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Total Flavonoids Content and Antioxidant Activity Encapsulation of Curcuma Extract Based on Variation of Coating Concentrations

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Abstract: Curcuma is a functional food that has active flavonoid compounds and functions as an antioxidant. An attempt to maintain bioactive compounds in Curcuma extract is encapsulation. Recently, application of the encapsulation technology in the extracting process and chitosan as a coating material has shown promising results for antioxidant levels and total flavonoids. This study aimed to determine the effect of variations in coating concentrations of Curcuma encapsulation on total flavonoid content and antioxidant activity. Chitosan was used for encapsulation as the main polymer and STPP (Sodium Tripolyphosphate) was used as a cross-linking agent through the ionic gelation method. This study used completely randomized design with several treatments. Comparison of variations in chitosan coating: STPP was F1 0.1:0.05, F2 0.2:0.1, F3 0.3:0.15, and F4 0.4:0.2. Total flavonoid content was measured using the colorimetric method, while the antioxidant activity was measured by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) method. The results show that the highest average total flavonoid content (74.790 ± 3.783 mg QE/g) and antioxidant content ($48.765 \pm 1.362\%$) were in formula 1. In conclusion, the lower the concentration of chitosan and STPP, the higher the levels of flavonoids and antioxidant activity.

Keywords: antioxidant activity, curcuma, encapsulation, flavonoids.

基于包衣浓度变化的姜黄提取物总黄酮含量及抗氧化活性封装

摘要: 姜黄是一种功能性食品, 含有活性黄酮类化合物, 具有抗氧化作用。在姜黄提取物中保持生物活性化合物的尝试是封装。最近, 在提取过程中使用封装技术和壳聚糖作为涂层材料在抗氧化水平和总黄酮方面显示出有希望的结果。本研究旨在确定涂层浓度的变化对姜黄的封装对总黄酮含量和抗氧化活性的影响。封装以壳聚糖为主要聚合物, 直通车 (三聚磷酸钠) 为交联剂, 采用离子凝胶法。该研究使用了具有多种治疗的完全随机设计。壳聚糖涂层变化的比较: 直通车为 F1 0.1:0.05、F2 0.2:0.1、F3 0.3:0.15 和 F4 0.4:0.2。总黄酮含量采用比色法测定, 抗氧化活性采用 DPPH (2,2-二苯基-1-苦基-胍基-水合物) 法测定。结果表明, 配方 1 的平均总黄酮含量 (74.790 ± 3.783 毫克量化宽松/克) 和抗氧化剂含量 ($48.765 \pm 1.362\%$) 最高。抗氧化活性也增加。

关键词: 抗氧化活性, 姜黄, 封装, 黄酮类化合物。

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1. Introduction

Degenerative disease is a health problem that is the leading cause of death in Indonesia. According to the Indonesia Basic Health Research 2018, stroke was the leading cause of death, with the prevalence of 10.9%, followed by diabetes mellitus 8.5% [1]. The higher a load of free radicals in the body will cause oxidative stress. This condition will cause cell, tissue, or organ damage that triggers degenerative diseases such as stroke, diabetes mellitus, and atherosclerosis that lead to heart disease [2]. Antioxidants are needed by the body to protect against and prevent oxidative stress [3].

Curcuma is one of the functional foods widely formulated into various food products and has antioxidant activity [4]. Components that act as antioxidants from Curcuma rhizomes are flavonoids included in phenolic compounds. However, phenolic compounds are sensitive to processing, pH, and temperature and are difficult to dissolve in water. Besides that, many active ingredient molecules have a bitter taste that limits their use for consumption [5]. An attempt to overcome this is by encapsulation.

Encapsulation technology is widely used for product development because it can increase the absorption of active compounds by the body and improve product quality [6].

