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Stabilization and Controlled Release of Curcumin from Temulawak by Spray-Drying Microencapsulation with Composite Wall Materials

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Abstract

Curcumin, is the main bioactive component of temulawak (*Curcuma xanthorriza* Roxb), which is a native Indonesian herbal plant. Although it has various health benefits, curcumin's stability and release time are very low, limiting its application. This study aimed to improve curcumin stability through spray-drying microencapsulation using various wall materials, including maltodextrin (MDE), gum Arabic (GAR), whey protein isolate (WPI), and their composites with β-cyclodextrin (βCD). The resulting curcumin microcapsules from temulawak extract had an irregular morphology with a rough surface and grooves, averaging 2 μm in diameter. The stability of curcumin during storage, against heat and various pH conditions increased significantly, especially in microcapsules prepared with composite wall materials. Curcumin release was faster in single wall material microcapsules, while composite wall material microcapsules achieved release times over 120 min. In conclusion, curcumin from temulawak extract can be prepared by spray drying. MDE-βCD composite wall materials are highly recommended because they produce curcumin microcapsules with improved stability and controlled release compared to single wall materials or other composite wall materials. This research can facilitate the utilization of curcumin from temulawak extract in industrial and food applications.

Keywords: Temulawak, Curcumin, Microencapsulation, Stability, Release study

Introduction

Temulawak (*Curcuma xanthorriza* Roxb.), also known as Java turmeric, is an herb that grows abundantly in Indonesia. Curcumin is the primary polyphenol found in temulawak, which has various pharmacological properties such as antioxidant, anti-inflammatory, antibacterial, anti-tumor neuroprotective, nephroprotective up to hepatoprotective [1]. Due to its health-enhancing properties, curcumin is increasingly in demand as an additive in new products and functional foods. However, the direct use of curcumin in the food and pharmaceutical industries has limitations. Curcumin suffers from low water solubility, thermal instability, and limited bioavailability [2].

Microencapsulation is a feasible method to overcome these curcumin problems. In particular, spray drying is a new approach that has been recommended for the curcumin microencapsulation process. In addition, this method is known to be very applicable on an industrial scale and produces commercially

competitive powder products [3,4]. In the microencapsulation process, functional components will be trapped in one or more layers of wall material, which will then form microcapsules with microns of diameter. The wall material will provide a physical barrier to prevent molecular diffusion and chemical reactions so that the stability of the functional components of the bioactive compounds increases. The protection mechanism is by forming a membrane, a wall system around the droplets or particles of the encapsulated material, namely the core. Simultaneously, microencapsulation will increase nutrition, cover unpleasant tastes, facilitate storage and extend shelf life [5,6].

The selection of a suitable wall material is one of the factors for the success of curcumin microencapsulation [7]. The selected wall material has a crucial role in properties such as compatibility, stability, availability and controlled release under certain conditions [3]. Wall materials such as MDE, GAR and WPI are recommended in the curcumin microencapsulation process [8-10]. Recently, the use of composite wall materials has been proposed for the microencapsulation process of bioactive compounds, including curcumin. This is related to the multifunctional ability of composite materials that can act as emulsifiers, film formers, matrix formers and fillers [11]. Composite wall materials can be prepared by adding βCD, as previously reported by researchers [7]. It has been confirmed that the use of composite wall materials can increase the stability and solubility of curcumin microcapsules from turmeric in water compared to single-wall materials that have lower performance [12].

The selection of adequate wall materials for microencapsulation of curcumin from temulawak extract is important because each substance has unique emulsifying and film-forming properties that determine its ability to function as an encapsulant. To our knowledge, no previous study has explored the use of composite microencapsulation to enhance curcumin stability from temulawak extract. Therefore, in this study, curcumin microcapsules were prepared from temulawak extract through a spray drying process using MDE, GAR and WPI wall materials and composite wall materials combined with βCD, which aims to increase higher protection against the effects of processing, storage and absorption. In addition, a release profile study with the Weibull model was also carried out to predict biological performance, which is an advantage of developing new products.

Materials and methods

Materials

Temulawak rhizome powder was sourced from regional farmers in Semarang, Indonesia. MDE with DE 10 - 12 from Lihua Starch (China), GAR from Ingredion (Thailand), WPI with 90 % protein content from Beyon Nutrisi (Indonesia), and βCD from Landor Trading Company (Thailand). Chemicals such as ethanol, n-hexane, methanol, curcumin standard and octanol from Sigma Chemical Company (Sigma-Aldrich, St. Louis, Missouri, USA).

