

SUPERCRITICAL FLUID EXTRACTION OF α - MANGOSTIN FROM MANGOSTEEN FRUIT RIND (*GARCINIA MANGOSTANA* LINN.)

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Received Date: January 17, 2013

Abstract

The extraction of α -mangostin from mangosteen fruit rind *Garcinia mangostana* Linn cultivated in Vietnam using supercritical carbon dioxide with ethanol as a modifier was studied in this work. Experimental design was used to investigate the effect of operating pressure (20-30MPa), temperature (313-333 K), and solvent flow rate (10-20g/min) on the recovery and the selectivity of α -mangostin. Statistical analysis of experiments indicated that pressure and flow rate of solvent affected on the recovery of α -mangostin; with the selectivity of α -mangostin, pressure, solvent flow rate, temperature and interaction between these factors significantly affected. The central composite design showed that the polynomial regression models were in good agreement with the experimental results with the coefficients of determination from 0.9037 to 0.9666. Based on the proposed model, the optimal condition for extraction that was within the experimental range was found to be at lowest temperature 313 K, highest pressure 30 MPa and the nearly lowest flow rate of solvent 12.71 g/min. It was proved that the model equation developed can be used for predicting the extraction of α -mangostin from *Garcinia mangostana* Linn. The maximum recovery and selectivity of α -mangostin achieved at the optimal conditions was 22.83 mg/g material and 32.77%.

Keywords: Alpha mangostin, *Garcinia mangostana* Linn., Mangosteen fruit rind, Supercritical fluid extraction

Introduction

Xanthenes is a family of polyphenolic compounds that contain a special chemical structure with three aromatic rings. Xanthenes have been reported to have antibacterial activity against a variety of microorganisms including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Enterococcus species*, *Mycobacterium tuberculosis* and *Propionibacterium acnes* [1-5]. Other studies have also proved that xanthenes can be used to induce mitochondrial dysfunction [6], cell-cycle arrest of human colon cancer cells [7]. Moreover, xanthenes and their derivatives are shown to have antifungal and bacterial microbes including antibiotic resistance against *Candida albicans* [8], anti-skin cancer [9] antimicrobial activity [2-4], antioxidant activity [10-12]. Although xanthenes can be synthesized from maclurin and 1,3,6,7 tetrahydroxy [13], a significant amount of xanthenes can be found in natural resources, e.g. mangosteen fruit rind which is widely available in South East Asia. Therefore, studies on extraction of xanthenes from *G. mangostana* Linn are

obviously necessary to utilise the natural supply as well as to enhance the efficiency in xanthonenes production.

Conventional extraction methods such as Soxhlet, maceration, microwave-assisted extraction have been used to extract xanthonenes from mangosteen fruit rind [14-18]. Despite of simple procedures, they suffer from many disadvantages, e.g. low selectivity, great solvent consumption and long extraction time.

In recent years, the supercritical fluid extraction (SFE) method using carbon dioxide (CO₂) has received a great deal of attention due to its highlighted advantages such as high extraction rate, low-temperature processing, minimal thermal degradation of components, minimal undesired reactions and ease of solvent separation [19]. Moreover, CO₂ is non-flammable, nontoxic and environmentally safe. For the extraction of xanthonenes, the SFE exhibits high selectivity [20, 21] which can be improved by the addition of ethanol [10]. Zarena et al [10] showed that the extract yield of xanthonenes was increased from 2.7×10^{-5} (g/g) to 6.3×10^{-3} (g/g) with the additional of ethanol as a co-solvent.

It has been documented that the selectivity as well as the extraction yield of SFE can be improved by adjusting the operating parameters [10, 20, 21]. Therefore, evaluating the effects of process parameters on desirable responses is highly necessary. It will provide not only a better understanding of the overall extraction process but also useful data for optimization and scale up. In this work, the response surface methodology was used to study the effects of operating conditions, i.e. the extraction pressure, the temperature and the solvent flow rate on the recovery and the selectivity of the extraction α -mangostin from *Garcinia mangostana* L. The aim of this study was to evaluate the effect of process parameters on the supercritical carbon dioxide with co-solvent extraction process, the recovery and the selectivity of α -mangostin were the response functions (empirical models) of the three factors that have an effect on extraction efficiency including: temperature, pressure and flow rate of solvent. The methodology applied in this work leads to a mathematical model which describes the effects of process variables on the studied response, and therefore the response behavior can be predicted over the whole experimental field.

