37 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 a 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 its 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 odor, so it needs to be processed into a product that is practical for consumption, such as in powder form.

45 Wariyah and Riyanto (2011) have studied the anti-oxidative properties of aloe vera extract and 46 powder. The Radical Scavenging Activity (RSA) of aloe vera extract is 35.17% and the inhibition of lipid 47 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 48 49 microencapsulated aloe vera powder with a 2.5% maltodextrin as an encapsulating agent and then dried 50 using a spray drier to produce instant aloe vera. This resulted instant aloe vera showing a solubility of 51 23.08 ± 0.97 s/g, meanwhile maintaining high RSA at 35.59 ± 2.65% and inhibition of lipid peroxidation at 52 16.15 ± 0.73 %.

53 Instant aloe vera produced from the spray dryer process can change its physicochemical properties, including a decrease in moisture content, hygroscopicity, water activity and antioxidative activity (Shishir 54 and Chen, 2017). The changes associated with antioxidative activity could reduce instant bioactive 55 56 properties. This decrease will be accelerated by the contact of oxygen and water vapor in the air. 57 (Fennema, 1996). Robert *et al.*, 2015 stated that flavonoid stability is greatly influenced by pH level, water 58 activity, radiation, oxygen, metals, antioxidants, temperature and enzyme activity. According to Minah and Astuti (2018), storage of instant tomato in polyethylene plastic at room temperature for 10 weeks 59 resulted in a decrease of antioxidant activity from 9% to about 4-5%. Until now, research is still rarely 60

61	done on the changes in the quality of instant aloe vera during storage. The purpose of this study was to				
62	evaluate the stability of the anti-oxidative properties of instant aloe vera during storage until critical				
63	conditions was reached and to determine the appropriate storage time.				
64					
65 66	2.	Materials and methods 2.1 Materials			
67		This study used aloe vera leaves with the variety of Aloe vera var. chinensis which was purchased from			
68		a farmer at Loano village in the Purworejo Regency of Central Java Province, Indonesia. The			
69		encapsulating agent of maltodextrin was obtained from Brataco Chemika, Yogyakarta. The chemicals			
70		for analysis of antioxidative activity from Merck, except the 1,1-Diphenyl-2-picrylhydrazil (DPPH) were			
71		purchased from Sigma-Aldrich Chemie.			
72					
73		2.2 Research Procedure			
74		2.2.1. Aloe vera analysis			
75		The aloe vera gel used for the study was analyzed for moisture content by static gravimetric method			
76		(AOAC, 1990), total phenol with the Folin-Ciocalteu method (Horax et al., 2005; Sensoy et al., 2006)			
77		by using spectrophotometer (UV-VIS Spectrophotometer Shimadzu 1240) to determine the			
78		aabsorption of the solution at 726 nm. A standard curve was prepared with gallic acid (Gallic acid CAS			
79		149-91-7 Sigma Aldrich). Furthermore, aloe vera leaves are used to produce instant aloe vera through			
80		the microencapsulation process.			
81		2.2.2. Aloe vera preparation for microencapsulation			
82		Instant aloe vera was processed from aloe vera powder which can be referred to Riyanto and Wariyah			
83		(2011) and the processing of instant aloe vera was referred to Wariyah and Riyanto (2016). The leaves			
84		of aloe vera were peeled, then washed with running water, then the clean gel sliced 3 mm thick, then			
85		arranged in a baking sheet to be dried in the oven (Memmert DIN 40050 IP 20) at a temperature of			

86	60-70 $^\circ \rm C$ until the moisture content is between 8-10%. The dried aloe vera is mashed in a blender
87	(Kirin KKB-210 GL1), then filtered using a 60 mesh sieve (ASTM E II Mesh 60). The resulting aloe vera
88	powder is made instantaneously by microencapsulation. The microencapsulation process was carried
89	out in the following steps: aloe vera powder was reconstituted with added distilled water at a ratio of
90	1/120 (w / v) and mixed with 2.5% concentrations of maltodextrin. The solution was stirred at 700
91	rpm for 45 min using a magnetic stirrer (Stir plate Nuova II) and then was dried into the spray dryer
92	(Lab Plan SD-05). The air flow rate of the spray dryer was set at 50 m3/h, and the solution flow rate
93	was 350 mL/h, the inlet temperature was 130°C and an outlet temperature of 103°C. The instant
94	powders obtained were kept at -10°C until analyzed and storage treatment.

