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Abstract

Aloe vera contains a phenolic compound that has bioactive activity. Previous research showed that microencapsulation of aloe vera powder with maltodextrin as an encapsulation agent produced instant aloe vera with high antioxidative activity. The problem was the hygroscopic instant caused rapid moisture and oxygen absorption during storage, therefore decreasing the instant aloe vera antioxidative activity periodically. The aim of this research was to evaluate the antioxidative activity stability of instant aloe vera during storage. The processing of instant aloe vera through a reconstituted aloe vera powder with water with a ratio of 1:120 and then added with 2.5% maltodextrin as the encapsulating agent. The solution was then inserted into a spray dryer with an inlet temperature of 130°C, an outlet temperature of 103°C, and the flow rate of the solution is 350.0 mL/h. The resulted instant aloe vera was divided into 15 packs with a weight of 25 g, and each sample was wrapped with polyethylene plastic film with 0.80 mm thickness and then was stored at 25°C with a relative humidity of 75%. The sample was conducted in triplicate. The moisture content, and antioxidative activity that was based on the ability to capture 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (RSA) and lipid peroxidation inhibition were analyzed every week until the critical condition was achieved at a moisture level of 12%. The research showed that the radical scavenging activity (RSA) and lipid peroxidation inhibition of instant aloe vera before storage were 16.34±1.22% and 39.33±1.68%, respectively, whereas in the critical condition the RSA was 3.63±0.04% and the lipid peroxidation inhibition was 22.31±0.02%. Based on their antioxidative activity, the appropriate storage time of instant aloe vera was about 12 weeks in polyethylene plastic film of 0.08 mm thickness.

6

1. Introduction

Aloe vera (*Aloe vera* var. *chinensis*) is a plant source of bioactive compounds that are beneficial to health. It has lance-shaped leaves containing clear gel in central mucilaginous pulp (Gangadharan *et al.*, 2019). Aloe vera contains polysaccharides (55%), sugars (17%), minerals (16%), proteins (7%), lipids (4%), and phenolic compounds (1%) (Kumar *et al.*, 2019). Phenolic compounds such as quercetin, myricetin and kaempferol are known for their antioxidant activity (Sultana and Anwar, 2008). However, as a source of antioxidants, fresh consumption from an aloe vera gel is impractical and has an unpleasant odour, so it needs to be processed into a product that is practical for consumption, such as in powder form.

Wariyah and Riyanto (2011) have studied the antioxidative properties of aloe vera extract and powder. The Radical Scavenging Activity (RSA) of aloe vera extract is 35.17% and the inhibition of lipid peroxidation is 49.53%, while the RSA aloe vera powder is 26.05% and the inhibition of fat peroxidation is 44.17%. However, in powder form it has low solubility, therefore Wariyah and Riyanto (2016) have microencapsulated aloe vera powder with a 2.5% maltodextrin as an encapsulating agent and then dried using a spray drier to produce instant aloe vera. This resulted in instant aloe vera showing solubility of 23.08±0.97 s/g, meanwhile maintaining high RSA at 35.59±2.65% and inhibition of lipid peroxidation at 16.15±0.73 %.

Instant aloe vera produced from the spray dryer

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process can change its physicochemical properties, including a decrease in moisture content, hygroscopicity, water activity and antioxidative activity (Shishir and Chen, 2017). The changes associated with antioxidative activity could reduce instant bioactive properties. This decrease will be accelerated by the contact of oxygen and water vapour in the air. (Fennema, 1996). Robert *et al.* (2015) reported that flavonoid stability is greatly influenced by pH level, water activity, radiation, oxygen, metals, antioxidants, temperature and enzyme activity. According to Minah and Astuti (2018), the storage of instant tomato in polyethylene plastic at room temperature for 10 weeks resulted in a decrease of antioxidant activity from 90% to about 4-5%. Until now, research is still rarely done on the changes in the quality of instant aloe vera during storage. The purpose of this study was to evaluate the stability of the anti-oxidative properties of instant aloe vera during storage until critical conditions were reached and to determine the appropriate storage time.