Material and Method

Reagent and Material

G. mangostana fruit rind was obtained from a local fruit farm at Binh Duong province, Vietnam in September 2012. The fruit rind was cut into two halves and air dried to the moisture content of 3.75% on dry weight basis. The dried rind was proceeded to hammer milling system to obtain the size distribution in the range of 0.5mm-2 mm. The processed fruit rind was kept away from direct sun light and stored in dry condition until used.

All organic solvents including ethanol, ethyl acetate, n-hexane and water were analytical grade (Merck, Germany). Methanol, acid acetic and water used for HPLC were chromatographic grade (Merck, Germany). α -mangostin standard was purchased from Sigma-Aldrich (Singapore).

Extraction

SC-CO₂ extraction was carried out in a Thar SFE – 100 supercritical fluid extraction systems consisted of a 100 ml extraction vessel, a back pressure, an extractor temperature controller and a temperature controller. The extraction process is illustrated in Figure 1. For each

experiment, 20g of dried mangosteen fruit rind was loaded into the extraction vessel. To distribute the material throughout the extraction chamber efficiently, the vessel was filled with 1mm glass bead. The temperature, the pressure and the flow rate of solvent were configured and maintained at desired values. The extraction process was carried out with 5% (wt. %) ethanol co-solvent with respect to the total mass flow rate of carbon dioxide. The extract was collected and loaded into a vacuum rotary evaporation system to remove ethanol. The removal of impurities was performed prior to the analysis of final extract. Each experiment was done in triplicate to ensure the reliability of the results.

