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FULL PAPER

Evaluation of total phenolic content, antioxidant activity, germination power, and yield of pigeon pea (*Cajanus cajan*) sprouts elicited using various Na-alginate levels with different elicitation duration

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Abstract

This study was aimed to investigate the total phenolic content and antioxidant activity of elicited pigeon pea (*Cajanus cajan*) sprouts prepared using various Na-alginate concentrations at different elicitation durations. The germination power and yield of the sprouts were also examined. Elicitation proved capable to improve the antioxidant capacity of legumes sprouts but the yield and germination rates were decreased due to the elicitation stress. In this study, elicitation conducted by immersing pigeon pea seeds in Na -alginate solutions (250, 300, and 350 ppm) for various duration (16, 20, and 24 hrs). The seeds were then germinated for 48 hrs to produce Na-alginate elicited pigeon pea sprouts. The results showed that the total phenolic content (TPC) and antioxidant activity (Trolox equivalent antioxidant capacity, TEAC, and Ferric reducing antioxidant power, FRAP) of elicited pigeon pea sprouts were significantly increased along with the increasing elicitation duration in all Na-alginate levels. The higher Na-alginate levels produce a higher level of TPC, TEAC, and FRAP values. On the other hand, germination power and the yield of the sprouts were significantly decreased along with increasing Na-alginate levels and elicitation duration. Elicitation using 350 ppm Na-alginate with an elicitation duration of 24 hrs produces elicited pigeon pea sprouts with the highest TPC and antioxidant activity, but lowest germination power and yield. These results have an important consequence in developing an elicitation technique to improve the antioxidant capacity of leguminous.

1. Introduction

Pigeon pea seed is proved as a potential source of bioactive molecules that promising to treat multiple diseases (Mathew *et al*., 2017). The health benefits of pigeon pea are mainly contributed by the phenolic compounds which are exhibited versatile bioactivities such as antitumor, antiviral, anti-inflammatory, antioxidant, hypocholesterolemic, and hypoglycemic activities (Gai *et al*., 2021). Ariviani *et al*. (2018) reported that pigeon pea seeds have the potential to be developed as an anti-diabetic functional drink related to their antioxidant capacity. It had been reported that the total phenolic content and radical scavenging activity of pigeon pea were higher than those of cowpea.

Germination is a common strategy to promote antioxidant compounds production as well as antioxidant activity of several legumes (Rajendra *et al*., 2019; Ariviani, Mudalifah, Ishartani *et al*., 2020). Several studies had reported improvement of antioxidant capacities such as total phenolic content (TPC) and radical scavenging activity (RSA) as well as the reducing power of pigeon pea through germination, which reported by Uchegbu and Ishiwu (2016), Uchegbu *et al*. (2017), and Sharma and Singh (2018).

Liu *et al*. (2019) stated that elicitation is a promising strategy rather than other conventional biotechnological techniques for increasing the bioactive compounds and biological activity of sprouts. Elicitors most widely used in the elicitation process for enhancing the accumulation of bioactive compounds in sprouts, among others are methyl jasmonate, salicylic acid, chitosan, sucrose, methionine, glutamic acid, NaCl, mannitol, and light. Elicitation proved capable to increase the antioxidant

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capacity of legumes sprouts. The UV elicitation increases TPC and RSA of lentil sprouts (Swieca *et al*., 2014). Elicitation using mannitol effectively enhance the TPC, RSA and reducing power of lentil sprouts depend on mannitol concentration (Swieca, 2015a). NaCl elicitation improves total flavonoid content, RSA and reducing power of cowpea sprouts (Rajendra *et al*., 2019) and pigeon pea sprouts (Kristiani *et al*., 2019) depend on NaCl concentration and the elicitation duration, respectively. Swieca (2015b) stated that elicitation trigger desirable secondary metabolites production, include antioxidant compounds, but the elicitor concentration and elicitation duration required for optimum secondary metabolite accumulation are a characteristic of each plant species. Therefore, Investigation of antioxidant capacities such as TPC and antioxidant activities of legumes sprouts elicited using various elicitor levels and elicitation duration is crucial.