One of the coating materials that can be used is chitosan. Chitosan as a coating material is non-toxic, safe for food products, and can form a gel [7], [8]. However, chitosan needs to be stabilized because it is brittle by cross-linking sodium triphosphate to increase the mechanical strength of the gel formed. The encapsulation is made by the ionic gelation method because the process is simple and can be controlled easily. The principle of this method is the occurrence of electrostatic interactions between the amine groups in chitosan and STPP polyanions to form a three-dimensional intramolecular structure [9]. Several factors could affect the coating material and the bioactive content. The type of encapsulation affects the characteristics of the microcapsules, such as encapsulation efficiency, solubility in water, and stability [10]. The characteristics and efficiency of encapsulation are also influenced by the concentration of the coating material [11]. This study aims to determine the effect of variations in coating concentration on total flavonoid levels and antioxidant activity in the encapsulation of Curcuma extract. Encapsulation requires incorporation with a nonionic surfactant, namely tween 80. Tween 80 serves to prevent combinations and increase emulsion stability [12]. This study aimed to determine the effect of variations in coating concentration on total flavonoid

content and antioxidant activity in the encapsulation of Curcuma extract.

2. Methods

2.1. Research Methods

This study was true experimental with a completely randomized design (CRD). The encapsulation of Curcuma extract was made at the Food Technology Laboratory, Soegijapranata Catholic University, Semarang. Antioxidant activity and total flavonoid content were tested at the Food Chemistry Laboratory, Faculty of Nursing and Health Sciences, Universitas Muhammadiyah Semarang.

The materials used in this study were Curcuma extract bought from PT Java Plant, Chitosan (Biotech Surindo No. Batch CHC_1119AC765.M) from Subur Kimia Jaya, STPP from Kimia Indrasari Semarang, Tween 80 from Kimia Indrasari Semarang, 1% acetic acid, pro-analysis ethanol, distilled water, aluminum chloride (AlCl₃) 10%, potassium acetate, quercetin, and DPPH.

The equipment used in this study were Freeze dryer (CUDDON FD 80), hot plate stirrer (cimarec 2), Vortex mixer (super-mixer), sonicator (ultrasonic homogenizer UP100H), spectrophotometer-UV vis (AMV 09), digital scale (Shimadzu/ATX 224), measuring cup, burette, micropipette, and aluminum foil.

2.2. Research Procedure

2.2.1. Preparation of Extract Ingredients

The preparation of Curcuma extract was re-purified to obtain the active compound content of the extract produced. The extract was purified using n-hexane under the method for extracting [13] with a ratio of ethanol extract: n-hexane 1:3. The liquid-liquid extraction was carried out twice for 30 minutes for each extraction. The solvent n-hexane was used to dissolve non-polar compounds and the fat component of the extract. This extraction resulted in two layers: the top layer containing the n-hexane phase and the bottom layer containing the ethanol phase.

2.2.2. Preparation of Coating Material

The first stage is making a chitosan solution by dissolving 0.2 grams in 100 mL of 1% (w/v) acetic acid, and then the chitosan solution is stirred with a magnetic stirrer until dissolved. The second stage involves making STPP solution from 0.04 grams of STPP by adding distilled water to obtain 40 mL. Tween 80 is in a liquid form; therefore, it can be added without any preparatory steps.

Table 1 Curcuma extract encapsulation formula

Formula	Curcuma Extract	Chitosan		STPP		Tween 80
		%	gram/ml	%	gram/ml	
F1	0.3 gram	0.1	0.1/100	0.05	0.02/40	200 μ L
F2	0.3 gram	0.2	0.2/100	0.1	0.04/40	200 μ L
F3	0.3 gram	0.3	0.3/100	0.15	0.06/40	200 μ L
F4	0.3 gram	0.4	0.4/100	0.2	0.08/40	200 μ L

2.2.3. Ingredients

The encapsulation of Curcuma extract was made based on the method developed by Sari et al. (2018) and modified using four formula variations (%), as shown in Table 1 [14].

2.2.4. Preparation of Chitosan Encapsulation of Curcuma Extract

The encapsulation procedure refers to the research described in [14] with some modifications. First, a total of 0.3 grams of Curcuma extract was dissolved in the prepared chitosan solution, and 200 μ L of Tween 80 0.1% (v/v) was added. Second, the STPP solution was added dropwise to the chitosan extract solution using a burette accompanied by stirring with a magnetic stirrer [14]. Third, the size of the Curcuma extract was reduced using a sonicator at a speed of 7500 rpm in 30 minutes and, lastly, dried using a freeze dryer at a temperature of -1000C.