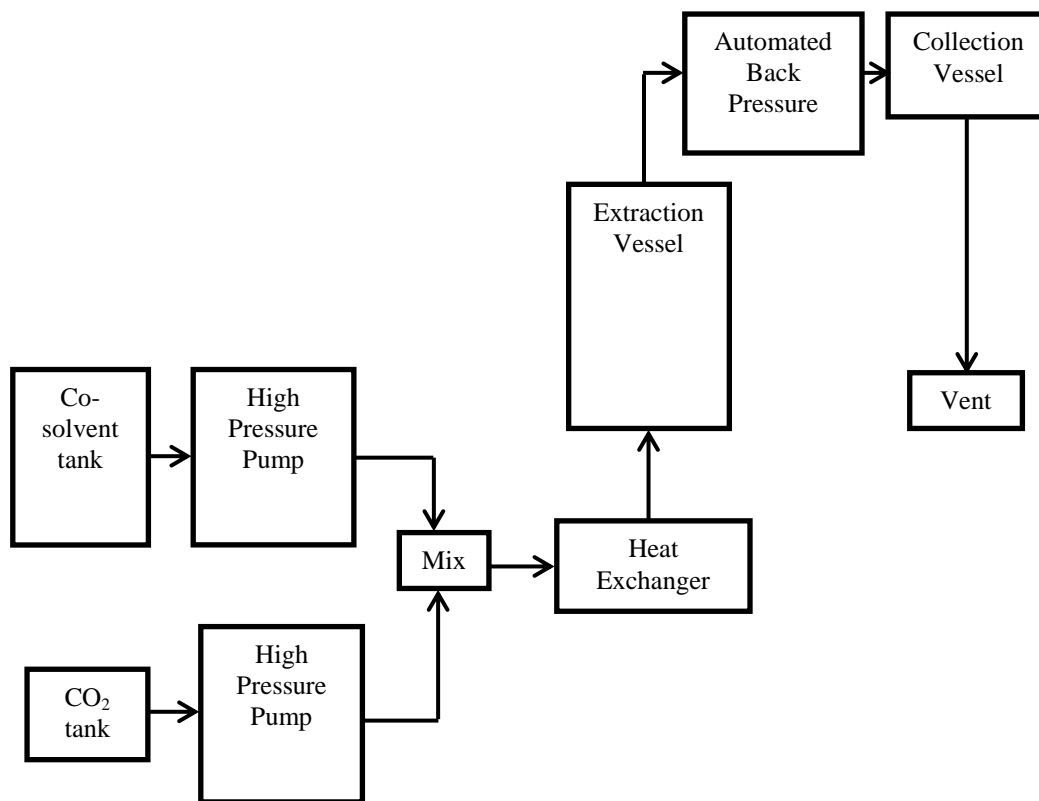


Figure 1. The schematic diagram of supercritical fluid extraction

Assessment of α -Mangostin Content

High performance liquid chromatography (HPLC) was employed to measure the concentration of xanthone in the extract. In this work, HPLC was performed with Agilent 1100 series system (USA) including Agilent 1200 Quaternary pump, Agilent 1200 series online vacuum degasser, Agilent 1200 series auto sampler, Agilent 1200 Thermostatted Column Compartment, Agilent 1200 series diode-array detector (DAD) and Agilent LC Chemstation 32bit software.

The chromatographic separation was accomplished with an Eclipse XDB C18 column (150mm x 4.6mm, I.D., 5 μ m) at room temperature. The mobile phase was methanol and 0.1 % acid acetic in water 85:15 (v/v). The flow rate of the mobile phase was adjusted to 1 mL/min and was applied in the isocratic elution. Each run was followed by an equilibration time of 30 minutes and the injection volume was 5 μ L. The chromatogram was determined at 320nm for α -mangostin by a diode-array detector. The calibration curve for α -mangostin was constructed from samples which concentration varied from 20 to 200 mg/L.

Optimization of SFE Conditions

In order to determine the appropriate extraction time, several extraction processes were performed in which the extraction end-point was varied. The combinations of two different levels of investigated factors, i.e. pressure P (X_1), flow rate of SC-CO₂ and co-solvent Q (X_2) and temperature T (X_3) were shown in Table 1.

The experimental data was fitted with quadratic model with interactions of polynomial response surface function, which has the following expression:

$$Y = b_0 + \sum_i b_i X_i + \sum_{ij} b_{ij} X_i X_j \quad (1)$$

With $i, j = 1, 2, 3$, where Y is the estimated response, X_i is the scaled independent process variable ($-1 = \text{low level}$, $0 = \text{central level}$ and $+1 = \text{high level}$) and the coefficients b_0, b_i, b_{ij} characterize the constant and the linear effects of the variable X_i and the interactions between X_i and X_j , respectively. Regression analysis of the data was carried out with a statistical design package ('Design-Expert' version 8.0.3, Stat Ease, Inc.).

Table 1. The Factors and Levels Tested for the Designed Experiment

X_i (i=1,2,3) Coded Variable	X_1	X_2	X_3
	P (MPa)	Q (g/min)	T (K)
-1	20	10	313
+1	30	20	333
0	25	15	323

The response functions Y_1 and Y_2 are the recovery and the selectivity of α -mangostin, and are defined as:

$$Y_1 \text{ (mg/g material)} = \frac{\text{the content of } \alpha\text{-mangostin (mg)}}{\text{the sample mass(g)}}$$

$$Y_2 \text{ (\%)} = \frac{\alpha\text{-mangostin in SFE extract (g)}}{\text{amount of extract (g)}} \times 100\%$$

The Initial Content of α -Mangostin in Material

In order to estimate the actual α -mangostin content in mangosteen fruit rind, 20g of processed material was loaded into the beaker and extracted using maceration method with 250mL ethanol solvent for 7 days. Every 24hours, ethanol was renewed. The extract was collected and loaded into a vacuum rotary evaporation system to remove ethanol. The removal of impurities was performed prior to the analysis of final extract.

