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The thermostability of temulawak extract encapsulation with several coating variations of sodium tripolyphosphate and chitosan

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Abstract

Temulawak (*Temulawak zanthorhiza Roxb*) is a rhizome plant that contains curcuminoid compounds (curcumin, demethoxycurcumin, and bisdemethoxycurcumin) and essential oils. Curcumin is unstable to heat, hence it is encapsulated to increase its thermostability of curcumin. Encapsulation was made using the freeze-drying method. The study used a completely randomized design method with two factors, namely variations in coating and temperature. This study aimed to produce encapsulation of Temulawak extract with the best thermostability. The variation of the coating used is the concentration of chitosan and sodium tripolyphosphate (STPP) (g/mL), (0.1%: 0.05%), (0.2%: 0.1%), (0.3% : 0 .15%), and (0.4%: 0.2%), and the addition of tween 80. The thermostability method used immersion samples at temperatures of 30°C,40°C,50°C,60°C,70°C, and 80°C with the parameter of the amount of curcumin released which was measured using a UV-VIS spectrophotometer at a wavelength of 425 nm. Encapsulation of Temulawak extract and heating temperature were significantly different from curcumin content. After 80°C heat treatment for 20 mins, Curcuma extract in formula 1 coating with 0.1% chitosan and 0.05% STPP had the highest residual curcumin.

1. Introduction

Temulawak (*Temulawak xanthorhiza Roxb*) is a spice plant that belongs to the *Zingiberaceae* family. Temulawak has a pungent, sharp aroma, and a bitter taste (Said, 2007). It contains protein, starch, curcuminoid yellow dye, and essential oil. Curcuminoids have functioned as antioxidants, anti-inflammatory, antibacterial, anti-tumour, hepatoprotective and antihyperlipidemic properties (Cho *et al*., 2017; Syamsudin *et al*., 2019). Curcuminoids in Temulawak consist of three homogeneous components: curcumin, demethoxycurcumin, and bisdemethoxycurcumin (Anggrahini *et al.*, 2007; Cahyono *et al*., 2019; Kementerian Kesehatan Republik Indonesia, 2017).

Curcumin is unstable in heat. Temperature and duration of heating significantly affect the degradation of curcumin (Tensiska *et al*., 2012). Therefore, curcumin can be protected by encapsulation. Encapsulation effectively protects sensitive compounds from degradation and can mask the unpleasant taste of specific compounds (Kumari *et al*., 2014). The number of active ingredients that can be coated and the particle size are affected by incorporating the coating used (Jayanudin and Rochmadi, 2017). One of the coating materials that can be used for food is Chitosan.

Chitosan has a powder texture, is white, has no odour, and has low toxicity (Agustina *et al*., 2015). Chitosan can form a film layer and improve texture but has brittle mechanical properties, therefore it must be stabilized with crosslinking agents, such as sodium tripolyphosphate (STPP).

STPP can interact with polymer chains of Chitosan, form a denser network, and increase the polymer's stability to be more resistant to high temperatures (Jayanudin *et al*., 2015). The process of making encapsulation also requires a homogeneous solution to get good encapsulation. The addition of tween 80 can prevent clumps from forming in the encapsulation

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process (Masitoh *et al*., 2016).

Thermostability is a test carried out to determine the encapsulation stability of Temulawak extract at various temperatures using curcumin content parameters. Good coating stability can protect the coated components, such as curcumin which is not heat resistant. Curcuma extract which has good thermostability properties shows Curcuma can be absorbed maximally in the body. Based on this description, the study was conducted to determine the effect of variations in coating and heating temperature on curcumin levels in the encapsulation of Temulawak extract.

2. Materials and methods

2.1 Study design

This type of research is a true experiment using a completely randomized design with two treatment factors and two replications. The first factor is the heating temperature which consists of 7 different temperatures. The second factor is the variation of the coating, which consists of 4 formulations. Research replication was determined based on the formula (t-1)(r-1) 15 and obtained a repeat value of 1.6, therefore two replications were carried out for each treatment. Thus, the data as a whole produces 56 combinations.

