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Contributed Paper

Experiments and Statistical Analysis of Supercritical Carbon Dioxide Extraction

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ABSTRACT

In this study, experimental design was employed in order to investigate the effects of operating condition on supercritical carbon dioxide extraction of astaxanthin from *Haematococcus pluvialis* and essential oils from *Amomum krevanh Pierre*. The factors investigated for astaxanthin were operating temperature (40-80 °C), the operating pressure (300-500 bar), and the extraction time (1-4 hours). The results showed that the main effect of operating pressure, the main effect of extraction time, and the interaction effect between operating temperature and operating pressure were significant factors for the astaxanthin yields. From the response surface model of the experimental data, an optimal condition for astaxanthin content was found to be at the temperature of 90 °C, the pressure of 640 bar, and the extraction time of 2.9 hours. This condition yields the highest amount of astaxanthin extract of 22.66 mg/g dry algae. The factors investigated for essential oil were operating temperature (30-70 °C), the operating pressure (90-260 bar), and the extraction time (20-70 min).

Keywords: Supercritical carbon dioxide, Haematococcus pluvialis, Amomum krevanh Pierre, Astaxanthin, Essential oil, Central composite design.

1. INTRODUCTION

Haematococcus pluvialis is one of the most important microalgae producing many kinds of carotenoids such as beta-carotene, zeaxanthin, lutein and astaxanthin. Of these carotenoids, astaxanthin is found in the largest amount. Astaxanthin is a powerful biological antioxidant as it exhibits strong free radical scavenging activity [1] and protects against lipid peroxidation and oxidative damage of LDL-cholesterol, cells and tissues, and cell membranes.

Amomum (*Amomum krevanh Pierre*) is one of the most commonly used spices and herbs. The oil from seeds of amomum is widely used for flavoring purposes in food. Medicinally, they are used for flatulent indigestion, carminative and to stimulate the appetite.

There has been increasing interest to extract astaxanthin from *H. pluvialis* and essential oil from *A. krevanh*, however the conventional extraction of astaxanthin content and essential oils from natural materials requires toxic organic solvents, such as hexane, acetone, or dichloromethane, and to separate these solvents, evaporation is employed which may cause product degradation. For this reason, in recent years, supercritical fluid extraction (SFE) has become an alternative to more conventional extraction procedures. Supercritical fluid extraction (SFE) is a modern technology with increasing applications in pharmaceutical and food processing industry [2]. The physicochemical properties of supercritical fluid are between those of a liquid and a gas [3]. The temperature and pressure that are above the critical values lead these solvents to possess special properties such as high diffusivity and low viscosity [4], allowing them to better diffuse through natural solid matrix, and thus better extract the natural compounds than the conventional liquid solvents. The most frequently employed supercritical solvent in processing of food and natural products is carbon dioxide (CO_2) due to its low toxicity and low critical temperature and pressure [5]. For extraction of carotenoids from marine materials, many recent studies have been carried out to investigate the effect of operating conditions and to determine the optimal conditions for the process [6]. In 1991, Narayanan et al. published their results on the extraction of volatile oil from cardamom seeds with Supercritical carbon dioxide (SC-CO_2). It has been reported that when CO_2-extracted cardamom oil has higher quality than the distilled oil [7].

In most of the previous studies, the process conditions have been optimized merely by conducting one-variable-at-a-time experiments. In such case, no interaction between process variables was assumed, and thus causing biased results. Statistical experimental design has been demonstrated to be a powerful tool for determining the factors effects and their interactions, which allow process optimization to be conducted effectively [8]. The central composite design (CCD) is probably the most widely used experimental design for fitting a second-order response surface. This design has the additional capability of intrinsic confirmation of results and estimation of experimental error.

The aim of the present work is to employ statistical analysis for the investigation of the effects of operating pressure, temperature and extraction time for the supercritical carbon dioxide extraction of astaxanthin from *H. pluvialis*. In addition, the comparison was made between the SC-CO_2 extraction and solvent extraction of essential oil from *A. krevanh*.

2. MATERIALS AND METHODS

2.1 Materials

The *Haematococcus pluvialis* strain powder samples were the commercial algae powder (NatuRose®), manufactured by Cyanotech, USA; they were stored in an oxygen free package and kept in a refrigerator at 4°C until use. Astaxanthin standard was obtained from Wako Chemical, USA. Dried fruits of *Amomum krevanh Pierre* were obtained by local market. The seeds of *Amomum krevanh Pierre* used in this experiment were obtained from dried fruits. The seeds were ground in a blender to produce fine powder. The average particle size was 0.3 mm. The ground samples were stored in a dry place until use. Standards for the identification of chromatographic peaks were from Fluka. SC-CO_2 was carried out with high purity carbon dioxide.

