



## Antioxidant Activity of Sprouting Mungbean (*Vigna radiata*) Variety VIMA-1

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### Abstract

The objectives of this work were to investigate the influence of germination times (24, 48, 72, 96, and 120 hours) and conditions (light and dark conditions) on the antioxidant activity and total phenolic compounds in mungbean sprout. Antioxidant activity and total phenolic content were analyzed using in vitro methods, with the antioxidant activity assessed using ABTS and DPPH methods. We observed a significant increase ( $p < 0.05$ ) in antioxidant activity using the ABTS method, which decreased after 48 hours of germination. In contrast, the DPPH method showed a significant decrease ( $p < 0.05$ ) followed by an increase in antioxidant activity after 48 hours of germination. Meanwhile, germination up to 48 hours significantly reduced ( $p < 0.05$ ) the total phenolic content in both conditions, while against that time, it increased significantly ( $p < 0.05$ ) up to 96 hours. The effects of germination time and conditions were significant ( $p < 0.05$ ) for both antioxidant activities and total phenolic content. Generally, germination under dark conditions resulted in lower antioxidant activities and total phenolic content during the germination process.

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### Introduction

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 phytochemical contents compared to seeds (Falcinelli, 2017). The grains undergo a change in composition during the germination process that affects the sprouts' 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).

Mungbeans are food item with affordable purchase value for everyone and easy to obtain. In Indonesia, mungbeans are a type of bean widely consumed by the public as a functional food. Mungbean Vima-1 variety is a superior quality green bean developed by the Indonesian Bean and Tuber Crops Research Institute. According to Sutrisno (2015), the Vima-1 variety is a form of fine seed because it is tolerant of climate change, simultaneous harvest, and high productivity. Nowadays, Vima-1 mungbeans are becoming the prima donna, such as in the Demak region (Prasetiaswati and Radjit, 2011) and East Nusa Tenggara (Seran, Kote and Benu, 2011).

Based on previous research on mungbeans, it is known that they contain secondary metabolites, including flavonoids, tannins, steroids/triterpenoids, and saponins which have the potential to be antioxidants (Djamil and Anelia, 2009). According to Guo, Li, Tang,

and Liu (2012), one of the most commonly consumed vegetables is the mungbean, a high amount of vitamins, minerals, and phytochemicals that may serve complementary nutrients and bioactive substances together with other fruits and vegetables. Antioxidants are chemical compounds that can protect several biological components such as proteins, lipids, vitamins, and DNA with mechanisms in the form of inhibition of damage, rancidity, and/or a change in color due to oxidation reactions. Antioxidants can be said to be electron donor compounds that can prevent radical formation. Antioxidants are substances that can restrain oxidation reactions by trapping free radicals and highly reactive molecules. As a result, breakage cell is inhibited. According to Winarsi (2007), an easy way to prevent or reduce the risk caused by free radical activity is by consuming foods or supplements that contain antioxidants. Phenolics are the most important dietary antioxidants, with their average dietary intake have been estimated to be around 1 g / day (Scalbert *et al.*, 2005). Naczka and Shahidi (2004) stated that one of the primary biochemical metabolism classes in plant originated from phenolic compounds. The phenolic compounds can act as antioxidants to protect food from oxidative rancidity even at a low concentration (Karakaya, 2004). The phenolic antioxidants disturb the oxidation process as free radical terminators and metal chelators (Shahidi and Ambigaipalan, 2015).

In Indonesia, there is no information about the potential local mungbean sprout var. VIMA-1 is a source of antioxidants and total phenolic compounds. So, this research provided information about antioxidant activity and total phenolic compounds in the local mungbean sprout from Indonesia, especially var. VIMA-1. Therefore, the objectives of this research were to evaluate the influence of germination times (24, 48, 72, 96, and 120 hours) on antioxidant activity and total phenolic compounds in mungbean sprouts. Different treatments were used in two storage conditions (light and dark conditions) to study the effect of light during germination.

## Materials and Methods

### Materials

The raw material used in this study was mungbeans VIMA-1 variety obtained from the Research Institute for Nuts and Tubers, Malang. The mungbean seeds are packaged in 200 g using plastic packaging.

### Methods

#### Sprouting method

The research was conducted from July to August 2020. This germination was performed to determine the pattern of antioxidant activity in VIMA-1 mungbean sprouts for five days. This germination method refers to the technique used by Puyanda (2015) with modifications. The first step is cleaning the VIMA-1 variety mungbean seeds with tap water to remove dirt

on the surface of the beans and as a sorting effort to remove bad seeds. Furthermore, the clean green bean seeds are soaked in warm water at 47°C for 7 hours and placed in a dark place. Soaking using warm water can soften the skin of the mungbeans, making it easier for water to penetrate the mungbean seeds. After 7 hours, the mungbean seeds are drained, then placed in a plastic filter stored in 2 conditions, namely dark and bright conditions, exposed to sunlight. On the second day, the mungbean sprouts were soaked in water for 5 hours as a further imbibition effort, then drained again and stored in 2 conditions, such as the first day. On the third to the fifth day, the sprouts are no longer soaked but only poured with running tap water three times a day.

