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Extraction of phytochemicals from saffron by supercritical carbon dioxide with water and methanol as entrainer

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ABSTRACT

Saffron (*Crocus sativus* L.) is a perennial herb that belongs to the family Iridaceae. Pigments obtained from saffron are widely used for dyeing and in coloring of food. They also have beneficial health effects. Being heat labile and highly reactive both enzymatically and chemically, the components in saffron such as picrocrocin, safranal, 4-hydroxy-2,6,6-trimethyl-4-hydroxy-1-carboxproducts (HTCC), crocin, and crocetin require mild extraction. In this study, we compared classical extraction with methanol and water and compared these results to supercritical CO_2 extraction performed with and without entrainer. The extracted components were analyzed using high performance liquid chromatography (HPLC). Higher yields of functional components (picrocrocin, HTCC, safranal, α-crocin, deglycosylated crocin) were obtained using supercritical carbon dioxide extraction than by using conventional organic solvent. Yields of picrocrocin and safranal were highest when methanol was used as an entrainer. α-Crocin was optimally extracted at 80 °C and 30 MPa using water as an entrainer.

1. Introduction

Crocus sativus L. (saffron) is a perennial herb that belongs to the family Iridaceae. It is popular because of its delicate aroma, pigment, and bitter flavor rendered by components, safranal, crocin, and picrocrocin, respectively. Saffron flower is composed of six purple petals, three golden-yellow stamens, and one red pistil. Spice saffron is obtained by drying the pistils which consist of three stigmas [1–5]. Saffron has mainly been cultivated in regions of Iran, Turkey, and Greece, but now, it is also successfully cultivated in European countries like Spain, Italy, France, and Switzerland, as well as, in Morocco, Egypt, Israel, Azerbaijan, Pakistan, India, New Zealand, Australia, China, and Japan [6]. To prevent deterioration of saffron, it is important to apply drying process, under controlled temperature and humidity, immediately after harvest [7]. A large proportion of spice saffron fails to dry and is classified as low-grade, as per the ISO3632 standards. The low-grade saffron is not utilized to its full potential and is discarded. Recently, the pigments in saffron have increasingly attracted attention because of their varied functions, such as in prevention of Alzheimer's disease [8–10], cancer [5,11], and neurodegenerative disorders accompanying memory impairment [12,13].

The main active ingredients of saffron are picrocrocin, 4-hydroxy-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde (HTCC), safranal, α-crocin, and deglycosylated crocin, including crocetin. These components tend to degrade in the cell during drying, storage, and extraction. Structural stability of a compound depends on several factors, such as concentration, pH, temperature, enzymes, and oxygen. Though, techniques for stabilization of extracted components have progressed recently, those for controlling the stabilization of components during extraction have not developed [14]. Fig. 1 depicts the scheme for structural changes of picrocrocin to HTCC and safranal. Picrocrocin is decomposed to HTCC, the aglycone, and glucose by the action of hydrolyzing enzymes like β-glucosidase. Thereafter, HTCC changes to stable safranal by dehydration under the influence of protons and heat. In addition, picrocrocin liberates glucose and safranal under the influence of the hydroxyl group or proton and heat. The scheme of the structural changes in α-crocin is depicted in Fig. 2. In an environment with light, oxygen, and moisture, α-crocin undergoes oxidation and hydrolysis, followed by the gradual dissociation

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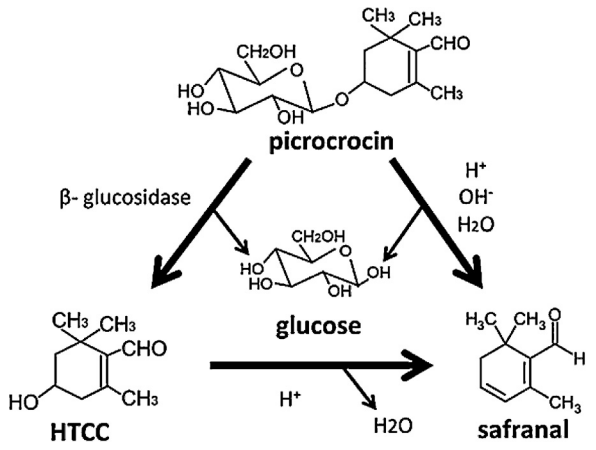

Fig. 1. Scheme of the chemical and enzymatic conversion of picrocrocin to 4-hydroxy-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde (HTCC) and safranal.

of four bound glucose molecules, to become crocetin, which is a deglycosylated crocin. Pharmacological activity of α-crocin is reduced due to the dissociation of glucose [15].

Lozano et al. [7] extracted functional components from three-quality grades of saffron, namely high-quality, moderate quality, and quality-deteriorated saffron. This grade has been classified by the commercial standards of Spain, which is different from the ISO. Water and organic solvents were used as extraction solvents. Crushed saffron was stirred in water or organic solvent for 1 h for extraction at temperatures of 25, 30, 40, and 50 °C. The amount of extracted components changed depending on the extraction solvent and conditions. Optimal extraction was observed when the extraction solvent contained a mixture of water and methanol at 25 °C. In addition, quality-deteriorated saffron contained small amounts of picrocrocin, HTCC, safranal, and α-crocin compared to the high-quality saffron. It was concluded in the above-mentioned study that by using different extraction methods and solvents, different composition of the extracted component could be obtained.

