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Influence of Exogenous Sucrose on Total Phenolic, Vitamin C and Antioxidant Enzymes of Soybean (*Glycine max* L.) Sprouts

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Abstract

This research explained the effect of exogenous sucrose on the levels of DPPH free radical scavenging ability, total phenolics and vitamin C as well as the L-galactono-1,4-lactone dehydrogenase (GalLDH), superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activities of soybean (*Glycine max* L.) sprouts. Soybean seeds were soaked in the various solutions prepared with 0, 1, 10 and 100 mM sucrose at 25°C for 12 hr and then sprayed with these solutions every 12 hr during the germination for 5 days. The higher concentration of exogenous sucrose decreased the L* value of soybean sprouts; whereas, the levels of DPPH free radical scavenging ability, total phenolic, vitamin C and GalLDH, SOD, CAT and APX activities increased. The soybean sprouts from 100 mM sucrose-treated seeds showed the maximum levels of DPPH free radical scavenging ability, total phenolics, vitamin C and GalLDH, SOD, CAT and APX activities, i.e. 85%, 164.95 mg GAE/g FW, 120.65 mg/100g FW, 25.32 U/g FW, 12.00 U/g FW, 25.36 U/g FW and 924 U/g FW, respectively. Thus, it suggested that the sucrose treatment helped to promote the soybean sprouts in containing high-levels of antioxidant activities.

Introduction

Soybean (*Glycine max* L.) sprouts are used for basic ingredients contained in Korean foods and popularly consumed in Southeast Asian countries (Ebert et al., 2017). They are composed of the significantly high contents of ascorbic acid, phenolic compounds, flavonoids, organic acids, amino acids and antioxidative properties (Guo et al., 2012). Tang et al. (2014) reported that soybean sprouts have the important functional ingredients used for human diets.

Sucrose extremely affects plant growth and

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metabolisms found in the cellular and organism levels (Couée et al., 2006). It is one of the important disaccharides available in most plants, regulates the photosynthesis and respiration, serves as a storage compound and maintains osmotic pressure in the cytosol (Eastmond, 2006; Eltayeb et al., 2007; Nishikawa et al., 2005). Thus, its main functions are the promotion of germination and seedling development (Nishikawa et al., 2005).

During germination or sprouting of soybean sprouts, a high number of monosaccharides and disaccharides is generated in soybean sprouts, which activates the sugar metabolism to stimulate the production of secondary metabolites, i.e. phenolic compounds (Chen et al., 2019). The application of exogenous sucrose tended to accumulate the ascorbic acid in harvested broccoli flowers due to regulate gene expression associated with vitamin C metabolism (Nishikawa et al., 2005). It not only played a role in improvement of the nutritional properties and antioxidative properties of broccoli flowers (Xu et al., 2016), but also greatly increased the contents of vitamin C, anthocyanins and phenolic compounds in broccoli sprouts (Guo et al., 2011). Wei et al. (2019) reported that the exogenous sucrose treatment of 0.5 g/L enhanced the levels of ascorbic acid, glucose, L-galactono-1,4-lactone dehydrogenase (GalLDH) activity and total phenolics as well as promoted the activities of antioxidant enzymes, e.g. superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) in mung bean sprouts. Recently, Yu et al. (2023) further suggested that the exogenous sucrose treatment at 30 g/L enhanced the higher levels of phenolic compounds, flavonoids and gamma-aminobutyric acid (GABA) in the treated mung bean sprouts as well as its extract inhibited alcohol-induced oxidative injury in HepG2 cells.

Consequently, the development of immerging and/or soaking solutions with the optimal sucrose concentration for soybean seeds to produce soybean sprouts is expected to lead to worthy scientific data based on the antioxidant enzymes and physicochemical characteristics. Besides, this may be a reference in helping to achieve a more comprehensive understanding for changes in the antioxidants as well as the antioxidant activities and enzymes of other cereal sprouts (treated with the soaking sucrose solution) during sprouting process.

