ANTIOXIDANT ACTIVITIES IN GERMINATING MUNGBEAN

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ABSTRACT

The objectives of this work were to investigate the influence of germination times (24,48, 72, 96, 120 h) and conditions (light and dark) on the moisture content and antioxidant activity in mungbean sprout. The antioxidant activity were analyzed using in vitro methods. ABTS and DPPH methods were used to measure the antioxidant activity. A significant increase (p-value ≤ 0.05) was observed under ABTS method at 72 and 96 h, while it decreased at longer germination times. In addition, using DPPH method decreased the antioxidant activity significantly (p-value ≤ 0.05) from 24 to 48 h, however, it increases again at 72 and 96 h. Germination times and conditions significantly affects (p-value ≤ 0.05) the antioxidant activities of mungbean sprout. Based on the results, germination under dark conditions enhanced lower antioxidant activities during germination process.

Keywords: mungbean; germination; moisture content; antioxidant activity.

1. INTRODUCTION

Mungbean (*Vigna radiata* L.) is an easy-obtained food commodities with affordable purchase value and widely consumed as a functional food in Indonesia. It contains high number of vitamins, minerals, phytochemicals, and also secondary metabolites including flavonoids, tannins, steroids, saponins, which make it a potential food as a source of antioxidant (Djamil and Anelia, 2009; Guo, Li, Tang, and Liu, 2012). Antioxidants are defined as protective chemical towards free radicals that can stimulate oxidation reaction. It can work as an electron donor to prevent radical formation or by disrupting free radical chain reaction, and as a result, breakage cell is inhibited (Damiani *et al.*, 2008). As it mentioned before, consuming food labelled as functional food or other supplements containing antioxidants are one of easy way to prevent or reduce the risk caused by free radical activities (Winarsi, 2007). Phenolic compounds, including phenolic acids, flavonoids, tannins, and others, are the origin of biochemical metabolism in plants. In plants, they act as pigments that contributes as protective agents against UV light, and response to stress conditions. The phenolic antioxidants have a role in disturbing the oxidation process as free radical terminators and metal chelators They considered as the most important dietary antioxidants with average estimated intake to be around 1 g/day (Scalbert *et al*., 2005; Naczk and Shahidi, 2004; Shahidi and Ambigaipalan, 2015). Even at low concentration, phenols can also serve as antioxidants to protect food from oxidative reaction such as rancidity (Karakaya, 2004). Several studies reported that some factors can affect the antioxidant capacity in seeds (Khattak *et al.*, 2007; Chen *et al.*, 2016). Rather than biologically modified factors, physical treatment is an easier path to optimize the antioxidant capacity of seeds.

Seed germination is a series of processes that usually occur before the root tip emerges from the seed layer (Mayerand and Shain, 1974). The emergence of these roots is a form of embryonic growth that begins after water absorption. Germination is a new trend in healthy food and nutraceuticals since it serves edible seedlings with degraded antinutrients and upgraded the phytochemical contents compared to seeds (Falcinelli, 2017). The grains undergo a change in composition during the germination process that affects the sprout nutritional content. For grain-type foods, the value and nutritional content change more after going through the germination process. During grain germination, the breakdown of the macromolecular components begins with the help of amylolytic, lipolytic, and proteolytic enzymes. The product of this breakage is used for seed growth and development. Moreover, light sources as a growing condition can improve the nutrient contents in sprouts (Liu *et al.*, 2016).

This research aim to investigate the potency of mungbean as a local antioxidant source potency. Moisture content and antioxidant activity mungbean will be determined, moreover, the influence of germination times (24, 48, 72, 96, 120 h) and dark (without sunlight) and light (under sunlight) conditions on antioxidant activity mungbean sprouts will also be discussed.

2. METHODS

2.1 Material

Mungbean (*Vigna radiata* L.) used in the present study was obtained from Research Institute for Nuts and Tubers, Malang, Indonesia. Mungbean seeds were packed in plastic bag (200 g/bag) and stored at room temperature until used.

2.2 Sampel preparation

This germination was carried out to determine the pattern of antioxidant activity in mungbean sprouts for five days (120 h). The germination method refers to the method used by Puyanda (2015) with modifications. Mungbean seeds were washed with tap water to remove surface dirt. Furthermore, the clean seeds were soaked in warm water at 47ºC and kept in a dark place for 7 h. Soaking using warm water can soften the skin of the mungbeans, making it easier for water to penetrate the mungbean seeds. After 7 h, the mungbean seeds were drained, then placed in a plastic filter stored in 2 conditions; namely dark condition, kept in the dark place, and light condition, exposed to sunlight. On the second day, the mungbean sprouts in each conditions were soaked in water for 5 h for further imbibition effort, then drained again and stored in the former conditions. On the third to the fifth day, the sprouts were no longer soaked but only poured with running tap water three times a day.

