Preparation and Characterization of Highly Water Soluble Curcumin – Dextrose Cocrystal

Katherine\textsuperscript{1,2*}, Denny Nugroho\textsuperscript{2} and Asaf Kleopas Sugih\textsuperscript{2}

\textsuperscript{1*} Department of Biotechnology, Indonesia International Institute for Life Sciences, Jalan Pulomas Barat Kavling 88, Jakarta, Indonesia  
\textsuperscript{2}Department of Chemical Engineering, Universitas Katolik Parahyangan, Jalan Ciumbuleuit 94, Bandung, Indonesia

*Corresponding email: katherine.k@i3l.ac.id

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ABSTRACT

Curcumin is a natural food colorant isolated from rhizomes of turmeric (\textit{Curcuma longa}). Despite its many favorable properties, curcumin is practically insoluble in water and relatively unstable, thus limiting its application. In this research, a potential method to improve curcumin solubility and stability, \textit{i.e.}, cocrystallization of curcumin with dextrose was investigated. The effect of curcumin content in the cocrystals on solubility and yield of the product was studied. The morphology of the cocrystals was observed using SEM. In addition, stability in different pH range was investigated. Crystal structure and curcumin–dextrose interaction was analyzed using FT-IR spectra and DSC thermograms. The result shows that curcumin–dextrose cocrystal is a potential food colorant that could be applied to water–based food at various pH range.

Keyword: cocrystallization, curcumin, dextrose, highly-soluble curcumin

INTRODUCTION

Curcumin (C\textsubscript{21}H\textsubscript{20}O\textsubscript{6}) is a yellow color polyphenol obtained from extraction of the rhizomes of turmeric (\textit{Curcuma longa}) that is widely used as natural food coloring agent. Due to its diverse pharmacological activity such as antioxidant, anti-inflammatory and anticancer, curcumin is also an active ingredient in traditional herbal remedy in Indonesia [1-2]. As an active compound with tremendous potential, curcumin is limited in application due to its low solubility and poor bioavailability in the aqueous environment. One study reported that the water solubility of curcumin is as low as 0.6 μg/mL [3]. In addition, curcumin application as food colorant is limited by its instability at different pH [4].

In the effort to increase its stability and efficacy, several methods have been proposed, including inclusions of curcumin in different nanoformulation systems [5], and in lipophilic matrix [6], and formation of curcumin cocrystals [7]. Cocrystallization offers more simple and cost-effective method compared to other techniques such as nanoparticle preparation and spray drying. A previous study reports cocrystallization of curcumin with resorcinol and pyrogallol as coformers via wet grinding which increased dissolution rate and solubility [7]. The increase in solubility and stability in pH was due to the formation of hydrogen bonding of phenol group in resorcinol and pyrogallol and keto–enol group in curcumin. Extensive usage of resorcinol and pyrogallol, however, was subject to further research due to the concern of its toxicity. Another study has successfully produced curcumin cocrystal with

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phloroglucinol using solvent evaporation [8]. However, the cocystal showed no significant improvement in dissolution rate due to instantaneous conversion into curcumin upon contact with dissolution medium.

This study investigates the potential of cocrystallization of curcumin with simple sugar, dextrose. Dextrose or D-glucose, a primary fuel source of the most organism, is proposed as coformer as it is a compound generally regarded as safe (GRAS) and is widely available. [9] With five hydroxyl groups serves as potential hydrogen bonding sites, it shows potential as a coformer for curcumin. It is hypothesized that the stability of curcumin in different pH and the solubility increase could be achieved through the formation of hydrogen bonding of curcumin with coformer. The existence of hydrogen bonding stabilize the lone pair electrons of oxygen located in the β-diketone linker, preventing the delocalization of the electrons, and stabilizing the C-H bonds along the linker. [7, 10] While wet grinding is commonly used to produce cocystal, in this study, solubilization of dextrose followed by cooling is chosen to decrease the size of cocrystals, which could facilitate curcumin dissolution. This method has been used previously to form sugar cocrystals with different active ingredients such as honey [11], yerba mate extract [12], and cardamom oleoresin [13].

