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<u>Abstract</u>

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Aloe vera powder Antioxidative activity Encapsulating agent Aloe vera powder contains of phenolic compound which has antioxidative properties. However, aloe vera powder has low solubility and unfavorable taste. Hence, the solubility of aloe vera powder needs to be improved by using microencapsulation technologies. Microencapsulation by spray dryer is usually done at a high temperature which is able to decrease the antioxidative activity. The purpose of this study was to evaluate the antioxidative activity of encapsulated aloe vera powder resulting from the process of microencapsulation. Microencapsulation was conducted by reconstituting *aloe vera* powder using aquadest at a ratio of 1 : 120. The solution was filtered and then maltodextrin added as an encapsulating agent with variations of: 2.5, 5.0, 7.5 and 10.0% (w/v). The solution was fed into a spray dryer at an inlet temperature of 130°C and an outlet temperature of 103°C, with the flow rate of solution being 350.0 mL/h. The resulting powders were analyzed for their moisture content, phenolic content and antioxidative activity based on an ability to capture free radicals of 1,1-Diphenyl-2-picrylhydrazil (DPPH) and lipid peroxidation inhibition using the ferrythiocyanate method. The research showed that the encapsulated powders' moisture contents were significantly different. Total phenolic content decreased with the increase of maltodextrin. The higher the concentration of maltodextrin, the lower the percentages of both RSA (Radical Scavenging Activity) and of lipid peroxidation inhibition. Microencapsulation of *aloe vera* powder with maltodextrin at 2.5% produced an encapsulated powder with a high antioxidative activity indicated by an RSA value of $35.59 \pm$ 2.65% and an inhibition of lipid peroxidation measuring $16.15 \pm 0.73\%$.

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Introduction

Aloe vera (Aloe vera var. chinensis) leaves contain phenolic compounds in the form of flavonoids which can function as an antioxidant. These compounds are contained inside the leaf in the clear gel (mucilage) (He et al., 2005). The flavonoid compounds in aloe vera gel are: kaempeferol, quercetin and merycetin, weighing in respectively at 257.7; 94.80 and 1283.50 mg/kg (Sultana and Anwar, 2008). The antioxidative properties of *aloe vera* extract are indicated by an ability to capture free radicals DPPH (1,1-Diphenyl-2-picrylhydrazil) (Hu et al., 2005). According to Riyanto and Wariyah (2012), aloe vera extract has an antioxidant activity with a percentage of Radical Scavenging Activity (RSA) of 35.17% and an inhibition of lipid peroxidation of 49.53%. Therefore, aloe vera is mostly used as the ingredients in health foods, cosmetics and pharmaceuticals (Chang et al., 2006).

Processing of aloe vera leaves into products has

already been carried out which resulted aloe vera gel drink (Rivanto and Wariyah, 2012), aloe vera powder (Miranda et al., 2009; Wariyah and Riyanto, 2011). The antioxidative activity of aloe vera powder expressed by percentage of RSA was 26.15% and the inhibition of lipid peroxidation, 44.17% (Riyanto and Wariyah, 2011). However, aloe vera powder is less favored due to its low solubility. Improving solubility requires further processing, for example by using microencapsulation. Microencapsulation is defined as the packaging of solid, liquid, and gaseous material in sealed capsules of sizes between nanometers and millimeters. The packaged material is the active or core material; the packaging material is known as the shell or wall material, or can also be said as carrier or encapsulant (Ozkan dan Bilek, 2014). Saenz et al. (2009) reported that the microencapsulation technique of spray drying on the cactus pear flavonoid compounds could increase the solubility of the powder and protect it from oxidation. It was supported by Goula and Adamopoulos (2008) who



stated that during the process of drying in a spray dryer there is a transformation of the material from liquid into powder in high solubility.