2.3 Moisture content analysis

Moisture content was analyzed following AOAC (2012) method using a drying method at 100 ± 2 ˚C until constant weight.

2.4 Antioxidant activities analysis

The extraction of mungbean sprouts followed the method described by Yuan *et al*. (2010) with modifications. Briefly, mungbean sprouts were crushed and mixed with 50% methanol (1:10, w/v) until completely dissolved. The Whatman filter paper no.1 was used to filter the mixture. The extracted solutions were pooled together and measured for antioxidant activity and total phenolic content analysis.

The antioxidant activity (AOA) analysis using ABTS method refers to method by Stratil *et al*. (2006) with modification. The stock solution of 2'azinobis (3)ethylbenzthiazoline-6-sulfonic acid ABTS (Sigma-Aldrich) was made by mixing 7 mM ABTS with 4.95 mM of potassium persulphate with the ratio 1:1 (v/v) for 12 h in the dark condition at room

temperature to form radical action $ABTS^{\bullet+}$. After that 40 µL of mung bean extract was mixed with 3 mL of $ABTS^{\bullet+}$ solution, then incubate for 10 min at room temperature in the dark place before optical density measurement at 734 nm using UV/Vis spectrophotometer. Distilled water was used as the blank. AOA was expressed as prosen Radical Scavenging Activity (%RSA).

The DPPH method was determined according to the method described by Leong and Shui (2002) with modification. Fresh 0.1 mM solution of DPPH (Sigma-Aldrich) in methanol was prepared. 100 µL of mungbean extract was mixed with 4.0 mL of DPPH solution, then incubated for 30 min at room temperature in the dark condition before optical density mesurement at 517 nm by UV/Vis spectrophotometer. AOA was expressed as prosen Radical Scavenging Activity (%RSA).

2.5 Statistical analysis

The experiment was designed as a split-plot design. The main-plot experiment unit was germination times (24, 48, 72, 96, 120 h), and the sub-plot experiment was the storage condition (dark and light conditions). Comparison of the means were conducted using analysis of variance (ANOVA). The significant differences between means were performed using Duncan's multiple range test (p-value < 0.05). Statistical analysis was run out using the SPSS statistic program (version 24.0).

3. RESULTS AND DISCUSSION

3.1 Moisture content

The moisture content in mungbean sprout was shown in Table 1. The moisture content increased during germination in both conditions. The moisture content in both conditions increased in parallel until 72 h of germination. However, after 72 h, mung bean sprout in light conditions losses its moisture content whereas the one in dark keep increasing until reached 62.05 ± 0.00 % at 120 h. This might happen because the sprouts were having different metabolism pathway after 72 h in the effect of light and dark conditions. This difference might lead to different production of enzyme and other metabolite, as well as different rates of each metabolite production.

Table 1. Moisture content of mung bean sprout during germination

Time (h)	Conditions	
	Light (%)	Dark (%)
24	46.97^{a} ± 0.06	44.95^{a} ± 0.38
48	44.65^{a} ± 3.83	44.94^{a} ± 4.22
72	57.90^{bc} ± 0.63	58.70^{c} ± 1.18
96	53.90^{b} ± 2.10	60.78^{c} ± 1.54
120	45.97^{a} ± 0.00	62.05^{c} ± 0.00

^{a,b,c} the superscript letter indicated significant different among the treatments with p-value < 0.05

3.2 Antioxidant activities

Antioxidant activity (AOA) of mung bean was unstable during germination time. AOA of mung bean sprouts were determined using ABTS and DPPH assays. Figure 1 shows the results of AOA using ABTS assay. The highest %RSA using ABTS was observed at 24 h of germination times in both dark and light conditions; which were 60.51 ± 4.29 % and 71.57 ±

1.79 %, respectively. Until 96 h, ABTS value of mung bean sprout in light condition was significantly higher than the one germinated in the dark condition. Prolonging germination time up to 120 h in both conditions significantly decreased the value. Although at 120 h dark-germinated mung bean showed higher ABTS value, the number still lower than the highest value of light-germinated sprout at 24, 48, 72 and 96 h. Mung bean germinated in dark conditions at 48 h showed the lowest ABTS value among all treatments.