Preparation of the microcapsule

Curcumin extract of temulawak was prepared by the liquid-liquid method using 70 % ethanol solvent (1:10 w/v) and purification using n-hexane (1:3 v/v) [13,14]. First, each structure-forming material (19 g) was dissolved in 100 mL of distilled water using a homogenizer (Daihan HG-15D) for 10 min (10,000 rpm). Next, βCD (1 g) was added and dissolved using a homogenizer for 10 min at 10,000 rpm with a temperature of 50 °C to achieve perfect emulsification. Then, 1 g of thick curcumin extract was mixed with the composite wall material solution and homogenized for 30 min at 10,000 rpm. Microencapsulation of temulawak curcumin with single wall material (MDE/GAR/WPI) was also prepared as a comparison using the same steps. All prepared solutions were dried using spray drying (Buchi Spray Dryer B-190) under the following conditions: Inlet temperature, outlet temperature, air flow rate, and air pressure were set to 150 ± 1, 80 ± 2 °C, 4 mL/min at 25 ± 2 °C, and 5 bar, respectively [4]. The microcapsules were immediately analyzed or stored in dark glass bottles at 4 °C (maximum 6 h) for further analysis.

Microcapsule characterization

Morphological and size analysis of curcumin microcapsules of temulawak went through scanning electron microscopy (SEM) type JSM-6510LA (Jeol Ltd. Japan). The prepared microcapsule samples were first fixed on brass stubs using double-sided adhesive

tape, then coated with gold with ion coating and observed at an accelerating voltage of 12 kV. Based on the inset scale bar in each SEM image, at least 200 particle sizes were measured using open-source image analysis software ImageJ [2].

Storage stability

The storage stability of curcumin microcapsules of temulawak was assessed for 40 days, and the retention of curcumin content was calculated [15]. A total of 5 g of microcapsules for each treatment were transferred into glass bottles lined with aluminium foil to protect the samples from light and stored at temperatures of 4, 16, and 27 °C. The amount of retained curcumin was measured every 10-day interval. The curcumin content was determined based on the previous method [14], where standard solutions of curcumin in ethanol were prepared at concentrations of 0, 2, 4, 6, 8, 10 and 12 ppm. The absorbance was measured at a wavelength of 425 nm using a UV-Vis spectrophotometer (UV-1800, Shimadzu Scientific Instrument, Japan) and used to obtain a standard curve equation. Each sample was weighed 1 g, then dissolved in 10 mL of ethanol and centrifuged at 5000 rpm for 5 min. Next, the absorbance was measured at a wavelength of 425 nm using a UV-Vis spectrophotometer. A calibration curve of the standard solution was developed to evaluate the remaining curcumin concentration during storage.

Heat stability

The heat stability of temulawak curcumin microcapsules was evaluated according to the method previously described [12]. A total of 0.5 g of microcapsules was dispersed in 10 mL of water mixed until completely dissolved. The solution was then heated at 50, 70 and 90 °C for 10 min using a water bath, respectively. After heat treatment, the sample was cooled to room temperature. One mL of sample was then taken and mixed with 9 mL of acetone, and the absorbance was measured at a wavelength of 425 nm using a UV-Vis spectrophotometer. Controls were prepared with the same treatment (25 ± 2 °C) without any heating process using a curcumin standard curve.

pH stability

Evaluation of the pH stability of curcumin microcapsules of temulawak was carried out based on the method described [2]. Microcapsules with known total curcumin content were dissolved in acetate buffer (pH 2), distilled water (pH 7) and phosphate buffer (pH 9). Aliquots of the suspension were then taken regularly every 10 min for 1 h to measure the amount of remaining curcumin using a standard curve of curcumin, and its absorbance was measured at a wavelength of 425 nm using a UV-Vis spectrophotometer.

Controlled release study

The release of curcumin from curcumin microcapsules of temulawak was measured by absorption analysis using a UV-Vis spectrophotometer at a maximum absorption wavelength of λ max = 429 nm ± 5 nm, and at a temperature of 37 °C. The calibration curve used a curcumin standard prepared in octanol (0.01 - 5 ppm). Curcumin microcapsules of temulawak (10 mg) were prepared in 10 mL of octanol at a temperature of 37 ± 2 °C with stirring. Octanol solvent was chosen because of its similarity to biological systems, such as biological membranes consisting of hydrophobic alkyl chains and polar groups. The enhancement and stability of curcumin microcapsules of temulawak were further evaluated continuously at 30-second intervals for 3 h at a wavelength of 429 ± 5 nm. The release profile of temulawak curcumin microcapsules was analyzed using the Weibull Model using the Excell for Windows instrument [3,4].