#### Extraction Method

The extraction of mungbean sprouts has followed the method described by Yuan *et al.* (2010) with some modifications. Briefly, mungbean sprouts were crushed and added to 50% methanol (1:10, w/v) and mixed well 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 and total phenolic analysis. Two assays of antioxidant activity (ABTS and DPPH) were analyzed.

#### Antioxidant activities (AOA) analysis

The ABTS assay was analyzed using Stratil *et al.*'s (2006) method with modifications. The stock solution of 2'azinobis (3) ethylbenzthiazoline-6-sulfonic acid ABTS (Sigma-Aldrich) was made by mixing seven mM ABTS with 4.95 mM of potassium persulphate with the ratio of 1:1 (v/v) for 12 h in the dark condition at room temperature to form radical action ABTS<sup>•+</sup>. 40 µl of JAT extract was mixed with 3 ml working ABTS<sup>•+</sup> solution, then incubated for 10 min at room temperature in the dark before measuring at 734 nm using UV/Vis spectrophotometer. AOA was expressed as mg Trolox equivalent per 100 g mungbean sprout (wet basis).

The DPPH method was determined according to the method described by Leong and Shui (2002) with modification. Freshly, 0.1 mM solution of DPPH (Sigma-Aldrich) in methanol was prepared, and 100 µl of JAT extract was mixed with 4.0 ml of DPPH solution and then incubated for 30 min at room temperature in the dark condition before analysis at 517 nm by UV/Vis spectrophotometer. AOA was expressed as mg Trolox equivalent per 100 g mungbean sprout (wet basis).

#### Total phenolic content (TPC)

TPC was analyzed following the method of Dewanto *et al.* (2002). The 125 µl of mungbean sprout extract and 250 µl of Folin-Ciocalteu (Sigma-Aldrich) reagent were mixed and followed by adding 3 ml of distilled water in a test tube. The mixture was incubated for 6 min and added 2.5 ml of 7% Na<sub>2</sub>CO<sub>3</sub> solution. The mixture was incubated for 90 minutes at room temperature before measuring at 760 nm using a UV/Vis spectrophotometer. TPC was expressed as mg gallic

acid equivalent (GAE) per 100 g mungbean sprout (wet basis).

### Statistical analysis

This research was designed as a split-plot design. The main-plot experiment unit was sprouting time (24, 48, 72, 96, and 120 hours), and the sub-plot was storage condition (dark and light conditions). ANOVA procedures measured analysis of variance. The significant differences between means were performed using Duncan's multiple range test ( $p \leq 0.05$ ). Statistical analysis was run out using the SPSS statistic program version 24.0.

### Results and Discussion

The statistical analysis using SPSS revealed that the interaction between storage time and condition affected AOA and TPC response ( $p \leq 0.05$ ). Regarding AOA, two assays were analyzed, ABTS and DPPH assay. Trolox was used as a standard for scavenging activities. The result showed that AOA was unstable during storage (Figure 1). AOA by ABTS assay significantly increased after a 48-hour germinating time (445.73 mg Trolox/100 g FW for dark conditions and 576.24 mg Trolox/100 g FW for light conditions). However, the decrease showed until 120-hour germinating time with no significant difference between dark and light conditions. Swieca *et al.* (2012) reported that the increased antioxidant activity in lentil sprouts was under the germinating time. The increase of AOA may induce the water imbibition that initiates grain metabolism to produce new compounds (Khang *et al.*, 2016).

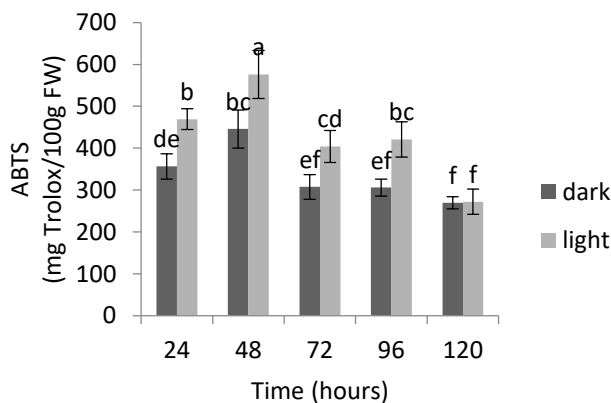


Figure 1. Antioxidant activity by ABTS+ scavenging assay was expressed as mg Trolox equivalent/100 g sample (FW). The superscript values with the different letters were significantly different ( $p < 0.05$ ) using Duncan's Multiple Range Test.

Moreover, germinated mungbean stored in light conditions was found to lower the loss of antioxidants with ABTS assay compared to dark conditions. This result showed similarity with the effect of light sources on major flavonoids and antioxidant activity in typical

buckwheat sprouts (Nam, Kim, and Eom, 2017). The antioxidant degradation may be responsible for a significant loss of pigments in the seed coats (Lin and Lai, 2006).