96 The stability of the anti-oxidative properties of instant aloe vera was determined by storing instant 97 aloe vera in a 0.80 mm polyethylene plastic package in a room with a relative humidity of 75% 98 regulated with saturated NaCl salt (Ranganna, 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 99 100 analysis was carried out periodically (once a week) during the storage which included analysis of 101 moisture content and antioxidant activity with the DPPH method based on the percentage of RSA (Radical Scavenging Activity) and the ferriticocyanate (FTC) method to determine the percent 102 103 inhibition of lipid peroxidation (Hu et al., 2003).

2.2.3. Determination of antioxidative stability of aloe vera instant during storage

- 104
 DPPH free radical scavenging activity was determined based on the absorbance of the sample

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 measured periodically from zero to 120 min with 15 min intervals at a wavelength of 517 nm, the RSA

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 value is calculated by the following formula:

 107
 Radical Scavenging Activity (%) = [1- (AT / Ao)] × 100,
- which Ao is the absorbance of the sample at t = 0 min, and AT is the absorbance of the sample at t
 = 30 min (initial steady state).

110	The antioxidant activity by ferric thiocyanate method (FTC) was determined based on the	
111	inhibition of lipid peroxidation with the ferric thiocyanate (FTC) method (Hu et al., 2003). Absorbance	
112	of the solution was measured at 500 nm every 24 h for 10 d using a spectrophotometer. The inhibition	
113	of lipid peroxidation was determined with the formula of Anesini et al., (2008):	
114	Inhibition of lipid peroxidation (%) = 100 - (A1 / Ao) x 100	
115	which Ao is the absorbance of control (blank) at t = 7 d, and A1 is the absorbance of the sample at t =	
116	7 d (when the current reaches its maximum absorbance).	
117		
118	2.3. Design of experiments	
119	This research used completely randomized design with the storage time as a factor. The	
120	differences among the treatments were determined by F test, and the significant difference between	
121	samples was examined by Duncan's Multiples Range Test (DMRT) (Gacula and Singh, 1984).	
122		
122 123	3. Results and discussion/Results	
122 123 124	3. Results and discussion/Results 3.1. Moisture and phenolic content of aloe vera gel	
122 123 124 125	 3. Results and discussion/Results <i>3.1. Moisture and phenolic content of aloe vera gel</i> The moisture content and total phenol of aloe vera gel and instant are shown in Table 1. The 	
122 123 124 125 126	 3. Results and discussion/Results <i>3.1. Moisture and phenolic content of aloe vera gel</i> 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±0.08% and the phenolic compound was 	
122 123 124 125 126 127	 3. Results and discussion/Results 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±0.08% and the phenolic compound was 25.31±0.56 mg GAE/100g (dry matter). DiScala <i>et al.</i>, (2013) found the moisture content of aloe vera 	
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122 123 124 125 126 127 128 129 130	3. Results and discussion/Results 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±0.08% and the phenolic compound was 25.31±0.56 mg GAE/100g (dry matter). DiScala <i>et al.</i> , (2013) found the moisture content of aloe vera was 98.93±0.06% and the phenolic 54.46±7.87 mg GAE/100g (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	
122 123 124 125 126 127 128 129 130 131	3. Results and discussion/Results 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±0.08% and the phenolic compound was 25.31±0.56 mg GAE/100g (dry matter). DiScala <i>et al.</i> , (2013) found the moisture content of aloe vera was 98.93±0.06% and the phenolic 54.46±7.87 mg GAE/100g (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, merycetin, with a concentration of 257.7; 94.80 and 1283.50 mg/kg,	
122 123 124 125 126 127 128 129 130 131 132	3. Results and discussion/Results 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±0.08% and the phenolic compound was 25.31±0.56 mg GAE/100g (dry matter). DiScala <i>et al.</i> , (2013) found the moisture content of aloe vera was 98.93±0.06% and the phenolic 54.46±7.87 mg GAE/100g (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, merycetin, with a concentration of 257.7; 94.80 and 1283.50 mg/kg, respectively (Sultana and Anwar, 2008). Wariyah dan Riyanto (2016) found that the anti-oxidation	

