Study on Precipitation of Astaxanthin in Supercritical Fluid

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Abstract

As health hazards of synthetic pigments are continuously found, more and more natural food pigments have been applied to the food industry. Astaxanthin is one of carotenoids that have been widely used in the food, cosmetic and pharmaceutical industries as natural colorants. In this paper, the micronization of astaxanthin in dichloromethane via the solution enhanced dispersion by supercritical fluids through prefiltering atomization (SEDS-PA) process has been successfully performed. Morphologies and particle sizes (PSs) of the astaxanthin microparticles were analyzed by scanning electron microscopy (SEM). The results of micronization show that astaxanthin crystals with PSs about 20–30 µm have successfully been micronized to the microparticles with PSs about 0.5–6.0 µm by the SEDS-PA process. The effect of experimental variables on micronization of the astaxanthin has been studied. With the increase of initial solution concentration, the PSs of astaxanthin microparticles decrease initially, then increase. With the increase of solution flow rate and temperature, the PSs of astaxanthin microparticles increase. With the increase of pressure, the PSs of astaxanthin microparticles decrease. The nubbly particles were obtained in SEDS-PA process, and the crystallinity change of astaxanthin was showed by X-ray powder diffraction patterns (XRD).

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1. Introduction

The increasing reports about health hazards and toxicity of synthetic pigments are leading to the substitution of synthetic pigments by natural food pigments. Carotenoids are the most common natural pigments responsible for the red, orange and yellow colors, including carotene, astaxanthin, lycopene, lutein, etc. In addition, carotenoids also have some pharmaceutical and nutritional applications due to their positive effect on human health [1–3]. Carotenoids have widely been used in the food, cosmetic and pharmaceutical industries as natural colorants.

Astaxanthin found in aquatic animals possess diversiform biological functions such as antioxidant, UV-light protection, anti-inflammatory and other properties. *Haematococcus pluvialis* is believed to accumulate the highest levels of astaxanthin in nature, which can reach more than 30 g astaxanthin per kg dry biomass [3]. The drug particle with a controlled particle size (PS) and particle size distribution (PSD) can deposit in the human respiratory tract selectively, which will maximize their effectiveness and minimize the adverse side effects [4]. The use of very small pigment particles can improve first of all the stability of pigment in the dispersion medium, but also the color strength, contrast, and transmittance can be enhanced [5]. Therefore, many researchers keep an increasingly interest in decreasing the size of drug particles.

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The solution enhanced dispersion by supercritical fluids (SEDS) process is a very convenient technique for meeting these requirements. Carbon dioxide (Tc = 304.1 K and Pc = 7.38 MPa), as an environmentally benign process solvent, offers the additional benefits of being nonflammable, nontoxic, inexpensive. The SEDS process can be carried out at near ambient temperature and in an inert atmosphere while CO2 is used as supercritical fluid (SF), thus avoiding the thermal degradation and the oxidation of astaxanthin particles. It is possible to obtain a solvent-free product without a complex purification process using high solubility of organic solvents in supercritical CO2. Additionally, the small particles with a controlled PSD can be obtained by controlling parameters of the SEDS process. Based on these advantages, the SEDS process has extensively been studied in the last years, of which include lactose [6], polymers [7], pigments [8,9], pharmaceuticals [10–18] and composite microparticles [19–25].

In SEDS-PA process, a prefiltering twin-fluid atomizer was used for increasing transfer rates between the SF and the solution. The liquid to be atomized is driven along the coaxial annular passage and formed to a thin swirl liquid film (thickness 10 µm) by the liquid distributor with spiral slots, inclined at an angle of 45° relative to the central axis of the atomizer. At the exit of the atomizer the atomizing dense gas stream impinges on the thin swirl film at 45° and interacts with it to generate shear force on it. Upon violent interaction with the jet stream, the annular liquid sheet is disintegrated into fine drops and the mixing of the SF and the solution is intensified for increased transfer rates.

A study on the precipitation of astaxanthin by SEDS-PA process has previously not been reported in the literature. The aim of this work is to develop a SEDS-PA process for the precipitation of astaxanthin. The
influences of operating variables on PSs and morphologies of the astaxanthin microparticles were discussed in detail in this paper.

2. Materials and methods

2.1. Materials

*H. pluvialis* containing 1.5% astaxanthin was purchased from Qingdao Samuels Industrial & Commercial Co., Ltd. Astaxanthin (3, 3'-dihydroxy-β, β-carotene-4, 4'-dione) was conventionally separated and purified from dry *H. pluvialis* by using solvent extraction, column chromatography and crystallization techniques in our laboratory. Its structure, as indicated in Fig. 1, was characterized by Elemental analysis, IR, UV and MS in our previous work [26]. The astaxanthin crystals used to the SEDS-PA process see Fig. 2, which are nubbly particles with mean sizes of about 25 µm. Dichloromethane in analytical grade was used as the solvent. Carbon dioxide in food grade (99.97%) was used as the SF.

