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Antioxidant activity of microencapsulated lemongrass (*Cymbopugon citratus*) extract

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Antioxidant activity of microencapsulated lemongrass (*Cymbopugon citratus*) extract

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Abstract. Lemongrass is a plant with various bioactive components including alkaloids, flavonoids, tannins and essential oils; which is potential to be developed into natural preservative. However, the components are susceptible to the environmental conditions, such as temperature or oxygen contact. Formulation of lemongrass extract (LE) into powder form through microencapsulation, able to retain its stability over temperature or oxygen contact, moreover in the form of powder its application on food ingredients become flexible. This study aimed to assess antioxidant activity of microencapsulated lemongrass extract. This study used Randomized Block Design (RBD) with 18 treatment combinations and conducted in two replications. Factors examined in this study include; lemongrass extract concentrations (K); 25, 16.7 and 12.5%; microcapsule ingredients (P); maltodextrin and β–cyclodextrin and heating temperature (T) of; 120, 130, and 140ºC. Result of the study showed that microencapsulation of 25% lemongrass extract using β–cyclodextrin heated at 120ºC gave the highest antioxidant activity of 14.14% and the highest total phenol value of 34.64 mg/100gr. This study resulted that the use of β–cyclodextrin produce microcapsule with better antioxidant activity.

1. Introduction

Lemongrass (*Cymbopogun citratus*) is a local plant from the family *Graminae / Poaceae*. This plant grows in areas with tropical and sub-tropical climates in Southeast Asia and Africa [1]. Lemongrass is known to be rich in bioactive compounds, such as; alkaloids, flavonoids, tannins and essential oils; which is known to have many benefits, especially in the fields of food, agriculture, pharmacy and health, through the use of its bioactive compounds. In Indonesia, utilization of lemongrass has not been commercially developed, most are only used for flavor ants and herbal drinks. Therefore, it is necessary to develop products from lemongrass so that their use is wider. In general, the use of lemongrass can be obtained from its bioactive compounds such as citral at 65 - 85% of the total essential oils components of as a result of lemongrass extraction [2,3]. Lemongrass essential oil is generally obtained through distillation process of lemongrass leaves, the oil is yellowish with a distinctive aroma of lemon (citral). In addition, citral also proven to have anti-fungal activity that can control post-harvest disease in oranges [4,5].

To optimize its utilization, the bioactive compounds contained in lemongrass need to be extracted. Microwave Assisted Extraction (MAE) is a simple and economical method used to extract bioactive compounds from plants [6]. By applying MAE method to Rosella flower extraction, was found increase the quality and quantity of extracted bioactive components, in addition reducing extraction time and saving production costs [7].

As it is understood that because of its structure, extract of bioactive compounds is unstable, not

resistant to changes in environmental conditions so that its utilization becomes limited and not flexible. For the purpose of maximizing and flexibility of utilization, extract formulations were carried out into solid form, using encapsulation techniques and suspension forms. One solution that can be used is coating the active compound using the encapsulation method. According to [8], microencapsulation is a method of coating a compound with a very small size with a particle diameter of 15-20 microns. The use of encapsulation technology can increase the distribution of stored active compounds and expand surfaces contact of particle [9].

2. Materials and methods

2.1. Extract preparation and microencapsulation formula

Ethanolic extract of lemongrass was prepared using fresh lemongrass stalk washed and sorted, then chopped into smaller size, followed by pounding so that the aroma of the lemongrass comes out. Sixty percent ethanol was used as solvent. Extraction was conducted using MAE method. The ratio of lemongrass to solvent was 1:10 extracted for 7 minutes. The extract obtained then evaporated to remove the remaining solvents.

Furthermore, microencapsulate formulation of lemongrass ethanolic extract was done in order to protect the bioactive components in the extract. The extract mixed with a solution of encapsulant agent (20% w/v of maltodextrin or β-cyclodextrin) which had been mixed with 1% w/v CMC as emulsifier. The suspension then stirred with a magnetic stirrer for 30 minutes and followed with hand mixer for 3 minutes. Then the suspension is ready for microencapsulation. The microencapsulation process using a spray dryer is carried out with various drying temperatures (120,130 and 140°C).

