Extraction of lutein from marigold flower using solvent extraction

P Kasemwattanarot¹, S Chuachang¹, P J Seepheng¹ and P Ramakul¹, ²

¹ Department of Chemical Engineering, Faculty of Engineering and Industrial Technology, Silpakorn University, Nakon Pathom 73000, Thailand
² Corresponding author. E-mail address: ramakul_p@su.ac.th

Abstract. The solvent extraction of lutein from marigold flowers was studied. The influence of three variables namely, the extraction temperature, ratio of the marigold flowers in the solvent, and ratio of ethanol in the solvent were investigated by a central composite design (CCD). The relationship between the yield and these variables was obtained by a regression model. The optimum condition was also determined. The highest yield for lutein (91.4%) was predicted by the regression model, which showed good agreement with the experimental results.

1. Introduction

Lutein is a yellow pigment that is synthesized from plants and can be found in green leafy vegetables in high quantities; such as, spinach, yellow carrots, and kale. Lutein is a member of the carotenoid group, which is used as a food additive in order to adjust the flavor and color. Lutein also provides benefits for human health because of its strong antioxidant properties, and can help maintain normal vision for humans [1,2]. Additionally, lutein significantly inhibits breast cancer cell growth [3]. Dwyer et al., 201 [4] ameliorating cardiovascular diseases. Lutein is also an accessory pigment by absorbing light energy in a blue light region and changing it into chlorophyll. Furthermore, carotenoids containing lutein are involved in photoprotection; that is, eliminating photooxidative damage because of over-illumination [5].

Marigold flower is a high source of lutein. As such, for extracting lutein from marigold flowers, the solvent extraction method is essential because lutein is bound with a light-harvesting complex (LHC). However, the extraction of supercritical carbon dioxide is not effective for the extraction of lutein from marigold flowers, as it is a polar molecule. Polar organic solvents; such as, ethyl ether, methanol, and ethanol can increase the extraction yield of lutein. Yen et al. [6] and Macias-Sanchez et al. [7] also reported that the extraction of lutein from Scenedesmus almeriensis by supercritical fluid extraction (SFE) provided a low yield of lutein, but a high yield of beta-carotene (less polar). In addition, Ruen-Ngam et al. [8] extracted lutein from Chlorella vulgaris by solvent extraction that resulted in a good efficiency of 53% of lutein recovery and 43% of selectivity with the lutein content of 7.9 g/kg dry weight. Therefore, the extraction technique from marigold should be a promising method for the extraction of lutein.

In this study, ethanol was selected as a cosolvent because it was a safe solvent with a low environmental impact. The mixture of water and ethanol was used to extract lutein from marigold flowers by a one-step extraction process. The samples were analysed by an ultraviolet-visible (UV-Vis) spectrophotometer. Although the product (lutein) was still in the solvent, this method was reliable because it was simple, rapid and economical. The method achieved the best balance between the extraction performance and the complicated apparatus. The influences of the composition of ethanol, solvent/solid ratio, and pH of the solvent were investigated. The optimum point was determined by a central composite design CCD.
2. Experiment

2.1 Materials, Chemical and Apparatus
Marigold flowers were collected from a shrine in Thailand. They were milled and sieved through a 200-mesh sieve (the size was in a range between 62-74 µm) for use in all experiments. The analytical standard of lutein was purchased from Sigma® (Germany). The main solvent was double-deionized water (Milli-Q; Millipore, Billerica MA, USA), with 18.2 MΩ·cm⁻¹ conductivity, and the ethanol (Labscan®, Ireland) was of an analytical grade. The concentration of lutein was determined by a UV-Vis spectrophotometer at 470 nm [9] by using a calibration curve between the absorbance and lutein concentration standard with a linearity of 0.9991.

2.2 Procedure
The procedure is shown in Figure 1. A total of 20 experiments were run in triplicate using 100 g of marigold flowers placed in a beaker. After that, 300 ml of a mixture between water and ethanol was added. The samples were mixed using a magnetic stirrer with a stirring speed of 100 rpm for 30 minutes to achieve the equilibrium. Finally, the marigold flowers part was removed by a 0.25 µm filter (Double-Ring; Hangzhou, China), and the extracted solutions were sampled into the UV-Vis spectrophotometer.

2.3 Experimental Design
The extraction temperature, solvent/solid ratio, composition of the ethanol in the water were varied between 41.6-58.4°C, 0.5-1.5, and 23.2-56.8% (v/v), respectively. A CCD [10] was performed to determine the influences of these factors on the extraction yield. The results were compiled on five levels: -α, 1, 0, +1, and +α, where $\alpha = 2^{n/4}$ and n was the number of the variables. Therefore, in this research, the $\alpha$-value for four variables was 1.68. The coded values and actual values are shown in Table 1, and there were 20 conditions of the experiment that are tabulated in Table 2.
### Table 1. Actual levels and CCD code levels.

