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Rice bran supercritical CO_2 extract microparticle preparation using an aerosol solvent extraction system and particles from gas-saturated solution processes

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ABSTRACT

To improve the solubility of rice bran supercritical CO_2 extract (RB-SCE) in water, RB-SCE microparticles were successfully prepared using a mixing process involving an aerosol solvent extraction system (ASES) and particles from a gas-saturated solution (PGSS). To improve the microparticle formation efficiency, RB-SCE, a sodium-caseinate saturated aqueous solution, and ethanol (as a co-solvent) (1:3:10 wt%) were used as raw materials, with supercritical CO_2 as the anti-solvent. The particle size distribution of the RB-SCE microparticles ranged from 1.25 to 346.15 μm; the median value of the particle size distribution and the mean particle size of the RB-SCE microparticles were 266.42 and 250.12 μm, respectively. 5% RB-SCE microparticles were stably dissolved in a test tonic product. Thus, RB-SCE microparticles prepared by a mixture process using an ASES and a PGSS can be used in the food industry and for cosmetic product applications.

KEYWORDS

Aerosol solvent extraction system (ASES);
Microparticle;
Particles from gas-saturated solution (PGSS);
Rice bran supercritical CO_2 extract (RB-SCE).

INTRODUCTION

Various techniques using supercritical fluids for particle formation from polymers and non-polymeric materials have been developed. The particle formation technology that uses supercritical fluids has taken various forms over the last 30 years, including the rapid expansion of supercritical solutions (RESS), gas anti-solvent (GAS) processes, supercritical anti-solvent (SAS) processes and various modifications, and particles from gas-saturated solution (PGSS) processes[1].

Among them, the aerosol solvent extraction system (ASES) utilizes the extraction properties of supercritical gases for the production of microparticles[2]. Extraction with supercritical gases is frequently used in the pharmaceutical, cosmetic, and food industries. In the ASES process, the drug

or polymer is dissolved or dispersed in an organic solvent, which is then sprayed into a supercritical gas phase (*e.g.*, carbon dioxide). The organic solvent is soluble in the supercritical gas phase and is extracted, resulting in the formation of solid microparticles.

Supercritical carbon dioxide (CO_2) also shows strong interactions with polymers and fats, which can be exploited to favor the impregnation of active compounds in these carriers, or to powderize them. In particular, the PGSS process is a particle formation technique based on the interactions of supercritical CO_2 with low-melting point polymers and fats[3].

Rice bran is the largest single by-product of the rice-milling process, representing 8% of the milled rice. Between 20 and 30% of this by-product is used for oil production, and the remainder is discarded or used as livestock feed or fertilizer[4]. Previous studies have reported that rice bran oil has various health benefits, including antioxidant[4], anticancer[5], and anti-hyperlipidemic[6] effects. In addition, rice bran extract has been shown to act as a 5-α-reductase inhibitor in cell lines *in vitro*[7] and to promote hair growth *in vivo*[8]. Rice bran oil extract has been used in the food industry[9] and for cosmetic applications as a skin moisturizer[10] and hair-growth promoting agent[8].

Supercritical CO_2 extraction (SCE), which is conducted at low temperatures using supercritical CO_2 as the solvent, has been introduced as an alternative one-step method for oil extraction. Extracting oil at a low temperature minimizes the thermal degradation of proteins, antioxidants, and other nutritionally valuable components. Additionally, supercritical CO_2 has the advantages of being environmentally friendly, non-toxic, non-ûammable, inexpensive, and easily removed from the final product[11].

Rice bran supercritical CO_2 extract (RB-SCE) is insoluble in water, indicating that its application to the food and cosmetic industries is limited, because water-insoluble materials are usually characterized by low absorption, poor bioavailability, as well as poor availability for making oil-in-water emulsions. These solubility limitations could be overcome to some extent by applying microparticle technology.

In this study, to improve the solubility of RB-SCEagainst water, RB-SCEmicroparticle formation was successfully performed using a combination of the ASES and PGSS processes; *i.e.*, microparticles produced using high-speed solvent dispersion via a high-pressure reactor and separator. The particle size distribution and inclusion efficiency of the prepared RB-SCEmicroparticles were measured.

MATERIALS AND METHODS

Reagents and materials

All chemicals and solvents used in this study were of analytical grade. Triple-distilled water was prepared in our laboratory. Pure CO_2 (99.99%) wasprovided by KOSEM Co., Ltd. (Yangsan, Korea).