Statistical analysis

All experiments were performed in triplicate, and the results obtained are presented as mean ± standard deviation. Significant differences between treatments were analyzed using analysis of variance (ANOVA) followed by further Least Significant Difference (LSD) comparison test at 5 % significance level using SPSS 22.0 for Windows software.

Results and discussion

Morphology structure

SEM instrument was used to analyze the morphological structure of temulawak extract microcapsules, as presented in **Figure 1**. In general, temulawak extract microcapsules have very diverse and irregular particle shapes. The use of GAR wall material produces microcapsules with wrinkled surfaces, many grooves and cracks, and roughness. These results were previously also observed in curcumin microcapsules

from turmeric extract prepared with GAR wall material [3]. The use of GAR as a wall material has been confirmed to produce irregular particles with many shrinkages and grooves on their surfaces [16]. High and rapid heat contact during drying causes microcapsules prepared with GAR to form large spaces that lead to the formation of structures as described. The low glass transition temperature of particles by GAR also supports the formation of wrinkles and grooves on the surface of microcapsules [17]. The surface morphology of microcapsules prepared with MDE and WPI wall materials tends to be the same, where a small portion of particles are perfectly round and oval, and there are fewer wrinkles with a smoother surface than GAR. These results strengthen previous findings that reported that curcumin microcapsules prepared with MDE had a smooth surface and only a few wrinkles [9]. While curcumin microcapsules prepared with WPI tended to produce microcapsules with a pot-like shape, namely round or slightly wrinkled, and some particles formed relatively large hole-like depressions [18], which was caused by solvent evaporation and solute diffusion in atomized droplets due to heat transfer [19].

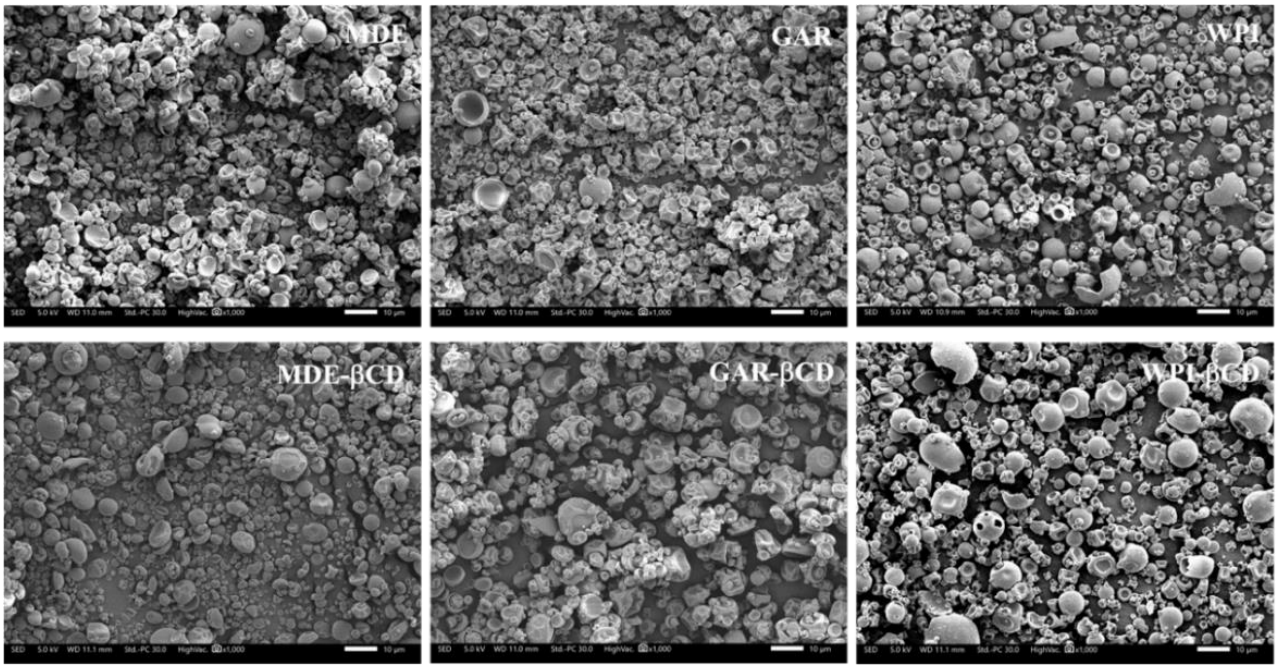

Figure 1 Morphological structure of curcumin microcapsules from temulawak extract made with different wall materials.