2.2 Supercritical fluid extraction and analysis

Supercritical carbon dioxide extraction was carried out using SFX-220 extraction system from ISCO. The equipment consisted of a 10 ml extractor, a syringe pump, a controller and a restrictor temperature controller.

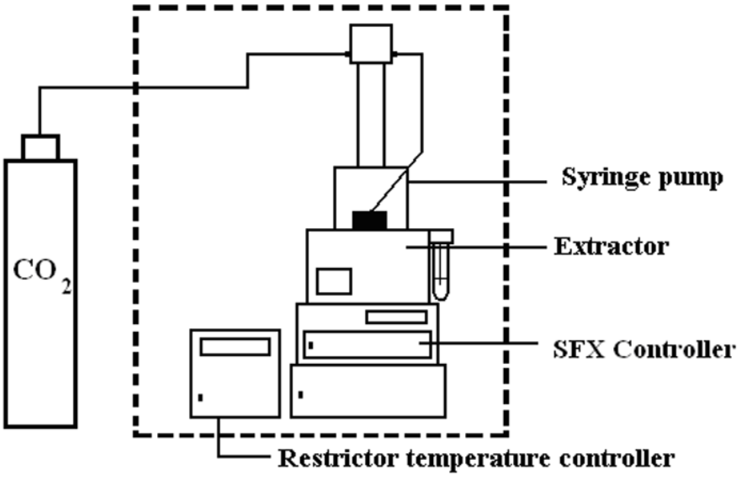

Figure 1. SFX-220 extraction system of supercritical carbon dioxide extraction

For each experimental run of astaxanthin extraction, 0.5 g of dried *H. pluvialis* algae was loaded in the extraction chamber. The extract was trapped in acetone and analyzed using a spectrophotometer, Genesys 20 (Thermo spectronic, USA) at the wavelength of 475 nm. For each experimental run of essential oil extraction, 3.0 g dried ground *A. krevanh* were loaded in the extraction chamber. The extract was trapped in a tube containing *n*-hexane. After extraction, *n*-hexane was removed with nitrogen stream at room temperature and analyzed using a gas chromatography. In addition, the solvent extraction was used for comparison.

2.3 GC analysis

GC analyses were performed using a Shimadzu GC-2010 gas chromatograph equipped with a DB-WAX capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). The SFE samples (1 μl) were injected using the split mode with a split ratio of 1/30. Oven temperature was programmed to increase from 80°C to 130°C at a rate of 5 °C/min. Injector and detector temperatures were held at 230°C and 250°C, respectively.

2.4 Experimental design and statistical analysis

In this study the experimental design was used to evaluate the main and interaction effects of the factors: temperature (X_1), pressure (X_2), and extraction time (X_3) on SC-CO_2 process of *H. pluvialis*. Seventeen experiments were performed with three experiments as the repeatability of the measurements at the center of the experimental domain. The three-level face-center central composite design was used to evaluate both the main and the interaction effects of the operating conditions for astaxanthin extraction, denoted as 0 and ±1. The ranges of experimental variables used in the investigation are temperature (40-80°C), pressure (300-500 bar), and extraction time (1-4 hours). All factors and levels tested were reported in Table 2.1.

Table 2.1 Factors and levels tested for the designed experiment of astaxanthin extraction.

Variables	Levels		
	-1	0	+1
X_1:Temperature (°C)	40	60	80
X_2: Pressure (bar)	300	400	500
X_3: Extraction time (hour)	1	2.5	4

The statistical analysis of variance (ANOVA) of the experimental results was employed to determine the main effect and interaction of the factor effects using SPSS program. The response surface equations were then proposed, from which the optimal conditions were determined.

3. RESULTS AND DISCUSSION

3.1 Supercritical carbon dioxide extraction of astaxanthin from *H. pluvialis*

The relations between each factor and the extract yields were modeled with a 2nd order polynomial model, using the statistical program, SPSS.

The response surface equation obtained from the analysis is:

$$Y = 10.107 - 0.199X_1 + 1.032X_2 + 0.972X_3 - 0.458X_1^2 - 0.393X_2^2 + 0.127X_3^2 + 0.651X_1X_2 + 0.0288X_1X_3 - 0.451X_2X_3 \quad (1)$$

where Y is the astaxanthin yields, X_1, X_2, and X_3 are the operating temperature, the operating pressure, and the extraction time, respectively. The response surface of astaxanthin extract is shown in Figure 2.

