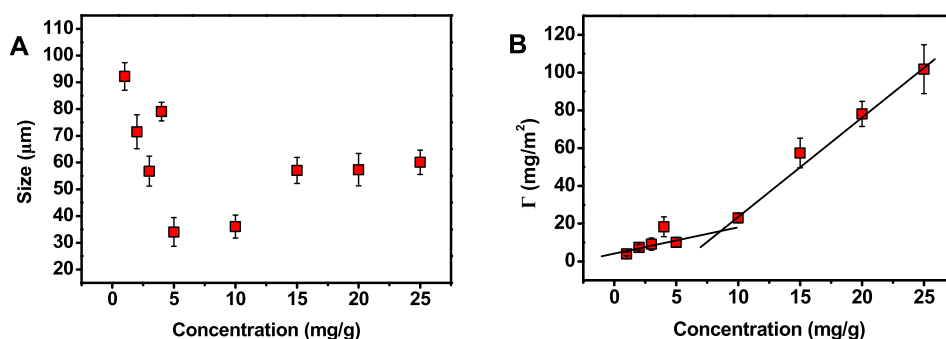


Fig. 4. Changes in the average droplet size (A) and surface load of BC stabilized W/O emulsions as a function of BC concentration in continuous phase.

compressed [20], BC tended to form a 3D film. Tighter the compression, thicker the formed film.

This phenomenon was similar with that observed by Mildner et al. [17] who choose various solvents to spread BC on the air-water interface. Although the spreading solvent was different, the isotherm curve and the BAM of the formed BC film on the air-water interface were similar, which were porous networks and no Langmuir monolayer film was formed. Mircheva [16] also had observed the morphology of BC spreading on air-water interface through AFM in which the BC films were transported onto the mica sheet before observation. The AFM images of BC films transferred at different surface pressures (9 mN/m and 25 mN/m respectively) showed the coexistence of two distinguishable phases: a net-like phase (brighter areas) composed of expelled BC molecules, surrounding a phase organized as monomolecular layer (the darker areas of image). The height difference between the two phases shown in their results was estimated about 1.0 ± 0.2 nm. These results were the same with our observation.

3.2. Formation of the W/O emulsions

BC nanoparticles could stabilize W/O emulsions as observed in Fig. 3A, and there was a growing tendency for the emulsion fraction (the sedimentation) with increasing BC concentration (Fig. 3B). The optical microscopic observation of the emulsions is presented in Fig. 3C. At a low BC concentration (e.g. 1 mg/g), the emulsion droplet size is shown to be polygon. With increasing BC concentration (e.g. 5 mg/g), the droplets became smaller and round, and the size became more homogeneous. Partially covering of the

droplets at low BC concentration might lead to coalescence and further result in larger droplets. Non-spherical droplets might be a result of either jamming of the interfacially trapped particles or the buckling and crumpling of the BC particle layer. Both scenarios would prevent the relaxation of the droplet to a spherical shape. With increasing BC concentration, more particles are available to cover the droplet surfaces, thereby preventing them from coalescing. With higher BC concentration (e.g. 15 and 25 mg/mL), there were observable flocculation and coalescence existing in the emulsion dispersions.

The droplet size of the W/O emulsion as a function of BC concentration is presented in Fig. 4A. The change of average droplet size ($d_{4,3}$) could be divided into two stages. At the first stage, the $d_{4,3}$ was decreased from 92.2 ± 5.1 μm to 34.0 ± 5.4 μm as the BC concentration increased from 1 mg/g to 5 mg/g. This should be attributed to the sufficient BC particles to stabilize the interface, and prevent the coalescence between the droplets as mentioned above. At the second stage, the increase in BC concentration from 5 mg/g to 25 mg/mL, led to a slight increase in the droplet size to 60.1 ± 4.5 μm, and then levelled off. This might be the results of bridged flocculation induced by the excessive BC particles existing in the continuous phase.