There are several coating materials or encapsulating agents used for the microencapsulation of antioxidants such as flavonoids. Krishnan et al. (2005) proposed several types of encapsulating agent such as Starch Hydrolysis Products (SHP) and gum (especially gum acacia) which protect materials from light, temperature, oxygen and humidity. Moreover, Robert et al. (2015) reported that the use of maltodextrin as an encapsulating agent could maintain the stability of flavonoids in the pulp of cactus pear (Opuntia ficus-indica). Maltodextrin is a starch hydrosylate with a Dextrose Equivalent (DE) of less than 20 (Wang and Wang, 2000). DE is a quantitative measure of the degree of hydrolysis of starch polymers and DE value is a common parameter which is used to characterize the maltodextrin molecular weight (Rong et al., 2009). It is known that the higher the DE value, the greater the amount of starch hydrolyzed would be. Even, Goula and Adamopoulos (2008) stated that maltodextrin could also reduce the stickiness and loss of material during the spray drying process. Maltodextrin is cold-water soluble, has a low sweetness level, bland taste and is generally applied as a spray-drying aid.

Mishra et al. (2014) reported that spray dried amla (Emblica officinalis) juice powder made from 7% maltodextrin and was processed at 175°C inlet temperature showed excellent water solubility. While Robert et al. (2015) informed the SEM (Scanning Electron Microscopy) of that encapsulated purple cactus pear (Opuntia ficus-indica) pulp with maltodextrin that showed microparticles with irregular shape and particles with indented surfaces, obviated their agglomerating tendency. The morphology of microcapsules with maltodextrin as an encapsulating agents was irregularly spherical in shape with an extensively dented surface. The formation of the dented surfaces of the spraydried particles was attributed to the shrinkage of the particles during the drying process (Saenz et al., 2009).

Stability is an important parameter when drying antioxidants in foods by using a spray dryer. Robert *et al.* (2015) stated that the stability of flavonoids is affected by pH, water activity, radiation, oxygen, metals, antioxidants, temperature and enzyme activity. Generally, drying using a spray dryer involves high temperatures and exposure to oxygen and light. This condition allows the destruction of antioxidant compounds. Therefore, the purpose of this study was to evaluate the effect of various maltodextrin concentrations on the antioxidative activity of encapsulated *aloe vera* powder; and determining the appropriate maltodextrin concentration to produce an encapsulated powder with a high antioxidative activity.

Materials and Methods

Materials

The *aloe vera (Aloe vera* var. *chinensis)* leaves used in the study were obtained from Loano village in the Purworejo Regency of Central Java Province, Indonesia. Maltodextrin as an ecapsulating agent was purchased from Brataco Chemika, Yogyakarta. The reagents used in this research were purchased from Merck, except the DPPH (1,1-Diphenyl-2picrylhydrazil) which came from Sigma-Aldrich Chemie.

Aloe vera powder preparation

Process of making *aloe vera* powder was referred to Riyanto and Wariyah (2011). *Aloe vera* leaves were peeled and washed simultaneously, and the clear gel (mucilage) sliced into 3 mm thickness and dried with an oven (Memmert DIN 40050 IP 20) at 70°C until a moisture content of between 8 -10% was reached. The dried *aloe vera* was then pulverized by using a blender (Kirin KKB-210 GL1), and was filtered through a 60 mesh sieve (ASTM E II Mesh 60). The resulting powder was packed in polyethylene plastic and stored at -10°C until encapsulated in maltodextrin.

Preparation of the microcapsules

Encapsulation of *aloe vera* powder in maltodextrin referred to Saenz *et al.* (2009) with minor modifications, and prepared as follows : *aloe vera* powder was reconstituted by using distilled water at ratio of 1/120 (w/v) to achieve a viscosity appropriate for spraying into the spray dryer and mixed with maltodextrin at various concentrations of : 2.5; 5.0; 7.5 and 10.0% (w/v) with constant stirring using a magnetic stirrer (Stir plate Nuova II) at 700 rpm for 45 minutes. Each preparation was fed into the spray dryer (Lab Plan SD-05) at an inlet temperature of 130°C and an outlet temperature of 103°C, an air flow rate of 50m³/h, and a solution flow rate of 350 mL/h. The powders obtained were kept at -10°C until analyzed.

