Response surface methodology optimization of α-mangostin extraction using betaine-1,2-propanediol deep eutectic solvent

Kamarza Mulia*, Irfan Faisal Pane and Elsa Krisanti
Chemical Engineering Department, Faculty of Engineering, Universitas Indonesia, Depok 16424, Indonesia

*Corresponding author's e-mail: kmulia@che.ui.ac.id

Abstract. Mangosteen pericarp contains α-mangostin, the most abundant xanthone that is useful as an antioxidant. Deep eutectic solvent (DES) was used in this study as a green solvent for extraction of α-mangostin. Our previous study shows that a DES, consisting of betaine as the hydrogen bond acceptor and 1,2-propanediol as the hydrogen bond donor, having the highest extraction yields compared to other betain-based DESs. In this study, the operating condition of the solvent extraction process has been optimized using the response surface methodology approach, based on the Box-Behnken design. The highest calculated α-mangostin extraction yield obtained was 4.2% (w/w), corresponding to an extraction temperature of 56.5 °C, mangosteen powder to DES mass ratio of 0.12, and extraction time of 6.9 hours. The optimum temperature was found to be the combined effect of enhanced leaching of the mangosteen pericarp matrix by the DES and α-mangostin degradation at higher temperatures.

1 Introduction
Mangosteen (*Garcinia mangostana* L.) is a tropical fruit known for its health benefits. The pericarp of the mangosteen contains phenolic compounds known as xanthones that have significant antioxidant, anti-cancer, anti-virus and anti-microbial properties [1]. The traditional method to extract bioactive compounds from mangosteen is solvent extraction [2,3] that uses a large amount of organic solvent that bring about air and water pollution due to their high vapor pressures and non-biodegradable nature [4]. Solvents that do not pollute the environment and cause health problems are desirable. Deep eutectic solvents (DESs) are promising alternative solvents, usually consist of a quaternary ammonium compound as the hydrogen bonding acceptor (HBA) and one or more hydrogen bonding donor (HBD) compounds. The intermolecular hydrogen bonding that form between the HBA and HBD molecules will decrease the melting point temperature of the mixture to an eutectic temperature much lower than the melting points of the DESs' components, hence, they usually stable as liquids at room temperature. DESs are known as designer solvents as many organic compounds are available to choose from as the HBD. DESs are also known as green solvents because they have low vapor pressures with excellent biocompatibility and biodegradability [5].

The xanthones in the pericarp of mangosteen was represented by α-mangostin, the most abundant xanthone. Extraction of α-mangostin from the mangosteen pericarp using choline chloride-based DESs with 1,2-propanediol, citric acid, glycerol, or glucose as the HBDs has been reported. The highest extraction yield of 2.6 % (w/w), α-mangostin in dried pericarp, was obtained using the a DES consisting of choline chloride and 1,2-propanediol in 1:3 mole ratio [6]. To increase the extraction
yield of α-mangostin, the operating condition of the solvent extraction process have been optimized using the response surface methodology (RSM) approach, based on the Box-Behnken design. RSM is a method of mathematical and statistical analysis used to determine the optimal state of a process in which the output of a process is determined by several independent variables [7]. In this study, these variables were extraction temperature, mangosteen powder to DES mass ratio, and extraction time.

2 Materials and methods

2.1 Chemicals
Betaine anhydrous (99% purity), analytical grade 1,2-propanediol (99% purity), analytical grade acetonitrile, phosphoric acid and ethanol (purity >99%) as HPLC eluents, were purchased from Sigma Aldrich.

2.2 Preparation of DES and mangosteen powder
The deep eutectic solvent was prepared by mixing betaine and 1,2-propanediol in a molar ratio of 1:3. The mixture was heated and stirred on a hot plate set at 60 °C until the solid betaine dissolved completely to form a clear DES solution. Mangosteen pericarp was cleaned with demineralized water, cut into small pieces, and crushed using a blender. The paste obtained was dried in a hot air oven at 40 °C until the weight become constant, the dried mangosteen powder was sieved using a 20 mesh, and, the mangosteen powder obtained was kept in a closed container away from direct sunlight.