The effect of various crystallization parameters during formation of curcumin–dextrose cocystal such as water to dextrose ratio, final heating temperature, and water bath temperature had been reported before. [14] With crystallization process parameters optimized, the cocrystals form agglomerated crystals, which provide a porous matrix to entrap and protect active ingredients while at the same time, provide a large surface that could increase a solubility of the active ingredient. This work examined the effectiveness of the cocrystallization by analyzing the effect of different curcumin–dextrose ratio on curcumin solubility in water and yield of curcumin.

EXPERIMENT

Chemicals

Curcumin and ethanol were obtained from Merck, Jakarta. Dextrose monohydrate was purchased from Bratacham, Bandung. All chemicals were of analytical grade and used without further purification.

Procedure

Effect of curcumin concentration in cocrystallization

Cocrystallization was performed by adding ethanolic curcumin solution into the sugar solution. Initially, 15 g of dextrose was mixed with 1 mL water. The mixture was heated at 100 ºC while stirring until all dextrose was dissolved. Afterward, the dextrose solution was placed in a water bath with the temperature of 35 ºC. The solution was cooled in the water bath while stirring until its temperature reached 50ºC. Curcumin solution (0.125 mg curcumin/mL ethanol) was then added to sugar solution under continuous stirring. The volume of curcumin solution was adjusted to obtain various curcumin concentration (up to 1% w). The curcumin concentration is defined as a mass of curcumin added divided by total mass of curcumin and dextrose used, expressed in percentage. The mixture was then poured into silicon mold placed in a water bath (temperature of 35 ºC) to further decrease the temperature of the solution. The crystal formed upon further cooling was removed from the silicon mold and placed in the evaporation cup. The remaining solvent was evaporated by drying in a vacuum oven for 24 hours at 40ºC. The dried cocystal was then stored in a tightly closed container to protect from moisture and light.
Morphology analysis
The morphology of pure curcumin, dextrose, recrystallized dextrose and curcumin-dextrose cocrystal was analyzed using Scanning Electron Microscopy (SEM), type JEOL JSM 6510 LA, Japan. Before analysis, the samples were spread on the surface of double-face carbon tape and coated with gold. In addition, brightfield and fluorescence microscopy images of pure curcumin, sugar, and cocrystal were also obtained using fluorescence microscope type Nikon Eclipse E800 at 400x magnification.

Curcumin content determination
Curcumin content was analyzed using UV–vis spectrophotometer (Genesys 20, Spectronic) at the peak absorbance wavelength for curcumin (430 nm). At this wavelength, dextrose posed no interference to curcumin because dextrose absorbed at 260 - 270 nm [15]. A series of a standard solution of known curcumin concentration in 80% ethanol–water medium was used to build a calibration curve. The ethanol–water mixture is chosen as the solvent for its ability to dissolve curcumin at higher concentration. To determine curcumin content in the cocrystal, 300 mg cocrystal was dissolved in a known amount of water. A 10 mL aliquot was taken and centrifuged for five minutes at 6000 rpm. The obtained supernatant (2 mL) was mixed with 8 mL of ethanol, and its absorbance was analyzed with the spectrophotometer. The concentration of curcumin was calculated using the calibration curve. A yield of curcumin is defined as the actual amount of curcumin in the cocrystal divided by the initial amount of curcumin added and expressed in percentage.

Solubility of curcumin – dextrose cocrystal
To determine the solubility of curcumin in cocrystal in water, it requires to conduct a small amount of cocrystal was added to 10 mL water while stirring. During the test, the temperature was maintained at 37°C. When the crystal has been completely dissolved, more crystal was added while stirring. The process was repeated until no more crystal dissolved in water. The aliquot (5 mL) was taken and centrifuged for 5 minutes at 6000 rpm. Ethanol (8 mL) was added to the supernatant (2 mL), and the solution was further analyzed for its curcumin content using a spectrophotometer. The solubility of curcumin in cocrystal form is defined as the mass of curcumin dissolved in the known volume of water.

