Antioxidant capacity of pigeon pea (*Cajanus cajan* L.) sprouts elicited using NaCl with various elicitation time

L S Kristiani, F M Rajendra and S Ariviani

Departement of Food Science and Technology, Faculty of Agriculture, Universitas Sebelas Maret, Jl. Ir. Sutami 36 A, Kentingan, Surakarta 57126, Indonesia

Email: setya_ariviani@yahoo.com; setyaningrum_ariviani@staff.uns.ac.id

Abstract. Previous research showed that pigeon pea has potential as an anti-diabetic functional drink related to its antioxidant capacity. Antioxidant capacity in legumes can be increased by elicitation. Improvement of antioxidant capacity in legumes elicited by NaCl was significantly higher than that of other abiotic elicitation. The objective of this study was to determine the antioxidant capacity of pigeon pea (*Cajanus cajan* L.) sprouts which were elicited using NaCl (50mM) with various elicitation times (8 hours, 12 hours, 16 hours) and germinated for 48 hours. The results showed that elicitation time did not have a significant effect on the total flavonoids content, but significantly increased antioxidant activity (DPPH radical scavenging, and reducing power) of pigeon pea sprouts along with increasing elicitation time. This study proved that elicitation using NaCl 50mM for 16 hours increases the total flavonoid content (69.36%), DPPH radical scavenging activity (134.18%), and reducing power (24.54%) of pigeon pea sprouts. Elicitation using 50mM NaCl with 16 hours elicitation time and 48 hours germination time can be considered as a technique to enhance antioxidant capacity in legumes sprouts.

1. Introduction

ROS naturally occurring as a result of energy synthesize reaction in mitochondria. Several conditions such as infection, respiratory burst, ischemia, and strenuous exercise lead to excessive ROS production. Air pollutants, tobacco smoke, radiation were further increased ROS accumulation in the body [1,2,3,4]. An Imbalance between ROS and antioxidant compounds in a human body leads to oxidative stress which further triggers various degenerative diseases [5]. Therefore, it is important to keep the balance of ROS and human antioxidant status through an intake of food which were rich in antioxidant compounds as one of the simple strategy.

Leguminous known as a natural source of antioxidant [6,7]. Previous researches had reported antioxidant capacity both in pigeon pea seed [8,9,10] and pigeon pea sprouts [11]. Previous studies reported that pigeon pea seed contains several antioxidant compounds, such as phenolic and flavonoids content [8,9,10], and has potential as anti-diabetic functional drink related to its antioxidant capacity [12].

Elicitation is one of the effective technique to improve antioxidant capacity in plants. Elicitation can cause stress so that the plants activated a series of defense system such as enzymatic and non-enzymatic antioxidant production as a response to the stress [13,14]. There are two major groups of elicitor used for elicitation, namely biotic and abiotic elicitor. Abiotic elicitor available as a chemical
compound that is safe, cheap, easy to be applied, and effective to increase phenolic compounds [14,15,16].

The use of NaCl as an abiotic elicitor in lentil proved to produce sprouts with a higher antioxidant capacity than other abiotic elicitors [14]. Several studies showed the antioxidant capacity improvement of mungbean [17] and common bean [18] by NaCl elicitation. Elicitation using various concentration of NaCl to enhance antioxidant capacity of legumes sprouts also have been reported [14,19,20,21]. The higher NaCl concentration produces legumes sprouts with higher antioxidant capacity. Na+ and Cl- are the most easily salt ions entered the plant cells, therefore induce environmental stress to the plants which further induce ROS production [22]. Swieka [14] stated that plants have developed an antioxidant defense system to reduce the ROS excess. Thus, the higher NaCl concentration can trigger higher stress so that generate a higher antioxidant defense through higher antioxidant compounds production. To the best of our knowledge, the study about the effect of NaCl elicitation time on the antioxidant capacity enhancement of pigeon pea sprouts hasn’t been reported. The authors hypothesized increasing NaCl elicitation time are going to increase NaCl accumulation thus provide higher environmental stress which further induces a higher antioxidant compounds production. This study aims to determine the antioxidant capacity of pigeon pea (Cajanus cajan L.) sprouts which were elicited using NaCl (50mM) with various elicitation times (8 hours, 12 hours, 16 hours) and germinated for 48 hours. In this study, the potential of NaCl elicitation in increasing the antioxidant capacity of pigeon pea sprouts was also examined. It was determined by comparing the antioxidant capacity of pigeon pea sprouts prepared using NaCl elicitation at the best elicitation time with that of control which was prepared using 0mM NaCl (without elicitation).