Results and Discussion

Effects of Extraction Time on the Recovery of α -Mangostin

Due to the fact that extraction is a dynamics process, estimating the appropriate extraction time is definitely a critical task. The recovery of α -mangostin corresponding to different extraction end-points are illustrated in Figure 2. It is obvious that the use of longer extraction time provides higher recovery of α -mangostin which is expected to reach the initial content in the material, i.e. 37.919mg/g. There is a dramatic increase of the recovery of α -mangostin within the first 3 hours of extraction. After 3 hours, only a slightly increase of the recovery is

observed. Therefore, the extraction time of 3 hours was chosen and applied to all designed experiments. HPLC chromatogram of standard α -mangostin and collected samples are shown in Figure 3. The retention time of α -mangostin in standard and samples are similar indicating that α -mangostin in the extract can be detected by such method.

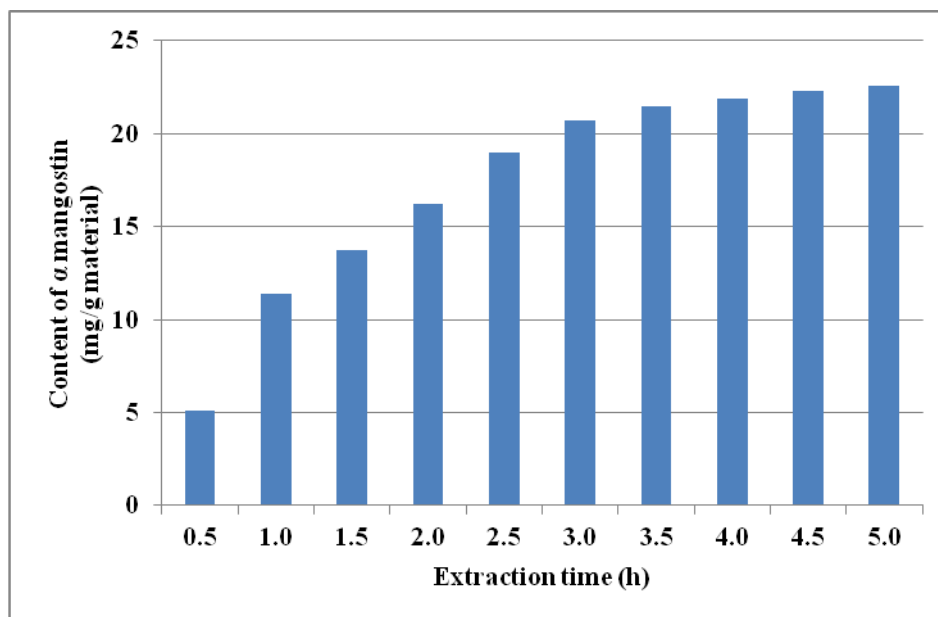


Figure 2. Recovery content of α -mangostin corresponding to different extraction times at 25 MPa, 15 g/min, 323K

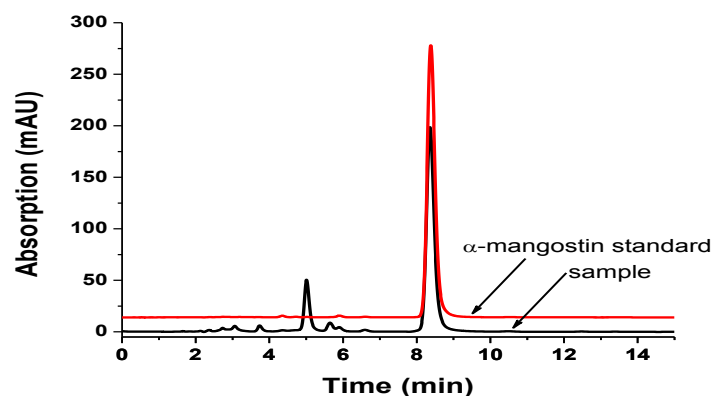


Figure 3. Chromatogram of α -mangostin in standard and sample

Optimization of SFE Conditions

To determine the optimal condition for an efficient extraction of α -mangostin, the extraction pressure, the flow rate of SC-CO₂ and co-solvent and the extraction temperature are the most important factors that need to be investigated. The experimental error was estimated by triplicating the central level experiment. The experimental matrix and the response results for each experiment are shown in Table 2 and sorted by standard order (StO) for an easier

