

Process development for the enrichment of curcuminoids in the extract of ionic type of NADES

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Abstract. Curcuminoids were successfully extracted from *Curcuma zeodaria* powder with Natural Deep Eutectic Solvents (NADES) as solvent. Ionic types of NADES such choline chloride-citric acid-water (CCCA-H₂O = 1:1:18, mole ratio) and choline chloride-malic acid-water (CCMA-H₂O = 1:1:18, mole ratio) gave the highest yield of extracted curcuminoids, ca. 0.270±0.025 and 0.355±0.055 mg/g respectively for CCCA and CCMA. However, the final product of the extracted curcuminoids was mixed in the NADES matrix. Hence, a separation process by means a solidification was mandatory. Isopropanol-n-hexane in both volume and molar ratio, i.e. 1:1, 1:1.5, 1:2, 2:1, and 2.5:1, were observed as a solvent in the solidification process as well as the solubility of curcuminoids in both isopropanol and n-hexane. Unfortunately, both of CCCA-H₂O (1:1:18) or CCMA-H₂O (1:1:18) and curcuminoids form a homogenous mixture with isopropanol. Therefore, all NADES constituents, i.e. choline chloride, citric acid/malic acid, and water, should be first removed before solidification process. Solubility of curcuminoids in isopropanol and n-hexane was, respectively, 3.2 mg/mL and 0.4 mg/mL at 40 °C. Isopropanol-hexane (1:1.5, v/v) give the highest recovery of curcuminoids, ca. 74.29%, by solidification process.

1. Introduction

Curcuma zeodaria or kunir putih (in Bahasa) contains bioactive compounds, curcuminoids. Curcuminoids consist of curcumin (C), desmethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC) which potentially act as antioxidant and anticancer. Due to the low solubility of curcumin in water (pH = 7.3), approximately 4 ppb of water (4µg / L) [1,2], curcumin is not yet approved as an anticancer agent. Therefore, a carrier which can release curcumin completely into the human body is needed [2,3]. Natural Deep Eutectic Solvent (NADES) solubilized and extracted curcumin is much better than commonly organic solvent such methanol, ethanol, as well as water at 40 °C within 24 hours of extraction time [4]. Natural Deep Eutectic Solvent (NADES) is an environmentally friendly solvent which is made of natural primary metabolites such amino-acids, monosaccharides, disaccharides, polysaccharides, acetic acids, lactic acids, choline chlorides, etc. in a certain molar ratio [5].

Ionic types of NADES, i.e. CCMA (Choline Chloride-Malic Acid) and CCCA (Choline Chloride-Citric Acid) with 1:1:18 in mole ratio yield the highest curcuminoids compared to other types of NADES [4]. The batch stirrer reactor is previously applied for the extraction curcuminoids from *Curcuma zeodaria* [4,6]. Therefore, extracted curcuminoids and NADES as solvent including the powder of *C. zeodaria* are completely mixed as a slurry. Therefore, the separation process following the purification process is needed to be developed to produce high purity of curcuminoids while maintaining both



physically and chemically stable curcuminoids for longer self-life. Hence, a solidification method is considered. It is done by purifying the target analytic simply by precipitation. It is expected that the curcuminoids can be directly purified and precipitated from the extracted solution, mixture of curcuminoids and NADES.

2. Materials and methodology

2.1. Reagents and chemicals

Standard Curcuminoids which contain 75.13%-w of curcuminoids (C), 21.29%-w of desmethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) was commercially purchased from Merck (Darmstadt, Germany) as well as solvents such as acetonitrile, acetic acid, and methanol. All the solvents were in High Pure Analytical grade. While individual components of NADES such as choline chloride and malic acid were purchased from Sigma Aldrich (St. Louis, MO, USA). Food grade of citric acid was purchased at Surabaya local market (Gajah, Jakarta, Indonesia) and buffer solution (pH = 2.0) from Mediss (India). Moreover, a filter paper (no. 589/2) within 4-12 μm of pore size was purchased from Whatman (Germany).