2.3 Quantitative analysis using high performance liquid chromatography (HPLC)
HPLC analysis was performed using the HPLC Shimadzu Prominence UFLC system equipped with LC-20AD pump, SPD-20A 230 V UV-Vis detector, Rheodyne injector with a 20 μL loop, and a C-18 column (250 mm long and 4.6 mm diameter). The sample injection volume was 8 μL with and the isocratic elution was used at a flow rate of 1 mL/min at room temperature. The mobile phase consisted of 0.1% (v/v) orthophosphoric acid-acetonitrile was filtered through a 0.45 μm filter and sonicated before use. Prior to injection, the sample was first diluted with methanol and filtered through a 0.45 μm membrane. The UV-vis detector was set at wavelengths of 244 and 320 nm to measure the absorbance of standard and sample solutions [8].

2.4 Pre-optimization screening of the solvent extraction variables
Before carrying out the full optimization scheme, the individual effect of each extraction process variable (extraction temperature, mass ratio of mangosteen powder and DES, and extraction time) on α-mangostin extraction yield extraction was determined. The objective of this pre-optimization step was to estimate the three levels of each independent variable used in the optimization of the independent variables. The mangosteen pericarp powder was mixed with DES in sealed tubes, in a mass ratio of 1:10. The mixture was put in a thermostaker set at a stirring speed of 500 rpm and temperature of 55 °C for 4 hours to extract the α-mangostin. The suspension was then centrifuged for 15 minutes at 2000 rpm and the residue separated using a 0.45μm filter to obtain the supernatant.

2.5 Optimization of the solvent extraction variables
Response surface methodology (RSM) based on the Box-Behnken design was used to obtain a quadratic model containing of the factorial trials and the central points which aim to estimate quadratic effects and the pure process variability with extraction yield as the response [9]. Overall, 15 pericarp powder samples were extracted using the prescribed combination of three levels of each variable, determined based on the results of the pre-optimization screening step. The extraction yield dependence on the process variables and the maximum yield that can be obtained using the optimized condition were obtained using Minitab statistical software. Statistical comparison was performed using one-way analysis of variance (ANOVA) where p-values <0.05 are considered statistically significant [9].
2.6 Scanning electron microscopy of the pericarp matrix

The scanning electron microscopy was also used to analyze the morphology of the plant matrix before and after extraction using DES.

3 Results and discussion

3.1 Pre-optimization results

The effect of temperature on $\alpha$-mangostin extraction yield is shown in figure 1a. The presence of a peak suggests that there is an optimum extraction temperature in the range of 45-65 °C. Figure 1b shows the monotonic increase of extraction yield as the mass ratio of mangosteen powder to DES increases. Although a peak expected, no peak is observed in figure 1b. This is due to the formation of a viscous suspension when mangosteen powder was mixed with DES in the ratio of 0.12 or higher, making centrifugation to separate to the suspended powder ineffective. The extraction yield increases almost linearly with extraction time until 8 hours and become constant afterward as shown in figure 1c, suggesting extraction time range of 4-8 h to be used in the optimization phase.

![Figure 1](image1.png)

Figure 1. Extraction yields as a function of temperature (a), mangosteen powder and DES mass ratio (b), and extraction time (c).

3.2 Box-Behnken RSM optimization results

The three levels of the independent variables used in the Box-Behnken optimization were set based on the pre-optimization results for temperature ($T$: 45, 55, 65 °C), mangosteen powder to DES mass ratio ($MD$: 0.08, 0.10, 0.12 g/g), and extraction time ($t$: 4, 6, 8 h). These levels were assigned as -1 (low), 0 (medium), and 1 (high) levels, respectively. Table 1 gives the $\alpha$-mangostin extraction yields given as weight percentage of $\alpha$-mangostin in dried mangosteen pericarp obtained from 15 samples. Based on the these results, the following second-order regression equation was obtained:

$$
\text{Extraction yield} = -36.7 + 0.80 T - 263 MD - 0.802 t - 0.00647 T^2 - 1273 MD^2 + 0.0717 t^2 + 0.037 T \cdot MD + 0.095 T \cdot t + 6.06 MD \cdot t
$$

(1)

The coefficient of determination ($R^2$) for the response was 92.5%. The regression equation was used to generate 3D surfaces and contour plots showing extraction yields as a function of two independent variables, while keeping the third variable at its central (0) level (figures 2-4).