2. Materials and methods

2.1 Materials

This study used aloe vera leaves with the variety of *Aloe vera* var. *chinensis* which was purchased from a farmer at Loano village in the Purworejo Regency of Central Java Province, Indonesia. The encapsulating agent of maltodextrin was obtained from Brataco Chemika, Yogyakarta. The chemicals for analysis of antioxidative activity from Merck, except the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Chemie.

2.2 Aloe vera analysis

The aloe vera gel used for the study was analyzed for moisture content by static gravimetric method (AOAC, 1990), total phenol with the Folin-Ciocalteu method (Horax *et al.*, 2005; Sensoy *et al.*, 2006) by using a spectrophotometer (UV-VIS Spectrophotometer Shimadzu 1240) to determine the absorption of the solution at 732 nm. A standard curve was prepared with gallic acid (Gallic acid CAS 149-91-7 Sigma Aldrich). Furthermore, aloe vera leaves are used to produce instant aloe vera through the microencapsulation process.

2.3 Aloe vera preparation for microencapsulation

Instant aloe vera was processed from aloe vera powder which can be referred to the procedure by Riyanto and Wariyah (2012) and the processing of instant aloe vera was referred to Wariyah and Riyanto (2016). The leaves of aloe vera were peeled, then washed with running water, then the clean gel sliced 3 mm thick, then arranged in a baking sheet to be dried in the oven

(Memmert DIN 40050 IP 20) at a temperature of 60-70°C until the moisture content is between 8-10%. The dried aloe vera is mashed in a blender (Kirin KKB-210 GL1), then filtered using a 60 mesh sieve (ASTM E II Mesh 60). The resulting aloe vera powder is made instantaneously by microencapsulation. The microencapsulation process was carried out in the following steps: aloe vera powder was reconstituted with added distilled water at a ratio of 1/120 (w/v) and mixed with 2.5% concentrations of maltodextrin. The solution was stirred at 700 rpm for 45 mins using a magnetic stirrer (Stir plate Nuova II) and then was dried into the spray dryer (Lab Plan SD-05). The airflow rate of the spray dryer was set at 50 m³/h, and the solution flow rate was 350 mL/h, the inlet temperature was 130°C and an outlet temperature of 103°C. The instant powders obtained were kept at -10°C until analysed and storage treatment.

2.4 Determination of antioxidative stability of aloe vera instant during storage

The stability of the anti-oxidative properties of instant aloe vera was determined by storing instant aloe vera in a 0.80 mm polyethylene plastic package in a room with a relative humidity of 75% regulated with saturated NaCl salt (Rangana, 1976) and a storage temperature of 25°C until it reaches a critical condition, namely at a moisture content of 12% (Wariyah dan Riyanto, 2015). The analysis was carried out periodically (once a week) during the storage which included analysis of moisture content and antioxidant activity via the DPPH method based on the percentage of RSA (Radical Scavenging Activity) and the ferriticyanate (FTC) method to determine the per cent inhibition of lipid peroxidation (Hu *et al.*, 2003).

DPPH free radical scavenging activity was determined based on the absorbance of the sample measured periodically from zero to 120 mins with 15 min intervals at a wavelength of 517 nm, the RSA value is calculated by the following formula:

$$\text{Radical Scavenging Activity (\%)} = [1 - (AT/A_0)] \times 100$$

Where A₀ is the absorbance of the sample at t = 0 min, and AT is the absorbance of the sample at t = 30 mins (initial steady state).

The antioxidant activity by ferriticyanate method (FTC) was determined based on the inhibition of lipid peroxidation with ferric thiocyanate (FTC) method (Hu *et al.*, 2003). The absorbance of the solution was measured at 500 nm every 24 hrs for 10 days using a spectrophotometer. The inhibition of lipid peroxidation was determined with the formula of Anesini *et al.* (2008):

11 Inhibition of lipid peroxidation (%) = $100 - (A1/A0) \times 100$

2 Where A0 is the absorbance of the control (blank) at t = 7 d, and A1 is the absorbance of the sample at t = 7 d (when the current reaches its maximum absorbance).

2.5 Design of experiments

1 This research used a completely randomized design with storage time as a factor. The differences among the treatments were determined by the F test, and the significant difference between samples was examined by Duncan's Multiple Range Test (DMRT) (Gacula and Singh, 1984).