2. Methods

2.1 Preparation of pigeon pea sprouts
Pigeon pea (Cajanus cajan L.) seeds were purchased from local market in Surakarta, Indonesia. The selected seeds were rinsed thrice with distilled water and soaked in 50mM NaCl solution (1:3 w/v) with different elicitation time (8, 12, 16 hours). Elicited seeds were germinated at room temperature for 48 hours on bamboo weaved (15x15 cm). Distilled water was sprayed every 12 hours.

2.2 Extract preparation
2 grams of dehulled pigeon pea sprouts were extracted with 20 ml methanol (methanol: distilled water of 80:20 v/v) for 2 hours at 50°C and 200 rpm using water bath shaker (HAAKE SWB 20, Fisher Scientific Haake, Germany). The mixture was centrifugated using a tabletop centrifuge (PLC-05 Centrifuge, Gemmy Industrial Corp, Taiwan) at 10,000 rpm for 15 minutes. The clear supernatant was collected in an amber bottle and stored at 10°C until further analysis.

2.3 Total flavonoid content analysis
Total flavonoid content was determined using a method previously described by Pekal and Pyrznska [23] and expressed as µM Quercetin Equivalent (QE)/g sample dry weight.

2.4 Radical scavenging activity analysis
Radical scavenging activity was analyzed using the DPPH method [24] and expressed as µM Gallic Acid Equivalent Activity (GAEA)/g sample dry weight.

2.5 Reducing power analysis
The reducing power was measured following the method described by Berker et al [25] and expressed as µM Ascorbic Acid Equivalent Activity (AAEA)/g sample dry weight.
2.6 Statistical analysis
The Total flavonoid content, reducing power, as well as DPPH radical scavenging activity data of pigeon pea sprouts were analyzed using the program IBM SPSS Statistics 22 (SPSS Inc., Chicago, USA) by analysis of variance (ANOVA) followed by Duncan Multiple Range test to evaluate the significant differences between mean (p<0.05). The t-test was used to evaluate the potential of NaCl elicitation for enhancing total flavonoid content and antioxidant activity of pigeon pea sprouts compared with control.

3. Results and Discussion

3.1 The effect of NaCl elicitation time on the antioxidant capacity enhancement of pigeon pea sprouts
Flavonoids are secondary metabolites that have the capability to reduce the production of and quench ROS. Flavonoids have low redox potential and can undergo single electron oxidation so it is capable to reduce strong free radicals. Flavonoids is also can directly quench free radical due to proton transfer from the A and or B ring [26]. Elicitation increases total flavonoid content and antioxidant activity of plants [13,14]. Total flavonoid content and antioxidant activity of pigeon pea sprouts elicited using 50mM NaCl with various elicitation time were presented in Table 1.

Table 1. Total flavonoid content and antioxidant activity of NaCl elicited pigeon pea sprouts with different elicitation time.

| Elicitation time (hours) | Total flavonoid content (µM QE/ g sprouts dry weight) | Radical scavenging activity (µM GAEA/ g sprouts dry weight) | Reducing power (µM AAEA/ g sprouts dry weight) |
|-------------------------|------------------------------------------------------|----------------------------------------------------------|-----------------------------------------------|
| 8                       | 395.19 ± 6.48a                                       | 464.01 ± 16.63a                                         | 7942.46 ± 105.30a                             |
| 12                      | 408.37 ± 11.14a                                      | 521.60 ± 10.31b                                         | 8375.56 ± 226.84b                            |
| 16                      | 413.38 ± 6.91a                                       | 551.85 ± 9.93c                                         | 9259.24 ± 272.28c                            |

