Optimization of xanthophylls extraction from *Marigold* extractum with supercritical CO_2 by response surface methodology

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Abstract: In this work, xanthophylls were extracted from *Marigold* using supercritical CO_2. A response surface experimental design was used to optimize operating conditions for extraction pressure (22-30 MPa), extraction temperature (50-60℃), CO_2 flow rate (9-15 kg/h). The maximum concentration of xanthophyll in extracts was determined to be 35.8% by high performance liquid chromatography (HPLC), and optimum xanthophylls extraction was obtained at 55℃, 26 MPa and 12 kg/h. The optimization results demonstrated that for xanthophylls extraction, all variables (temperature, pressure and CO_2 flow rate) were to be the influential variables, with the statistical significant effect p-value was smaller than 0.05.

Keywords: xanthophylls, supercritical CO_2 extraction, extraction rate constant, *Marigold* extractum
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1 Introduction

Marigold (*Tagetes erecta L.*) is widely used as colorant in the food and animal feed industry[1]. *Marigold* is commercially cultivated and flower extracts are used as poultry feed supplements for the coloring of egg yolk[2]. The principal pigment of its flower is xanthophyll, a fat-soluble carotenoid. Carotenoids are widespread pigments in plants in which they are involved in photosynthesis and photoprotection, but they are also found in animal tissues where they may act as antioxidants or as tumor-preventing, antimutagenic and immunomodulating agents. The application of carotenoids in medicine and cosmetics is well documented as is their utilization as food additives (colorants and antioxidants). Marigold has been considered as an excellent antioxidant and has been widely used as ingredients for nutritional, cosmetic, and pharmaceutical applications. It is reported in the literature that the risk of chronic disease, such as heart disease, cancer and age-related eye diseases might be significantly reduced by diets rich in xanthophyll[3-6].

Supercritical fluid extraction is an environmentally benign alternative to conventional industrial solvent extraction, with the important advantages of giving products that are completely free from toxic residues. One of the most frequently used supercritical fluid is carbon dioxide (CO_2), because it is neither toxic nor flammable, and also available at low cost and high purity. Because of its moderate critical temperature, CO_2 can be used to extract thermally labile compounds[7-9]. Taking into account these characteristics, CO_2 is an ideal solvent in food, dye, pharmaceutical and cosmetic industries [10-12]. Previous work on supercritical CO_2 extraction of xanthophylls from *Marigold* have been reported in the

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literature[13-16], but the optimization of supercritical CO_2 extraction conditions from *Marigold* extractum have not been investigated. They reported that the materials extracted by supercritical CO_2 were *Marigold* flower petals.

Development of *Marigold* xanthophylls extraction with supercritical CO_2 implies the necessity for optimization of the variables involved in supercritical CO_2 extraction process, i.e. temperature, pressure and CO_2 flow rate. Therefore, the research work presented here involved taking the experimental data and carrying out a response surface experimental design in order to analyze the effects of above-mentioned variables on the xanthophyll yield and the xanthophyll content.

2 Materials and methods

2.1 Materials and chemicals

Samples obtained from Sindchem Qingdao Co., China were *Marigold* extractum and produced through *Marigold* flower petals extracted by hexane and the extracts were dissolved. Standard xanthophyll (99%), HPLC grade of acetone, methanol and acetonitrile were purchased from Sigma Industries Ltd., China. CO_2 (99.99%) was obtained from Xinhua Co., Zibo, China.

2.2 Supercritical CO_2 extraction

The SFE system used in this study was purchased from Huali Supercritical Supplement Ltd. model HL121-50-40 had the following features: one 1 L extractor and two 0.5 L separators.

Fifty grams of samples of Marigold were loaded into a 1 L extraction vessel and the remaining volume was filled with supercritical CO_2. The extraction started until the extraction temperature and pressure reached set condition. Samples were collected every 30 min in 250 mL glass flasks from separation vessel which was 8 MPa of pressure, 40℃ of temperature, and samples were weighed immediately after collected.

2.2.1 Effect of extraction condition on xanthophyll yield or content

Experiments of extraction condition are single factor experiments of three parameters including extraction pressure, extraction temperature and flow rate, the range of which are 18-34 MPa, 45-65℃ and 9-18 kg/h, respectively. Operating conditions of extraction pressure experiments are 55℃ of extraction temperature and 12 kg/h of CO_2 flow rate. Operating conditions of extraction pressure experiments are 26 MPa of extraction pressure and 12 kg/h of CO_2 flow rate. Operating conditions of CO_2 flow rates experiments are 55℃ of extraction temperature and 26 MPa of extraction pressure.