comparison. Wide distributions of response functions, i.e. the recovery and selectivity, can be observed from Table 2. The significant effect factors for the recovery of α -mangostin are the extracting pressure and the flow rate of SC-CO₂ and co-solvent. On the other hand, the extraction pressure, the flow rate of SC-CO₂ and co-solvent, and the temperature introduce complex impacts on the selectivity of α -mangostin. The highest content of α -mangostin (23.88 mg/g material) is obtained at the pressure of 25 MPa, the flow rate of 15 g/min and the extraction temperature of 323K. The corresponding operating conditions for highest selectivity of α -mangostin, i.e. 35.54%, are 30MPa, 10 g/min and 313 K, respectively.

Table 2. Experimental Matrix and Values of Observed Responses

StO.	X ₁	X ₂	X ₃	Extraction Time (h)	Y ₁	Y ₂
1	20	10	313	3	8.86	17.01
2	30	10	313	3	21.33	35.54
3	20	20	313	3	15.26	17.58
4	30	20	313	3	18.27	22.26
5	20	10	333	3	11.11	21.79
6	30	10	333	3	12.21	19.40
7	20	20	333	3	17.81	33.43
8	30	20	333	3	21.13	23.37
9	16.6	15	323	3	11.61	18.80
10	33.4	15	323	3	23.78	25.91
11	25	7	323	3	7.46	20.04
12	25	24	323	3	23.13	24.84
13	25	15	306	3	19.58	23.92
14	25	15	340	3	20.63	24.99
15	25	15	323	3	23.36	27.93
16	25	15	323	3	22.87	27.95
17	25	15	323	3	23.88	27.32
18	Maceration extraction method			-	37.92	15.50

Statistic Data Analysis

The statistical analysis of experimental results was carried out to calculate the estimated coefficients of the polynomial functions of response surfaces for the recovery of α -mangostin. The results pointed out that the response functions with the quadratic model were statistical significance. The value of p-value or “Prob > F” was < 0.05 at 95% confidence. Furthermore, the F-value of the model was of 7.3 and 22.5 implying the model were significant. The polynomial regression models were appropriated with the experimental data with the coefficients of 0.9037 and 0.9666 for the recovery and the selectivity of α -mangostin.

Effects of Operating Conditions on the Recovery of α -Mangostin

In the response surface methodology, the effects of factors on the response functions can be determined by the value of coefficients of coded factors and their significance. The great value of coefficient illustrates the significant effect of the factor on the response function and the

value of "Prob > F" less than 0.05 indicates the mean model. The coded and actual regression functions are shown as following.

$$\text{Code regression function: } Y_1 = 23.47 + 2.96X_1 + 3.32X_2 - 2.36X_1^2 - 3.21X_2^2$$

$$\text{Actual regression function: } Y_1 = -1753.3 + 14.79P - 4.49Q - 0.09P^2 - 0.13Q^2$$

The results showed that X_1 , X_2 , X_1^2 and X_2^2 were significant model terms ("Prob > F" < 0.05) which indicated that the extraction pressure and the flow rate of SC-CO₂ and co-solvent were the main factors affecting the recovery of α -mangostin.