Interestingly, the use of composite wall materials tends to produce microcapsules with smoother surfaces, with only a few cracks, without changing the shape of the particles. The presence of βCD is known to increase the glass transition temperature of particles [20]. In addition, the ability of βCD to form inclusion complexes provides a suitable environment for trapping curcumin compounds, thereby reducing the wrinkled particle structure [21]. The formation of hydrogen bonds between βCD and molecules of the core compound is known to form a more stable inclusion complex [22]. It can be concluded that the encapsulation agent is the main factor that affects the morphological structure of microcapsules, as previously explained by researchers [8].

Particle size distributions

$D_{[10]}$, $D_{[50]}$, and $D_{[90]}$ representing 10, 50, and 90 % of the volumetric diameter of the accumulated particles, and all microcapsules had a unimodal distribution pattern (**Figure 2**), with a particle size range of 0.21 - 9.59 μm, where the average microcapsule size ($D_{[4,3]}$) ranged from 1.19 - 2.23 μm (**Table 1**). These results confirm previous studies that curcumin microcapsules prepared by spray drying have particle sizes up to 10 μm [7], with an average size of around 3.31 μm [12]. Based on the type of wall material, the use of WPI produced particles with a smaller size distribution (0.21 - 3.84 μm) than GAR (0.38 - 6.07 μm) and MDE (0.45 - 7.07 μm). On the other hand, the use of composite wall materials

resulted in a more extensive particle size distribution for all treatments (**Table 1**). An increase in the average particle size with the presence of βCD has also been reported [21]. Particle size is greatly influenced by the ability of the wall material to form emulsion viscosity [23]. Higher emulsion viscosity will form larger droplets during the atomization process, thus tending to produce larger particle sizes [24]. Particle size greatly influences the dispersibility of microcapsules, microcapsules with smaller particle sizes tend to be more easily dispersed in water. Smaller particle sizes improve dispersibility due to a higher surface area-to-volume ratio, which enhances interaction with water and reduces sedimentation during storage [21]. Microcapsules with smaller particle sizes are known to have better techno-functional properties [25].

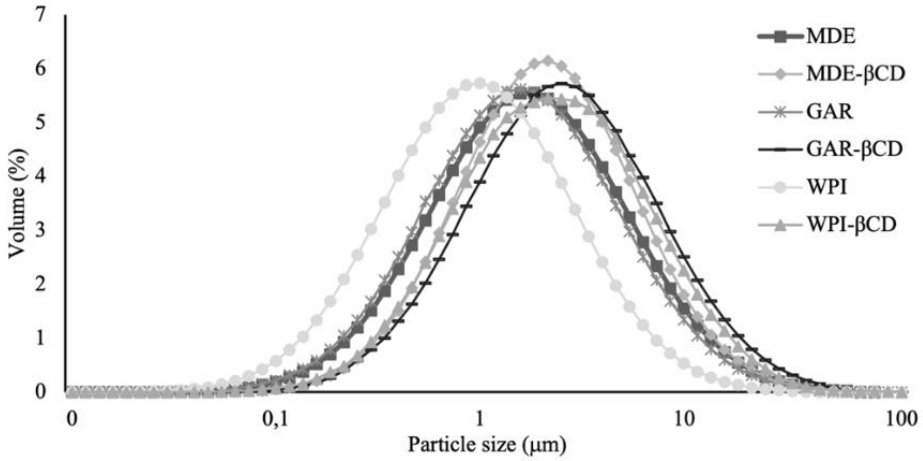

Figure 2 Particle size distribution pattern of curcumin microcapsules from temulawak extract made with different wall materials.

Table 1 Particle size distribution of curcumin microcapsules from temulawak extract made with different wall materials.

Coating materials	$D_{[10]}$ μm	$D_{[50]}$ μm	$D_{[90]}$ μm	$D_{[3,2]}$ μm	$D_{[4,3]}$ μm
MDE	0.45	1.79	7.07	1.79	1.99
MDE-βCD	0.72	2.83	9.93	2.08	2.38
GAR	0.38	1.57	6.07	1.62	1.68
GAR-βCD	0.61	2.43	9.59	1.85	2.23
WPI	0.21	0.97	3.84	1.13	1.19
WPI-βCD	0.55	2.08	8.23	1.53	1.82