2.2.2 Process optimization

In process optimization for xanthophyll, the effects of three main variables on supercritical CO_2 extraction were simultaneously studied using a three-factor design, with three levels for each factor (low (-1), medium (0) and high (+1)). The 15 experiments were performed and run at random. The selected factors were supercritical CO_2 extraction pressure (*P* in MPa), extraction temperature (*T* in ℃) and supercritical CO_2 flow rate (*F* in kg/h). Table 1 lists the matrix for the experimental design, as well as the values given to each factor, which were selected according to the results of preliminary experiments and single experiments.

Table 1 Factor levels and design matrix

Run	*P*	*T*	*F*	Xanthophyll content/%
1	-1(22)	-1(50)	0(12)	14.4
2	-1	0(55)	-1(9)	22.2
3	-1	0	1(15)	19.8
4	-1	1(60)	0	24.7
5	0(26)	-1	-1	25.2
6	0	-1	1	26.7
7	0	1	-1	28.7
8	0	1	1	32.7
9	1(30)	-1	0	23.9
10	1	0	-1	25.3
11	1	0	1	29.1
12	1	1	0	27.1
13	0	0	0	35.8
14	0	0	0	35.7
15	0	0	0	35.8

Extraction processes were the same to 2.2. Thesection responses were considered to evaluate the concentration of xanthophyll. The extraction yield was calculated according to the ratio of weight of xanthophylls to weight of *Marigold* extractum sample feed into extraction vessel.

2.3 Chemical composition analysis

Xanthophyll was determined by high performance liquid chromatography (HPLC) equipped with Intelligent UV/VIS Detector (VWD, Agilent, USA). Extract dissolved in acetone was injected through a 20 μL loop and separated with an Eclipse XDB-C_{18} HPLC column (5 μm; 4.6 mm× 250 mm) at 30℃. The mobile phase was a binary solvent consisting of 10:90 acetonitrile: methanol flowed at a flow rate of 0.8 mL/min. The xanthophyll was monitored at 470 nm. Peak identification of xanthophyll profile was based on retention times of xanthophyll standard. Weight of xanthophyll in the extract was calculated according by the peak area and calibrations curves of xanthophylls standard.

2.4 Statistical analysis

To determine possible interactions of process variables and their effects on the xanthophyll content of extracts, ANOVA was carried out to analyze the experiment results according to SAS 9.0 program, by SAS Inc (North Carolina, America). The significance level was stated at 95%, with p-value 0.05.

3 Results and discussion

3.1 Effect of extraction condition on xanthophyll yield or content

Figure 1 shows a chromatogram of xanthophyll at 26 MPa, 55℃ and 12 kg/h for 60 min.

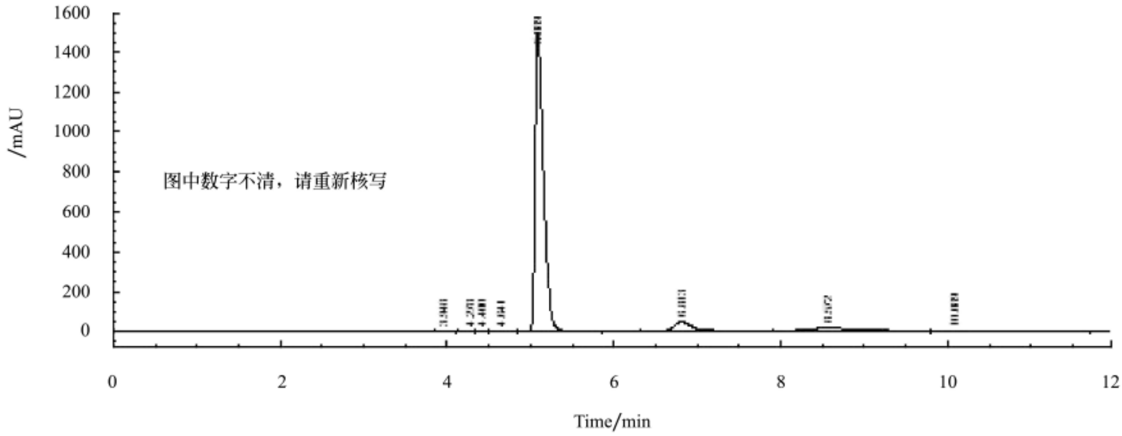
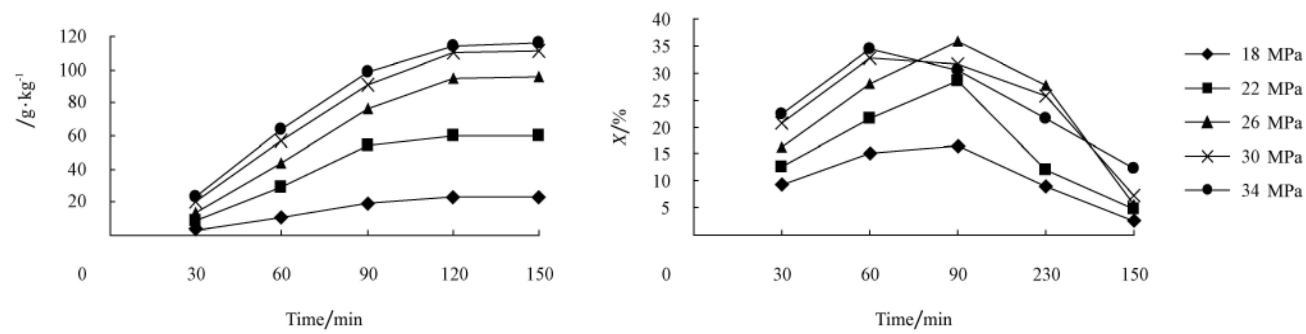