2.2. Natural Deep Eutectic Solvents (NADES) preparation

All NADES used in this study were prepared based on the method by Rachmaniah et al. [6], which is a modified method of [7]. Each constituent of NADES was accurately weighted in molar ratio; mixed with water and subsequently stirred in sealed bottle equipped with water bath at 70 °C. The agitation was continued till all the solid compounds of NADES melted, forming a homogeneous mix of liquid. The formed liquid was directly stored in drank.

2.3. Solubility test of curcuminoids in isopropanol-Hexane

Solubility test of curcuminoids was conducted by gradually adding an accurate weight of curcuminoids in mixture of different molar and volume ratio of isopropanol-hexane at 40 °C. The addition was stopped until saturated condition of curcuminoids was reached, Indicated by a precipitation of curcuminoids. Subsequently, samples were suddenly cooled at 9 °C, vacuum filtered, dried at dessicator. A dried sample of curcuminoids was weighted and its percentage of recovery was calculated.

$$\text{recovery} = \frac{\text{Weight of curcuminoids collected after the experiment}}{\text{initial weight of curcuminoids that was used}} \times 100\% \quad (1)$$

2.4. Sample preparation for solidification process

An accurate weight of curcuminoids standard was dissolved in CCMA-H₂O (1: 1: 18) or CCCA-H₂O (1: 1: 18) at 40 °C within S/F ratio of 5/10 (mL NADES/mg curcuminoids). Subsequently, isopropanol-hexane was added, suddenly cooled at 9 °C, vacuum filtered, dried at dessicator. A dried sample of curcuminoids was weighted and its percentage of recovery was calculated following the equation (1).

3. Result and discussion

Purifying and having curcuminoids in solid form from extract mixture of NADES is required. Curcuminoids in the solid form is more storable than in liquid. The keto structure of curcumin is commonly found in the solid phase [8]. While in the liquid phase, the keto-enol structure of curcumin is more stable than its diketone structure [9]. Enol enthalpy of formation of curcumin is lower than its keto tautomer; implying that the enol tautomer is more stable (Figure 1) [8]. Curcumin in diketone structure obviously does not have active methylene group. This C-H bond has higher enthalpy of dissociation than O-H group at phenolic; therefore, O-H bonds more reactively active follows antioxidant mechanism [9].

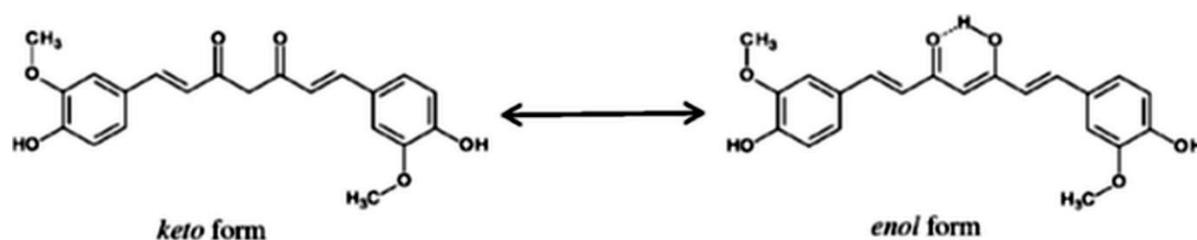


Figure 1. Keto-enol form of curcuminoids [10].

Therefore, firstly, a solubility test of curcuminoids in mixture of both of volume and molar ratio of isopropanol-hexane at 40 °C was conducted (Table 1 and 2). Mixture of isopropanol-hexane as solvent was chosen since it was the most suitable solvent to solidify the curcuminoids [11], increasing the solubility and simultaneously avoiding the degradation of curcuminoids. Therefore, 40 °C was settled.