Elicitation effectively increased antioxidant capacity and had a significant impact on the sprouting percentage of germination rate of common bean (*Phaseolus vulgaris*) sprouts (Ampofo *et al.*, 2020). Different elicitors used in the elicitation process produced different impacts on the antioxidant capacity and germination rate of kidney beans sprouts (Limón *et al*., 2014), and lentil sprouts (Peñas *et al*., 2015). Increasing elicitor concentration was necessary for maximizing the TPC of chickpea sprouts but it significantly reduces the germination power (León-López *et al*., 2020). The author's previous study showed that the antioxidant capacity of cowpea sprouts increased along with increasing NaCl concentration used as an abiotic elicitor (Rajendra *et al*., 2019). Other studies such as Swieca (2015a) and Saha *et al*. (2010) reported improvement of the antioxidant capacity of lentil and mung bean sprouts elicited with NaCl along with increasing NaCl levels. The antioxidant capacity of pigeon pea sprouts increases along with increasing NaCl elicitation duration (Kristiani *et al*., 2019). To the best of our knowledge, investigation on TPC and antioxidant activity, as well as germination power and yield of pigeon pea sprouts elicited using Na-alginate with various concentrations and elicitation durations have not been reported yet. The study aims to evaluate the TPC, antioxidant activity which includes RSA (Trolox Equivalent Antioxidant Capacity, TEAC) dan Ferric Reducing Antioxidant Power (FRAP) (Ascorbic Acid Equivalent Activity, AAEA), germination power and yield of pigeon pea sprouts elicited using Na-alginate (250, 300, 350 ppm) as biotic elicitor for different elicitation durations (16, 20, 24 hrs).

2. Materials and methods

The materials used in this study were pigeon pea obtained from Gunung Kidul, Yogyakarta, Indonesia; folin-ciocalteau's phenol reagent, ABTS (2,2'Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), potassium persulfate, Trolox (6-Hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid), ascorbic acid were obtained from Sigma-Aldrich Co. (St.Louis, MO, USA). Na-alginate, sodium carbonate, sodium dihydrogen phosphate dihydrate, disodium phosphate dihydrate, potassium ferricyanide, hydrochloric acid, Iron (III) chloride hexahydrate, trichloroacetic acid (TCA), Ethanol, and Methanol were purchased from Merck Millipore Co. (Darmstadt, Germany). All reagents used in this study were analytical grade.

2.1 Pigeon pea sprouts preparation

Pigeon pea sprouts were prepared as previously described (Kristiani *et al*., 2019; Rajendra *et al*., 2019; Ariviani, Mudalifah, Ishartani *et al*., 2020) with slight modification. Briefly, the sorted pigeon pea seeds were rinsed three times using distilled water. Furthermore, the seeds were exposed to elicitation by submerging in Na-alginate solution (250, 300, and 350 ppm) with a ratio of 1:3 w/v for various elicitation durations (16, 20, 24 hrs), then germinated for 48 hrs.

2.2 Preparation of pigeon pea sprouts extract

The pigeon pea sprouts extract preparation was conducted according to Ariviani, Lainuna, and Fauza (2020) with slight modification. Briefly, the dehulled and mashed pigeon pea sprouts (2 g) were extracted with 20 mL of methanol (80%) using a water bath shaker (SWB 20, Fisher Scientific Haake, Germany) at 200 rpm, 50°C for 2 hours. The methanol extract was separated using PLC-05 Centrifuge (Gemmy, Taiwan) at 10000 rpm for 15 minutes. The clear supernatant was collected in dark bottles and stored at 10ºC until further analysis.

2.3 Total phenolic content determination

The total phenolic content of pigeon pea sprout extracts was analyzed using Follin-Ciocalteu Reagent as described by Singleton *et al*. (1999). The total phenolic content was expressed as Gallic Acid Equivalent (μM GAE/g sample dry weight).