3. Results and discussion

3.1. Moisture and phenolic content of aloe vera gel

The moisture content and total phenol of aloe vera gel and instant are shown in Table 1. The moisture content of aloe vera gel was about $98.74 \pm 0.08\%$ and the phenolic compound was 25.31 ± 0.56 mg GAE/100g (dry matter). DiScala *et al.* (2013) found the moisture content of aloe vera was $98.93 \pm 0.06\%$ and the phenolic 54.46 ± 7.87 mg GAE/100 g (dry matter). The phenolic differences are caused as a result of the variety of *Aloe barbadensis* Miller, while in this study *Aloe vera* var. *chinensis* was used. The phenolic compounds in aloe vera are a group of flavonoids, namely kaempferol, quercetin, myricetin, with a concentration of 257.7, 94.80 and 1283.50 mg/kg, respectively (Sultana and Anwar, 2008). Wariyah and Riyanto (2016) found that the anti-oxidation activity of aloe vera gel was based on the ability to capture DPPH radicals with RSA $12.09 \pm 1.79\%$ and inhibition of lipid peroxidation $12.70 \pm 2.30\%$, whereas Hes *et al.* (2019) reported that RSA aloe vera gel is 13.52% and hydroxyl radical scavenging at 11.74%.

6 Table 1. Moisture and phenolic content of *Aloe vera* gel and instant

Sample	Moisture (%)	Total phenol mg GAE/100 g (dry matter)
<i>Aloe vera</i> gel	98.74 ± 0.88	25.31 ± 0.56
<i>Aloe vera</i> instant	6.28 ± 0.05	2.43 ± 0.10

Instant aloe vera has a moisture content of approximately $6.28 \pm 0.05\%$, according to SNI 01-4320-1996 (Indonesian National Standard), moisture content for instant beverage products is 3-5%. The spray drying process results in a decrease in moisture content (Shishir and Chen, 2017). However, the moisture content achieved in each product is not the same because it is influenced by the stability of the substances contained in the material and also the inlet and outlet temperatures of the spray drying equipment suitable for each product.

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12 3.2 DPPH radical scavenging activity of aloe vera instant during storage

The antioxidative activity of instant aloe vera was determined by the ability to scavenge the free radical DPPH and inhibit lipid or fatty acid peroxidation. The purple colour intensity of the DPPH free radical decrease if these radicals are captured by antioxidants. Therefore, the lower the purple colour intensity, the higher ability to capture free radicals. Zou *et al.* (2020) reported, that like other flavonoid compounds, quercetin, myricetin and kaempferol have 0-3 hydroxyl groups on ring B and double bonds on ketone groups that are capable of capturing free radicals. The antioxidative activity of aloe vera instant during storage is shown in Figure 1.