2.2 Materials

Temulawak extract from PT. Java Plant, Chitosan (Biotech Surindo No. Bacth CHC_1119AC765) from Subuh Kimia Jaya, STPP (sodium tripolyphosphate) from Kimia Indrasari Semarang, tween 80 from Kimia Indrasari Semarang, 1% acetic acid, ethanol and n-hexane from Merck, standard curcumin, distilled water.

2.3 Equipment

Analytical balance (Shimidzu/ATX 224), magnetic stirrer (Cimarec 2), sonicator (ultrasonic homogenizer UP100H), freeze dryer (CUDDON FD 80), water bath (Biobase), UV-Vis spectrophotometer (AMTAST AMV09), separating funnel.

2.4 Temulawak extract

Temulawak extract (v) was extracted using the liquid: liquid method with a separating funnel. Temulawak extract was dissolved in ethanol and n-hexane (1 : 3). Two phases formed: the ethanol phase containing the temulawak extract and the n-hexane phase. The ethanol phase is in the lower layer due to its density (p: 0.7893 g/mL) which is higher than n-hexane (p: 0.6606 g/mL) (Rosidi, 2020). Temulawak ethanol extract was separated and processed to obtain thick temulawak extract.

2.5 Temulawak extract encapsulation

The encapsulation of Temulawak extract was carried out based on the method by Sari *et al*. (2018) using the ionic gelation method. Modifications were made to the coated material in the form of Temulawak extract and four variations of the ratio of Chitosan and STPP, (0.1%: 0.05%), (0.2%: 0.1%), (0.3%: 0.15%), (0 ,4% : 0.2%) and the addition of tween 80. First, Chitosan was dissolved in 100 mL of 1% acetic acid (w/v) using a magnetic stirrer then the temulawak extract was gradually added. The solution was stirred until the ingredients were well mixed. Next, STPP was dissolved in 40 mL of distilled water, and then 200 µL of tween 80 (0.1%) was added. Next, the particle size was reduced using a homogenizer at 7500 rpm for 30 mins. Last, the solution was dried using a freeze dryer at a temperature of -100^{o}C for approximately eight days for each formula to obtain the encapsulation of the Temulawak extract. The comparison of the formula variations of the four coatings can be seen in Table 1.

2.6 Thermostability test

The thermostability test of curcumin was analyzed based on the method used by Sun (2017) with several modifications (Sun *et al*., 2017). First, the encapsulated sample of Temulawak extract was dissolved in ethanol with a concentration of 100 ppm. A total of 5 mL of the sample solution was put into a test tube. Then, each sample was heat treated with temperatures of 30^{o}C, 40^{o}C, 50^{o}C, 60^{o}C, 70^{o}C, and 80^{o}C for 20 mins using a water bath. After heat treatment, the samples were cooled in cold water to room temperature. Before heating, absorbance measurements were taken as control data for curcumin at room temperature. Furthermore, the analysis of curcumin with a UV-Vis spectrophotometer at a wavelength of 425 nm was carried out.

2.7 Analysis of curcumin levels

Preparation of standard curcumin solution in ethanol with a concentration of 100 ppm and diluted into several concentrations, 0, 2, 4, 6, 8, 10, and 12 ppm. The absorbance of standard curcumin was measured using a wavelength of 425 nm. The results of the absorbance of standard curcumin were used to obtain the standard curcumin calibration curve equation. Each sample of encapsulated Temulawak extract treated with thermal treatment was put in a cuvette, and then its absorbance was measured at a wavelength of 425 nm. Then, the absorbance data is substituted into the standard calibration curve equation to obtain the reading results (ppm). Next, the curcumin content was calculated using the following formula (Rosidi, 2020):

$$Curcumin\ Total\ \left(\%\frac{w}{w}\right) = \frac{Concentrate\ (ppm) \times volume\ (mL)}{sample\ weight\ (g)}/1000$$

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Table 1. Comparison of various coating formulas.