The surface load of BC in the W/O emulsions had also been analyzed (Fig. 4B). High concentration of BC was shown to be beneficial for its enrichment on the interface, as the surface load increased gradually when the BC concentration increased from 1 to 5 mg/g. When the BC concentration in the continuous phase increased above 10 mg/g, a rapid increase in the surface load was observed. Multilayer adsorption and bigger particle size of the BC

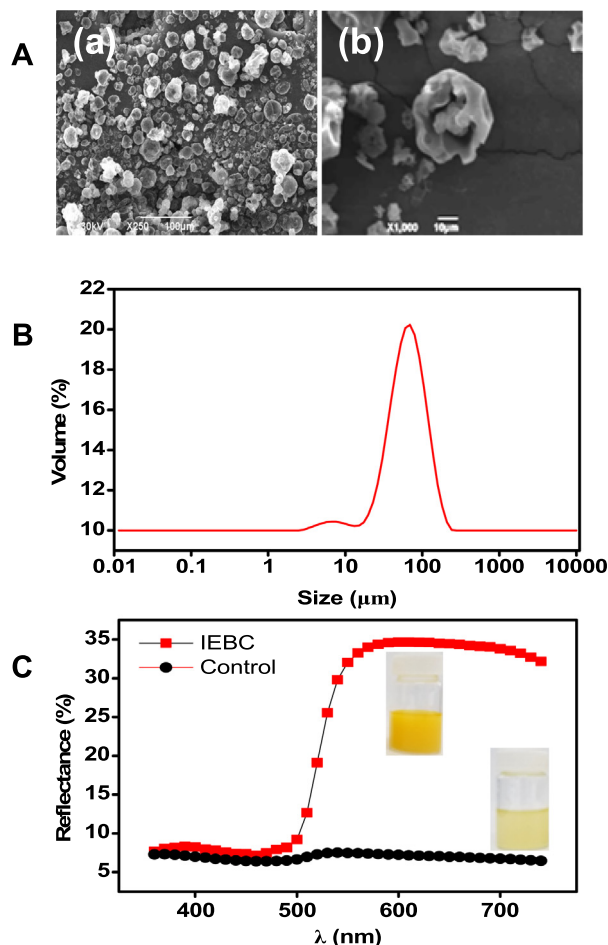


Fig. 5. SEM images observed at different magnifications (A), particle size distribution (B) and reflectance (C) of the IIBC powder dispersed in deionized water. Inset in C: the aqueous dispersions of the control and IIBC powder with the same BC content of 0.1 mg/mL (control was 1% commercial BC powder). Scale bars in A (a) and A (b) are 100 μm and 10 μm, respectively.

at higher concentration might be the main reasons of the increase in the surface load. As can be seen in Fig. 2A, the final surface tension was not dependent on the concentration of BC, indicating the equal coverage ratio of the interface. Dilatational rheology of the BC film also showed that at a high concentration BC, the modulus increased deeply at the final stage of adsorption while the surface pressure did not show an apparent increase (data not shown). These results implied that the adsorption of BC at a latter stage did not contribute to the surface coverage, but forming a multilayer film. This coincides with the observation in Fig. 2B that compressing did not produce a complete film but a porous multilayer film.

3.3. Spray-drying and characterization of the powder

The BC content in the IIBC powder was $0.39 \pm 0.08\%$ (w/w) and the microstructure of the powder was characterized by SEM (Fig. 5A), ((a) and (b) were the samples observed at different magnification). The spray-dried granules were shown spherical on the whole. The core-shell structure could be observed clearly from the enlarged image (Fig. 5A(b)), where the κ -carrageenan gel as the core was encapsulated in the shell composed of the mixture of NaCS/gelatin. The surface of the κ -carrageenan gel was relatively smoother than that observed by others [21], in which a porous framework had been observed. This might be caused by the coverage of BC on the surface. The particle size distribution of the IIBC