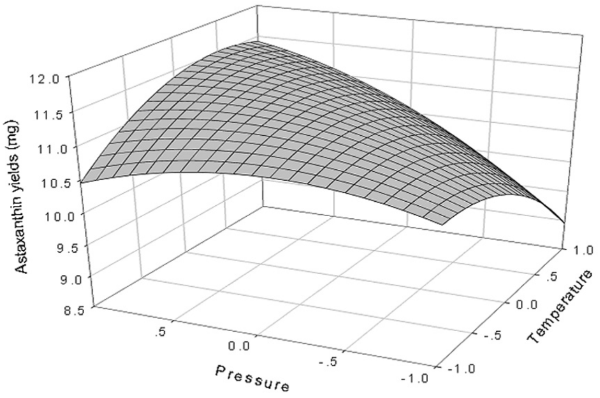

Figure 2. Response surface of astaxanthin yields.

Figure 3 shows the main effect of operating pressure to astaxanthin yields and the results show that the astaxanthin yields were higher when the pressure was operated in the range of 300-500 bar. This agrees generally with theory, which relates the compound solubility in SC-CO_2 with the solvent density. Because the supercritical solvent density increased when the pressure increased, this leads to the increase in the solvent power to dissolve the substances.

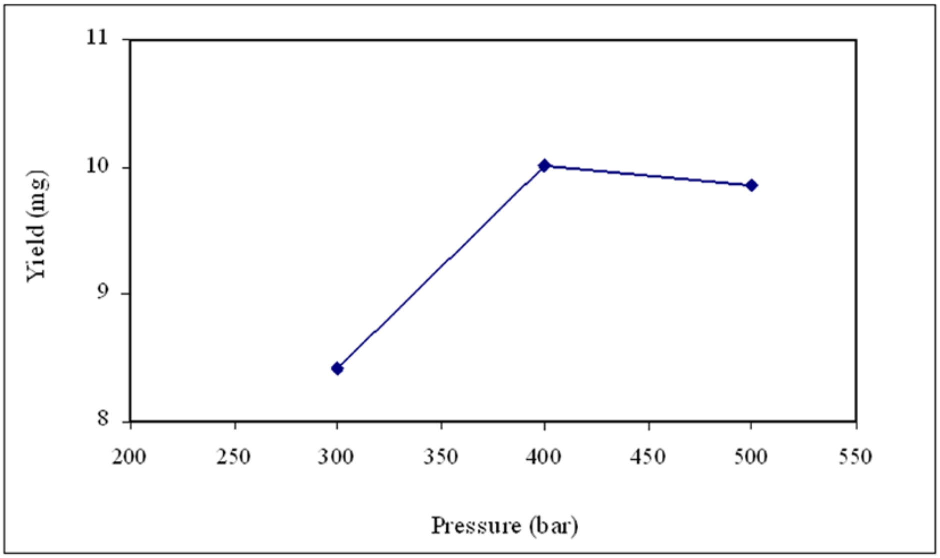

Figure 3. Main effect of operating pressure to astaxanthin yields.

3.1.2 Main effect of extraction time to astaxanthin yields

The main effect of the extraction time to the astaxanthin yields is shown in Figure 4. The results show that the astaxanthin yields were higher with increasing extraction time between 1-4 hours. This is because increasing the time of extraction increases the contact time between the supercritical solvent and the solute.

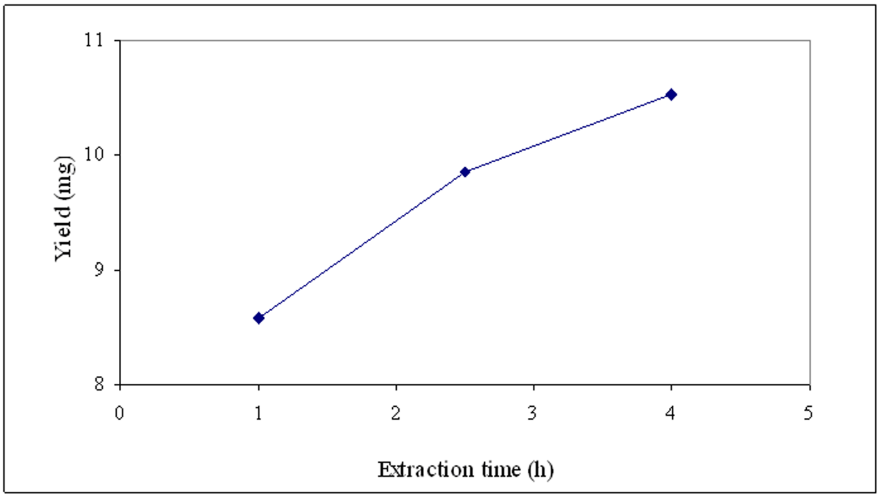

Figure 4. Main effect of extraction time to astaxanthin yields.