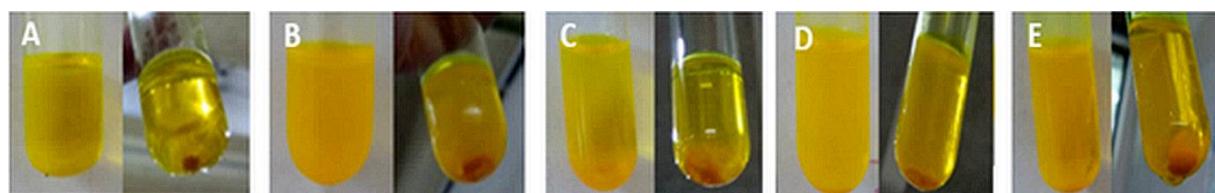


Figure 2. Solidification of curcuminoids in various volume ratio of isopropanol-n-hexane: (A) 1:1, (B) 1:1.5, (C) 1:2, (D) 2:1, and (E) 2.5: 1.

Mixture of isopropanol-hexane (1:1.5, v/v) gave the highest recovery of curcuminoids, ca.74.29% (Table 1). Moreover, solid form of curcuminoids in volume ratio was more coarse compare to the molar ratio variable (Figure 2 and 3). Interestingly, volume ratio of isopropanol-hexane at 1:2 (v/v) and 1:1 (v/v) resulted in the finest form of curcuminoids. Therefore, the solubility of curcuminoids in isopropanol and n-hexane were also observed. There were 3.2 mg/mL and 0.4 mg/mL of curcuminoids solubility, respectively, in isopropanol and n-hexane at 40 °C. Curcumin and desmethoxycurcumin could partly be dissolved in n-hexane [12]. Hence, by increasing volume of n-hexane, curcuminoids would more likely precipitate. As a nonpolar solvent, n-hexane acted as anti-solvent in this solidification process of curcuminoids, precipitating the curcuminoids [13]. Meanwhile, isopropanol with medium-polarity selectively dissolved curcuminoids. Hence, the optimum solidification of curcuminoids could be done by combining solvent and anti-solvent.

Table 1. Curcuminoids recovery by solidification with isopropanol-hexane (v/v).

Isopropanol-Hexane (v/v)	1:1	1:1.5	1:2	2:1	2.5:1
Curcuminoids Solubility (mg/mL)^a	0.81±0.020	1.41±0.021	1.19±0.026	1.62±0.014	1.93±0.021
Recovery (%)^b	29.41%	74.29%	55.56%	46.94%	44.93%

^a Solubility of curcuminoids at 40°C

^b Recovered curcuminoids was obtained by using isopropanol-n hexane solvents (v/v) at 9 °C

Solidification of curcuminoids using molar ratio of isopropanol-hexane was also observed (Table 2). Unfortunately, the results of molar ratio were not as good as the volume ratio. The solid form of curcuminoids was finer, creating some difficulties in the filtering process. It might be explained by the smaller volume of n-hexane added as it was counted in molar ratio. Hence, it affected the precipitation.

Table 2. Solubility of curcuminoids in isopropanol-hexane (molar ratio).

Isopropanol-Hexane (molar ratio)	1:1	1:1.5	1:2	2:1	2.5:1
Solubility (mg/mL) ^a	6.40±0.141	6.10±0.472	4.90±0.331	6.60±0.141	6.80±0.354

^aSolubility of curcuminoids in Isopropanol-Hexane at 40 °C

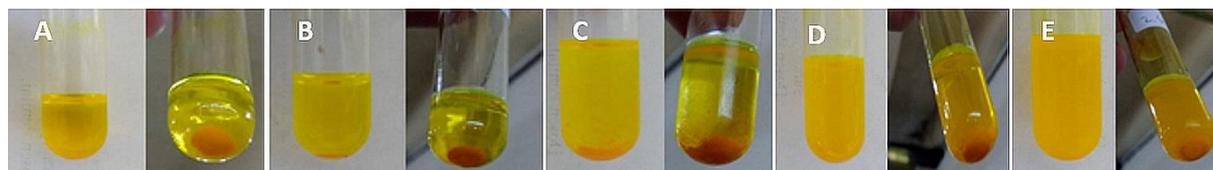


Figure 3. Solidification of curcuminoids in various molar ratio of isopropanol-n-hexane: (A) 1:1, (B) 1:1.5, (C) 1:2, (D) 2:1, and (E) 2.5: 1.