Storage stability

The results showed that the rate of curcumin degradation was directly proportional to the storage temperature. Curcumin in microcapsules stored at room temperature (27 °C) degraded faster during 40 days of storage. Curcumin degradation in single-wall materials ranged from 68.12 - 75.18 % (WPI > GAR > MDE), higher than composite wall materials, which were 66.19 - 71.26 % (GAR-βCD > WPI-βCD > MDE-βCD) as presented in **Figure 3**. Without encapsulation, pure curcumin stored at room temperature in the absence of light has been reported to be completely degraded after 12 days of storage [2]. This means that the encapsulation process can significantly prevent the loss of curcumin during storage. The MDE-βCD composite wall material exhibited the best stability, with only 10.94 % degradation after 40 days. Previous researchers reported that microcapsules with surface morphology without cracks or gaps were confirmed to slow down the curcumin degradation process during storage [15]. This information can be confirmed when associated with the SEM results of the microcapsules in **Figure 1**. The

presence of βCD in the composite wall material significantly reduced cracks on the surface of the microcapsules, especially microcapsules prepared with MDE-βCD wall materials. The addition of βCD in the wall material formula has been reported to be able to prevent cracks on the surface of the microcapsules [26]. βCD is known to be able to form good inclusion complexes and protect particles from physical damage during the drying process [21].

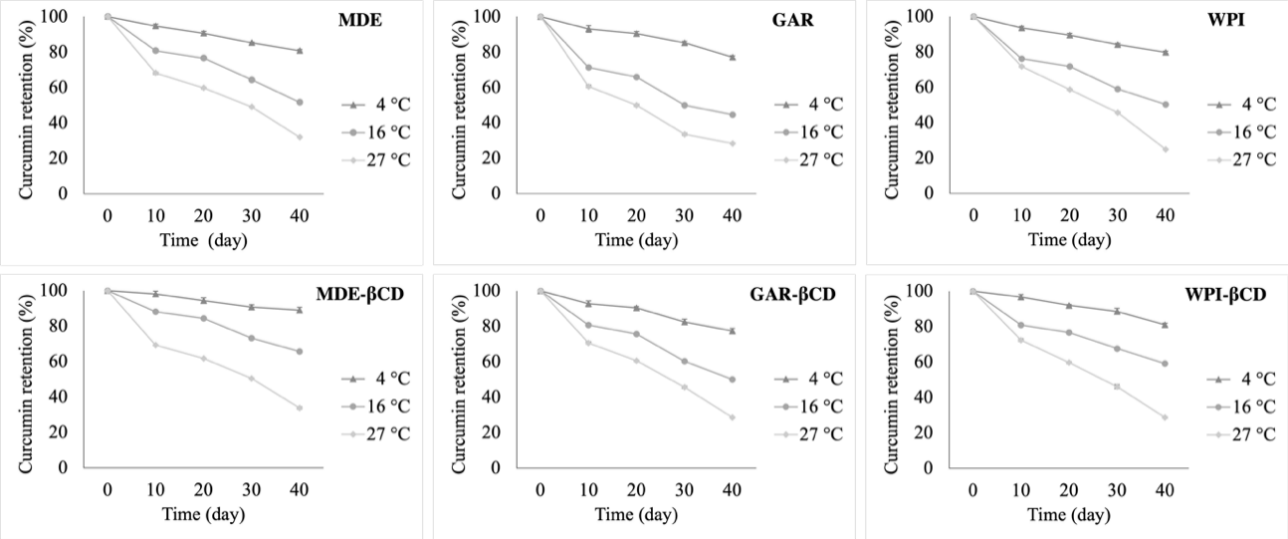

Figure 3 Degradation of curcumin from temulawak extract microcapsules made with different wall materials during storage.

Heat stability

Curcumin degradation from temulawak extract microcapsules prepared in aqueous solution was heated for 10 min and then measured, the results are presented in **Figure 4**. The stability of curcumin decreased with increasing temperature, previous researchers also found a similar phenomenon [26]. Curcumin has been confirmed to undergo significant degradation during processing under high-temperature exposure, even for a short time [27]. When the microcapsules prepared with single-wall material were heated to a temperature of 90 °C, the rate of curcumin degradation increased significantly by about 46.66 - 57.94 % (164.21 - 167.78 mg/100 g). Meanwhile, the degradation of curcumin in microcapsules prepared with composite wall material was better, at about 40.86 - 41.87 % (125.50 - 164.86 mg/100 g). These results are much better than previous researchers, who reported that curcumin degradation from microcapsules prepared with MDE wall materials and modified starch reached 87.07 % [12]. Based on these findings, the thermal stability of MDE wall materials is relatively better than WPI, especially at very high temperatures. In contrast, GAR produces the worst thermal stability. GAR has been reported to produce microcapsules with a reasonably low glass transition temperature [17]. This can explain the high degradation of curcumin prepared with GAR wall materials. Meanwhile, the addition of βCD in the composite wall material is quite effective in preventing curcumin loss. This means that βCD is able to increase the thermal stability of curcumin microcapsules from temulawak extract. When curcumin molecules are embedded into the βCD cavity, there is an increase in the melting point, boiling point and sublimation point so that its thermal stability becomes better [28]. This increase can certainly be attributed to the ability of βCD to produce microcapsules with better glass transition temperatures [20]. Curcumin-βCD complexation produces microcapsules that are pretty stable against isothermal heating.