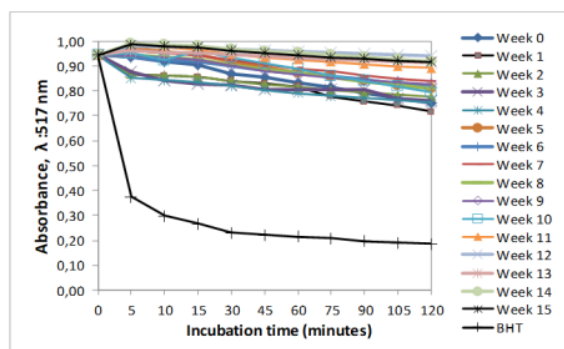


Table 1. Moisture and phenolic content of *Aloe vera* gel and instant

Figure 1 shows that the absorbance of DPPH solution containing aloe vera instant which was stored during 0 (fresh aloe vera instant) until 15 weeks and synthetic antioxidants (BHT, Butylated hydroxytoluene) decreased as a result of the length of the incubation period. It indicates that aloe vera instant and BHT has antioxidative activity by capturing DPPH free radicals. And the greater the decrease in absorbance, the higher the antioxidative activity. Table 2 shows the RSA of aloe vera instant during storage. The RSA were significantly different between samples with different storage times. The RSA value of fresh aloe vera instant was $16.34 \pm 1.22\%$ and after it was stored until the critical condition (at the moisture content of 12%) it decreased into $3.63 \pm 0.04\%$. The antioxidant activity of aloe vera is determined by phenolic compounds. Flavonoids are susceptible to oxidation which is influenced by pH level, water activity, radiation, oxygen, metals, antioxidants, temperature and enzyme activity (Robert *et al.*, 2015). In this study, instant aloe vera was stored in polyethylene plastic packaging of 0.80 mm thickness, at a temperature of 25°C and relative humidity of 75%. According to Keller and Kouzes (2017), polyethylene plastic has a permeability coefficient to water vapour of 0.39-0.59 g·mm/m²·d at 25°C, while the permeability to oxygen

has a diffusion coefficient of $98\text{-}453\text{ cm}^3\cdot\text{mm}/\text{m}^2\cdot\text{d}\cdot\text{atm}$, thus allowing phenol oxidation to occur. Therefore, instant aloe vera antioxidant activity decreases during storage. However, the BHT synthetic antioxidant shows the highest antioxidative activity, while the instant aloe vera was indicated lower. Yunut *et al.* (2017) stated that the IC_{50} of BHT was lower than flavonoids such as pelargonin, silychirstin and callistegin, meaning that the antioxidant activity of BHT was higher. In addition, BHT has tert-butyl groups which cause extreme activity of capturing radicals (Yehye *et al.*, 2015).

The critical condition of instant aloe vera is determined by the increase in water content up to 12% and is characterized by the clumping of the powder. Table 2 shows that the critical condition of instant aloe vera stored at 25°C with polyethylene plastic with a thickness of 0.80 mm occurred at the 12th week of storage, namely at a moisture content of $11.99\pm 0.07\%$. In this condition, the RSA value is already very low, namely $3.63\pm 0.04\%$. According to Yunut *et al.* (2017), the antioxidant activity is highly dependent on the dose of flavonoids, whereas Jia *et al.*, (2020) stated that the flavonoids of vine tea extract found that the longer the storage, the lower the antioxidant effectiveness. Zhao *et al.* (2020) show that there is a positive correlation between antioxidant activity and the content of polyphenol compounds, the higher the polyphenols, the higher the antioxidative effect. Thus, this study should be complemented by changes in polyphenols during

storage, so that the relationship between the two parameters is clearer.

3.3 Inhibition of lipid peroxidation of encapsulated- aloe vera powder

The lipid oxidation reaction begins with the formation of free radicals from unsaturated fatty acids in the presence of heat or light initiators. Furthermore, the fatty acid radicals undergo peroxidation to produce peroxy radicals. These radicals can be captured by antioxidants by donating hydrogen to produce peroxide (Fennema, 1996). The flavonoid compound in aloe vera is one of the antioxidants that can scavenge radicals by donating hydrogen to block free radicals (Heř *et al.* 2019). The inhibition of lipid peroxidation by instant aloe vera during storage can be seen in Figure 2.

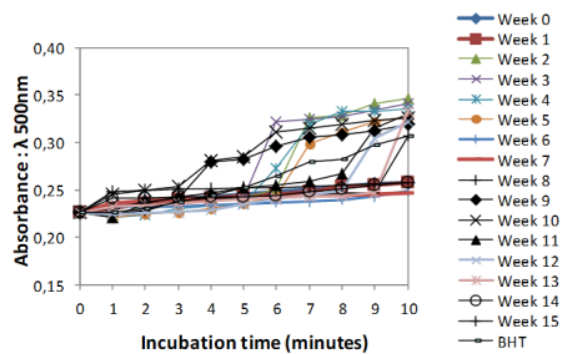


Figure 2. Antioxidative activity (inhibition of lipid peroxidation) of aloe vera instant during storage.