Figure 2 shows the dependency of extraction yield on $T$ and $MD$, keeping $t$ constant at 4 h. At this short extraction time, very probably the extraction has not yet reached the equilibrium state, and, mass transfer or diffusion is the rate limiting step. It is well known that decreased viscosity at a higher temperature would lead to enhanced diffusion and mass transfer of DES in the plant matrix, and also, bioactives in the DES [10]. Along a constant $MD$ value, the extraction yield increased with the
increase of $T$, reached the optimum, and then decreased. It is the combined effect of enhanced degradation of the mangosteen pericarp matrix by DES and $\alpha$-mangostin degradation at higher temperatures [11]. The negative effect of excessive $T$ on extraction yield is magnified by extending the extraction time, clearly shown in figure 3. The synergistic effect of extended extraction time and high $MD$ values on extraction yield, at a moderate temperature of 55 °C, is shown in figure 4.

**Table 1.** Experimental BBD with experimental value of response on extraction process.

| Run | Coded level | Actual value | Dependent variable |
|-----|-------------|--------------|--------------------|
| # | $T$ | $MD$ | $t$ | $T$ (°C) | $MD$ (g/g) | $T$ (h) | Extraction yield (<, %, w/w) |
| 1 | -1 | -1 | 0 | 45 | 0.08:1 | 6 | 1.52 |
| 2 | 1 | -1 | 0 | 65 | 0.08:1 | 6 | 1.90 |
| 3 | -1 | 1 | 0 | 45 | 0.12:1 | 6 | 3.45 |
| 4 | 1 | 1 | 0 | 65 | 0.12:1 | 6 | 3.80 |
| 5 | -1 | 0 | -1 | 45 | 0.10:1 | 4 | 2.53 |
| 6 | 1 | 0 | -1 | 65 | 0.10:1 | 4 | 3.68 |
| 7 | -1 | 0 | 1 | 45 | 0.10:1 | 8 | 2.48 |
| 8 | 1 | 0 | 1 | 65 | 0.10:1 | 8 | 2.87 |
| 9 | 0 | -1 | -1 | 55 | 0.08:1 | 4 | 2.21 |
| 10 | 0 | 1 | -1 | 55 | 0.12:1 | 4 | 3.21 |
| 11 | 0 | -1 | 1 | 55 | 0.08:1 | 8 | 2.36 |
| 12 | 0 | 1 | 1 | 55 | 0.12:1 | 8 | 4.33 |
| 13 | 0 | 0 | 0 | 55 | 0.10:1 | 6 | 3.81 |
| 14 | 0 | 0 | 0 | 55 | 0.10:1 | 6 | 3.89 |
| 15 | 0 | 0 | 0 | 55 | 0.10:1 | 6 | 3.77 |

**Figure 2.** Contour (a) and surface (b) plot showing the effect of temperature and mangosteen powder to DES mass ratio on $\alpha$-mangosteen extraction yields, for a constant extraction time of 4 h.

The statistical analysis, given as ANOVA (table 2), confirms the qualitative features of the response surfaces where $P<0.05$ indicates that the model term is significant. The extraction yield dependence on mangosteen to DES mass ratio was linear ($P=0.002$) and dependence on extraction temperature was quadratic ($P=0.024$). While linear and quadratic dependence of extraction yield on time were not statistically significant, the lower $P$ values for the interaction terms involving
time ($T^*t$ and $MD^*t$) compared to $T^*MD$ term indicate that extraction time is also a factor to be considered in the optimization. Based on the Box-Behnken scheme, the optimal operating condition for extraction corresponding to the highest $\alpha$-mangosteen extraction yield was obtained and the results are given in table 3.

![Figure 3](image1.png)

**Figure 3.** Contour (a) and surface (b) plot showing the effect of temperature and extraction time on $\alpha$-mangosteen extraction yields, for a constant mangosteen powder to DES mass ratio of 0.1.