Stability of curcumin – dextrose cocrystal
Pure curcumin and curcumin – dextrose cocrystal were analyzed at pH range of 1 until 9 according to the method employed by Wang et.al.[16]. The pH of water as solvent was adjusted with 1 M HCl or NaOH. Pure curcumin and curcumin–dextrose cocrystal was added into each pH-adjusted water to obtain 0.5% w/v solution. The solution was stirred for 30 minutes, and 10 mL aliquot was taken and centrifuged for five minutes at 6000 rpm. Afterward, 2 mL supernatant was collected and added to 8 mL of ethanol. The solution was then analyzed using a spectrophotometer.

Analysis with FTIR and DSC
DSC analysis was performed using Netzsch STA 449 F1 Jupiter, Germany. Cocystal sample (20 mg) was placed in crimped aluminum pans and heated up to 500 °C at a rate of 20 °C/ min. During the analysis, the sample was continuously purged with dry nitrogen flowing at a speed of 20 mL/min.
Cocrystals were grounded and sieved using mesh size -100. The finely ground samples were then dispersed in KBr pellets, and its IR spectra were recorded using Prestige 21 FTIR (Shimadzu, Japan) apparatus.

RESULT AND DISCUSSION

Morphology of pure curcumin, pure dextrose, recrystallized dextrose, and curcumin–dextrose cocrystal were presented in Figure 1a–1d. Pure curcumin exhibits irregular morphology with size ranging from 1 μm to 20 μm. Pure dextrose was irregular with average size about 50 μm. The recrystallized dextrose is also irregular with a smaller size range from 5–20 μm. On the other hand, the shape of curcumin dextrose–cocrystals are more distinguished and could be divided into two shapes, long flakes and round, with some aggregates. The length of the flakes spans from 10 to 20 μm with the width of 2.5 μm. Analysis using fluorescence microscope, which makes use of the fluorescent property of curcumin, indicates that the curcumin is distributed unevenly in the aggregates.

Figure 1. SEM images of (a) pure curcumin (1500x magnification), (b) pure dextrose (500x magnification), (c) recrystallized dextrose (1500x magnification), and curcumin–dextrose cocrystals (1500x magnification)

The yield of curcumin from cocrystal produced of different initial curcumin concentration was presented in Figure 2. At low initial curcumin concentration (0.2% curcumin), the yield was high (more than 90%). However, when initial curcumin concentration was increased, the yield of curcumin decreased rapidly. At higher initial curcumin concentration, the yield leveled off at 30%.
Figure 2. Effect of initial curcumin concentration on curcumin yield

Curcumin content in the cocrystal can be calculated by multiplying yield of curcumin with initial curcumin concentration and the values are given in Figure 3. Figure 3 shows a slow and steady increase in curcumin content from 0.18% to 0.29% as initial curcumin concentration was increased from 0.2% to 1%. Both Figure 2 and Figure 3 indicate that the cocrystallization process is more efficient at low concentration.

Figure 3. Effect of initial curcumin concentration on curcumin content in cocrystal