Figure 1 HPLC chromatogram of xanthopyhll extracted at 26 MPa, 55℃ and 12 kg/h for 30 min of extraction time

3.1.1 Effect of pressure

The effects of pressure on the yield of xanthophyll are shown in Figure 2. The title of y axis, accumulated yield of xanthophyll (g Xanthophyll/kg Feed) and Xanthophyll content in the extract/%, are abbreviated to “g/kg” and “X/%”, respectively. The yield of xanthophyll significantly increased with increasing pressure. The dependence on the pressure was expected as the supercritical CO_2 solubility increases with increasing pressure, and therefore the solvent power increases. But the concentration of xanthophyll was highest at 26 MPa and 90 min, but it decreased with increasing pressure. This was accounted for most of the xanthophyll in extractor to be dissolved in supercritical CO_2. After this time, the concentration of xanthophyll was significantly decreased with increasing time. The less concentration of xanthophyll in extractor was, and the more difficulty to extract them was.



a. Xanthophyll yield b. Xanthophyll content

Figure 2 Effects of pressure on xanthophyll yield and content at 55℃ and 12 kg/h

3.1.2 Effect of temperature

Figure 3 shows the effect of temperature on the yield of xanthophyll. As shown in Figure 3, the yield of xanthophyll significantly increased with increasing temperature, however the concentration of xanthophyll slightly decreased with increasing temperature after 90 min. Increase in temperature enhances the solubility of the xanthophyll, which results in higher yields. Instead of that the increase in temperature contributed to the damage of the particle cell wall, xanthophyll availability for extraction was increased.

3.1.3 Effect of CO_2 flow rate

The effect of CO_2 flow rate on the yield of xanthophyll is shown in Figure 4. It is evident from the figure that the yield of xanthophyll significantly increased with the increase of CO_2 flow rate. But content decreased after 90 min and became lower with increasing CO_2 flow rate at 90 min. The increasing CO_2 flow rate caused the increasing intermolecular interaction between the xanthophyll and CO_2, thus increased the solute dissolution. However the more CO_2 flow rate was, the shorter residence time for CO_2 in extract vessel was; it resulted in decreasing yield of xanthophyll. Based on the experimental result, the higher CO_2 flow rate was, the higher extraction efficiency was. Extraction process can be conducted at shorter time with higher extraction yield.

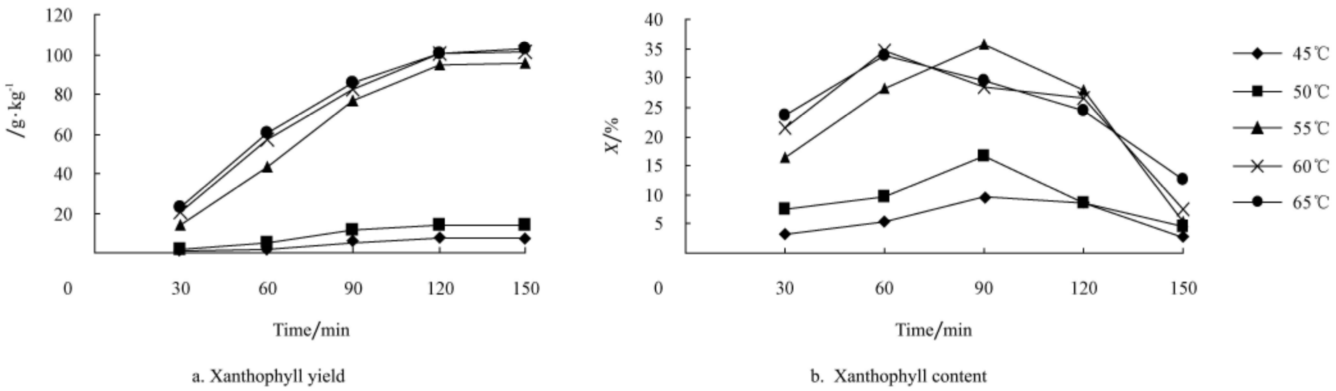


Figure 3 Effects of temperature on xanthopyhll yield and content extracted at 26 MPa and 12 kg/h

