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Supercritical CO_2 extraction of lutein esters from marigold (*Tagetes erecta* L.) enhanced by ultrasound

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ABSTRACT

As a novel technique, supercritical CO_2 (SC-CO_2) extraction enhanced by ultrasound was applied to the extraction of lutein esters from marigold and the extraction curves were described by Sovová model. The mass transfer coefficient in the solid phase (k_s) increased from 3.1×10^{-9} to 4.3×10^{-9} m/s due to ultrasound. The effect of extraction parameters including particle size of matrix, temperature, pressure, flow rate of CO_2, and ultrasonic conditions consisting of power, frequency and irradiation time/interval on the yield of lutein esters were investigated with single factor experiments. The results showed that the yield of lutein esters increased significantly with the presence of ultrasound ($p < 0.05$). The maximal yield of lutein esters (690 mg/100 g) was obtained for a particle size fraction of 0.245–0.350 mm, extraction pressure of 32.5 MPa, temperature of 55 °C and CO_2 flow rate of 10 kg/h with ultrasonic power of 400 W, ultrasonic frequency of 25 kHz and ultrasonic irradiation time/interval of 6/9 s.

1. Introduction

As one of richest sources of natural carotenoids, whose biological functions and commercial value are widely accepted, marigold (*Tagetes erecta* L.) has been of great interest to the food processing and pharmaceutical industries [1–4]. Lutein ester, identified as the principal colouring component of marigold, has been reported to have beneficial effects on human health [5,6]. Research and epidemiological investigations have shown that the risk of age-related macular degeneration, heart disease, lung cancer and skin cancer might be reduced by higher intake of lutein [7–10]. These findings have made it attractive to an expanding international market for dietary supplements.

Conventionally, lutein is extracted from natural matrices using organic solvent processing [11], which is time-consuming and has solvent residues. Interest in alternatives to conventional solvent extraction such as supercritical carbon dioxide (SC-CO_2) extraction is increasing since CO_2 is non-flammable and friendly to environment [12]. Previous literature has explored the possibility of extracting lutein with SC-CO_2 from different matrices [13–15], for example, Wu et al. [16] described lutein extraction process from chlorella using SC-CO_2 combined with 50% ethanol as co-solvent. Rao [17] made the investigation into the SC-CO_2 extraction of lutein diesters from marigold (*T. erecta* L.) flowers. Furthermore, the models of the lutein ester extraction with SC-CO_2 have been reported in literature [18–19].

Several studies have shown that ultrasonic techniques can enhance SC-CO_2 extraction. Riera et al. [20] reported that the yield of almond oil was increased by 20% with the present of ultrasound (US) compared to traditional SC-CO_2 extraction. Balachandran et al. [21] found that the yield of pungent compounds from ginger increased dramatically under the influence of US, with an improvement up to 30% compared with traditional SC-CO_2 extraction. Hu et al. [22] pointed out that at less severe operating condition, the yield for oil and coixenolide from adlay seed could increase by 14% when US was coupled with SC-CO_2 extraction. Luo et al. [23] also showed that US significantly accelerated SC-CO_2 reverse microemulsion extraction of ginsenosides from ginseng.

The objective of the present study was to describe the process of SC-CO_2 extraction of lutein esters from marigold with and without US using Sovová model [24] and compare the differences of model parameters. Moreover, an investigation was made into the effects of extraction parameters including particle size of the matrix, temperature, pressure, flow rate of CO_2 and US conditions such as power, frequency, and irradiation time/interval on the yield of lutein esters.

2. Materials and methods

2.1. Raw material

Fresh marigold pedals were dried under room temperature in dark to obtain a water content of 50–70%, placed in a pool, sprayed with a special fermentation enhancing liquid, pressed

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Nomenclature

a	specific interfacial area (m^2/m^3)
D_{12}	diffusion coefficient (m^2/s)
d	screen size (mm)
d_0	representative particle size, where R = 36.8% (mm)
k_f	mass transfer coefficient in the fluid phase (m/s)
k_s	mass transfer coefficient in the solid phase (m/s)
$\dot{m}_f$	the mass flow of the fluid (kg/s)
m_s	the amount of the raw material (kg)
n	uniformity factor
q	easily accessible solute fraction in the surface of the particles
Q	dimensionless model parameter
R	sum of sieve residue (%), from the sieve experiments
Re	Reynolds number
S	dimensionless model parameter
Sc	Schmidt number
Sh	Sherwood number
u	integration variable in (11)
x_0	initial solute concentration in the solid phase (kg/kg)
y^*	loading concentration of soluble components in CO_2 (kg/kg)
Y	extraction yield (kg extract/kg solid matrix)
Z_k	the dimensionless length coordinate of the boundary between the two extraction sections

Greek letters

ϑ	dimensionless time
ϑ_k	dimensionless time when the soluble material runs out from the external surface
δ	bulk density of raw material (kg/m^3)
ε	void fraction in bed (m^3/m^3)
η	viscosity of the fluid phase (Pa s)
ρ_f	density of the fluid phase (kg/m^3)
ρ_s	density of the solid phase (kg/m^3)
τ	the minimal extraction time (s)

tightly, sealed up with a film and fermented in dark for about 10 days. Fermented marigold petals were purchased from SaiTe Company of Natural Pigments (Qingdao, China) with a moisture content of 6.76 ± 0.05%. The raw material was ground into small particles with a size range of 0.198–0.833 mm and stored in the dark until use.