To conclude, Isopropanol-hexane (1:1.5, v/v) is the best combination for solidifying curcuminoids. Furthermore, the volume ratio of Isopropanol-hexane is studied to solidify the curcuminoids in NADES matrix.

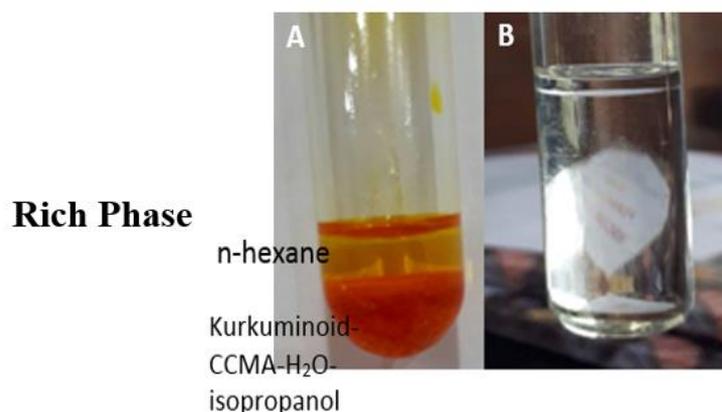


Figure 4. (A) Two organic layers observed from mixture of curcuminoids-NADES-isopropanol-n-hexane, (B) A homogenous mixture of NADES (CCMA-H₂O)-isopropanol-n-hexane.

A sample of curcuminoid-NADES (CCMA-H₂O = 1:1:18) 20.6 mL was added with 2.5 mL Isopropanol-hexane (1:1.5, v/v). A resulted mixture was suddenly cooled at 9 °C. Two organic layers were observed (Figure 4A). Considering the densities of isopropanol and hexane, the upper layer mainly contained hexane while the lower layer contained isopropanol. Unfortunately, though upper layer was sharply separated, curcuminoids in lower layer still could not be precipitated by adding either more isopropanol or hexane. In addition, observing this phenomenon, we mimic the lower layer by accurately mixing (in percentage of weight) curcuminoids-choline chloride-malic acid-water-isopropanol. A homogenous mixture of curcuminoids-choline chloride-malic acid-water-isopropanol was formed (Figure 4B) such a eutectic mixture. Isopropanol is commonly used as a solvent for many natural products such as curcuminoids. There is presumably a competition in dissolving curcuminoids between isopropanol and NADES. Moreover, choline chloride-polyalcohol, i.e. 1, 2-propanediol, isopropanol, in certain molar ratio can form a NADES [7]. Choline chloride also has high solubility in isopropanol, ca. 58.8 mg/mL at 40 °C. Consequently, curcuminoids cannot be successfully solidified using hexane as anti-solvent when NADES is still present in mixture.

Due to the phenomenon, curcuminoids in NADES matrix cannot be precipitated directly by solidification process using isopropanol-hexane. The NADES ingredients should be minimized first or even eliminated to precipitate curcuminoids by solidification.

4. Conclusion

Isopropanol-hexane (1:1.5, v/v) is the best combination for solidifying curcuminoids. However, curcuminoids in NADES matrix cannot be precipitated directly by solidification process using isopropanol-hexane. A homogenous mixture of curcuminoids-choline chloride-malic acid/citric acid-water-isopropanol was formed. Hence, the NADES ingredients should be minimized first or even eliminated to precipitate curcuminoids by solidification.

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