2.4 Antioxidant activity analysis

Antioxidant activity analysis conducted by measuring the radical scavenging activity (RSA) and Ferric reducing antioxidant power (FRAP). RSA determined using the ABTS•+ free radical method (Re *et al*., 1999) and expressed as Trolox Equivalent

Antioxidant Capacity (mM TEAC/g sample dry weight). FRAP measured using the method described by Berker *et al*. (2007) and expressed as Ascorbic Acid Equivalent Activity (µM AAEA/ g sample dry weight)

2.5 Germination power and yield determination

The germination power was determined by calculating the ratio of the number of pigeon pea seeds that sprouted to the total number of the seeds and expressed as a percentage (%). The yield was determined based on the ratio of dry weight of pigeon pea sprouts to the initial pigeon pea seeds and expressed as a percentage (%)

2.6 Statistical analysis

The Data was presented as a mean±standard deviation from three replication. IBM SPSS Statistics 22 (SPSS Inc., Chicago, USA) program with the General Linear Model (Univariate Analysis of Variance) was used for data analysis. Duncan's Multiple Range Test (DMRT) was used to evaluate the significant difference between means ($p<0.05$) for each factor.

3. Results and discussion

3.1 Total phenolic content (TPC)

TPC of pigeon pea sprouts prepared with various Na-alginate concentrations and elicitation durations was presented in Figure 1A. The TPC values (µM GAE/g sample dry weight) of pigeon pea sprouts elicited using various Na-alginate levels with elicitation duration of 16, 20, and 24 hrs were 9729.2±105.4, 10611.4±70.6, and 11374.2±97.8 for 250 ppm Na-alginate, 10328.1±95.8, 11172.7±85.9, and 12066.5±101.8 for 300 ppm Na-alginate, 11864.5±63.1, 12659.1±100.9, and 13587.5±90.3 for 350 ppm Na-alginate.

Based on the statistical analysis, the TPC was affected by Na-alginate concentration and elicitation duration. Increasing of elicitation duration produce significantly higher TPC in all Na-alginate concentration. For each elicitation duration, a higher Na-alginate concentration produces pigeon pea sprouts with significantly higher TPC. The highest TPC of pigeon pea sprouts produced by elicitation using 350 ppm Na-alginate for 24 hrs. This is possible because the higher either the Na-alginate concentration or the elicitation duration induced more plant stress levels. The higher stress levels stimulate higher secondary metabolite production, including enzymatic and non-enzymatic antioxidant production such as phenolic biosynthesis as the stress response (Swieca, 2015a; Swieca, 2015b; Swieca, 2016). Several studies reported a similar result, the total phenolic and total flavonoid content of elicited legumes sprouts increases along with increasing elicitor concentrations, such as in cowpea and lentil (Swieca, 2015a; Rajendra *et al*., 2019).