Figure 2. describes the antioxidant activity using the DPPH assay. It showed that the number of antioxidants decreased significantly after 24 hours of germinating time ( $p \leq 0.05$ ) in both conditions. The antioxidant activity was reported to be stable from 48 h to 96 h and decreased at 120h with 20.99 mg Trolox/100 g FW in the dark condition. On the other hand, the antioxidant activity in light conditions reached a high amount, up to 66.53 mg Trolox/100 g FW at 96 hours of germinating time. The increase in antioxidant activity impacted the biochemical production of seeds during germination (Vale *et al.*, 2014). Furthermore, Lin and Lai (2006) reported that the antioxidant activity rose during the germination period in other legume products.

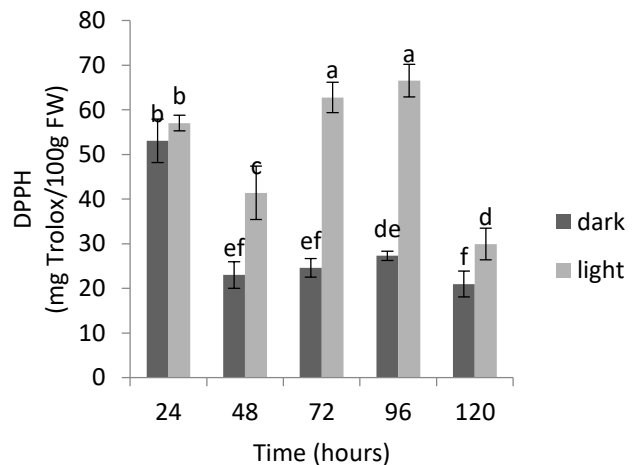


Figure 2. Antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging assay was expressed as mg Trolox equivalent/100 g sample (FW). The superscript values with the different letters were significantly different ( $p \leq 0.05$ ) using Duncan's Multiple Range Test.

This research used two methods to analyze the antioxidant—the difference between those methods in scavenging activity. Following Huang, Boxin, and Prior (2005), the scavenging activity of ABTS takes the role of trapping via electron donation from the antioxidant. On the other hand, Charles, D.J. (2013) stated that DPPH acts as a proton receiver from the antioxidant. This research showed that the antioxidant activity in mungbean sprouts was higher when analyzed using ABTS than the DPPH method. It described that antioxidants in mungbean sprout could scavenge the free radical via electron donation.

The results of the TPC are shown in Figure 3. In both dark and light conditions, TPC decreased after germinating for 24h from 126.36 to 43.33 and 145.60 to 50.14 mg GAE/100g FW, respectively. Compared to the previously published (Guo, Li, Tang, and Liu, 2012) on

phenolic content in mungbean sprouts, the concentration of TPC was lower than that of research. According to Pajak *et al.* (2014), the differences in phenolic compounds among mung bean sprouts may cause the dissimilarity of varieties, growing conditions, and extraction methods. However, the TPC increased after 48h germination and decreased at 120h germinating time. Germination caused significant changes in the phenolic composition due mainly to the activation of endogenous enzymes and the production of complex biochemical metabolism in seeds and germination (Duenas *et al.*, 2009).

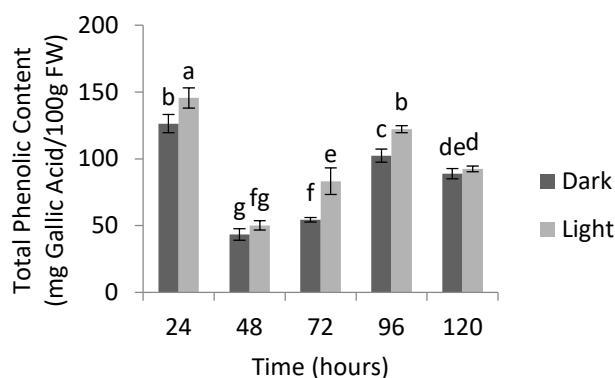


Figure 3. Total phenolic content (TPC) was expressed as mg Gallic acid equivalent/100 g sample (FW). The superscript values with the different letters were significantly different ( $p \leq 0.05$ ) using Duncan's Multiple Range Test.

Meanwhile, the decrease in the phenolic compound was caused by the influence of their free radical scavenging activity. The relationship between phenolic and antioxidant activity during germination has been reported previously in Canary sprouts (Chen *et al.*, 2016) and sweet corn sprouts (Xiang *et al.*, 2017). Meanwhile, germinating in dark conditions promoted a more significant loss than in light conditions. Khattak *et al.* (2007) reported that the light presence influenced the total phenolic in chickpea sprouts. The light as a growing condition could provide the nutrient contents in sprout (Liu *et al.*, 2016).

### Conclusion

AOA and TPC in mungbean sprouts were unstable during the 120-hour germination. However, the storage under light conditions improved AOA and TPC preservation. Moreover, the AOA analysis used ABTS showed higher antioxidant activity than DPPH in mungbean sprouts.

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