Oryza sativa Bran Preparation

Rice (*Oryza sativa* LINN. var. *japonica*; the Korean cultivars, Dongjin; Gramineae) used in this study was harvested in Gijang, Busan during the fall of 2011. The rice bran was milled and provided by PN RICE Co., Ltd. (Kimhae, Gyeonsanggnamdo, Korea) in March of 2012.

Preparation of RB-SCE

Supercritical CO_2 extractions of rice bran were performed in a semi-continuous flow-type apparatus with a 3-L extractor as per Choi *et al*. (2013)[12]. Briefly, CO_2 was pumped into the extractor using positive displacement-controlled volume-metering pumping. A flow rate of 135-g CO_2 min^{-1} was used for extraction. The pressure in the extractor was controlled using a back-pressure regulator. The extraction vessel was loosely packed with glass wool. A 1-kg rice bran sample was added and distributed throughout the packing. A small plug of glass wool was then placed in the outlet end of the tube before closure to reduce entrainment. The extract was collected in a separator and chilled with ice by expanding the loaded solvent to ambient pressure. Extractions were performed at 32°C and 270 bar for 240 min. RB-SCEwas stored at “80°C until use.

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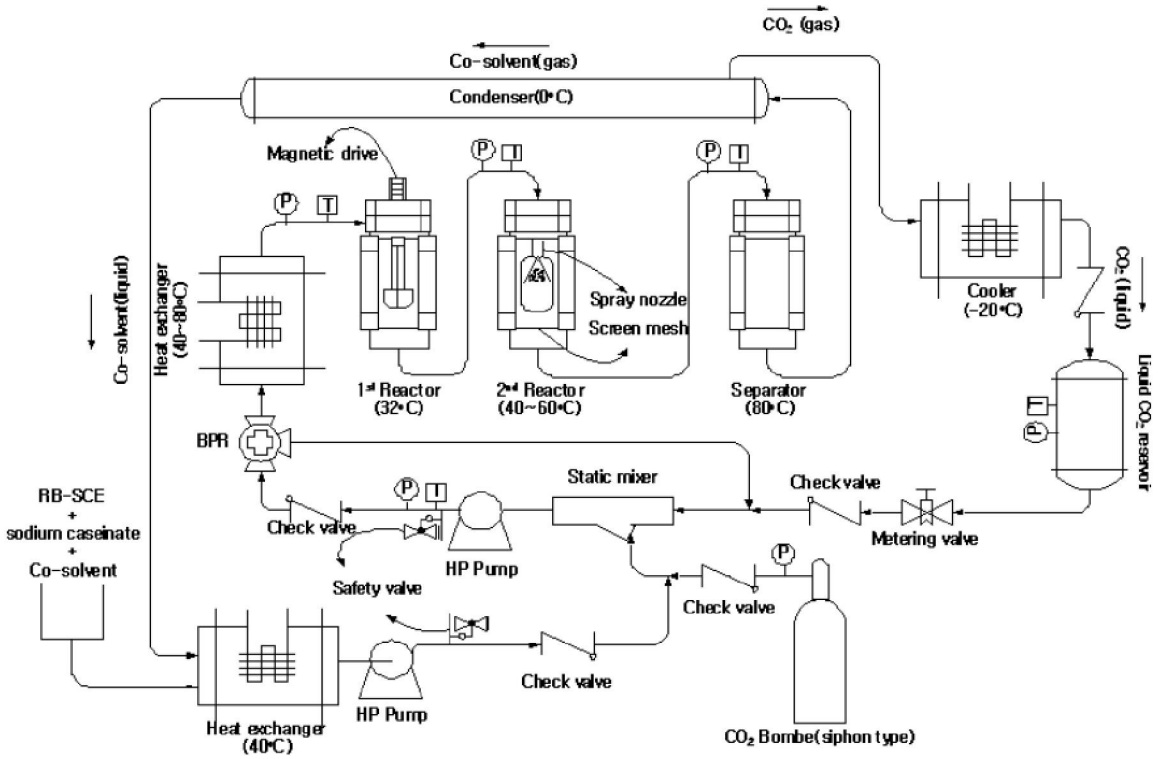

Figure 1 : Flow chart of the continuous-type RB-SCE microparticle formation process using an aerosol solvent extraction system (ASES) and particles from gas-saturated solution (PGSS) processes

Apparatus

Figure 1 shows a schematic diagram of the apparatus used for the ASES and PGSS. It consisted mainly of a CO_2 cylinder, a solution vessel, two high-pressure pumps, a stainless steel precipitator (diameter: 7 cm; height: 30 cm), and a separator positioned after the precipitator to recover the liquid solvent.