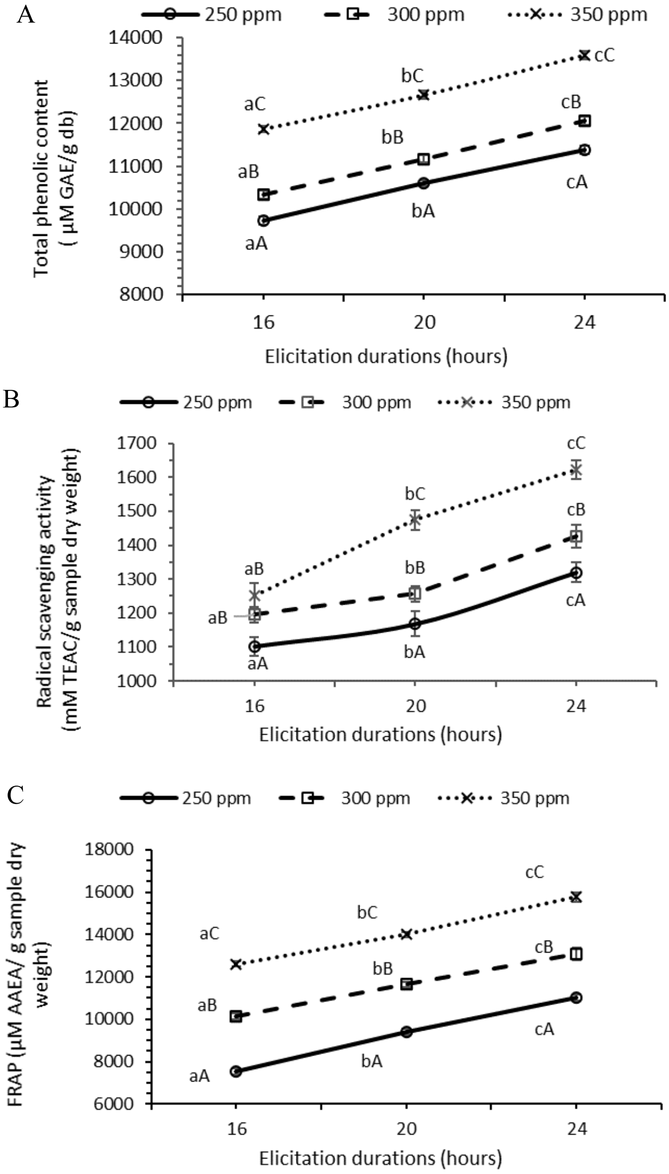

Figure 1. Total phenolic content (A), radical scavenging activity (B) and Ferric Reducing Antioxidant Power (FRAP) (C) of elicited pigeon pea sprouts prepared using various Na-alginate concentration with different elicitation durations. Different capital letters mean significant differences ($p<0.05$) for different Na-alginate concentration with same elicitation duration, whereas different lowercase letters mean significant differences for different elicitation duration with the same Na-alginate concentration.

Na-alginate is one kind of carbohydrate biotic elicitor that capable to improve the production of plant secondary metabolites (Thakur *et al*., 2019). Carbohydrate is classified as "general elicitors" which induce non-specific mechanisms for the trigger of defense response in different plant cultures (Baenas *et al*., 2014). Elicitors act as plant stress inducer, which will promote the biosynthesis of specific compounds that have an important role in the plant's defense system to overcome the stress conditions (Ramirez-estrada et al., 2016). Specific elicitors such as salt (NaCl) induce salinity stress which further triggers osmotic stress tolerance through proline formation as an osmolyte. Proline synthesis is related to overproduction of erythrose-4-phosphate that plays a role in the shikimate pathway which stimulate phenolic compounds synthesis (Shetty, 2004; Thakur and Sharma, 2005; Saha *et al.,* 2010)

3.2 Antioxidant activity

The antioxidant activity of pigeon pea sprout extracts was determined by measuring the radical scavenging activity (TEAC) and the reducing power (FRAP). The results were presented in Figure 1B and 1C. The TEAC values (mM TEAC/ g sample dry weight) of pigeon pea sprouts elicited using various Na-alginate levels for 16, 20, and 24 hrs elicitation durations were 1102.4±27.4, 1168.5±37.3, 1319.8±29.2 (250 ppm Na-alginate), 1195.9±22.9, 1256.5±23.0, 1426.5±34.3 (300 ppm Na-alginate), and 1252.0±34.9, 1474.4±29.5, 1622.5±28.8 (350 ppm Na-alginate), respectively. While the FRAP values (µM AAEA/ g sample dry weight) of the same sample were 7539.4±134.1, 9406.3±153.5, 11042.1±147.1 (250 ppm Na-alginate), 10149.0±132.9, 11641.5±127.8, 13087.2±304.9 (300 ppm Na-alginate), and 12612.8±133.6, 14004.9±113.4, 15778.1±216.4 (350 ppm Na-alginate).