2.2. Methods

2.2.1. Preparation of astaxanthin microparticles

Astaxanthin microparticles were produced by the SEDS-PA process from dichloromethane solution. The schematic diagram of the equipment used in the SEDS-PA process [17] is shown in Fig. 3. SC-CO2 was pumped into the top of the particle formation vessel (inside diameter of 56 mm, length of 200 mm, internal volume of 450 cm³) through the inner capillary (internal diameter of 80 µm) of the atomizer, using diaphragm compressor I (G447-420). After the particle formation vessel reached steady state (temperature and pressure), the astaxanthin solution was introduced into the vessel using a high-performance liquid chromatography pump (LB-10) through the coaxial annular passage of the atomizer. Meanwhile, the SC-CO2 continued to flow through the vessel to maintain the steady state. Precipitated microparticles were collected on a filter with an aperture of smaller than 5 µm at the bottom of the vessel. The fluid mixture (SC-CO2 plus dichloromethane) exited from the vessel and flowed to a depressurization tank (internal volume of 600 cm³) and two separation vessels (internal volumes of 300 cm³) where gas-liquid separation was allowed under the conditions (T=298 K, P=4 MPa). The separated dichloromethane solution remained in the depressurization tank and the two separation vessels, and the separated CO2 gas was decompressed to 0.6 MPa by a pressure reducing valve and recycled using diaphragm compressor II (G44-220). After the liquid solution delivered to the particle formation vessel was interrupted, the diaphragm compressor II was turned off and drain tap was opened, then pure SC-CO2 continued to flow through the vessel for an additional 60 min to remove residual solvent from the particles. The flow rate of CO2 was fixed at 25 mL/min while the experiments were performed at the standard state (T=273 K, P=101.325 kPa).

In the experiments, the operating pressure, temperature and flow rate of CO2 were measured by the diaphragm pressure meter (Y-100B/-Z/MH), transmitter (STT-R-10 K) and the mass flow meter (D08-8BM/ZM), respectively.

2.2.2. Particle characterization

The morphologies of microparticles were visually studied by scanning electron microscopy (SEM) (Hitachi S-4800), and the minimum and maximum sizes among the rod-like particles on a SEM image were estimated (using the ruler of SEM) as the mean particle sizes (PSs).

![Fig. 2. SEM micrograph of unprocessed astaxanthin.](image_url)

![Fig. 3. Schematic diagram of experimental facility.](image_url)

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<tr>
<th>Table 1</th>
<th>Particle sizes and morphologies of the astaxanthin precipitates by SEM</th>
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<tr>
<td>Experiment</td>
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Microparticle samples were manually dispersed on an aluminum stub with a thin self-adhered film. Then the samples were covered with gold (layer thickness 250 Å) using a sputter coater.

The X-ray power diffraction patterns were collected by a Bluker X-ray diffractometer, Model D8 Advance (German) with Cu Kα radiation. All samples were measured in 2θ angle range between 10° and 50°. The step scan mode was performed by a step size of 0.02° at a rate of 5°/min. The current used was 40 mA and the voltage was 40 kV.

The residual solvent in the microparticle samples was quantified by the headspace gas chromatography.

### 3. Results and discussion

The micronization of astaxanthin with different operating temperatures (T), pressures (P), initial solution concentrations (C) and solution flow rates (F) have been performed by the SEDS-PA process for studying the influence of operating parameters on product characteristics. A summary of all the experiments is presented in Table 1. Meanwhile, the SEM micrographs in Fig. 4 distinctly showed the change of morphologies and sizes of astaxanthin microparticles obtained by different process parameters. From Table 1 and Figs. 2, 4, it

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**Fig. 4.** SEM micrographs of processed astaxanthin microparticles by SEDS process. (a) \( T=313 \text{ K}, P=8 \text{ MPa}, C=0.5 \text{ g/L}, F=2 \text{ mL/min} \); (b) \( T=313 \text{ K}, P=8 \text{ MPa}, C=1.0 \text{ g/L}, F=2 \text{ mL/min} \); (c) \( T=313 \text{ K}, P=8 \text{ MPa}, C=2.0 \text{ g/L}, F=2 \text{ mL/min} \); (d) \( T=313 \text{ K}, P=20 \text{ MPa}, C=1.0 \text{ g/L}, F=4 \text{ mL/min} \); (e) \( T=313 \text{ K}, P=20 \text{ MPa}, C=1.0 \text{ g/L}, F=2 \text{ mL/min} \); (f) \( T=323 \text{ K}, P=20 \text{ MPa}, C=1.0 \text{ g/L}, F=2 \text{ mL/min} \).
can be observed that SEDS-PA process could successfully be used for micronizing astaxanthin.

The residual amounts of dichloromethane in the samples are less than 38 ppm in all cases.

3.1. Effect of the initial solution concentration

The initial solution concentration is a major experimental variable that affects sizes of particles in the SEDS-PA process, and influences on PSs and morphologies of astaxanthin microparticles (Table 1 and Experiments 1–4). Initial concentration of astaxanthin solution were varied between 0.5 and 2 g/L, with a constant value of other process parameters, in order to study the effect of initial solution concentration. From Table 1 and Fig. 4a–c, it can be observed that an increase of the initial solution concentration causes the decrease of PSs from 0.5–4 to 0.5–2 µm, and the increase of PSs from 0.5–2 to 1–2 µm while the concentration range is 0.5–1.0 and 1–2 g/L respectively. Very large particle agglomerates of nubbly particles are observed by higher initial concentrations in Fig. 4c.