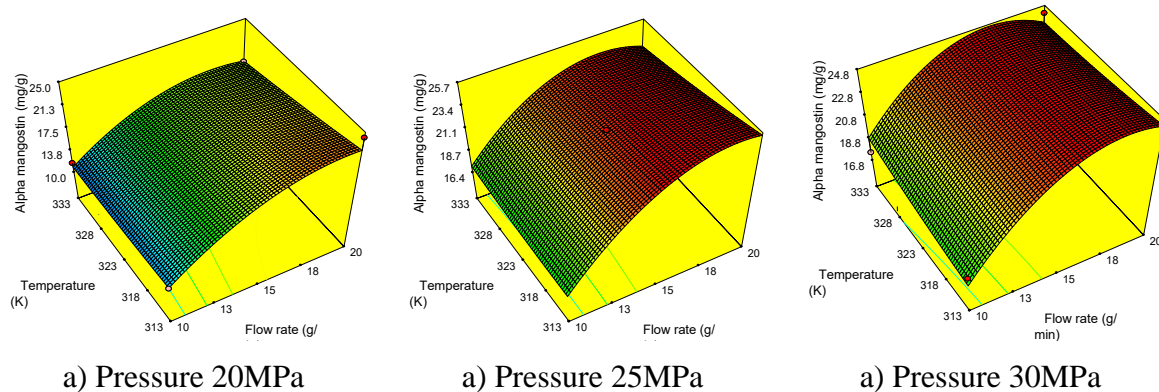


Figure 4. Three-dimensional plot of the response surfaces for the recovery of α -mangostin

Figure 4 illustrated the three-dimensional plots of the response surfaces for the recovery of α -mangostin calculated from the empirical model. The main feature of Figure 4 was that the recovery of α -mangostin was enhanced by the increase of solvent flow rate. It was demonstrated clearly the value of coefficients of coded factors and the figure that at the low operating pressure, the recovery increased whilst increasing pressure, however at the high pressure, increased pressure led to the decrease of the recovery. It was read from the predicted model that the extraction pressure and the flow rate of SC-CO₂ and co-solvent were the main factors affecting the response functions whilst the extraction temperature did not exhibit significant effect. This behaviour can be explained that for the supercritical fluid extraction, the solubility of natural compounds is increased with the increase of the extraction pressure. In other words, for such high molecular weight and non-volatile organic compound as α -mangostin, its solubility in SC-CO₂ is increased when pressure increases.

Effects of Operating Conditions on the Selectivity of α -Mangostin

The coefficient values of the coded and actual factors of the response surfaces corresponding to the selectivity of α -mangostin are presented in Y_2 function. The value of "Prob > F" less than 0.05 indicates that the model terms are significant, i.e. X_1 , X_1X_2 , X_1X_3 , X_2X_3 , X_1^2 , X_2^2 for the selectivity of α -mangostin. It means the selectivity of α -mangostin is affected by three factors and their interactions. The coded and actual regression functions are expressed as following:

$$\text{Code regression function: } Y_2 = 27.67 + 1.67X_1 - 2.69X_1X_2 - 4.46X_1X_3 + 3.54X_2X_3 - 1.68X_1^2 - 1.65X_2^2$$

$$\text{Actual regression function: } Y_2 = -1451.05 + 34.11P - 0.11PQ - 0.09PT + 0.07QT - 0.07P^2 - 9.36 \times 10^{-3}T^2$$

Figure 5 shows the three-dimensional plots of the calculated selectivity of α -mangostin as functions of the actual process variables.

The value of the coefficients of the coded factors as well as the results in Figure 5 illustrates complicated effects of the operating conditions on the selectivity of α -mangostin. It can be seen that the selectivity is increased with the increase of the temperature at low pressure and high flow rate. However, at high operating pressure and low flow rate, the increase of the extraction temperature decreases the selectivity of α -mangostin. In general, high selectivity are obtained at high pressure and high temperature. The greater solvent flow rate leads to the higher value of the selectivity.