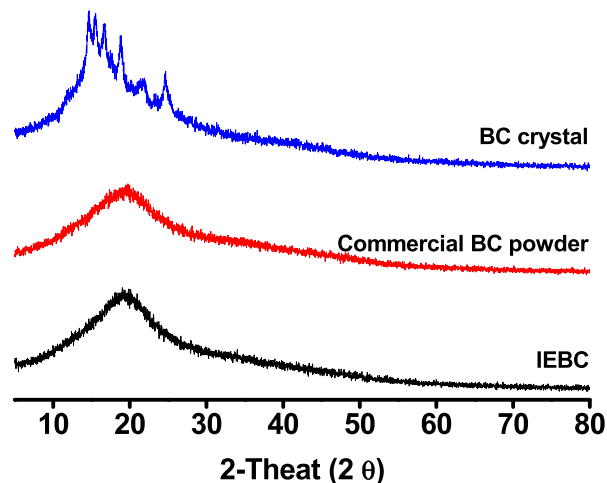


Fig. 6. X-ray diffraction of BC samples.

powder is shown in Fig. 5B, and it's monodispersed with the average particle size $69.1 \pm 5.9 \mu\text{m}$.

Reflectance of the IIBC powder had been measured by a spectrophotometer, as shown in Fig. 5C. With the same BC content (0.1 mg/mL), IIBC showed a stronger reflectance compared with the commercial BC powder (control), especially at the wavelength region from 500 nm to 700 nm. This region of wavelength represented the color range from green, yellow to red. The difference on reflectance was consistent with their appearance (Fig. 5C inset). $L^*a^*b^*$ system was applied to characterize the optical properties. The $L^*a^*b^*$ values of IIBC and commercial BC powder were presented in Table 1. Compared with the commercial BC, IIBC was more red (a^* value) and more yellow (b^* value). Moreover, the IIBC was brighter (L^* value) than the commercial BC powder. The difference in yellow color (b^*) was more significant than other two index, which were 2.77 and 43.72, respectively.

Since the variation of the $L^*a^*b^*$ values between commercial BC powder and IIBC powder were not proportional, the crystal types of the BC in different samples might have been changed. Therefore we analysed the crystal type of BC by X-ray diffraction (Fig. 6). The crystal BC showed three diffraction peaks (2θ), at 25° , 20° and 18° while both commercial BC powder and IIBC powder did not show any clear diffraction peak, indicating the BC in these two kinds of powders were almost amorphous. Therefore, the changes in optical properties were not induced by the changes of crystal type, but by the migration of BC from the internal phase to the interface.

To better understand the mechanisms of how IIBC changes the color properties, we show it schematically in Fig. 7. For the commercial spray-dried BC powder, most of BC was encapsulated in the internal phase, and aggregated seriously. The available BC molecules irradiated with incident light was very limited. By enriching onto interface, available BC molecules for irradiation increased, therefore their color intensity was sufficiently improved.

4. Conclusion

Changes of the structural properties of food colloids on different length scales will result in different functionality. For food colorants, molecular scale modification could be used to enhance their chemical stability and optical properties, but this approach had received more and more negative perspectives. Modulating the optical properties of food colorants on colloidal scale, such as regulating their self-assembly or distribution in food matrix, can also be an effective choice. In this research, through the emulsification, BC nanoparticles could be enriched onto oil-water interface