Table 2. Percentage of RSA and inhibition of lipid peroxidation of Aloe vera instant during storage

Sample with Storage time (weeks)	RSA (%)**	Inhibition of lipid peroxidation (%) **	Moisture (%)
0	16.34±1.22 ^g	39.33±1.68 ^c	6.28±0.05 ^a
1	13.55±1.82 ^f	24.35±0.18 ^{cd}	6.85±0.25 ^b
2	13.45±1.77 ^f	23.66±0.39 ^{cd}	7.87±0.08 ^c
3	13.28±2.55 ^f	23.73±0.45 ^{cd}	8.63±0.12 ^d
4	11.20±3.05 ^{ef}	23.78±1.82 ^{cd}	9.22±0.03 ^e
5	10.46±0.42 ^{de}	23.55±0.54 ^{cd}	10.27±0.05 ^f
6	8.38±0.73 ^{cd}	23.26±2.29 ^{cd}	10.34±0.01 ^{fg}
7	7.58±0.76 ^c	23.48±0.47 ^{bcd}	10.58±0.05 ^{gh}
8	7.48±0.69 ^c	23.47±0.42 ^{cd}	10.73±0.01 ^{hi}
9	6.85±0.58 ^c	22.99±0.21 ^{abcd}	10.89±0.29 ⁱ
10	5.92±0.29 ^{bc}	22.57±0.54 ^{abcd}	11.29±0.03 ^j
11	6.46±0.18 ^c	22.30±1.36 ^{abcd}	11.40±0.29 ^j
12	3.63±0.04^{ab}	22.31±0.02^{abcd}	11.99±0.07^{k***}
13	3.60±0.89 ^{ab}	22.16±0.40 ^{abc}	12.22±0.26 ^{kl}
14	2.81±0.70 ^a	21.14±0.71 ^a	12.75±0.20 ^{lm}
15	2.34±0.59 ^a	21.34±0.15 ^{ab}	12.52±0.24 ^m
BHT*	78.65±1.69	24.10±1.25	-

*Sample weight: 1 g (dry matter), except BHT weight: 0.1 g (dry matter), ** Mean in a column with similar superscript are not significant different at $\alpha = 0.05$, *** The antioxidative activity of aloe vera instant in the critical condition at 12% moisture content.

The absorbances of aloe vera instant samples with different storage times were different. The longer the storage time, the lower the absorbance intensity with longer incubation. It means that the antioxidative activities were decreased. The sample containing BHT also showed differences in intensity with longer incubation. BHT showed a lower absorbance than aloe vera instant at various storage time. This indicated that the antioxidative activity of BHT in inhibiting peroxide formation was higher. Table 2 showed the quantitative data of the inhibition of lipid peroxidation.

Table 2 shows that the lipid peroxidation inhibition of aloe vera instant during storage was significantly different. The longer the storage time aloe vera instant caused the lower the inhibition of lipid peroxidation. This was due to a decrease in the ability of instant aloe vera to capture peroxide radicals. According to Jia *et al.* (2020) during storage the ability to catch radicals by flavonoid is getting low, this is because phenol compounds are not stable which will experience a decrease in their activities. The decrease in inhibition of lipid peroxidation decreased rapidly in the first week, then slower in the following week. In critical conditions, at 12% moisture content, the inhibition of lipid peroxidation was still quite high, namely $22.31 \pm 0.02\%$. However, when compared to synthetic antioxidant BHT, the inhibition of aloe vera lipid peroxidation was lower. This is because BHT is a homogeneous material, while instant aloe vera is composed of several components with phenolic compounds as micro-parts that are easily oxidized with longer storage time. Compared with RSA, the percent inhibition of lipid peroxidation was higher in critical conditions. This is because the lipid peroxidation inhibition test is a measurement of total antioxidant activity including metal chelating capacity, radical scavenger and reducing power, while RSA only measures the ability to capture free radicals individually (Huyut *et al.*, 2017). Based on the inhibition of lipid peroxidation, the antioxidant capacity is still 56.73%, so that in this critical condition it is still feasible as a source of antioxidants.

4. Conclusion

Instant aloe vera antioxidant activity during storage decreases with storage time. In storage until a critical condition, namely 12% moisture content, DPPH radical scavenging activity is only $3.63 \pm 0.04\%$, while the inhibition of lipid peroxidation is still quite high, namely $22.31 \pm 0.02\%$. Storage of instant aloe vera for 12 weeks in polyethylene plastic with 0.80 mm thickness is still possible as an antioxidant-rich beverage product.

Conflict of interests

The authors declare no conflict of interest.

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