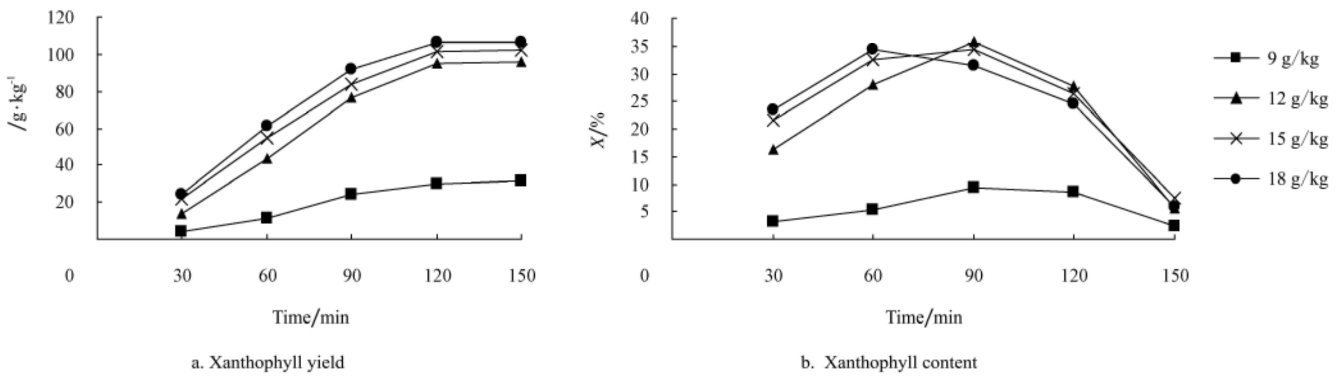


Figure 4 Effects of CO_2 flow rate on xanthophyll yield and content at 26 MPa and 55℃

3.2 Process optimization and analysis of experimental design

As shown in Table 1, the maximum concentration of xanthophyll was 35.8%. The results from the analysis of the experimental design for process optimization are shown in Table 2. The degree of significance of each factor and interactions among variables are represented in the Table 2 by its p-value; when a factor and an interaction among variables have a p-value smaller than 0.05, it significantly influences the extraction process[9]. The results obtained show that the most influential variable on the concentration of xanthophyll was pressure. Flow rate had a less influence on the concentration of xanthophyll. However, there is no possible interaction between temperature and flow rate on the concentration of xanthophyll (*p*-value >0.05), but the other variables had significant influence on xanthophyll extraction (p-value <0.05).

Table 2 Statistical significant effects (p-value) for all variables and possible interactions among variables

P	T	F	P×P	T×T	F×F	P×T	P×F	T×F
0.0002	0.0003	0.0411	<0.0001	0.0002	0.0015	0.0102	0.0186	0.2201

To determine the optimum condition in the range of variables process, concentration of xanthophyll was correlated with temperature, pressure and CO_2 flow rate based on the influential variables and interaction among variables. The empirical mathematical model was obtained by fitting the experimental data using solver in SAS 9.0 program and described in Equation (1). Y is the concentration of xanthophylls in extract, P is the pressure (MPa), T is the temperature (℃) and F is the CO_2 flow rate (kg/h). The resulting correlation coefficient R^2 is 0.9925. The importance of the equation is a model to predict concentration of xanthophylls in extract, thus the yield can be calculated according to the equation and does not need to do experiments.

$$Y = 35.776+3.053P+2.881T+0.86F-8.71825P^2-4.54725T^2-2.91325F^2-1.7845PT+1.5265PF+0.6235TF \quad (1)$$

4 Conclusions

Extraction process of xanthophyll from *Marigold* extractum with supercritical CO_2 has been optimized by response surface methodology. The optimum extraction condition of xanthophyll was found at 55℃, 26 MPa and 12 kg/h. Under this operation condition, xanthophylls content in extract is 35.8%. Otherwise, an empirical mathematical model for the concentration of xanthophylls was set up.

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