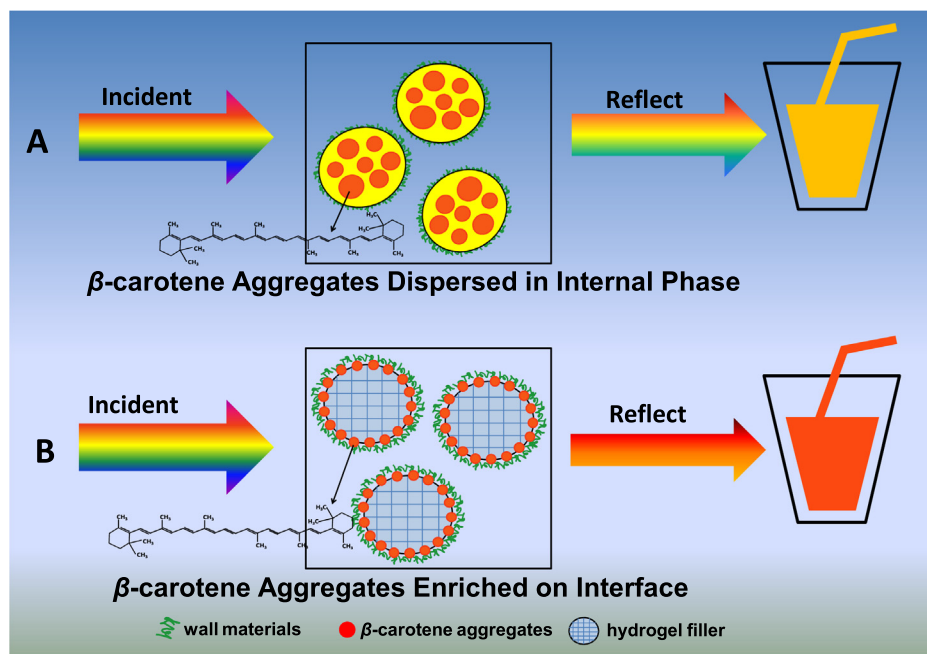


Fig. 7. Mechanisms of enhancing the color intensity by enriching BC onto the interface. In the commercial spray-dried BC powder (A), most BC particles seriously aggregated and encapsulated in the internal phase. The interaction of the light with BC particle is limited. After enriching onto the interface (B), BC particles could interact effectively with the incident light, therefore their color properties are enhanced.

Table 1

Comparison of the $L^*a^*b^*$ values of the commercial BC powder and the IEBC powder dispersed in water at the same BC concentration of 0.1 mg/mL. The IEBC powder was prepared from the 5 mg/g BC in oil phase.

Sample	BC content	L^*	a^*	b^*
Commercial BC	$1 \pm 0.12\%$	32.32 ± 5.21	-0.79 ± 0.08	$+2.77 \pm 0.06$
IEBC	$0.39 \pm 0.08\%$	58.71 ± 4.32	$+7.71 \pm 0.36$	$+43.72 \pm 0.57$

and stabilize an W/O emulsion, in which κ -carrageenan is dispersed as the aqueous phase. The spray-dried IEBC had a core-shell structure in which the BC covered polysaccharide gel was encapsulated in the shell of NaCS/gelatin. The enrichment of BC at the surface could enhance the color intensity of the IEBC to be brighter, more red, and more yellow. This research showed a potential strategy to enhance the color properties of the hydrophobic natural food pigments.

CRedit authorship contribution statement

Zhiming Gao: Conceptualization, Investigation, Writing-Original Draft. **Binxian Wu:** Investigation, Validation, Formal analysis, Data curation. **Bing Hu:** Validation, Formal analysis, Data curation. **Shaohua Cui:** Resources, Visualization. **Longquan Xu:** Resources, Visualization. **Ke Zhang:** Data curation, Visualization. **Katsuyoshi Nishinari:** Visualization, Writing - review & editing. **Glyn O. Phillips:** Visualization, Supervision. **Yapeng Fang:** Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