2.2. Characterization

2.2.1. Total phenolic analysis. To 0.4 mL of extract 1.5 mL of Folin-Ciocalteu reagent (10% v /v) was added, then incubated for 5 minutes. Afterward, the mixture was mixed with 1.5 mL of 7.5% (w/v) Na_2CO_3 solution, followed with incubation at room temperature for 90 minutes at dark. The absorbance was measured using UV-Vis spectrophotometer at 765 nm. In this study, Gallic acid is used as a standard. The results obtained were represented as mg gallic acid equivalent (GAE)/g of material.

2.2.2. Antioxidant activity analysis, DPPH methods. DPPH solution was prepared by weighing 1.97 mg DPPH, dissolved in methanol to 25 mL (concentration of 0.2 mM). Then the sample solution was prepared for 50 mg / L. Then to 1.5 mL sample solution 0.75 mL of DPPH 0.2 mM was added, the mixture is homogenized and left in a dark for 30 minutes; measured using UV-Vis spectrophotometer at 517 nm. The measurement was conducted in triplicates for each sample solution. Control solutions used were BHT and ascorbic acid at concentrations of 2, 4, 6, 8 and 10 mg / L. The inhibition calculated using the formula:

$$\% \text{ inhibition} = \frac{C-S}{C} \times 100\%$$

With C is control absorbance and S is sample absorbance

2.2.3. pH measurement. pH measurements were carried out using a digital pH meter which was first calibrated with pH 4 (acetate buffer) and pH 7 (phosphate buffer) solutions.

2.3. Statistical analysis

Data were analyzed using the F test (variance test) at the 95% confidence level and further test (DMRT) to differentiate between treatments**.**

3. Results and discussions

3.1. Total phenolic

The study showed that the highest total phenol value was obtained from the treatment of β-cyclodextrin encapsulant with 25% LE of 34.64 mg/100g, and the lowest was obtained from maltodextrin encapsulant 25% LE of 17.19 mg/100g (Figure 1). In the use of β-Cyclodextrin as encapsulants, it shows that the total phenol obtained is directly proportional to the amount of extract used; whereas the use of maltodextrin is inversely proportional. This effect occurs due to the nature of each encapsulant. Maltodextrin, has been reported to have a higher protective effect on phenolic and anthocyanin compounds than soy protein isolates for microencapsulating pomegranate juice [10]. Therefore, maltodextrin (P1) shows that the higher amount of encapsulant used, the more the protection provided for bioactive compounds from lemongrass.

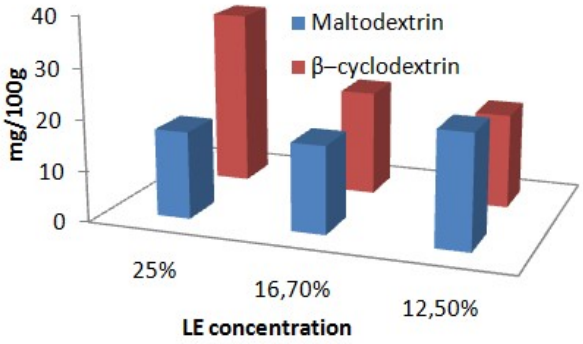

Figure 1. Total Phenolic content of MLE

β-cyclodextrin is a cyclic oligosaccharide, composed of glucose units linked by α-1,4 glycoside bonds derived from enzymatic degradation of starch by certain bacteria such as *Bacillus macerans*. The center of the β-cyclodextrin cavity is hydrophobic, while the periphery around the wall is hydrophilic [11]. The properties of LE are hydrophobic compounds so that the extract is protected in the cavity β-cyclodextrin. This shows that β-cyclodextrin is capable to trap the LE much better than the maltodextrin. Furthermore, the efficiency of encapsulation process was calculated as total phenolic content of LE used divided by total phenolic content of MLE [14] (Table 1)