Aloe vera powder and microecapsules powder analysis

Aloe vera powder and microecapsulated powder were analyzed for their moisture content using a gravimetric method (AOAC, 1990), total phenolic content were determined according to the Folin-Ciocalteu method (Horax *et al.*, 2005; Sensoy *et al.*, 2006)). The antioxidative activities of the *aloe*

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vera powder and microencapsulated powder were analyzed based on their ability to scavenge free radicals DPPH (Hu *et al.*, 2003) and the inhibition of lipid peroxidation by the ferry thiocyanate method (FTC) (Masuda and Jitou, 1994).

Phenolic content

The total phenolic content of the samples were determined by using the Folin-Ciocalteu method (Horax *et al.*, 2005; Sensoy *et al.*, 2006). Absorption of the solution at 726 nm was measured by using spectrophotometer (UV-VIS Spectrophotometer Shimadzu 1240). A standard curve was prepared with gallic acid (Gallic acid CAS 149-91-7 Sigma Aldrich).

DPPH free radical scavenging activity

The DPPH was prepared in absolute ethanol and the concentration of the resulting solution was 6.10-5 M. The stock solution was kept in a refrigerator. Sample preparation referred to Hu *et al.* (2003) and the solution absorbance was determined at 517 nm using the spectrophotometer and measured every 15 minutes to obtain a constant absorbance (usually over 120 minutes). DPPH Radical Scavenging Activity (RSA) was calculated using the formula of Yen and Duh (1994), which is:

Radical Scavenging Activity (%) = $[1 - (A_r / A_s)] \times 100$

where A_o is the absorbance of the sample at t = 0min, and A_T is the absorbance of the sample at t = 30min (initial steady state).

Total antioxidant activity determination by ferric thiocyanate method (FTC)

Inhibition of lipid peroxidation was determined by the ferric thiocyanate (FTC) method (Hu *et al.*, 2003). Absorbance of the solution was measured at 500 nm every 24 hours for 10 days using a spectrophotometer. Inhibition of lipid peroxidation was calculated by the formula of Anesini *et al.* (2008), which is:

Inhibition of lipid peroxidation (%) = $100 - (A_1 / A_2) \times 100$

where A_0 is the absorbance of control at t = 7 days, and A_1 is the absorbance of the sample (containing *aloe vera* instant) at t = 7 days (when the current reaches its maximum absorbance).

Design of experiments

This study used completely randomized design with the concentration of maltodextrin as a factor. The

differences among the treatments were determined by F test, and the significant difference between samples was examined by Duncan's Multiples Range Test (DMRT) (Gacula and Singh, 1984)

Result and Discussion

Phenolic content of aloe vera

The result of the moisture content and total phenol of encapsulated aloe vera powder are shown in Table 1. It can be seen that the moisture content of the powder and encapsulated aloe vera powder at various maltodextrin concentrations were significantly different. The moisture content of aloe vera powder was relatively higher than that of the encapsulated powder. The difference in moisture contents directly distinguished the total solid of the *aloe vera*, therefore, the phenolic content of aloe vera powder was relatively higher than that of the encapsulated powder. Varying the concentration of maltodextrin significantly affected the total phenolic content. It is concluded that the higher the maltodextrin concentration, the lower the phenolic content was. According to Cilek (2012), microencapsulation with a high concentration of maltodextrin caused a reduction in the proportion of phenol in the ancapsulated powder. It was estimated that decreasing the phenolic content might affect the antioxidative activity of the encapsulated powder since the antioxidant compounds in aloe vera was phenolic compounds flavonoids (Sultana and Anwar, 2008).