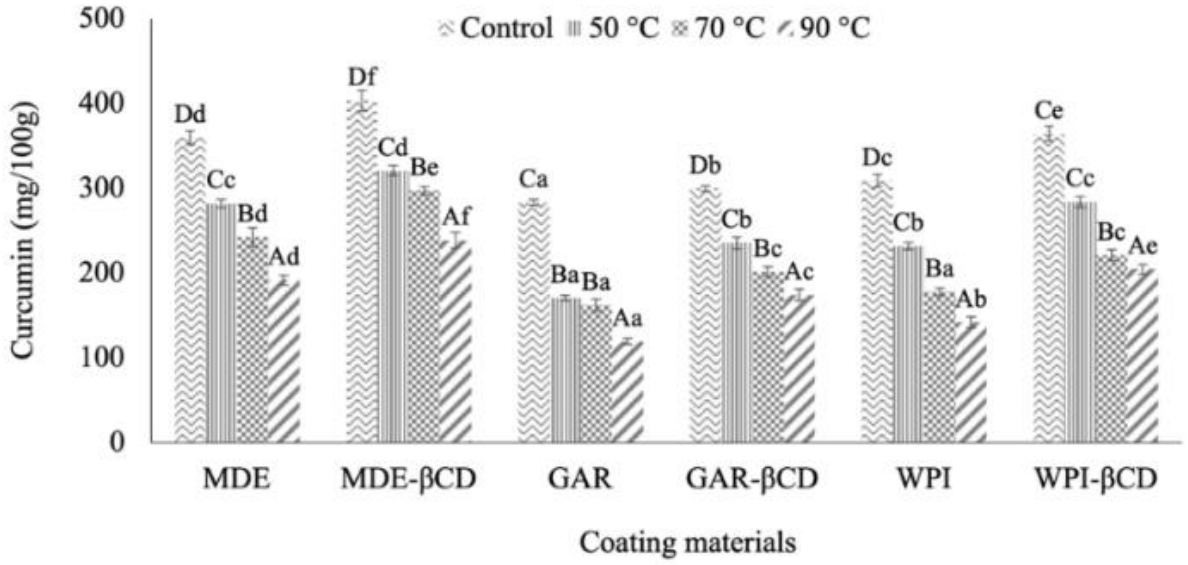

Figure 4 Degradation of curcumin from temulawak extract microcapsules made with different wall materials at various temperature.

pH Stability

Curcumin exhibits chemical instability in medium and high-pH media due to hydrolytic degradation and oxidation, which disrupt its keto-enol tautomeric structure [29]. The main purpose of the microencapsulation process is to protect curcumin from temulawak extract from these conditions. Based on previous studies, without the microencapsulation process, the rate of curcumin degradation occurs rapidly when the pH of the media changes from acidic to basic. Although relatively stable at pH 2, it is completely degraded at pH 9 after 20 h of observation [2]. In this study, curcumin microcapsules exhibited high stability across different pH conditions, with retention above 80 % after 60 h in acidic solutions (pH 2) and around 60 % in neutral conditions (pH 7) (**Figure 5**). Based on pH conditions, the stability of curcumin in acidic conditions (pH 2) is the best and tends to decrease as the pH of the solution increases. Previous studies also observed the same thing, the rate of curcumin degradation increased significantly at higher pH levels [30]. The results of this study are in line with previous reports where curcumin is more chemically stable in acidic conditions [31]. The higher percentage of curcumin degradation in alkaline conditions is caused by hydrolytic degradation, which has an impact on decreasing the stability of curcumin [28]. In contrast, the mechanism of curcumin stability in acidic solutions is caused by the protonation of carbonyl drops in the curcumin molecule, which produces a keto-enol form with a stable conjugated structure. The conjugated structure can be damaged in alkaline solutions due to the deprotonation of 1 hydrogen atom on the central carbon atom in the curcumin framework, and the keto-enol form is converted into a bis-keto form [2,31].