Table 1. Encapsulation efficiency

Treatment	Total phenolic content in		Encapsulation efficiency
	LE used	MLE	
P2K1T3	44,7 mg/100g	67,89 mg/100g	66%
P2K3T1	7,8 mg/100g	67,89 mg/100g	11%

Result of this study showed that the treatment of β-cyclodextrin encapsulant, 25% LE and drying temperature of 140ºC (P2K1T3) gave the highest total phenol value of 44.7 mg / 100g. While the lowest total phenol of 7.8 mg / 100g was produced from treatment of β-cyclodextrin encapsulant; 12.5% extract and drying temperature of 120ºC (P2K3T1). This can be explained in terms of the phenol compounds trapped or encapsulation efficiency; namely the number of bioactive compounds that can be protected by encapsulation. This shows that the larger the encapsulant used, the more phenol compounds that can be trapped [12] (Table 1).

In term of heating temperature, it showed that the higher the temperature the higher the total phenolic [13]. In addition, each encapsulant agent provides unique properties in protecting bioactive compounds. According to [14], the appropriate proportion of encapsulation will provide good emulsification and drying properties so as to enhance retention of core material during microencapsulation with a spray dryer.

3.2. Antioxidant activity

This study resulted that the highest antioxidant activity of 14,14% resulted from treatment 25% LE with β – cyclodextrin (P2K1); while the lowest of 5,9% resulted from treatment 25% LE with maltodextrin (P2K1) (Figure 2). This study resulted that encapsulation agent maltodextrin gave lower antioxidant activity microencapsulate compare to that of β – cyclodextrin. This because β – cyclodekstrin poses better antioxidant protective capability. β – cyclodextrin has been used to improve bioavailability through increasing water solubility on its hydrophobic cavity, improving stability and increasing permeability of water-soluble component in its hydrophobic cavity [15]. When compared to the nature of maltodextrin, the properties possessed by maltodextrin are only form a matrix with low browning possibility, inhibit crystallization, strong and stable binding power in o/w emulsions [16]. In the maltodextrin encapsulant, the treatment of 16.7% LE was higher than 25% and 12.5% extract. So it can be concluded that the treatment of 16.7% extract is the optimal point of encapsulant to protect antioxidants. This happens because related to the level of LE which capable to be protected in MLE. The more bioactive compounds that are saluted, the proportion of coating material used is also needed. A stable emulsion can provide considerable encapsulation efficiency, while the results at high oleoresin concentrations cause a decrease in yield, because at the concentration of the capsule wall is unable to hold the oleoresin so the capsule will break and the oleoresin will come out, which will ultimately reduce encapsulation efficiency.

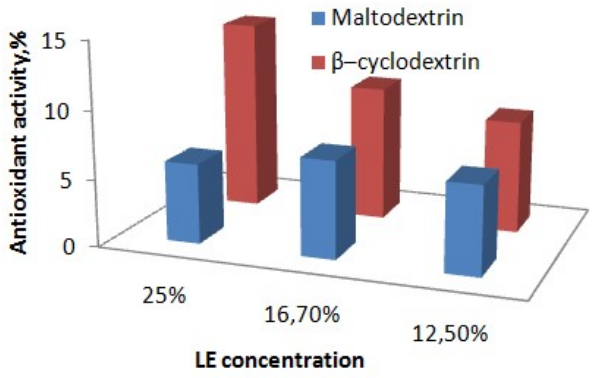

Figure 2. Antioxidant activity of MLE

The decrease in antioxidant activity as the proportion of encapsulant increases is caused by the ability of encapsulant to cover oil droplets. It is assumed that in higher proportions a thicker encapsulant matrix is formed so that it is more optimal in protecting oil droplets. Good coating material matrix provides a good influence on encapsulation products, especially protecting from oxidation and heat [17]. In addition, a decrease can occur due to the viscosity of the coating material. The more proportion of coating material used, the more viscosity of the suspension. High viscosity will reduce yield, which in turn will reduce protection ability of coating material on the active compound. High viscosity will cause atomization process to be disrupted and decrease the drying

speed hence decreases of microcapsules yield [18]. This causes a decrease in the value of antioxidant activity in the β-cyclodextrin encapsulant.