The particle size distribution was determined by screen analysis. Dry sieving was performed for 20 min with a vertical vibratory sieve shaker (Labortechnik GmbH, Ilmenau). About 60–80 g loading was used at each sieving. The raw material size distribution was determined using a nest of 7 sieves of aperture sizes 0.1, 0.2, 0.315, 0.4, 0.5, 0.63 and 0.8 mm. The mass of fragments remaining on each sieve after the process was used to calculate the distribution of fragments, which was then normalized with respect to the total mass.

For the evaluation of sieve analysis results the Rosin–Rammler–Bennet (RRB) distribution [25] was chosen. The percentage by mass of particles (R) greater than screen size (d) is given as:

$$R = 100 \exp\left[-\left(\frac{d}{d_0}\right)^n\right] \quad (1)$$

where d_0 represents the particle size corresponding to the 36.8th percentile of the cumulative probability distribution (size constant), and n controls the shape of the distribution (uniformity coefficient). The function of the sum of sieve residue (R) was fitted to the experimental data changing the representative particle size d_0 and the uniformity coefficient n, minimizing the sum of the mean square error using STATISTICA 7.1 software.

2.2. Chemicals and reagents

Carbon dioxide with a purity of 99.5% was supplied by Jingcheng Gas Co. Ltd. (Beijing, China). All the solvents (analytical grade) were purchased from Beijing Chemical Co. Ltd. (Beijing, China).

2.3. Equipment and procedure

The schematic diagram of the pilot-scale apparatus used for SC-CO_2 extractions with and without US (Huali Pump Co. Ltd., Hangzhou, China) is shown in Fig. 1. The ultrasound equipment was designed by South China University of Technology (Guangzhou, China). The probe with Langevin type transducer is installed in the upper part of the extractor, and driven by electrical signals from an ultrasound generator, which gives adjustable continuous power outputs at dual frequency (25 and 33 kHz). The ultrasound generator consists of a power amplifier and a special electro circuit designed to justify the power outputs at a constant level during the SC-CO_2 extraction process. The ultrasonic power outputs could be set from 0 to 400 W. The maximal resistant pressure and temperature of the ultrasonic system was 35 MPa and 55 °C, respectively. About 100 g of ground marigold powder was packed into the extraction vessel (350 mm long × 60 mm i.d.). Liquid carbon dioxide was pumped first through the evaporator (a coil can be heated or cooled), then into the extractor by a three-plunger type pump up to the given pressure. A back pressure regulator located at the outlet of extractor was manually adjusted to maintain the desired pressure within an accuracy of ±0.5 MPa. The extractor was enveloped by an electrical heating jacket with a thermocouple attached into the top and the water bath to monitor and control the temperature inside the vessel. The inside vessel temperature was controlled to an accuracy of ±0.5 °C. Following the extraction, CO_2 loaded with extract entered into the separator I (set at 40 °C and 6–8 MPa), where the marigold extract precipitated. Water and volatile components precipitated in separator II (set at 20 °C and 4–6 MPa). The relief valve at the outlet of separator II was kept open to ensure that the CO_2 could be recycled. The flow rate of the CO_2 was measured by a Coriolis mass flow-meter. The extract containing lutein esters was collected from separator I and stored in the dark at 4 °C until sample analysis.

2.3.1. Comparison of SC-CO_2 extraction with and without ultrasound

About 100 g of ground marigold powder was set in the extractor and extracted at 35 MPa, 55 °C without and with ultrasound (US) (power of 240 W, frequency of 25 kHz and irradiation time/interval of 6/9 s) for 6 h. Those extraction parameters were mainly chosen as the moderate values which the equipment could achieve. Total yield (%) at each hour was determined separately. The experimental data were compared with those calculated by mathematical model.

2.3.2. Effect of extraction parameters on lutein ester yields

Extractions were conducted with varying particle size (0.198–0.245 to 0.350–0.833 mm), temperature (35–55 °C), extraction pressure (17.5–32.5 MPa), and flow rate of CO_2 (5–10 kg/h) to evaluate the enhancement of US. Ultrasonic parameters of ultrasonic power varied from 100 to 400 W, ultrasonic frequency from 25 to 33 kHz and US irradiation time from 3 to 9 s with an interval of 9 s between each irradiation. The present work aimed to emphasize the effect of US, therefore, yields of lutein esters for the first 3 h were compared since US exhibited significant