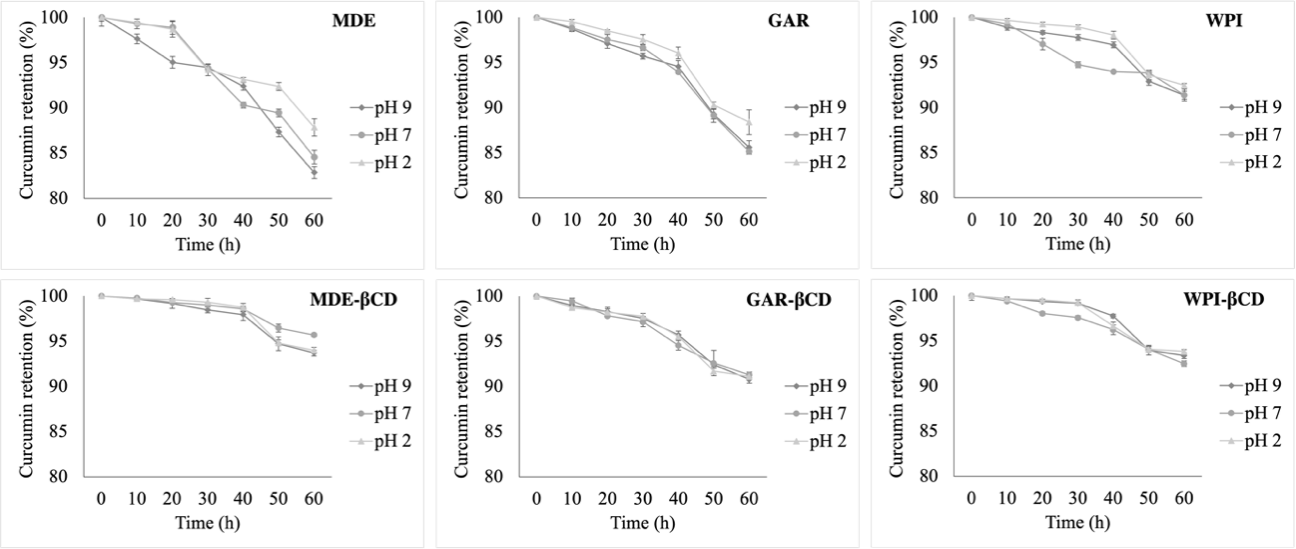

Figure 5 Degradation of curcumin from temulawak extract microcapsules made with different wall materials at various pH.

Based on the type of wall material, the pH stability of curcumin is different from that of other wall materials. WPI wall material tends to produce microcapsules with the best pH stability compared to MDE and GAR. This result shows that each curcumin constituent has different pH stability, so it is crucial to understand the development of an effective delivery system for this bioactive component [31,32]. Compared to single-wall materials, the use of composite wall materials significantly increases the pH stability of curcumin. This is because, in addition to being able to increase the glass transition temperature of particles, the presence of βCD effectively forms inclusion complexation with curcumin [20,33]. The complexation formed has good stability against pH changes [34]. Curcumin retention above 80 % illustrates the success of a microencapsulation process [7]. Since most food products have a pH value of around 2, curcumin microcapsules from temulawak extract can certainly be applied to very acidic foods with excellent resistance.

Controlled release

One of the most significant advantages of the microencapsulation process is the protection and controlled release capability. Controlled release allows the release of compounds under desired conditions thus the effectiveness of the compound can be enhanced. Therefore, the controlled release profile was studied.

Figure 6 shows the release profiles obtained for curcumin microcapsules from curcumin extract prepared with various wall materials. The results are expressed as a percentage of release (amount released at time t, normalized by the total amount released). In general, microcapsules prepared with single wall materials (MDE, GAR and WPI) showed different release profiles from each other. The fast release observed for microcapsules with GAR (50 min) may be attributed to the porous surface structure and lower glass transition temperature, which accelerate curcumin diffusion. In comparison, the same release profile was observed in microcapsules prepared with composite wall materials (MDE-βCD, GAR-βCD and WPI-βCD). However, some differences were observed, especially in the total release time. The curcumin extract microcapsules prepared with MDE-βCD and WPI-βCD required a similar total release time of 158 min, while those prepared with GAR-βCD were slightly faster at 135 min. The wall material is one of the most important factors affecting the curcumin release profile from microcapsules produced by spray drying. This is supported by previous studies that reported the total curcumin release time with chitosan wall material was around 80 min, while with alginate, it was more than 350 min [4]. Other researchers reported that the total curcumin release time with GAR wall material was only around 120 min [3].