In term of heating temperature used, this study showed that the highest antioxidant activity of 13.93% resulted from treatment at 120^{0}C with β-cyclodextrin encapsulant, while the lowest of 4.92% from treatment at 130^0 C with maltodextrin encapsulant (Figure 3) Again, that the highest antioxidant activity resulted from β-cyclodextrin encapsulant treatment, with the highest the heat temperature used the lowest the antioxidant activity. This is consistent with the previous study [19] about antioxidant activity of coffee found that high temperatures maintained during cooking can cause a high reduction of cinnamic hydroxy acids when compared to that at room temperature. However, with the addition of β-cylodextrins to the sinamat hydroxy acids, produce a decrease on cinnamic hydroxy acids reduction. This indicates that the microencapsulation method using β-cylodextrins can provide protection and stability. The higher the temperature used, the faster the oxidation process takes place, oxidation can occur due to the reaction between unsaturated triglycerides with oxygen from the air [20]. This reaction is accelerated because of the presence of heat, light and metals in very small concentrations, especially copper.

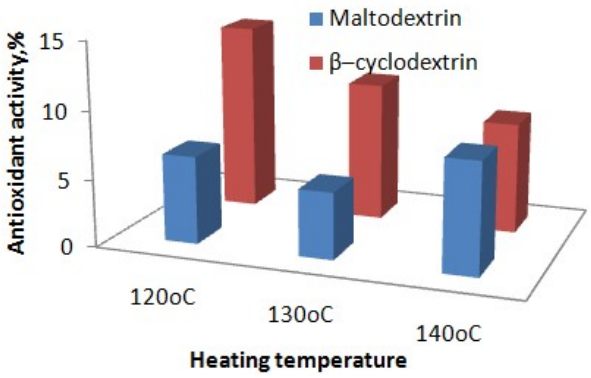

Figure 3. Effect heating on MLE antioxidant activity

Furthermore, effect of heating temperature on maltodextrin encapsulant showed the graph decreases at a temperature of 120^{o}C to 130^{o}C. Then the antioxidant value rises again at 140^o C (Figure 3). This is because the viscosity of the maltodextrin encapsulant affects the drying temperature. Very high viscosity can provide fast protection for protected bioactive compounds. According to [21], higher viscosity will cause the formation of layers that surround the nucleus quickly so that the nucleus is immediately protected. The higher drying temperature will help the drying process faster as explained earlier. Therefore, the viscosity properties of the maltodextrin encapsulant and drying temperature variations will have an effect on the value of the MLE antioxidant activity.

That the β-cyclodextrin encapsulant is better in encapsulated the lemongrass bioactive compounds compare to maltodextrin as previously discussed (Table 1). A good encapsulant when is capable in protecting and controlling the release of active compounds in the oil. The material chosen must depend on the nature of the oil to be encapsulated and the desired characteristics of the final microcapsules. Ideally, encapsulants must be soluble in water, biodegradable, form solutions with low viscosity, produce powder with certain properties (not hygroscopic, non-porous, soluble, stable, etc.), inexpensive, easily dried and not reactive [21].

Then when compared with the MLE total phenolic content, then the influence of the interaction of MLE antioxidant activity is not correlated with the MLE total phenolic value. This indicates that the MLE antioxidant activity is negatively correlated with its total phenolic content because antioxidant compounds are not all derived from phenol compounds. This is consistent with the study

of [22, 23] which states that the results of antioxidant activity have a negative correlation with amylase and total phenol.

4. Conclusions

In conclusion microencapsulates formulation of LE using encapsulant of β-cyclodextrin produced lemongrass extract microcapsule (MLE) with antioxidant activity of 14.14% and the highest total phenol value of 34.64 mg/100gr. This study resulted that the use of β–cyclodextrin produce microcapsule with better antioxidant activity.

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