2.3. Colorimetric Method of Total Flavonoid Test

2.3.1. Establishment of Standard Quercetin Curve

This procedure refers to the research described in [15]. A standard solution of 1000 ppm quercetin was prepared by weighing 25 mg of standard quercetin and then dissolved with ethanol to a volume of 25 mL, then made several concentrations, namely 20 ppm, 30 ppm, 40 ppm, 50 ppm, and 60 ppm [15]. Each concentration was added to a 0.5 mL pipette with 1.5 mL of ethanol, 0.1 mL of $AlCl_3$, 0.1 mL of 1 M potassium acetate, and made up to volume with 2.8 mL of distilled water. The solution was homogenized with a vortex for 3 seconds and allowed to stand for 30 minutes at room temperature. Next, the test solution measured its absorbance at a maximum absorption wavelength of 415 nm.

2.3.2. Determination of Flavonoid Content

This procedure refers to the research discussed in [15]. First, a total of 0.02 grams of the encapsulated sample was weighed and then dissolved in 100 mL of 70% ethanol. Then 0.5 mL of the solution was pipetted, then 1.5 mL of ethanol, 0.1 mL of $AlCl_3$, 0.1 mL of 1 M potassium acetate were added, and the volume was made up to 10 mL with distilled water. The mixture was then incubated for 30 minutes at 250C, and the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 415 nm. The total flavonoid calculation is as follows:

$$TFC \text{ (mg QE/g)} = \frac{C \left(\frac{\text{mg}}{\text{L}} \right) \times V \text{ (L)}}{m \text{ (g)}} \times FP$$

where C - concentration of flavonoid solution;
V - extract volume;
m - sample weight.

2.4. Antioxidant Activity Test DPPH Method

2.4.1. Determination of Antioxidant Activity

This procedure refers to the research of Xu and Chang [16]. Analysis of antioxidant activity using the DPPH method (1.1-diphenyl-2-picrylhydrazyl) [16]. Testing of antioxidant activity was carried out three times using ethanol as a blank with four variations in the concentration of different formulas. Preparation of the sample solution was weighed as much as 0.02 grams of encapsulated ginger dissolved in 200 mL of ethanol. Samples were taken 0.2 ml, added 3.8 ml of 0.1 M DPPH solution, and homogenized using a vortex for approximately 60 seconds. They were then incubated for 30 minutes. After incubation, absorbance readings were carried out with a spectrophotometer by adjusting the wavelength to 517 nm. A color change will indicate the presence of radicals from purple to yellow.

The control treatment was used as a controller and treated the same as the sample. Blank (control) used ethanol as a sample substitute. Free radical scavenging activity was assessed as reduced DPPH color. Free radical scavenging power is expressed in percent (%) RSA (% Radical Scavenging Activity) is % DPPH bleaching. The formula used to calculate the results of the antioxidant activity analysis is as follows:

$$\% \text{ RSA} = \left(\frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \right) \times 100\%$$

2.5. Data Processing and Analysis

Data analysis was performed using the ANOVA test to determine the effect of variations in coating concentration on Curcuma encapsulation on total flavonoid levels and antioxidant activity.

3. Results and Discussion

3.1. Extract Turmeric Encapsulation

The results of encapsulation of Curcuma extract with variations of chitosan and STPP are shown in Figure 1.