- [1] G. Klein, Light sources, types of colorants, observer, Industrial Color Physics, Springer, New York, 2010.
- [2] H. Auweter, H. Haberkorn, W. Heckmann, et al., Supramolecular structure of precipitated nanosize β -carotene particles, *Angew. Chem. Int. Ed.* 38 (1999) 2188–2191.
- [3] P. Amchova, H. Kotolova, J. Ruda-Kucerova, Health safety issues of synthetic food colorants, *Regul. Toxicol. Pharm.* 73 (3) (2015) 914–922.
- [4] M. Carochi, M. Barreiro, P. Morales, I. Ferreira, Adding molecules to food, pros and cons: a review on synthetic and natural food additives, *Comprehensive Rev. Food Sci. Food Safety* 13 (4) (2014) 377–399.
- [5] D. Rodriguez-Amaya, Natural food pigments and colorants, *Curr. Opin. Food Sci.* 7 (2016) 20–26.
- [6] F. Delgado-Vargas, O. Paredes-López, Natural colorants for food and nutraceutical uses, other natural pigments, in: *Natural colorants for food and nutraceutical uses*, CRC Press LLC, New York, 2003, pp. 221–251.
- [7] N. Martins, C. Roriz, P. Morales, et al., Food colorants: challenges, opportunities and current desires of agro-industries to ensure consumer expectations and regulatory practices, *Trends Food Sci. Technol.* 52 (2016) 1–15.
- [8] L. Yonekura, A. Nagao, Intestinal absorption of dietary carotenoids, *Mol. Nutr. Food Res.* 51 (2007) 107–115.
- [9] G. Dias, F. Camões, L. Oliveira, Carotenoids in traditional Portuguese fruits and vegetables, *Food Chem.* 113 (3) (2009) 808–815.
- [10] L. Cornacchia, Y. Roos, Stability of β -carotene in protein-stabilized oil-in-water delivery systems, *J. Agric. Food. Chem.* 59 (13) (2011) 7013–7020.
- [11] A. Mensi, P. Borel, A. Goncalves, et al., β -Lactoglobulin as a vector for β -carotene food fortification, *J. Agric. Food. Chem.* 62 (25) (2014) 5916–5924.
- [12] Y. Ma, J. Liu, F. Ye, et al., Solubilization of β -carotene with oat β -glucan octenylsuccinate micelles and their freeze-thaw, thermal and storage stability, *LWT - Food Sci. Technol.* 65 (2016) 845–851.
- [13] G. Gomes, M. Sola, L. Marostegan, et al., Physico-chemical stability and in vitro digestibility of beta-carotene -loaded lipid nanoparticles of cupuacu butter

- (Theobroma grandiflorum) produced by the phase inversion temperature (PIT) method, *J. Food Eng.* 192 (2017) 93–102.
- [14] A. Shibata, Y. Kiba, N. Akati, et al., Molecular characteristics of astaxanthin and β -carotene in the phospholipid monolayer and their distributions in the phospholipid bilayer, *Chem. Phys. Lipids* 113 (1) (2001) 11–22.
- [15] O. Horovitz, G. Tomoaia, C. Racz, et al., Surface properties of some carotenoids spread in monolayers at the air/water interface. experimental and computational approach, *Cent. Eur. J. Chem.* 4 (3) (2006) 489–501.
- [16] K. Mircheva, M. Gonnet, K. Balashev, et al., Properties of β -carotene and retinoic acid in mixed monolayers with dipalmitoylphosphatidylcholine (DPPC) and Solutol, *Colloids Surf. A* 460 (2014) 209–218.
- [17] J. Mildner, P. Dynarowicz-Latka, β -Carotene does not form a true Langmuir monolayer at the air/water interface, *Colloids Surf. B* 90 (2012) 244–247.
- [18] A. Gaonkar, Interfacial tensions of vegetable oil/water systems: effect of oil purification, *J. Oil Fat Industries* 66 (8) (1989) 1090–1092.
- [19] L. Cornacchia, Y. Roos, Stability of beta-carotene in protein-stabilized oil-in-water delivery systems, *J. Agric. Food. Chem.* 59 (13) (2011) 7013–7020.
- [20] D. Sowah-Kuma, M. Paige, The influence of surfactant head group on miscibility in mixed hydrocarbon- perfluorocarbon monolayers, *Coll. Surf. A* 556 (2018) 157–166.
- [21] G. Sason, A. Nussinovitch, Characterization of κ -carrageenan gels immersed in ethanol solutions, *Food Hydrocolloids* 79 (2018) 136–144.