Procedure

RB-SCE, a sodium caseinate-saturated aqueous solution, and ethanol (co-solvent) (1:3:10, wt%) as raw materials were mixed; the raw material mixture was then transported to a static mixer via a heat exchanger using a high pressure-controlled volume pump. The raw material mixture and liquid CO_2 were pre-mixed in the static mixer and pumped through the heat exchanger into the first reactor at 32°C and 130 bar, with a flow rate of 70 mL min^{-1}. In the first reactor, the raw material mixture and supercritical CO_2 were strongly stirred at 1,250 rpm by a magnetic-drive impeller, converting the raw-material mixture into micelle (*i.e.*, the first microparticlization step). The ethanol used for dispersion of the RB-SCEand sodium caseinate was evaporated to the supercritical phase. The first microparticlization particles (primary particles) were passed through a spray nozzle into a second reactor with supercritical CO_2, resulting in the formation of finer secondary microparticles by shearing force and cavitation as the residual ethanol evaporated. Thus, supercritical CO_2acted as an anti-solventin the reaction path between the first and second reactors. The microparticles prepared after completion of the secondary reaction were passed through a 0.5-μm-meshmetal frit filter located in the lower portion of the second reactor, forming a precipitate in the reactor. The mixture of supercritical CO_2 and ethanol were fed into a separating tank and converted to the gaseous phase under a reduced pressure and high temperature (80°C) within the separation vessel. The gaseous ethanol and CO_2 gas were then delivered to a condenser, in which the ethanol was condensed to liquid ethanol. Gaseous CO_2 was liquefied through a cooler and transferred to a CO_2 storage tank. The separated ethanol and liquid CO_2 were then recycled.

Scanning electron microscopy (SEM) analysis

TABLE 1 : Formulation of the test tonic water containing rice bran supercritical CO_2 extract (RB-SCE) microparticles

INCI	Percentage of components (W/W)
Water(aqua), demineralized	as100.00
Ethanol	30.00
RBOmicroparticles	5.00
Hyaluronic acid (1%)	1.00
HCO 60	0.50
BHT	0.02
Menthyl lactate	0.30
Tocopheryl acetate	0.15
Grapefruit seed extract	0.20
E-NAPRE	0.80
Sodium salicylate	0.20
Lavender oil	0.10
Tea tree oil	0.10
Patchouli oil	0.10
German chamomile	0.10
TEA (100%)	0.80
Flavoring	0.05

The particle size and morphology of samples were observed using field-emission scanning electron microscopy (FE-SEM, JSM-6700F, JEOL, Japan). Samples were sputter-coated with OsO_4 using a sputter coater for 30 s before analysis. The particle size of the samples was obtained with IMAGE-PRO PLUS 6.0 particle analytical software.

Particle size distribution of RB-SCEmicroparticles

The particle distribution of RB-SCEmicroparticles was determined using a laser-diffraction particle size analyzer (LS 13320, Beckman Coulter, USA). The particle size distribution and average particle size were analyzed in quadruplicate with the particles suspended in water. Before taking the measurements, the suspended samples were sonicated for 1 min; the analyses were carried out under constant stirring.

Inclusion efficiency of RB-SCE

The inclusion efficiency of the RB-SCE was calculated as follows:Inclusion efficiency of RB-SCE = (RB-SCE content (g) / RB-SCE microparticle powder (g)) × 100%.

Preparation of test tonic water product with RB-SCE microparticle

To confirm the solubility of RB-SCE microparticles, a test tonic water product was produced with the RB-SCE microparticles. The test tonic water product was kindly supplied by ECMINE Co., Ltd. (Busan, Korea). The formulations studied (TABLE 1) were prepared in a PRIMIX RM homomixer (PRIMIX Co., Ltd., Osaka, Japan) at 500 rpm for 10 min, and supplemented with 5% RB-SCE microparticles (as a dried solid material).

RESULTS AND DISCUSSION

RB-SCE microparticle

The microparticle formation process of RB-SCE used in this study combined an ASES and a PGSS to produce microparticles using high-speed solvent dispersion in a high-pressure reactor and separator. Food-grade sodium caseinate was used as an inclusive agent. Organic solvents having a partition coefficient of $0.31 < K_D$ (the distribution coefficient) <0.77 include ethanol, methanol, and ethyl acetate. Among them, ethanol was selected for this study, due to its low dermal toxicity. The molecular structure of sodium caseinateused as an inclusive agent in this study has amphoteric surfactant properties, allowing it to surround the RB-SCE via an annular structure.