Formula	Temulawak Extract	Chitosan		STPP		Tween 80
		%	g/mL	%	g/mL	
F1	0.3 g	0.1	0.1/100	0.05	0.02/40	200 µL
F2	0.3 g	0.2	0.2/100	0.1	0.04/40	200 µL
F3	0.3 g	0.3	0.3/100	0.15	0.06/40	200 µL
F4	0.3 g	0.4	0.4/100	0.2	0.08/40	200 µL

2.8 Data analysis

The data were processed using the SPSS statistic 17.0 application by testing its normality first using the Kolmogorov-Smirnov test at a significance level of 0.05. The data is declared normally distributed if the significance is more than 0.05. If the data is normally distributed, the analysis is carried out using a two-way analysis of variance (two-way ANOVA). The interaction analysis of coating and temperature variations on curcumin levels was tested using one-way ANOVA.

3. Results and discussion

3.1 Temulawak extract encapsulation

The concentration ratio of chitosan: STPP was made with 4 different variations, (0.1%: 0.05%), (0.2%: 0.1%), (0.3%: 0.15%), (0,4%: 0.2%). The results of the encapsulation of the coating variations can be seen in Figure 1. In terms of colour, formula 1 has a more yellow colour than formulas 2, 3, and 4 (Afandi, 2014).

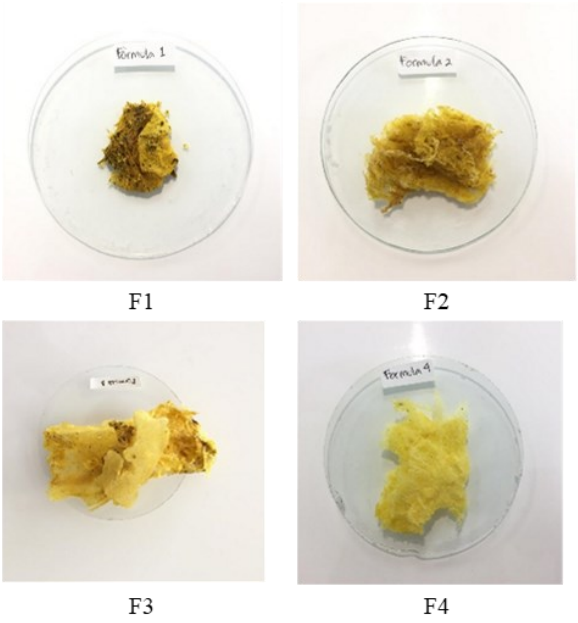

Figure 1. Temulawak extract encapsulation.

The distribution of the yellow pigment in each formula is different. For example, the yellow pigment formula 4 looks the most spread and evenly distributed but produces the palest yellow colour. On the other hand, the yellow pigment in formula 1 was observed to be the most concentrated. However, the distribution was not even compared to formula 4 and others, because some parts look very yellow and others look yellow. This result could be due to the less homogeneous encapsulation solution during the encapsulation process.

The encapsulation weight of Temulawak extract was obtained from 1 L of encapsulated solution. Based on the comparison of the encapsulation weights produced from the four formulas, formula 4 has the highest weight at 6.118 g. This result could be due to the highest concentration of Chitosan and STPP used in formula 4.

3.2 Curcumin standard calibration curve

Curcumin content in the encapsulated Temulawak extract can be calculated based on the standard curcumin calibration curve equation. The standard curcumin calibration curve equation is obtained from the ratio of pure curcumin concentration to its absorbance. Therefore, the curcumin concentrations made were expressed on the x-axis, while the absorbance obtained from the UV-Vis spectrophotometry measurements was expressed on the y-axis.