Supercritical fluid extraction (SFE) is a separation method that uses supercritical fluid as an extraction solvent. Supercritical CO_2 ($SC\text{-}CO_2$) is the most used extraction solvent for SFE which is neither toxic, nor flammable. It exhibits selectivity as a result of low viscosity, high diffusivity, and liquid-like density [16–18]. Generally,

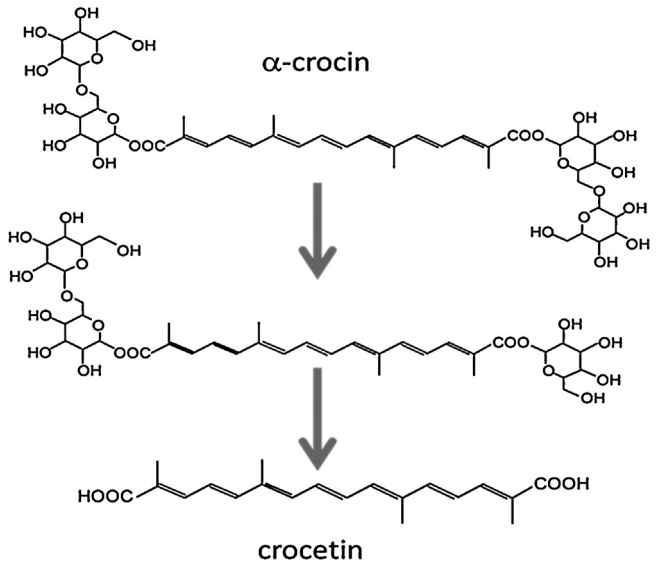

Fig. 2. Deglycosylation process of α-crocin.

less polar components can be extracted by using $SC\text{-}CO_2$. To extract polar components, water or an organic compound such as ethanol is used as an entrainer. Machmudah et al. [19] extracted caffeine and chlorogenic acids from green coffee beans by using liquid water and $SC\text{-}CO_2$ at the same time in the extractor in a hybrid process. They principally focused on obtaining both water- and oil-soluble components by using the entrainer and $SC\text{-}CO_2$ in conjunction during extraction [19].

Ivanovic et al. reported that higher moisture content of plant material of *Helichrysum italicum* resulted in the increase of the extraction rate at the beginning of extraction as well by higher yield of extraction. The moisture content in the *H. italicum* flowers was about three times larger (28.4%) compared to the measured moisture of dry flowers (10.4%). Humidification of *H. italicum* flowers by water presoaking led to increase of extraction rate for 40% and consequently to 25% less $SC\text{-}CO_2$ consumption for achieving same extraction yield at same pressure and temperature (2.45% at 10 MPa; 40 °C) [20]. For the effect of the $SC\text{-}CO_2$ extraction with water, there is a possibility as follows. (a) The dissolution of water into the supercritical phase enhance solubility of solute through an entrainer type mechanism, and/or (b) the water-swelling of plant tissue influence the internal or solid mass transfer resistance and movement of solute to the particle surface [21,22].

In the previous report [23,24], The $SC\text{-}CO_2$ extraction from saffron focused on safranal, which is low polarity compound. In this study, $SC\text{-}CO_2$ extraction was investigated, not only for the improving of safranal extraction rate, also for the recovery of other water-soluble pigments. The effects of parameters viz., temperature (from 40 to 80 °C), pressure (from 20 to 40 MPa), and entrainer (methanol or water), on the extraction of phytochemicals from saffron, were studied.

2. Material and methods

2.1. Materials and chemicals

Spice saffron (native to Iran) was supplied by Sumimoto Packaging Consultant Office (Japan). It has been classified into three stages (A, B and C) by quality. In this study, Grade A that is classified to the highest quality was used. It also meets the criteria of ISO 3632-1. Samples were milled to around 10 μm using a laboratory mill, MF10 basic (Ika Co., Japan). 4-nitroaniline used as an internal standard and HPLC-grade methanol used for analysis were purchased from Wako Pure Chemical Industries, Ltd. (Japan). CO_2 was obtained from Sogo Co. (Japan).

2.2. Equipment

Extraction experiments were carried out using the apparatus shown in Fig. 3. It consisted of a high-pressure pump for CO_2 and entrainer (Jasco PU-2086 Plus, Japan), a heating chamber WFO-400W (Eyela, Japan), a 10 mL extraction vessel (Thar Tech, Inc., USA), a back-pressure regulator (Jasco BP-2080, Japan), a number of collection vials, and a wet-gas meter (Sinagawa Co., Japan).

2.3. Liquid extraction using water or methanol

Saffron was milled to a size of 10 μm using a cutter mill. Using a batch extractor, 100 mL solvent was heated while mixing on a hot stirrer (320–420 rpm) at 40 °C and ambient pressure. A sample of milled saffron (0.5 g) was added when the predetermined temperature condition was achieved and the extraction was begun. 1 mL extraction liquid was collected at 10, 20, 30, 40, 50, and 60 min for to analyze the extracted components.