3.2. Effect of the solution flow rate

The experiments 5–8 in Table 1 were performed in the range of 2 to 5 mL/min to explore the effect of the solution flow rate, and the other operating conditions were T=313 K, C=1.0 g/L, P=20 MPa. From Table 1 and Fig. 4d, e, it can be noted that PSs of the particles increase, when solution flow rate increases. This trend was also observed by Miguel et al. [27] for lutien particle formation. In the SEDS-PA process, the solution was sufficiently atomized via the impingement of flowing SC-CO2 on liquid film in the prefilming atomizer. With increasing solution flow rate, the energy per unit mass of liquid gained from SC-CO2 to disintegrate the liquid sheet into drops became gradually weak for constant SC-CO2 flow rate. As a result, the sizes of liquid drops became gradually larger, which resulted in the precipitation of bigger particles with 1–6 µm PSs (see Fig. 4d).

Moreover, the variation of solution flow rate also changes the global composition solvent/CO2. The ratio of CH2Cl2/CO2 also increases with the increasing of solution flow rate, which decreased the driving force for the mass transfer of CH2Cl2 into the CO2. The decreased mass transfer rates decreased the supersaturation ratio, which then caused a decrease in the nucleation rate. A decrease in the nucleation rate would then lead to larger particles.

3.3. Effect of temperature

The effect of temperature was analyzed in experiments 5, 9–11 in Table 1. These experiments were performed in the range of 308–323 K while the operating conditions were C=1.0 g/L, F=2.0 mL/min, P=20 MPa. An increase of the PS with temperature was found. From Fig. 4e, f, it can be seen that the sizes of astaxanthin microparticles obtained at 308 K are smaller than those obtained at 323 K. Meanwhile, a larger tendency of particles agglomeration was found with increasing temperature. This trend was also observed by Cocero et al. [28] for crystallization of β-carotene. The morphology change of the microparticles was probably caused by the fierce collision among the microparticles under higher temperature.

3.4. Effect of pressure

The effect of pressure was studied by experiments 2, 5, 12 and 13 of Table 1. An increase of the pressure caused a slight decrease in particle size, from approximately 1–4 µm at 8 MPa to 0.5–3 µm at 20 MPa. The decrease in particle size was more noticeable in the pressure intervals 12–20 Mpa rather than in the intervals 8–12 MPa. The tendency of PS decrease with pressure is related to the volumetric expansion of the carrier solvent induced by SC-CO2. With the increase of pressure, the CH2Cl2 solution with astaxanthin can rapidly be diluted by SC-CO2, and the CH2Cl2 dissolving power to the solute is reduced, which lead to higher supersaturation of the expanded liquid solution that subsequently results in formation of smaller microparticles. On the other hand, diffusivity of CO2 decreased as pressure increased. In general, reduced diffusivity hinder mass transfer between the droplets and the surrounding CO2, so particle size must be increased with increasing pressure. As a result, increasing pressure led to a slight decrease in particle size.

3.5. X-ray diffraction patterns

The X-ray diffraction patterns of the astaxanthin before/after SEDS process were shown in Fig. 5. The 2θ of patterns a and b, c, d show the characteristic diffraction peaks of unprocessed astaxanthin at 11.0, 13.0, 13.5, 14.2, 15.0, 16.4, 17.6, 18.5, 20.7, 25.1°, and processed astaxanthin at 10.7, 12.1, 14.6, 15.0, 15.4, 16.4, 17.6, 20.7, 22.8, 24.0, 24.4°, respectively. As shown in Fig. 5, the most 2θ of astaxanthin characteristic diffraction peaks after SEDS process are near to those of unprocessed astaxanthin, however, the relative integrated intensity of the peaks is reduced, and the width of peaks is relative narrow. The 2θ of new diffraction peaks appear at 12.1, 22.8, 24.0 and 24.4°, and the diffraction peaks disappear at 18.5 and 25.1°. The 2θ position, intensity and width changes of peaks indicate that the crystallinity of astaxanthin have been altered after SEDS process, and the appearance of new diffraction peaks show that there are new crystalline planes in astaxanthin microparticles.

4. Conclusions

This work has shown that the SEDS-PA process can successfully be used for micronization of astaxanthin. The precipitates at all experimental conditions are nubbly particles, and the morphologies of particles have no obvious change as compared with unprocessed particles. A large effect of solution concentration variations on the PSs of particles was observed. With the increase of solution flow rate and temperature, the PSs of astaxanthin microparticles increase. A larger tendency of particles agglomeration was found at higher temperature. With increase of pressure, the PSs of astaxanthin microparticles decrease. The XRD analysis shows a change in the crystalline form and the appearance of new crystalline planes before/after SEDS process.

Acknowledgement

The authors gratefully acknowledge the financial supports of the National Natural Science Foundation of China (Grant No. 20666003).
References