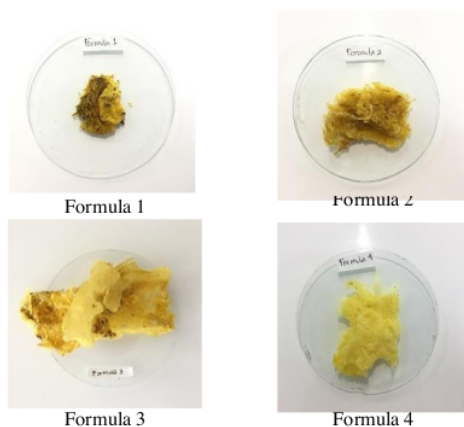


Fig. 1 Curcuma encapsulation solid product

The product produced from formula 1 was brownish from the extract precipitate attached to the chitosan bonds formed. The product of formula 2 was yellowish-brown in color, and the product of formula 3 was yellowish. Formula 4 was yellow. The difference in the encapsulated product's color is due to interactions that result in changes in electron transitions [17]. Increasing the chitosan concentration in the

encapsulation process can increase the clarity and dispersion formed [18]. The addition of STPP as a cross-linking agent can increase the encapsulation ability of chitosan by loosening the bonds between chitosan, causing particle size to increase [19].

3.2. Total Flavonoids Contents

Determination of flavonoid levels was carried out to determine the amount of flavonoid bioactive compounds contained in the encapsulation of Curcuma. Determination of total flavonoid levels using quercetin equivalent in units of mg QE/g. The absorbance of the quercetin obtained was then plotted against the concentration to obtain a calibration curve. Based on the study results, the absorbance data was

obtained by the equation of the flavonoid standard curve line $y = 0.0055x + 0.0532$ with an R^2 value of 0.9995. The R^2 value obtained close to 1 indicates that the calibration curve is linear, and there is a strong relationship between the concentration of the quercetin solution and the absorption value. The results of the measurement of total flavonoid levels are presented in Table 2.

Table 2 Total flavonoids content and antioxidants level encapsulated curcuma extract

Formula	Flavonoid Content		Antioxidant Level	
	Mean \pm SD	p-value	Mean \pm SD	p-value
F1	74.790 \pm 3.783 ^d	0.000	48.765 \pm 1.362 ^d	p<0.05
F2	33.577 \pm 2.775 ^c	0.000	33.358 \pm 1.214 ^{b,c}	0.169
F3	23.270 \pm 1.820 ^b	0.000	31.298 \pm 1.247 ^{b,c}	0.169
F4	6.303 \pm 2.780 ^a	0.000	8.843 \pm 2.493 ^a	0.000

Notes: Different letter notations indicate significant differences ($p < 0.05$).

The data presented in Table 2 shows that the average total flavonoid content in the encapsulated Curcuma extract ranged from 6.303 to 74.790 mg QE/g. Based on the analysis of variance (ANOVA) test, the difference in coating concentration affected the total flavonoid content ($p < 0.001$). Based on the LSD test, there was a significant difference in the total flavonoid levels in each formula at the 5% level.

The results of the determination of total flavonoid levels showed that formula 1 using a concentration of chitosan (0.1): STPP (0.05) had the highest mean total flavonoid content of 74.790 mg QE/g (1 g encapsulation of Curcuma extract was equivalent to 74.790 mg of quercetin). Formula 4 uses chitosan concentration (0.4%): STPP (0.2%) has the lowest total flavonoid content, which is 6.303 mg QE/g. This happened because the molecular weight of chitosan and the concentration of STPP affected the encapsulation efficiency that could trap the extract. The lower use of chitosan will increase the encapsulation efficiency of the extract so that the flavonoid content will increase.

Chitosan, which has a low molecular weight, will have a higher encapsulation efficiency. The chitosan chain is shorter so that it is easily protonated, making it

easier to interact with the extract. The encapsulated molecules will be larger than chitosan, which has a long chain [10]. Wu et al. reported that increasing the chitosan concentration can reduce the encapsulation efficiency of nanoparticles of ammonium glycyrrhizinate compound by the ionic gelation method [20]. The use of STPP as a cross-linking agent also affects the number of active ingredients coated. Increasing the concentration of STPP will reduce the value of encapsulation efficiency because it can cause rapid solidification formation so that there is little active ingredient coated [21]. The study results [22] reported that the optimal STPP concentration used was 0.1%, where the STPP concentration had to be controlled to prevent rapid droplet solidification during the ionic gelation reaction process.