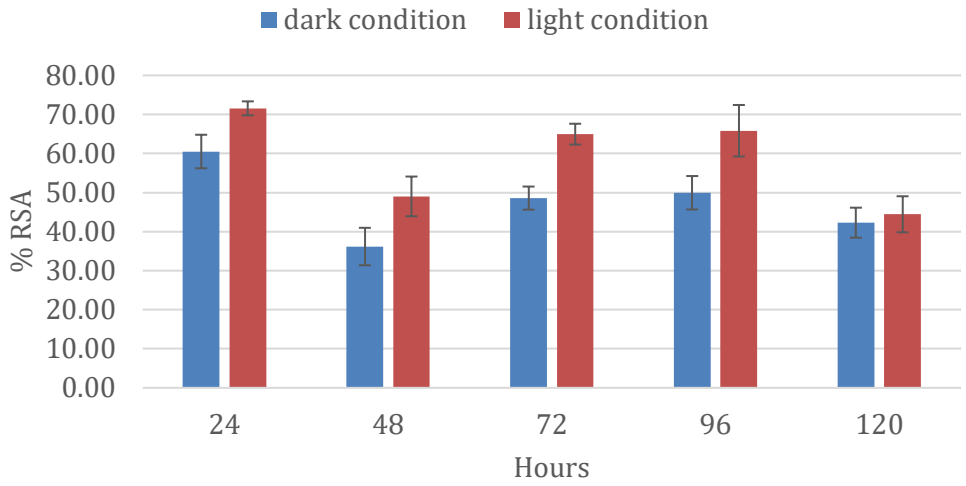

Figure 1. Antioxidant activity by ABTS$^{\bullet+}$ scavenging assay was expressed %RSA.

Figure 2 showed AOA of mung bean using DPPH assay. A significant decreased of DPPH value was observed at 48 h of germination time in both dark and light conditions. A significant increase was observed in light-germinated mung bean at 72 h and stable up until 96 h, after that a significant decreased, up to 62%, was observed at 120 h. On the other hand, statistically reported, there was no significant changes in DPPH value of dark-germinated VIMA-1 after 24 h. The highest % RSA using DPPH value was 14.90 ± 0.94 %, observed at 96 h of light-germinated mung bean.

According Wisaniyasa and Darmayanti (2019), the storage time affected the antioxidant activity in the germination process. The increase of antioxidant activity impacted the biochemical production of seeds during germination (Vale *et al.*, 2014). Antioxidant activity in the plant can be sourced from the phenolic compound that naturally available in the plant organs. Furthermore, Lin and Lai (2006) reported that the antioxidant activity rose during the germination period in other legume products.

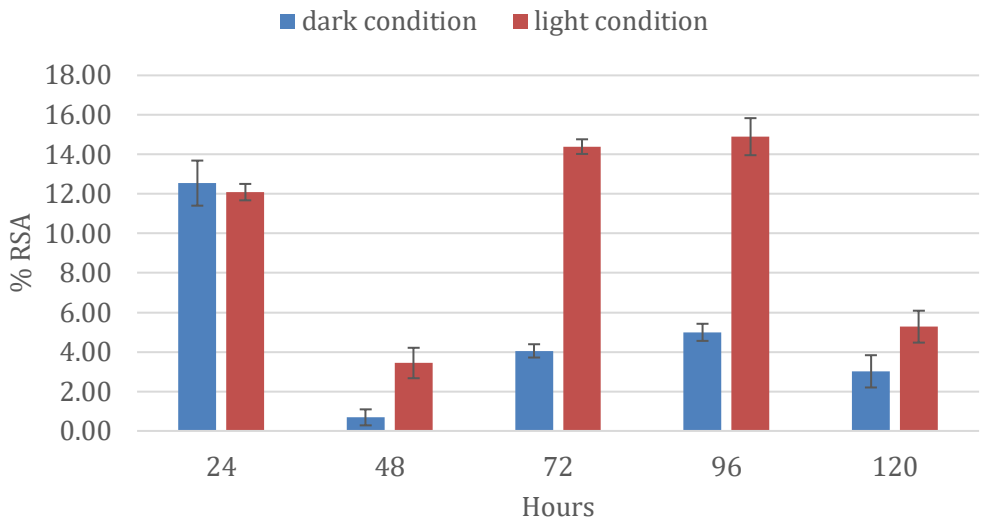

Figure 2. Antioxidant activity by1,1-diphenyl-2-picrylhydrazyl radical (DPPH•) scavenging assay was expressed as %RSA

In the present study, two methods were used to measure AOA of mung bean seeds germinated in different conditions. The difference of the two methods used was the interpretation of the scavenging activity. Huang *et. al.* (2005) described the scavenging activity in ABTS assay as the activity of ABTS in trapping the electron from the antioxidant. However, Charles, D.J. (2013) stated that DPPH acts as a proton receiver from antioxidant. Based on the results from this study, AOA of the mung bean sprouts was higher when determined using ABTS methods compared to DPPH. This describe that the antioxidants in mungbean sprouts could scavenge the free radical via electron donation.

4. CONCLUSION

The germinating time and condition of mung bean gave the different results on moisture content and antioxidant activity. The moisture content of mung bean sprout in the dark condition showed higher than light condition. In addition, germination under dark conditions enhanced lower antioxidant activities during germination process. Germination times and conditions significantly affects the antioxidant activities of mungbean sprout.

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