| Variable                                      | Unit | -1.68 | -1   | 0    | 1    | 1.68 |
|-----------------------------------------------|------|-------|------|------|------|------|
| Temperature (A)                               | °C   | 41.6  | 45.0 | 50.0 | 55.0 | 58.4 |
| Solid/solvent ratio (B)                       | g/100 mL | 0.5  | 0.7  | 1.0  | 1.3  | 1.5  |
| Composition of the ethanol in the water (C)   | %(v/v) | 23.2 | 30.0 | 40.0 | 50.0 | 56.8 |

### Table 2. Experimental conditions by CCD and the experimental results.

| Exp. no. | Extraction Temperature (°C) (A) | Solid/solvent ratio g/100 ml (B) | Composition of the ethanol in the water (%) (C) | %Yield |
|----------|---------------------------------|----------------------------------|-----------------------------------------------|--------|
| 1        | 45.0                            | 0.7                              | 30.0                                          | 63.21  |
| 2        | 55.0                            | 0.7                              | 40.0                                          | 63.90  |
| 3        | 45.0                            | 1.3                              | 30.0                                          | 58.90  |
| 4        | 55.0                            | 1.3                              | 30.0                                          | 68.83  |
| 5        | 45.0                            | 0.7                              | 50.0                                          | 65.37  |
| 6        | 55.0                            | 0.7                              | 50.0                                          | 85.00  |
| 7        | 45.0                            | 1.3                              | 50.0                                          | 61.77  |
| 8        | 55.0                            | 1.3                              | 50.0                                          | 89.30  |
| 9        | 41.6                            | 1                                | 40.0                                          | 71.69  |
| 10       | 58.4                            | 1                                | 40.0                                          | 74.00  |
| 11       | 50.0                            | 0.5                              | 40.0                                          | 77.61  |
| 12       | 50.0                            | 1.5                              | 40.0                                          | 64.78  |
| 13       | 50.0                            | 1                                | 23.2                                          | 52.51  |
| 14       | 50.0                            | 1                                | 56.8                                          | 69.10  |
| 15       | 50.0                            | 1                                | 40.0                                          | 77.37  |
| 16       | 50.0                            | 1                                | 40.0                                          | 75.40  |
| 17       | 50.0                            | 1                                | 40.0                                          | 75.93  |
| 18       | 50.0                            | 1                                | 40.0                                          | 76.02  |
| 19       | 50.0                            | 1                                | 40.0                                          | 75.69  |
| 20       | 50.0                            | 1                                | 40.0                                          | 76.37  |
2.4 Lutein Content and % Yield Determination

Firstly, the total amount of lutein in the marigold flowers had to be determined. The marigold flowers (100.0 g) were soaked by a solvent (water + ethanol) for 20 minutes to achieve the equilibrium. When the extraction was completed, the solvent was gathered and stored in a bottle, while the marigold flowers were taken to be extracted again by using the virgin solvent. The extraction was done 10 times to be certain that the concentration of the lutein in the solvent was zero. Finally, the total lutein content in the marigold flowers was determined by summing up the lutein content in all 10 solvent beakers. It was found that the total content of the lutein in 100.0 g of marigold flowers was 19.04 mg. This ratio of the value of 19.04 mg/100g was used as the initial content of lutein for all the experiment samples. The extraction yield is defined in Eq.(1) as a ratio between the weight of the lutein extracted into the solvent (Wc,ex) and the initial content of lutein in the marigold flowers (Wc,i). For example, when 100.0 g of marigold flowers was extracted, the initial content of the lutein was 19.04 mg.

\[
\%Yield = \frac{W_{c,ex}}{W_{c,i}} \times 100
\]  

3. Results and Discussion

From the experimental results by the central composite design (CCD), the effects of the temperature (X1), solid/solvent ratio(X2), and the composition of the ethanol in the water (X3) were investigated (Figures 2-4).

3.1 Effect of the Temperature

As can be seen in Figure 2, the yield of the lutein gradually increased as the extraction temperature was increased from 41.6°C to 58.4°C because a high temperature could reduce the viscosity and increase the diffusivity of the solvent into the marigold flowers’ cells. Simultaneously, this enhanced the solubility of the lutein in the cells, which resulted in a higher rate of dissolution into the solvent. However, when the temperature was above 55°C, the yield started to decrease due to the disintegration of the solid matrix, which prevented the lutein molecules from penetrating and dissolving into the solvent [11].
3.2 Effect of the Solid/Solvent Ratio

As shown in Figure 3, the yield was lower with an increasing solid/solvent ratio. It was noted that the solid/solvent ratio increased by decreasing the volume of the solvent. This could be explained by the mass transfer in Eq.(2).

\[ J = k'(C_s - C_B) \]  

Where \( J \) is the flux of the lutein transferred from the solid matrix (mg/cm²-s), \( k' \) is the mass transfer coefficient (cm/s), and \( C_s \) and \( C_B \) (mg/cm³) are the concentrations of lutein in the solvent that are very near the solid matrix and in the bulk solvent, respectively. From the experimental result, when the solvent volume was decreased, the ratio of the solid part (marigold flower) rose, and there was a lower difference between \( C_s \) and \( C_B \). This caused a lower mass transfer because when the volume of the solvent was decreased, \( C_B \) increased, and as can be seen from Eq.(2), this resulted in a lower flux.
6

3.3 Effect of the Composition of the Ethanol in the Water

It can be observed in Figure 4 that the yield of the lutein enhanced when the composition of the ethanol in the water increased. This could be described by the fact that ethanol could penetrate the cell walls. Therefore, this made a higher contact area between the solvent and the solids. The solvent could then easily gain access to leach the lutein out and dissolve it [11].