The statistical analysis of the experimental results shows that the interaction between operating temperature and operating pressure affect significantly the astaxanthin yield. The interaction effect between the operating temperature and the operating pressure is plotted in Figure 5. The result shows that at pressure 300 bar, the extraction yields were lower when the temperature increased, while at the pressure of 400 and 500 bar, the extraction yields were slightly higher and significantly higher when the temperature increased. The reason of this observation is that the solubility of organic compound depends on a complex balance between supercritical fluid density and solute vapor pressure, which are both controlled by the fluid pressure and temperature.

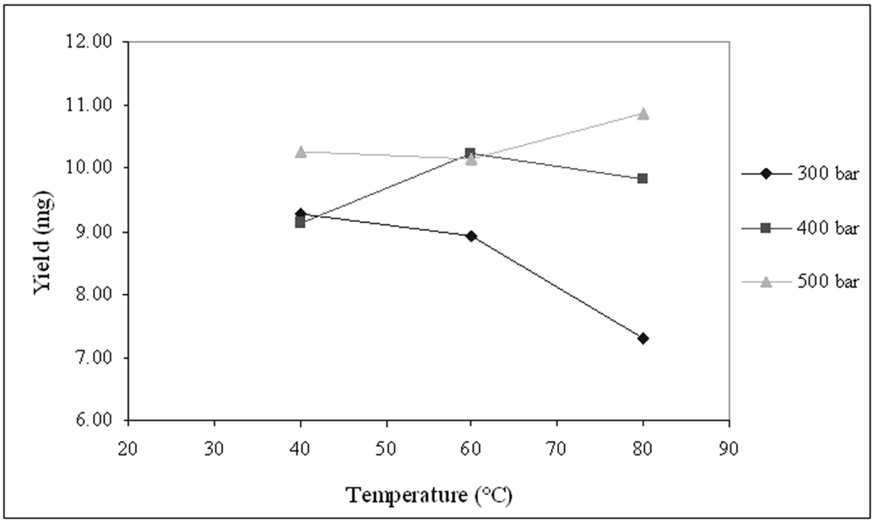

Figure 5. Interaction effect between temperature and pressure for astaxanthin yields

3.2 Supercritical carbon dioxide extraction of *A. krevanh*

Two major components of *A. krevanh* extract obtained by SC-CO_2 measured in this study were 1,8-cineole (3.74%) and beta-pinene (0.48%). The chromatogram of the extract is shown in Figure 6. Compared with the solvent extraction, the extract was found to have lower yields which were 2.96% for 1,8-cineole and 0.36% for beta-pinene, respectively.

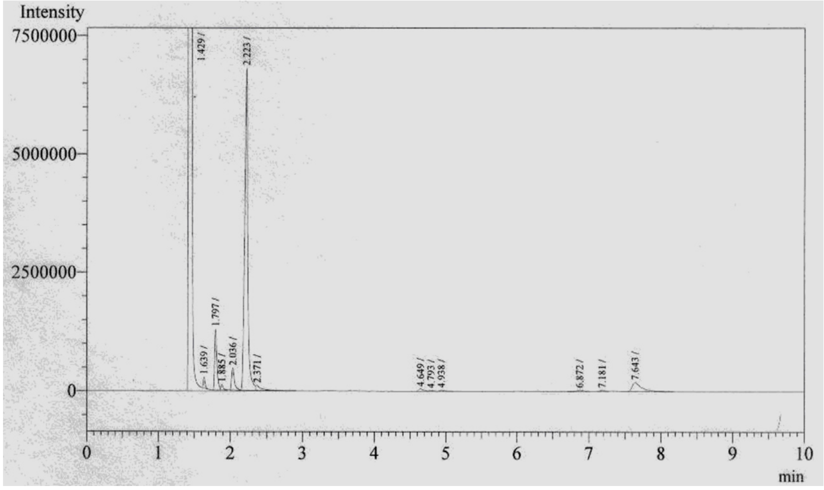

Figure 3.5 Chromatogram of amomum oil by SC-CO_2
(1. beta-pinene, 2. limonene, 3. 1,8-cineole)

4. CONCLUSIONS

In this study, the main effect of pressure (X_2), extraction time (X_3), and the interaction effect between temperature and pressure (X_1X_2) were significant factors affecting astaxanthin yields in SC-CO_2. The optimal condition was proposed within the range of this experiment to be at the temperature of 70 °C, the pressure of 500 bar, and the extraction time of 4 hours. At this condition, the predicted astaxanthin yield was 23.04 mg/g dry algae (or 83.78% recovery). For the extraction of essential oil, the amount of 1,8-cineole and beta-pinene recovered by SC-CO_2 was higher than those obtained with solvent extraction. The detailed statistical analysis will later be presented.

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