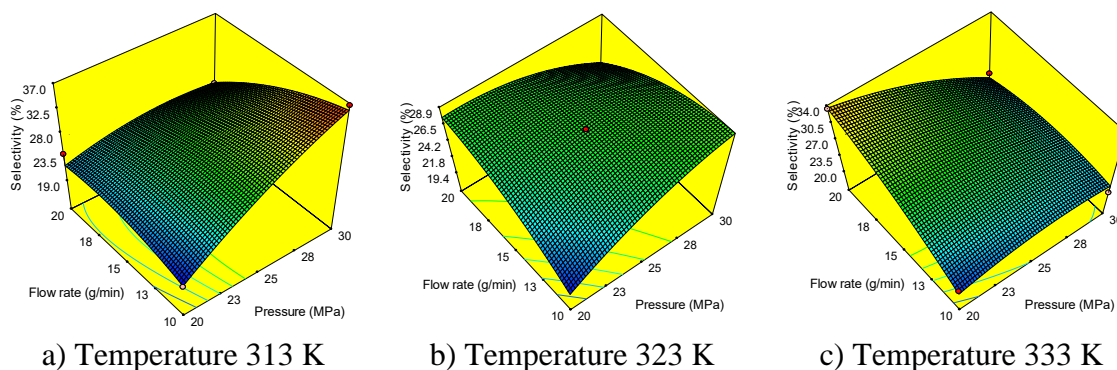


Figure 5. Three-dimensional plot of the response surfaces for the selectivity of α -mangostin

Optimal Operating Conditions of the SFE of α -Mangostin

The optimization of the response functions with multivariate factors was done with the desire of maximum content and selectivity of α -mangostin within the experimental range. The number solution were performed by using a statistical design package ('Design-Expert' version 8.0.3, Stat Ease, Inc.). Figure 6 plots the values of desirability depending on the operating parameters.

The optimal operating conditions and the corresponding response functions are shown in Table 3. The optimal condition in the investigated is the extraction pressure of 30MPa, the solvent flow rate of 12.71 g/min and the temperature of 313K. The recovery and the selectivity obtained at corresponding condition are 60.2% and 32.77%, respectively. Comparing with the maceration method in which the selectivity of α -mangostin is 15.5%; the SFE promises a simple purification process.

Table 3. Optimal Operating Condition

	X_1	X_2	X_3	Y_1	Y_2
Prediction	30	12.71	313	22.83	32.77

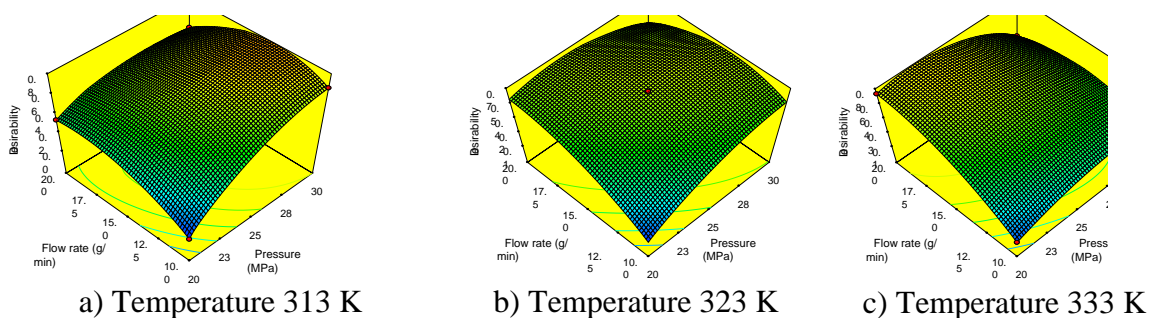


Figure 6. The plot of optimal desirability vs. the operating parameters

Conclusions

The SFE of α -mangostin from *Garcinia mangostana* L. was performed at different operating conditions, i.e. the extraction pressure of 20-30MPa, the flow rate of SC-CO₂ and co-solvent range of 10-20g/min and temperature of 313-333K. The significant parameters for the recovery of α -mangostin were the extraction pressure and the solvent flow rate. The developed model was used for predicting the recovery of the extraction of α -mangostin from *Garcinia mangostana*. A quadratic regression model was proposed to reasonably describe the experimental results. Basing on the proposed model, the optimal conditions to obtain the highest content and selectivity of α -mangostin were determined at the extraction pressure of 30Mpa, the solvent flow rate of 12.71 g/min and the extraction temperature of 313 K.

Acknowledgement

This research is funded by University of Technology under the grant number T-KTHH-2013-39.

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