Both the TEAC and FRAP of Na-alginate elicited in pigeon pea sprouts were increased along with increasing Na-alginate concentration in all elicitation duration. It was also observed that the longer elicitation duration produce higher levels of the TEAC and FRAP in all Na-alginate concentrations. The highest TEAC and FRAP of pigeon pea sprouts produced by elicitation using 350 ppm Na-alginate for 24 hrs. The improvements of the TEAC and FRAP observed were in line with the total phenolic content enhancement due to the increasing of Na-alginate levels as well as elicitation durations as presented in Figure 1A. Plant phenolic compounds can act as a radical scavenger by donating hydrogen atom or electrons and also as reducing agents (Craft *et al*., 2012; Gulcin, 2020). Several studies were reported the significant positive correlation between TPC and TEAC as well as TPC and FRAP of legumes extract. Lu *et al*. (2018) reported the significant correlation between TPC and TEAC (r = 0.897, $p<0.01$), TPC and FRAP (0.943, $p<0.01$) of faba bean (*Vicia faba* L.) seed extract. The TPC of grass pea (*Lathyrus sativus*) seed extract was correlated to the TEAC (r = 0.881) and the FRAP (r = 0.781) (Rybiński *et al*., 2018). There was significant correlation between TPC and TEAC (r = 0.901 – 0.933, $p<0.05$), TPC and FRAP (0.786 – 0.877, $p<0.05$) of non-oil seed legumes extracts (Diniyah *et al*., 2020). The results on TPC and antioxidant activity indicated that the best Na-alginate elicitation technique for improving the antioxidant capacity of pigeon pea sprouts through submerged in 350 ppm Na-alginate for 24 hrs before germination.

3.3 Germination power and yield

The germination power and yield of the pigeon pea sprouts presented in Figure 2A and 2B indicated that both the germination power and yield of the sprouts decreased along with increasing Na-alginate concentrations and elicitation durations. The lowest germination power and yield of pigeon pea sprouts were observed in elicitation using 350 ppm Na-alginate for 24 hrs. The result possible due to more severity of the stress effect as the consequence of higher elicitor concentration and longer elicitation durations as mentioned in section 3.1. The stress due to biotic elicitation inhibits the legumes plant's growth and development and further impact the yield decreases (Rasool *et al*., 2015). The higher level of elicitor concentration significantly reduces the germination power of lentil, mung bean, and chickpea sprouts (Dutta and Bera, 2014; Ouji, 2015; León-López *et al*., 2020). The research results suggested that the optimization study was needed to develop the Na -alginate elicitation technique as an effective strategy for antioxidant capacity improvement of pigeon pea sprouts that has a minimally impact on the inhibition of germination rate and the yield reduction.

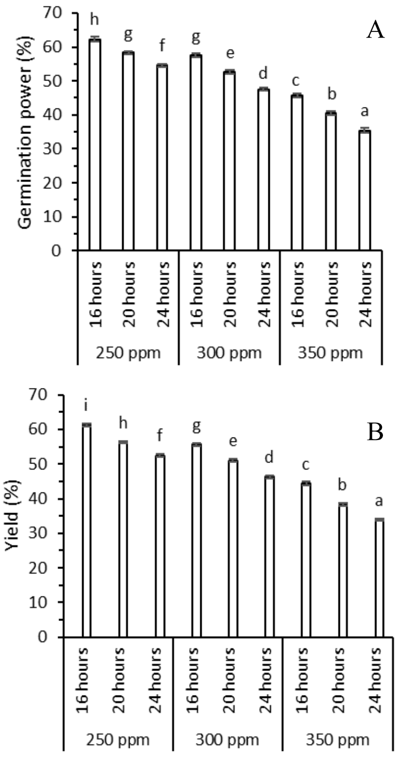

Figure 2. Germination power (A) and yield (B) of elicited pigeon pea sprouts prepared with different Na-alginate concentration and elicitation durations. Different letters mean significant differences ($p<0.05$).

4. Conclusion

Increased Na-alginate levels as well as the elicitation durations proved capable to significantly increase the TPC, RSA and FRAP but reduce the germination power and yield of Na-alginate elicited pigeon pea sprouts. Elicitation by submerging pigeon pea seeds in 350 ppm Na-alginate for 24 hrs produces pigeon pea sprouts with the highest TPC, RSA and FRAP but the lowest germination power and yield. The research results have an important consequence in developing carbohydrate as general elicitors to increase antioxidant compounds as well as antioxidant activity of legumes sprouts.

Conflict of interest

The authors declare no conflict of interest.

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