From the absorbance results obtained from each concentration, the equation for the curcumin calibration curve was obtained, namely $y = 0.1395x + 0.0189$ with a correlation coefficient (R^2) of 0.9994 (Figure 2). Thus, the correlation coefficient obtained is close to 1, meaning that the relationship between concentration and absorbance is linear.

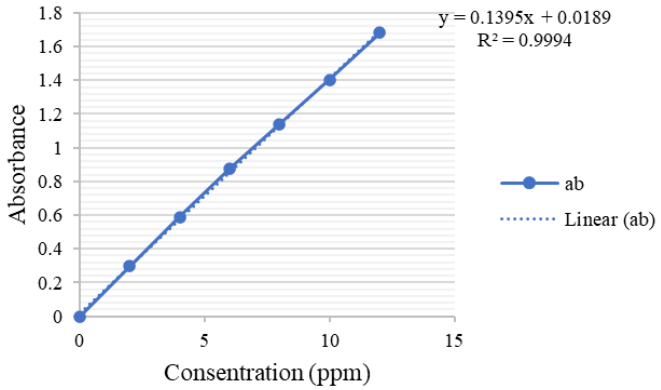

Figure 2. Calibration curve of curcumin standard.

3.3 Curcumin levels in the encapsulation of Temulawak extract

Curcumin levels in the encapsulated Temulawak

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extract showed that the average curcumin levels in each formula were significantly different ($p<0.05$) (Table 2). The average levels of curcumin in formulas 1, 2, 3, and 4 were 4.56%, 1.12%, 0.2%, and 0.17%, respectively. The highest curcumin content in the encapsulated Temulawak extract was found in formula 1, amounting to 4.56%, while the lowest curcumin content was found in formula 4, amounting to 0.17%. The curcumin content in the encapsulated Temulawak extract was lower than the curcumin content in the Temulawak extract.

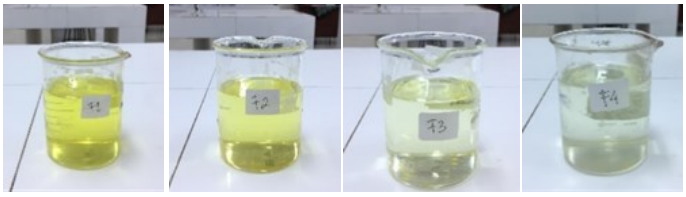
Table 2. Curcumin levels before heat treatment.

Formula	Curcumin level	p-value
F1	4.56	<0.001
F2	1.12	
F3	0.20	
F4	0.17	

Rosidi's research on Temulawak extract found curcumin levels of 27.19% (Rosidi *et al*., 2016). According to Hsieh *et al*. (2006), the thicker the concentration of Chitosan, the thicker the capsule membrane that will form in the encapsulation, and the smaller the pores between the chitosan molecules, the coated substance takes longer to release from the encapsulation (Hsieh *et al*., 2006). Curcumin substances with high concentrations of Chitosan will be more difficult to release, and the readable curcumin levels when the absorbance measurement becomes smaller. In addition, high concentrations of Chitosan and STPP can result in large encapsulation weights and curcumin that spreads throughout the encapsulation, thus the same sample weight of encapsulation formulas 1, 2, 3, and 4 will be different from each other.

3.4 Temulawak extract encapsulation thermostability

The sample used for the thermostability test was the encapsulation of Temulawak extract dissolved in ethanol. The encapsulated sample solution of Temulawak extract can be seen in Figure 3. In the sample-making process, encapsulated fibres are difficult to dissolve in each sample, hence they cannot be 100% soluble. As a result, formula 4 has more fibre residue than formula 3, 2, and 1. This result could be caused because Chitosan can dissolve well in acidic pH solutions such as acetic acid, and the chitosan content in formula 4 is the highest. Chitosan is insoluble in water with a neutral pH but soluble in acidic pH and in an acidic solution (pH <6.5) can convert glucosamine units into the soluble form of $R\text{-}NH_3^+$ (Shweta and Sonia, 2013; Pratiwi, 2015).