RB-SCE, a saturated aqueous solution of sodium caseinate (three times the RB-SCE amount) and ethanol (as a co-solvent) was mixed with CO_2 in the static mixer; this mixture was then introduced to the

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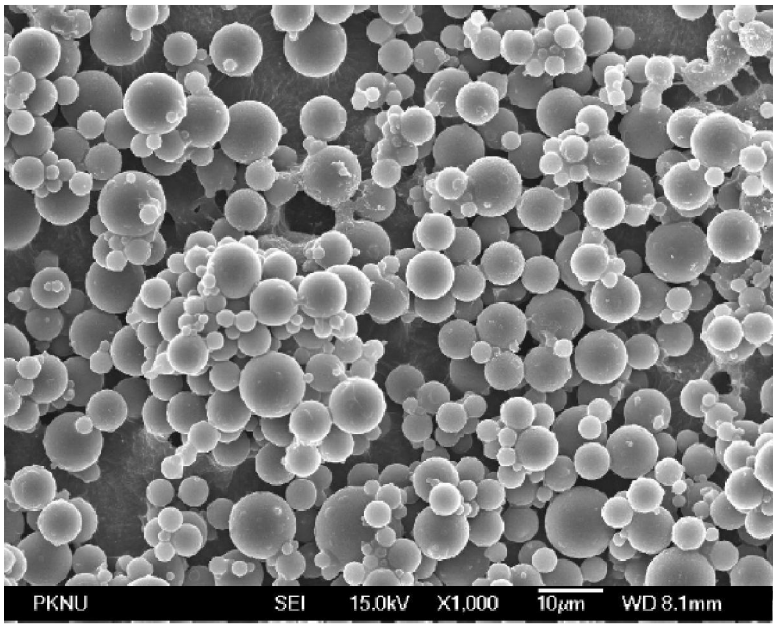

Figure 2 : Scanning electron microscopy (SEM) images of RB-SCE microparticles, Magnification: ×1,000

primary reactor. Within the primary reactor, the fine crystal nuclei of RB-SCE/sodium caseinate inclusion bodies were primarily generated by supercritical CO_2 acting as an anti-solvent. The crystal growth was initiated by internuclear cohesion between the generated nuclei; at this point in the process, the size of the inclusion body was determined by the rotating force of the magnetic drives. These inclusion bodies were transferred through the spray nozzle from the first reactor into the secondary reactor; their size decreased due to the shear force inside the narrow nozzle duct and cavitation caused by rapid diffusion. Figure 2 shows the prepared RB-SCE microparticles in this study.

Shape and particle size distribution of RB-SCE microparticles

The microparticle shape of the RB-SCE was globular, as shown in Figure 3. The particle size distribution of RB-SCE microparticles was 1.25 to 346.15 μm (Figure 4); the median value of the particle size distribution and the mean particle size of RB-SCE microparticles were 266.42 and 250.12 μm, respectively. Because RB-SCE microparticles were prepared using sodium caseinate as an inclusive agent, the particle size of the RB-SCE microparticle was relatively high; this was attributed to the strong cohesiveness of sodium caseinate during recrystallization.

Tonic product with RB-SCE

The filling rate of RB-SCE in this study was 33%. A test tonic product with 5% RB-SCE microparticles was prepared to confirm the RB-SCE microparticle solubility, as shown in Figure 4. The RB-SCE microparticles stably dissolved in the test tonic product.

In previous studies, RB-SCE, which contains linoleic acid and γ-oryzanol, exhibited outstanding hair growth-promoting potential, suggesting the use of these substances as hair-loss treatments. Additionally, rice bran extract has a skin moisturizing effect[10]. In our previous studies, there was no indication of *in-vitro* cytotoxicity of RB-SCE on RAW 264.7 cells, single-dose oral toxicity in Sprague–Dawley rats, or single-dose and 4-week repeated dose dermal toxicity of RB-SCE in Sprague–Dawley rats[12, 13]. Thus, RB-SCE is generally thought to be safe for cosmetic applications. Certain biological and pharmaceutical compounds that are oilic or waxy may be more difficult to process[14]. RB-SCE is oilic and insoluble in water. Therefore, for commercial applications, the solubility of RB-SCE must be improved.