Different superscript within a similar column means significant differences (p<0.05)

Although elicitation time did not have an effect on the total flavonoid content (p>0.05), however, the higher elicitation time produces significantly higher both radical scavenging activity and the reducing power of elicited pigeon pea sprouts. This results indicated that might be antioxidant compounds other than flavonoids were produced as a response to the NaCl stress. So even though the total flavonoid contents of elicited pigeon pea sprouts were not significantly different but it showed significantly different in both radical scavenging activity and the reducing power. Accumulation of Na⁺ and Cl⁻ ions lead to ionic and osmotic stresses as well as water deficit [22]. The plants activated a series of defense system as a response to stress [13] among others through increasing both enzymatic and non-enzymatic antioxidant production [14].

Phenolic acid, flavonoid, and tannin were naturally synthesized to protect plants from stress during the growth [27]. The antioxidant compounds such as phenolic and flavonoids have a high correlation to the antioxidant activities [7,14,28]. Table 1 showed that pigeon pea sprouts prepared using 50mM NaCl elicitation for 16 hours have the highest antioxidant capacity in term of radical scavenging activity and the reducing power. Based on this result, the potential of NaCl elicitation in enhancing the antioxidant capacity of pigeon pea sprouts was conducted by comparing it with the control which was prepared without elicitation (soaked using 0mM NaCl) for 16 hours. The data were presented in Table 2.
3.2 The potential of elicitation using NaCl in increasing antioxidant capacity on pigeon pea sprout

| Table 2. Antioxidant capacity of NaCl elicited pigeon pea sprout compared to control. |
|----------------------------------------------------------|
| Pigeon pea sprout | Total flavonoid content (µM QE/g sprouts dry weight) | Radical scavenging activity (µM GAEA/g sprouts dry weight) | Reducing power (µM AAEA/g sprouts dry weight) |
|------------------|-----------------------------------------------------|---------------------------------------------------|--------------------------------------------|
| Control          | 244.09 ± 6.99<sup>a</sup>                           | 235.66 ± 7.10<sup>a</sup>                   | 7434.60 ± 276.85<sup>a</sup>               |
| Elicited         | 413.38 ± 6.91<sup>b</sup>                           | 551.85 ± 9.93<sup>b</sup>                   | 9259.24 ± 272.28<sup>b</sup>               |

Different superscript within a similar column means significant differences (p<0.05)

The results presented in Table 2 showed that total flavonoid content and antioxidant activity (radical scavenging activity and reducing power) of pigeon pea sprouts elicited by 50mM NaCl were significantly higher than that of pigeon pea sprouts prepared without elicitation. This result was relevant to previous research [14] which reported that total flavonoid, antiradical activity, and the reducing power of the NaCl elicited lentil sprouts were higher than that prepared without elicitation (0mM NaCl) [14]. Total flavonoid content, radical scavenging activity, and the reducing power of NaCl elicited pigeon pea sprouts were 69.36%, 134.18%, and 24.54% higher compared to pigeon pea sprout without elicitation. This study proved that germination technique using NaCl elicitation significantly increase the antioxidant capacity of legumes sprouts.

4. Conclusion

Elicitation time had no significant effect on the total flavonoid content of NaCl elicited pigeon pea sprouts but significantly affect the radical scavenging activity and reducing power. The radical scavenging activity and reducing power of elicited pigeon pea sprouts were increased along with increasing of elicitation time. The research result exhibited that elicitation using 50mM NaCl for 16 hours improves the total flavonoid content, radical scavenging activity and the reducing power of pigeon pea sprouts reached to 69.36%, 134.18%, and 24.54% respectively, compared to control which was elicited using 0mM NaCl for 16 hours.

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