134	and inhibition of lipid peroxidation 12.70 \pm 2.30%, whereas Hes <i>et al.</i> (2019) stated that RSA aloe
135	vera gel is 13.52% and hydroxyl radical scavenging at 11.74%.
136	Instant aloe vera has a moisture content of approximately 6.28 \pm 0.05%, according to SNI 01-4320-
137	1996 (Indonesian National Standard), moisture content for instant beverage products is 3-5%. The
138	spray drying process results in a decrease in moisture content (Shishir and Chen, 2017). However,
139	the moisture content achieved in each product is not the same because it is influenced by the stability
140	of the substances contained in the material and also the inlet and outlet temperatures of the spray
141	drying equipment suitable for each product.
142	
1/2	2.2. DDDH radical scavenging activity of alog yerg instant during storage
145	5.2. DPPH Taultur scovenging activity of alloe vera instant auring scorage
144	
145	The antioxidative activity of instant aloe vera was determined by the ability to scavenge the free
146	radical DPPH and to inhibit lipid or fatty acid peroxidation. The purple colour intensity of the DPPH
147	free radical decrease if these radicals are captured by antioxidants. Therefore, the lower the purple
148	colour intensity, the higher ability to capture free radical. Zhou et al. (2020) stated, that like other
149	flavonoid compounds, quercetin, myricetin and kaempferol have 0-3 hydroxyl groups on ring B and
150	double bonds on ketone groups that are capable of capturing free radicals. The antioxidative activity
151	of aloe vera instant during storage is shown in Figures 1.
152	Figure 1 shows that the absorbance of DPPH solution containing aloe vera instant which was
153	stored during 0 (fresh aloe vera instant) until 15 weeks and a synthetic antioxidants (BHT, Butylated
154	hydroxytoluene) decreased as a result of the length of incubation period. It indicates that aloe vera
155	instant and BHT has antioxidative activity by capturing DPPH free radicals. And the greater the
156	decrease in absorbance, the higher the antioxidative activity. Table 2 shows the RSA of aloe vera
157	instant during storage. The RSA were significantly different between samples with different storage
158	time. The RSA value of fresh aloe vera instant was 16.34±1.22% and after it was stored until critical
159	condition (at the moisture content of 12%) it decreased into 3.63±0.04%. The antioxidant activity of

160	aloe vera is determined by phenolic compounds. Flavonoids are susceptible to oxidation which is
161	influenced by pH level, water activity, radiation, oxygen, metals, antioxidants, temperature and
162	enzyme activity (Robert et al., 2015). In this study, instant aloe vera was stored in polyethylene
163	plastic packaging of 0.80 mm thickness, at a temperature of 25 $^{\circ}\!C$ and a relative humidity of 75%.
164	According to Keller and Kouzes (2017), polyethylene plastic has a permeability coefficient to water
165	vapor of 0.39-0.59 g \cdot mm/m ² .d at 25°C, while the permeability to oxygen has a diffusion coefficient
166	of 98-453 cm ³ .mm/m2.d.atm, thus allowing phenol oxidation to occur. Therefore, instant aloe vera
167	antioxidant activity decreases during storage. However, the BHT synthetic antioxidant shows the
168	highest antioxidative activity, while the instant aloe vera was indicated lower. Yunut et al., (2017)
169	stated that the IC50 of BHT was lower than flavonoids such as pelargonin, silychirstin and callistegin,
170	meaning that the antioxidant activity of BHT was higher. In addition, BHT has tert-butyl groups which
171	causes extreme activity of capturing radicals (Yehye et al., 2015).