DPPH radical scavenging activity of encapsulatedaloe vera powder

The antioxidative activity of encapsulatedaloe vera powder was measured by the ability to capture the free radical DPPH and to inhibit fatty acid peroxidation. The DPPH free radical was purple in color which might have a decrease in the colour intensity if these radicals are captured by antioxidants. According to Bozzi *et al.* (2007), antioxidant compounds in *aloe vera* leaves are phenolic compounds with hydroxy and ketone group that are capable of capturing free radicals. The hydroxy and ketone group captures free radicals through the lone pair electron (Benavente-Garcia *et al.*, 1997). The antioxidative activity of encapsulated *aloe vera* powder is shown in Figures 1.

Figure 1 shows that the absorbance of DPPH solution containing synthetic antioxidants (BHT), or *aloe vera* samples, decreased as a result of the length of incubation period. It proves that BHT and samples of *aloe vera* had antioxidative properties

Sample	Moisture (% wb)**	Total phenol (µg/g (dry matter)
Aloe vera powder	$14.07 \pm 0.08c$	$2.64 \pm 0.105d$
Instant with maltodextrin 2.5%	7.55 <u>+</u> 0.11b	$2.52 \pm 0.190d$
Instant with maltodexrin 5.0%	$5.85 \pm 0.25a$	$2.17 \pm 0.065c$
Instant with maltodexrin 7.5%	$5.60 \pm 0.62a$	1.64 <u>+</u> 0.085b
Instant with maltodexrin 10.0%	6.75 ± 0.05 ab	1.01 <u>+</u> 0.185a

Table 1. Moisture and phenolic content of microencapsulated aloe vera powders*

* Mean in a coulom with similar superscript are not significant

different at $\alpha = 0.05$.

**source: Wariyah (2014)

 Table 2. Percentage of RSA and inhibition of lipid peroxidation of microencapsulated aloe vera powders

Sample	RSA (%)**	Inhibition of lipid peroxidation (%) **
BHT*	$95.00 + 2.04^{f}$	$21.89 + 3.09^{d}$
Aloe vera gel	12.09 <u>+</u> 1.79 ^a	12.70 ± 2.30^{b}
Aloe vera powder	43.32 ± 0.11^{e}	25.96 ± 0.41^{e}
Instant with maltodextrin 2.5%	35.59 ± 2.65^{d}	$16.15 \pm 0.73^{\circ}$
Instant with maltodextrin 5.0%	$19.02 \pm 0.40^{\circ}$	10.29 ± 0.92^{ab}
Instant with maltodextrin 7.5%	$18.91 \pm 0.21^{\circ}$	10.33 ± 1.24 ^{ab}
Instant with maltodextrin 10.0%	15.76 ± 2.16^{b}	9.52 ± 0.12^{a}

* Sample weight: 1 g (dry matter), except BHT weight : 0,1 g (dry matter)

** Mean in a coulom with similar superscript are not significant different at $\alpha = 0.05$

that had ability to capture DPPH free radicals. It means that the greater the decrease in absorbance, the higher the antioxidative activity. Antioxidative activity of aloe vera powder and encapsulatedaloe vera was sufficiently high. Table 2 showed the calculated results of the quantitative data of RSA (Radical Scavenging Activity). RSA value of fresh aloe vera was lowest at approximately 12.09% due to the high water content which caused the total phenol low, while the encapsulated-aloe vera powder with higher concentrations of maltodextrin have a lower antioxidative activity. The addition of maltodextrin caused the level of total phenol of the encapsulated powder became lower. Sensone et al. (2011) also stated that increasing maltodextrin or the encapsulating agent used in microencapsulation Fadogia ancylantha, Melissa officinalis and Tussilago farfara, might lowered the phenol content of the encapsulated powder.