3.3. Antioxidant Level

The data obtained from the determination of the antioxidant activity of the encapsulated Curcuma extract are presented in Table 2. Table 2 shows that formula 1 has an average antioxidant content in the range of values from 48.765% to 8.843%. Based on analysis of variance (ANOVA), the difference in

coating concentration affected the antioxidant activity ($p = 0.000$). The follow-up test results showed that the coating concentrations in formula 1 (0.1:0.05) and formula 4 (0.4:0.2) were significantly different at a significant level of 5%. On the other hand, formula 2 (0.2:0.1) and formula 3 (0.3:0.15) showed antioxidant activity that was not significantly different from the encapsulation of Curcuma extract.

Based on research by Saifuddin [23], antioxidant activity is classified into 3, namely, low ($< 20\%$), moderate (20-50%), and high ($> 50\%$). The results determined the highest average antioxidant content of encapsulated Curcuma extract in formula 1 with a concentration of chitosan (0.1): STPP (0.050) of 48.765% included in the medium category. The antioxidant content of formula four chitosan (0.4): STPP (0.2) was included in the low category of 7.703%, with the same concentration of ginger in each formula. The magnitude of the reducing power of an antioxidant extract shows its ability as an electron donor. Therefore, it can react with free radicals to turn it into a stable one and end the radical chain reaction [23]. The cause of this reaction is that the higher the total phenolic content of the microcapsules, the higher the antioxidant activity [24]. According to [25], phenolic compounds contribute to antioxidant activity.

3.4. Relationship between Flavonoid Levels and Antioxidant Activity

Based on Figure 2, the linear regression results show a relationship between total flavonoid levels and antioxidant activity with a correlation value of 0.8489. Figure 2 explains that for every 1% addition of flavonoids, the antioxidant activity increases by 0.520.

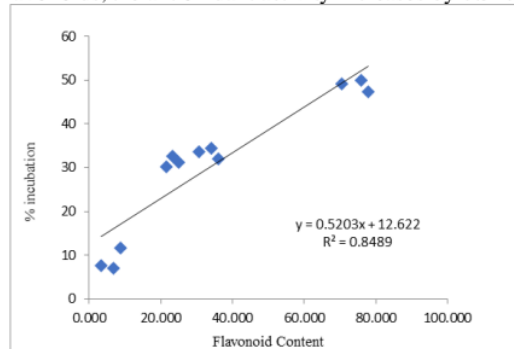


Fig. 2 Relationship between total flavonoid levels and antioxidant level

As reported in [23], the total flavonoid content contributed to the antioxidant activity of the cinnamon ethanol extract. The antioxidant activity decreased along with the reduced total flavonoid content that was absorbed in the encapsulation process. The percentage of antioxidant activity in the encapsulation is influenced by flavonoids included in the phenolic compound group. Phenolic compounds can bind free radicals [25]. Flavonoid compounds have potential as

antioxidants because they have a hydroxyl group bound to the carbon of the aromatic ring to capture free radicals resulting from fat peroxidation reactions. In addition, flavonoid compounds will donate one hydrogen atom to stabilize lipid peroxide radicals [26].

4. Conclusion

This study found that differences in the concentration of chitosan and STPP affect flavonoid levels and antioxidant activity. The best combination formula is the use of a coating with a concentration of chitosan (0.1): STPP (0.05), resulting in an average antioxidant activity value of 48.765% and a total flavonoid content of 74,790 mg QE/g. The level of antioxidant activity is influenced by the level of flavonoids, where the antioxidant activity decreases along with the reduction in the total content of flavonoids absorbed in the encapsulation process.

Compared to other studies, Curcuma extract in previous studies did not use the encapsulation process.

The manufacture of Curcuma extract through the encapsulation process shows high levels of antioxidants. The recommendation for further research is to make various formulas for encapsulation to obtain better results and perform other bioactivity tests because Curcuma extract has many benefits and thermostability tests to determine the encapsulation stability of Curcuma extract from various temperatures.

However, this study had a limitation, as the results of this study cannot be applied to food products. Further research is needed with various formulas, aiming to find the best formula that can be applied to food products.

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