Fig. 3. Plot of the effect of the solid/solvent ratio on the %yield of the lutein.

Fig. 4. Plot of the effect of the composition of the ethanol in the water on the %yield of the lutein.
Fig. 4. Plot of the effect of the composition of the ethanol in the water on the %yield of lutein.

3.4 Regression Model and Statistical Analysis

A second-order polynomial regression model was employed for modeling the yield of the lutein. The experimental results were statistically analysed using Minitab 17 statistical software (Minitab Inc., State College, PA, USA).

As a result, the quadratic regression equation for the independent and dependent variables could be expressed by the correlation coefficient (R²) of 0.92 as follows:

\[ Y = 76.07 + 4.52A - 1.48B + 5.45C - 0.80A^2 - 1.38B^2 - 5.05C^2 \]  
\[ \text{(3)} \]

The value of the coefficients characterized the magnitude of the effect. The sign (+,-) indicated the way that this would respond to the %yield of the lutein. From the ANOVA, the P-values of B, A², B², AB and BC were higher than 0.05. This inferred that these effects were not significant with a 95% confidence [10]. Therefore, the B, A², B², AB and BC terms were omitted from Eq.(3), and the regression equation was shortened with the R² of 0.85 as follows:

\[ Y = 76.07 + 4.52A + 5.45C - 5.05C^2 + 4.57AC \]  
\[ \text{(4)} \]

3.5 Optimisation and Model Validation

The significant factors; namely, extraction temperature (A) and the composition of the ethanol in the water (C), were optimized using a response surface. Figure 5 shows the 3-D response surface plot, which was created by Eq. (4). From the observation to the response surface plot, the extraction temperature of 55°C (A=1.00) and composition of the ethanol in the water of 56.8% (C=1.68) were selected to be the optimal operating conditions. The highest yield of 91.48% was predicted by Eq.(4).

In order to validate the optimized conditions, the extraction temperature (A) and solid/solvent ratio were set at the optimized conditions of 55°C and 56.8%, respectively, in the various amounts for the initial mass of marigold flowers of 150 g, 200 g, and 250 g, respectively. The results are shown in Figure 5 and indicate that the yields were approximately 90% when the initial mass of marigold flowers was increased, which demonstrated that the optimized conditions predicted by the CCD and Eq.(4) were satisfactory.
4. Conclusion

A simple, rapid, and economical solvent comprising ethanol/water could extract lutein from marigold flowers. High yields of lutein were obtained. The extraction temperature of 54°C and the composition of the ethanol in the water of 56.8%(v/v) were the optimal conditions, and the yield of 91.4% was predicted by the regression equation. The validity of the equation was evaluated by being compared to the experimental data, which resulted in achieving a good agreement.
5. Acknowledgment

The authors greatly appreciate the financial support by the Silpakorn University Research, Innovation and Creative Fund. Thanks is also given to the Department of Chemical Engineering, Faculty of Engineering and Industrial Technology, Silpakorn University, Thailand, for the chemical and apparatus support.

6. References

[1] Perry A, Rasmussen H, Johnson E J, 2009 J. Food Compos. Anal. 22 9

[3] Barbara D A, William W A 2002 Science. 298 2149

[3] Gong X, Smith J R, Swanson H M, Rubin L P, 2018 Molecules. 23, 905

[4] Dwyer J H, Navab M, Dwyer K M, Hassan K, Sun P, Shircore A, Hama-Levy S, Hough G, Wang X, Drake T, Merz C N, Fogelman A M 2001. Circulation 103 2922

[5] Frank H A, Cogdell R J, 1996 Photochem. Photobiol. 63 257

[6] Yen H W, Chiang, W C, Sun C H 2012 J. Taiwan Inst. Chem. Eng. 43 53

[7] Macias-Sanchez M D, Fernandez-Sevilla J M, Fernandez F G A, Garcia M C C, Grima E M, 2010 Food Chem. 123 928.

[8] Ruen-Ngam D, Shotipruk A, Pavasant P, Machmudah S, Goto M 2012. Chem. Eng. Technol. 35 255

[9] Goula A M, Ververi M and Adamopoulou A 2017 Ultrason. Sonochem. 34 821

[10] Montgomery D C 2013 Design and analysis of Experiment. (John Wiley and Sons Inc.)

[11] Calvo M M, Dado D, Santa-Maria G 2007. Eur. Food. Res. Technol., 224 567