F1 F2 F3 F4

Figure 3. Temulawak extract solution in ethanol solvent.

The thermostability of encapsulated Temulawak extract can be determined from the amount of curcumin remaining after being heat-treated at various temperatures (Figure 4). The analysis of curcumin levels showed that the average levels of curcumin at each temperature were significantly different ($p<0.05$). The average percentage of curcumin content after heat treatment can be seen in Table 3.

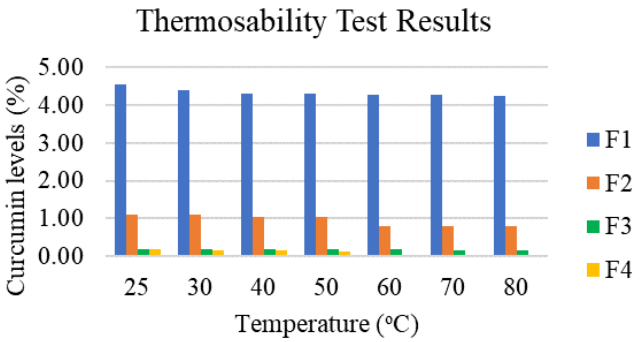


Figure 4. Curcumin levels after heat treatment.

Table 3. Percentage (%) of average curcumin content after heat treatment.

Temperature (°C)	F1	F2	F3	F4
25	4.56	1.12	0.20	0.18
30	4.41	1.12	0.19	0.17
40	4.32	1.06	0.19	0.15
50	4.31	1.05	0.19	0.13
60	4.29	0.81	0.18	0.00
70	4.27	0.81	0.17	0.00
80	4.24	0.80	0.16	0.00

The interaction analysis results of coating variations and heating temperature on curcumin levels were significantly different ($p<0.05$). With the decrease in the release of curcumin levels in the encapsulation of temulawak extract after being heat-treated, all formulas began to experience a decrease in curcumin levels at a temperature of 60°C. Formulas 4, 3, and 2 experienced a significant decrease in curcumin levels after heating at 80°C. Research conducted by Hsieh *et al*. (2006) reported that heating chitosan oil encapsulated with micro-size Chitosan: STPP coating at 80°C for 50 mins causes the chitosan molecular chain to gradually shrink in order that the space between molecules is reduced (Hsieh *et al*., 2006). The increase in heating time causes the pores in the chitosan encapsulation membrane to become tighter. As a result, the coated substance becomes challenging to release, thereby reducing the coated substance's release rate. Research by Ermawati *et al*. (2021) found that the curcuminoid levels in the tamarind turmeric herbal medicine after being stored in

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the refrigerator (4±2°C) and freezer (-10±2°C) temperature showed that the storage temperature affected the decrease in curcuminoid levels (Ermawati *et al*., 2021). The lower the storage temperature can slow down the degradation of curcuminoids.

Formula 1 has the best stability after heating at 80°C with a residual curcumin content of 93%. The levels of curcumin in formulas 2, 3, and 4 after heating at 80°C had the remaining percentage of curcumin content of 71.5%, 80.3%, and 0%. The research of Sun *et al*. (2017) showed that pure curcumin, which was heat-treated at 80°C for 20 mins, experienced a high decrease in curcumin levels, with residual curcumin of 15% (Sun *et al*., 2017). This result shows that coating variations in formulas 1, 2, and 3 can increase the thermostability of curcumin from Temulawak extract at a temperature of 80°C, therefore there is an effect of coating variations and heating temperature on the curcumin content of Temulawak extract.

4. Conclusion

The best thermostable curcumin extract in the treatment is formula 1. The concentration ratio of chitosan and STPP (g/mL) 0.1%: 0.05% was the best formula with a residual curcumin content of 93% after 80°C heat treatment for 20 mins.

Conflict of interest

The authors declare no conflict of interest.

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