Total phenol of the *aloe vera* powder and encapsulated powder in maltodextrin 2.5% was not significantly different; however, the antioxidative activity of encapsulated *aloe vera* powder in 2.5% maltodextrin was lower than *aloe vera* powder. Mishra *et al.* (2014) reported that spray drying of amla *(Emblica officinalis)* juice powder made

from 7% maltodextrin resulted lower free radical scavenging activity. It is probably caused by the structural modification of the compound during drying at high temperature. Saad et al. (2014) stated that the structural modification which took place during desorption or drying could lower the ability of the compound for binding or trapping. Encapsulated powder was resulted from twice drying during pocessing; the bioactive compounds were degraded during drying (Saenz et al., 2009). Therefore, the antioxidative activity of the powder was lower than aloe vera powder. However, compared to the synthetic antioxidant BHT (Butylated hydroxytoluene), the antioxidative activity of the aloe vera product was considered lower. Sharma et al. (2008) found that flavonoids in tea had a lower antioxidative activity than BHT due to a component in the BHT that was being more homogeneous than the aloe vera products. It provided an active group in the BHT that can be useful for capturing free radicals.

Inhibition of lipid peroxidation of encapsulated- aloe vera powder

The stage of fat oxidation through fatty acid peroxidation formed fatty acid and peroxide radicals. These radicals can be captured by antioxidants.

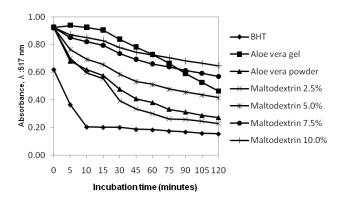


Figure 1. Radical Scavenger Activity of microencapsulated *aloe vera* powders

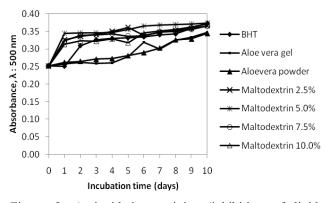


Figure 2. Antioxidative activity (inhibition of lipid peroxidation) of microencapsulated *aloe vera* powders

Flavonoid in *aloe vera* is one of the antioxidants that can capture radicals which result from fatty acid peroxidation (Hu *et al.*, 2005). The inhibition of lipid peroxidation by encapsulated-*aloe vera* powders are shown in Figure 2. The absorbance of samples containing BHT or *aloe vera* products showed differences in intensity with longer incubation. *Aloe vera* powder and BHT showed a lower absorbance than encapsulated-*aloe vera* powder at various maltodextrin concentrations. This result indicated that the antioxidative activity of *aloe vera* powder and BHT in inhibiting peroxide formation were higher. Table 2 showed the quantitative data of inhibition of lipid peroxidation.

The higher the concentration of maltodextrin in the encapsulated-*aloe vera* powder caused the lower of the inhibition of lipid peroxidation. This was due to a decrease in the proportion of phenolic compounds in the encapsulated-*aloe vera* powder (Table 1). In a comparison with the synthetic antioxidant BHT, the inhibition of lipid peroxidation of *aloe vera* products was much lower.

According to Wariyah (2014), the solubility of encapsulated-*aloe vera* powder with maltodextrin 2.5% was about 23.76+1.42 seconds (expressed as the time required for the material to dissolve completely), while the *aloe vera* powder could not

dissolve completely in distilled water. Thus, based on the solubility and the antioxidative activity of the encapsulated-*aloe vera* powder, microencapsulation of *aloe vera* powder with maltodextrin 2.5% was an appropriate concentration for encapsulating *aloe vera* powder.

Conclusion

Microencapsulated *aloe vera* powder that used maltodextrin as an encapsulating agent could produce an encapsulated *-aloe vera* powder with a high antioxidative activity. More specifically, it was concluded that the use of the encapsulating agent maltodextrin in higher concentrations produced powder with lower antioxidative activity; and, the use of 2.5% maltodextrin resulted in an encapsulated powder with high antioxidative activity.

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