172 The critical condition of instant aloe vera is determined by the increase in water content up to 173 12% and is characterized by 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 174 175 the 12th w 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 ± 0.04%. According to Yunut et al., (2017) the antioxidant activity 176 177 is highly dependent on the dose of flavonoids, whereas Jia et al., (2020) stated that the flavonoids 178 of vine tea extract found that the longer the storage, the lower the antioxidant effectiveness. Zhao 179 et al. (2020) shows 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 180 study should be complemented by changes in polyphenols during storage, so that the relationship 181 182 between the two parameters is clearer.

184 3.3. Inhibition of lipid peroxidation of encapsulated- aloe vera powder

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

The absorbances of aloe vera instant samples with different storage time were different. The longer 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 inhibition of lipid peroxidation.

Table 2 shows that the lipid peroxidation inhibition of aloe vera instant during storage was 197 198 significant different. The longer the storage time aloe vera instant caused the lower of the inhibition 199 of lipid peroxidation. This was due to a decrease in the ability of instant aloe vera to capture peroxide 200 radicals. According to Jia et al. (2020) during storage the ability to catch radicals by flavonoid is 201 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, 202 203 then slower in the following week. In critical conditions, at 12% moisture content, the inhibition of 204 lipid peroxidation was still quite high, namely 22.31 ± 0.02%. However, when compared to synthetic 205 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 206 207 compounds as micro-parts that are easily oxidized with longer storage time. Compared with RSA, the

208	percent inhibition of lipid peroxidation was higher in critical conditions. This is because the lipid	
209	peroxidation inhibition test is a measurement of total antioxidant activity including metal chelating	
210	capacity, radical scavenger and reducing power, while RSA only measures the ability to capture free	
211	radicals individually (Huyut et al., 2017). Based on the inhibition of lipid peroxidation, the antioxidant	
212	capacity is still 56.73%, so that in this critical condition it is still feasible as a source of antioxidants.	
213		
214	4. Conclusion	
215	Instant aloe vera antioxidant activity during storage decreases with storage time. In storage until a	
217	critical condition, namely 12% moisture content, DDPH radical scavenging activity is only 3.63 \pm 0.04%,	
218	while the inhibition of lipid peroxidation is still quite high, namely 22.31 \pm 0.02%. Storage of instant	
219	aloe vera for 12 weeks in polyethylene plastic with 0.80 mm thickness is still possible as an antioxidant-	
220	rich beverage product.	
221		
222	We declare no conflict of interest.	
223		
224	Acknowledgments	
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228		
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- 297
- 298

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

Sample	Moisture (%)	Total phenol
		mg GAE/100g (dry matter)
Aloe vera gel	98.74±0.88	25.31±0.56

Aloe vero	a instant	6.28±0.05	2.43±0.10
Table 2. Percentage	of RSA and inhibit	ion of lipid peroxidation of	of <i>aloe vera</i> instant durin
Sample with	RSA (%)**	Inhibition of lipid	Moisture (%)
Storage time		peroxidation (%) **	
(wooks)			

0	16.34±1.22 ^g	39.33±1.68 ^e	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±005 ^{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±0.70ª	21.14±0.71ª	12.75±0.20 ^{Im}
15	2.34±0.59ª	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 coulom with similar superscript are not significant different at α =0.05.



3	2	5	

- Figure 1. Radical Scavenging Activity (RSA) of aloe vera instants during storage.



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356	
357	Figure 2. Antioxidative activity (inhibition of lipid peroxidation) of aloe vera instant during
358	storage.
359	

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Title of Manuscript	:	The Antioxidative-Activity Stability of Aloe vera (<i>Aloe vera</i> var. <i>chinensis</i>) Instant During Storage

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Bukti korespondensi jurnal The antioxidative-activity stability of aloe vera (Aloe vera var. chinensis) instant during storage



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