In a previous study[9], the formation and stability of rice bran oil-in-water emulsions produced using various emulsifiers (*e.g.*, whey protein, gum Arabic, and modified starch) were investigated. How-

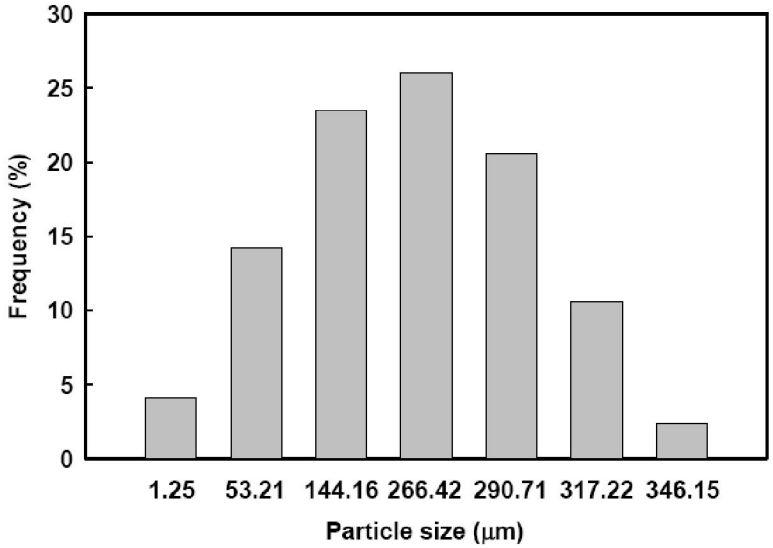

Figure 3 : Particle size distribution of RB-SCE microparticles prepared using the optimal conditions

Figure 4 : Test tonic product with 5% RB-SCEmic roparticles

ever, the results showed that a more effective process was needed to improve the solubility of RB-SCE.

Crystallization with supercritical fluids is a promising new technique. The use of supercritical fluids as solvents or anti-solvents in particle production has been shown by numerous researchers to be useful in modifying particle properties, such as particle size, size distribution, crystal habit, and morphology. Another advantage is the easy separation of the anti-solvent from the particles after precipitation[15].

Several processes involving supercritical fluids and the rapid depressurization of spraying devices, such as the rapid expansion of supercritical solutions (RESS), have been proposed and investigated. In this process, a solid is dissolved in a supercritical fluid that expands adiabatically through a capillary nozzle to a gaseous state. The resulting high supersaturation causes rapid nucleation and precipitation of the desired product as fine particles. In the PGSS approach, the supercritical fluid is solubilized in a molten substance and this solution is expanded in a nozzle. The cooling effect during expansion solidifies the desired substance.

In the GAS method, the supercritical fluid is used in batch mode. The solid is first dissolved in a conventional solvent. The solution is then introduced into a supercritical fluid (anti-solvent), leading to rapid volume expansion of the solution. As a result, the solvent power of the conventional solvent decreases and supersaturation triggers the precipitation of particles. After the solid has precipitated, fresh anti-solvent is used to flush away the solvent.

The semi-continuous gas anti-solvent techniques include the ASES[16], SAS precipitation[17] and solution enhanced dispersion by supercritical fluids (SEDS)[18]. In the ASES and SAS techniques, the solid is dissolved in a conventional solvent and the

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solution is sprayed continuously through a nozzle into the supercritical fluid. The dispersion of solution in the supercritical fluid leads to an expansion of the droplets and the simultaneous extraction of the liquid into the fluid. The solvent power of the conventional solvent decreases dramatically and supersaturation leads to particle precipitation. The SEDS technique resembles ASES and SAS techniques; however, in SEDS, the solution is sprayed continuously and co-currently with a compressed gas or supercritical fluid anti-solvent through a two-fluid nozzle.

In this study, to improve the solubility of RB-SCE, RB-SCE microparticle formation was attempted using the combination of the ASES and PGSS. RB-SCE, food-grade sodium caseinate saturated aqueous solution, and ethanol (co-solvent) (1:3:10, wt%) as raw materials and ethanol as an anti-solvent were used to improve the microparticle formation efficiency. As a result, RB-SCE microparticles, ranging in size from 1.25 to 346.15 μm, were prepared (Figure 4). The median value of the particle size distribution and the mean particle size of the RB-SCE microparticles were 266.42 and 250.12 μm, respectively.

According to Shin *et al.*, (2011)[19], the inclusion efficiencies of the omega-3 fatty acid ethyl ester of fish oil were 17.83%, 16.06%, and 13.99%, respectively, when beta-cyclodextrin, chitosan, and starch were used as carriers. The inclusion efficiency of RB-SCE using sodium caseinate as a carrier in this study was 33%; thus, higher than that of previous studies. RB-SCE microparticles (5%) were stably dissolved in a test tonic product (Figure 4). Further research is needed to confirm the physical and chemical stability of the remaining RB-SCE microparticles with respect to the shelf life of the test tonic product.

CONCLUSION

In conclusion, our results suggest that the combination of the ASES and PGSS is effective for RB-SCE microparticle preparation, and improved the solubility of RB-SCE. Therefore, RB-SCE microparticles prepared using the proposed method should be applicable to food and cosmetic manufacturing.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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