![Figure 4](image2.png)

**Figure 4.** Contour (a) and surface (b) plot showing the effect mangosteen powder to DES mass ratio and extraction time on $\alpha$-mangosteen extraction yields, for a constant temperature of 55 °C.

| Table 2. Analysis of variance for regression model to optimize response. |
|---|
| Source | DF | Adj SS | Adj MS | F | P |
| Linear | 3 | 0.6641 | 0.6641 | 4.30 | 0.093 |
| $T$ | 1 | 5.78 | 5.78 | 38.58 | 0.002 |
| $MD$ | 1 | 0.021 | 0.021 | 0.14 | 0.723 |
| $t$ | 1 | -0.0064 | 1.544 | 10.31 | 0.024 |
| Quadratic | 3 | -1273 | 0.9572 | 6.39 | 0.053 |
| $T^*T$ | 1 | -0.0717 | 0.3034 | 2.03 | 0.214 |
| $MD^*MD$ | 1 | 0.037 | 0.49 | 0.0002 | 0.971 |
| $t^*t$ | 1 | 0.0095 | 0.495 | 0.1444 | 0.371 |
| Interaction | 3 |  |  |  |  |
Table 3. Result of alpha-mangosteen extracted response optimizer.

| Parameter                          | Value   | Extraction yield |
|----------------------------------|---------|-----------------|
| Temperature (°C)                 | 56.5    |                 |
| Mangosteen powder to DES mass ratio (g/g) | 0.12    | 4.25%           |
| Time (h)                         | 6.9     |                 |

The SEM pictures of mangosteen powder show the morphology of the mangosteen pericarp matrix before (figure 5) and after (figure 6) DES extraction. It can be seen that more cavities were formed after the extraction process, indicating that the bioactive substances have been extracted.

**Figure 5.** SEM result of mangosteen pericarp mangosteen powder before extraction with scale 500x (a), 1000x (b), and 2000x (c).

**Figure 6.** SEM result of mangosteen pericarp mangosteen powder after extraction with scale 500x (a), 1000x (b), and 2000x (c).

4 Conclusion
Optimal operating conditions for the extraction of α-mangosteen from the pericarp of mangosteen using a deep eutectic solvent consisting of betaine and 1,2-propanediol, in a molar ratio of 1: 3, has been obtained based on the Box-Behnken design. The extraction yield dependence on mangosteen to DES mass ratio was linear (P=0.002) and dependence on extraction temperature was quadratic (P=0.024). A second-order regression equation was obtained with a coefficient of determination of 92.5% with the highest calculated α-mangostin extraction yield of 4.2% (w/w), based on an extraction temperature of 56.5 °C, mangosteen powder to DES mass ratio of 0.12, and extraction time of 6.9 hours. The maximum mangosteen powder to DES ratio The optimum temperature was found to be the combined effect of enhanced leaching of the mangosteen pericarp matrix by the DES and α-mangostin degradation at higher temperatures.
5 Acknowledgment
The authors wish to thank the financial support provided by Universitas Indonesia through the PITTA grant number 2465/UN2.R3.1/HKP.05.00/2018.

6 References
[1] Pedraza-Chaverri J, Cárdenas-Rodríguez N, Orozco-Ibarra M, Pérez-Rojas JM, Food Chem Toxicol. 46 (10) 3227-39 (2008).
[2] Jung HA, Su BN, Keller WJ, Mehta RG, Kinghorn AD, J Agric Food Chem. 54 (6) 2077-82 (2006).
[3] Asai F, Tosa H, Tanaka T, Iinuma M. Phytochemistry 39 (4) 943-944 (1995)
[4] Constable JC, Curzons D, Cunningham, VL, Green Chem. 4 521 (2002).
[5] Dai Y, Sprosen J van, Witkamp G-J, Verpoorte R, Choi YH, Anal.Chim.Acta 766 61-68 (2013)
[6] Mulia K, Krisanti E, Terahadi F, Putri S, International Journal of Technology 7 1211-1220 (2015).
[7] Bi W, Tian M, Row KH, J Chromatography 1285 22-30 (2013).
[8] Pothitirat W, Chomnawang MT, Supabphol R, Gritsanapan W, Fitoterapia, 80 442-447 (2009).
[9] Jeganathan PM, Venkatachalam S, Karichappan T, Ramasamy, S, Prep. Biochem. Biotech. 44 1 56-67 (2014)
[10] Zhang Q, De Oliveira Vigier K, Royer S, Jerome F, Chem. Soc. Rev. 41 7108-7146 (2012)
[11] Satong-aun W, Assawarachan R, Noomhorm A, J Food Sci Eng 1 85-92 (2011).