The curcumin solubility in water from various curcumin – dextrose cocrystal produced at different initial curcumin concentration is presented in Figure 4. The solubility of curcumin is inversely related to initial curcumin concentration. By converting curcumin to curcumin–dextrose cocrystal, the solubility increases up to 23 mg/mL (at 0.2% initial curcumin concentration). We hypothesized that the increase in solubility is due to the formation of hydrogen bonding of hydroxyl group of dextrose with either the carbonyl group or phenolic group of curcumin. The increase in solubility of curcumin in cocrystal form due to hydrogen bonding has also been observed in several other studies. [7-8]
Solutions of pure curcumin and curcumin–dextrose cocrystals in solution at pH 1–9 were compared (Figure 5). Pure curcumin is highly dependent on pH and exhibits very low solubility from pH 7 to pH 9. Below pH 7, the solubility increased as pH increased, with peak absorbance observed between pH 4–6, and lower absorbance value observed at pH 1–3. On the other hand, curcumin–dextrose cocrystals was stable in the range between pH 1–7 and showed a 33% decline in absorbance value starting from pH 8. This trend suggests protection of curcumin from precipitation in acidic pH range. Curcumin instability in acidic pH range is mainly due to delocalization of unpaired electron of oxygen in the carbonyl bond [10]. The delocalization weakens the C-H bond of the C₄ carbon of the heptadione linkage. By forming hydrogen bond with the curcumin’s carbonyl group, the effect of electron delocalization could be reduced, which in turns increase the stability of curcumin.

To observe a chemical interaction between curcumin and dextrose, FT–IR spectra of pure curcumin, pure dextrose, and curcumin–dextrose cocrystal were investigated (Figure 6). A peak between 3200–3600 cm⁻¹ observed in the three samples belongs to O–H stretch in respective molecules. The peak for dextrose is broad and strong due to many O-H bonds in dextrose and also the existence of hydrogen bondings. On the other hand, curcumin shows a
sharp peak at 3500 cm$^{-1}$ which indicates the presence of free O-H bonds on its methoxy phenyl rings. Curcumin–dextrose cocrystal has a peak with comparable size and width as dextrose. However, there is a slight shift in the peak to the lower wavenumber, suggesting that hydrogen bonding interaction might occur between curcumin and dextrose.[17] In the fingerprint region, FT-IR pattern of curcumin-dextrose cocrystals shows different pattern compared to dextrose and curcumin.

![FT-IR absorption spectra of pure curcumin (red), pure dextrose (black), and curcumin–dextrose cocrystal (blue)](image)

**Figure 6.** FT-IR absorption spectra of pure curcumin (red), pure dextrose (black), and curcumin–dextrose cocrystal (blue)

The thermal stability of pure curcumin, dextrose, and curcumin–dextrose cocrystals were examined using differential scanning calorimetry and thermogravimetric analysis (Figure 7). Curcumin shows a single sharp endothermic peak with an onset temperature of 172.8°C corresponding to the melting point of crystalline curcumin [7]. The following two broad-indistinct coupled peaks with loss of mass at a temperature above 300°C (Figure 7 left, red) suggested thermal degradation of curcumin [18]. Three endothermic peaks can be observed in pure dextrose monohydrate sample. The first broad peak with an onset temperature of 75.8°C combined with a loss of mass (as shown in TGA curves in Figure 7 right) suggested that at this point water was eliminated and dextrose monohydrate was transformed into anhydrous form. The dextrose was melted at 164°C, as indicated by the second endothermic peak [19]. On further heating, the dextrose starts to decompose at 217 °C. On the introduction of curcumin to curcumin–dextrose cocrystals, the melting point was shifted to slightly lower temperature compared to 159.4 °C. In addition, the cocrystals exhibited another endothermic peak with an onset temperature at 216.8°C. Coupled with the corresponding thermogram, it indicated the degradation temperature of curcumin–dextrose cocrystals.
Figure 7. DSC (left) and TGA (right) thermograms of pure curcumin (red), pure dextrose (black), and curcumin–dextrose cocrystal (blue)

CONCLUSION

Curcumin–dextrose cocrystals with high solubility and high stability at acidic pH range were successfully obtained. The lower initial concentration of curcumin in cocrystallization results in higher yield and higher solubility of curcumin–dextrose cocrystals. The FT-IR analysis indicates hydrogen bond formation between curcumin and dextrose. DSC/TGA analysis indicated that the curcumin–dextrose cocrystals have lower melting point. The cocrystals could be potentially used as a colorant for water–based food between pH 1 to 7. The process is scalable and economically feasible due to its simplicity and mild operating process parameters.

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