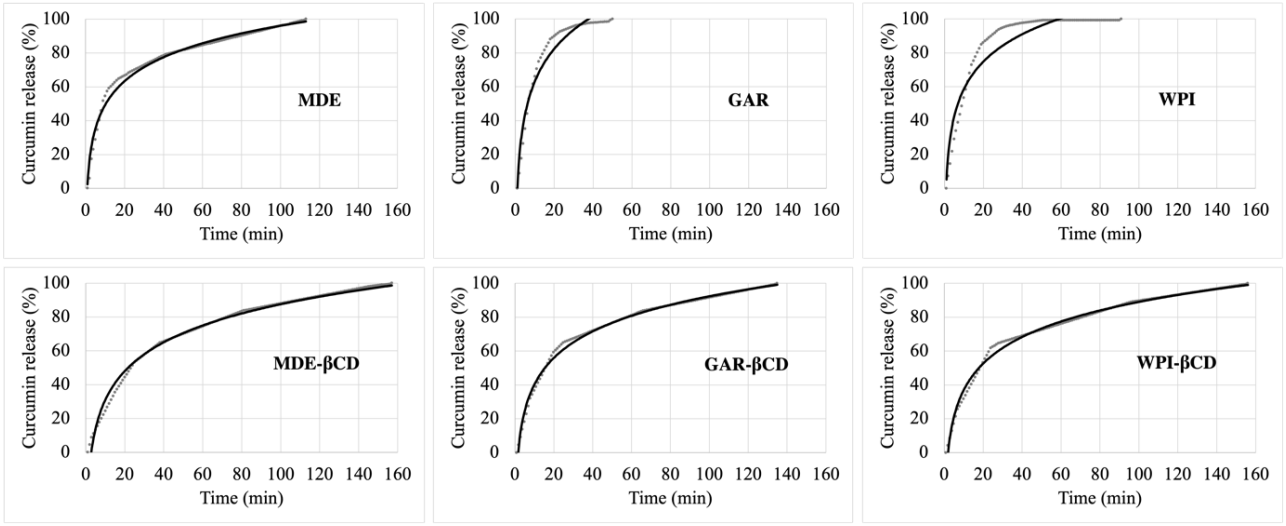

Figure 6 Release of curcumin from microcapsules of temulawak extract made with different wall materials.

The controlled release profile of microcapsules prepared with this composite wall material is like the release profile of curcumin [4] and the release profile of vitamin A [35] prepared with different wall materials. According to previous studies, controlled release can be associated with particle morphology [36]. In this study, microcapsules with finer particle structures tend to have longer release times. This is possible due to the excellent protection of the matrix wall. From the results of this study, if a fast release of curcumin is desired, the best option is to microencapsulate curcumin with GAR. If a slow release of curcumin is desired, the use of composite wall materials with the addition of βCD is the right choice. Selecting appropriate wall materials is essential to meet specific product requirements. For example, rapid-release microcapsules may suit functional beverages, while slow-release formulations are advantageous for supplements or pharmaceuticals requiring sustained delivery. Thus, all *in vitro* release studies and mathematical models can help improve the selection of the best solution for *in vivo* studies and finally for the selection of the final product.

Conclusions

In this study, microencapsulation of curcumin-rich temulawak extract using single wall material and composite wall material by spray drying can be done. Differences in the morphological structure and size of microcapsules have been observed. Microcapsules prepared with single wall material tend to have irregular shapes, cracks and rough surfaces with an average diameter of about 2 μm were observed for all wall materials tested. In the case of microcapsules prepared with GAR, the surface was very rough, with many wrinkles, grooves and cracks. While a smoother particle surface was observed for all microcapsules prepared with composite wall material. Increased stability during storage against temperature, acidic, neutral and basic pH was successfully obtained with composite wall material. Various release profiles were obtained, the release time of total amount of curcumin prepared with single wall material was relatively fast (50 min for GAR, 90 min for WPI and 110 min for MDE), while that prepared with composite wall material was about 135 min (for GAR-βCD) and 158 min (for WPI-βCD and MDE-βCD), and the curves were in good agreement with the Weibull model. In conclusion, the findings demonstrate the potential for applying curcumin microcapsules in food and pharmaceutical industries, especially those prepared with MDE-βCD composite wall material.

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