The formation of reactive oxygen species (ROS) in plants exposes to destroy firstly the cellular organelles and properties of cell membrane caused by the lipid peroxidation of membrane, then degradation of the biological macromolecules and lastly cell death (Alscher et al., 1997). There are several antioxidant enzymes (such as SOD, APX and CAT) presented during growing plants and simultaneously eliminating ROS in plants (Ebert et al., 2017). Besides these enzymes remaining in the various substance of cells from the damage of ROS, they help to regulate not only plant growth, cell elongation, senescence and cell death, but also the cell differentiation, cell growth/division and detoxification of xenobiotics. Hence, these antioxidant enzymes, protecting the systems of plants and humans (Dumont & Rivoal 2019) have been of interest to study in different *in vitro* systems.

However, until now the effect of sucrose on color and the biological effects, based on GalLDH, SOD, APX and CAT of soybean sprouts has not been studied. Therefore, this study focused on the different concentrations at 0, 1, 10 and 100 mM sucrose affecting the color, total phenolic content, vitamin C content and bioactive activities in terms of the DPPH free radical scavenging ability, GalLDH, SOD, APX and CAT of soybean sprouts.

Materials and methods

1. Seed sprouting conditions

Soybean (*Glycine max* L.) seeds were obtained from a local market in Bangkok, Thailand. They were cleaned and separated into 4 treatments. They were soaked in 0, 1, 10 and 100 mM of sucrose solution (pH 7.0) at 25°C for 12 h. After the incubation, the seeds were placed into sterile plastic cups (cotton wool thickness of 2.5 cm placed on the bottom) containing many holes to drain water and stored in darkness at 25°C for 5 days. The treated seeds were sprayed by an electric sprayer with a delivery volume of 260 mL/min and the previous soaking solutions were used every 12 hr for 5 days of germination (Qiu et al., 2015.). Every treatment comprised of 60 seeds of soybean and carried out in triplicate. The soybean hypocotyls were harvested on day 5 and then rapidly frozen in liquid nitrogen. The frozen hypocotyls were kept at -100°C prior to analysis (Fig. 1).

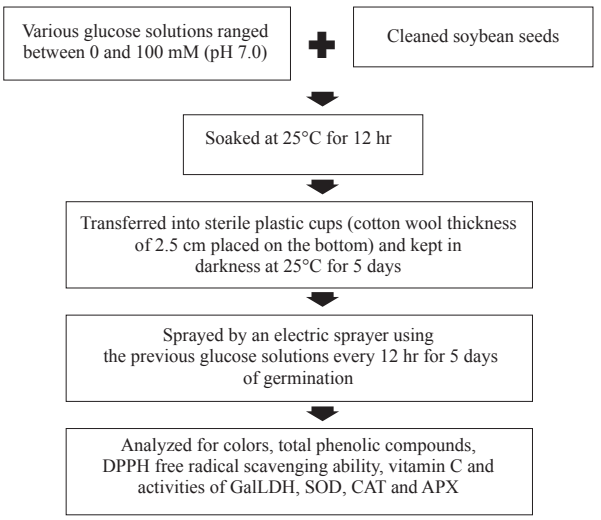

Fig. 1 Overview diagram of this experiment: preparation of soybean sprouts treated with exogenous sucrose solutions at 0-100 mM and their property analysis

2. Color analysis

The color analysis was performed using a colorimeter (Minolta CR-410, Minolta, Japan). The calibration was done by a standard plate. The means of L*, a* and b* were obtained from five points on the sample surface.

3. Total phenolic content

For the total phenolic content, a 2 g of the sample was transferred into 10 mL of 85% ethyl alcohol and then centrifuged at 4,500 g for 20 min. The supernatant was analyzed to find total phenolic content following the Folin-Ciocalteu method, described by Wang et al. (2017). Gallic acid was used as a standard. The phenolic level was calculated as milligrams gallic acid equivalent per gram of fresh weight (mg GAE/g FW).

4. DPPH free radical scavenging ability

To analyze the DPPH free radical scavenging ability, 2.0 g of the sample was put into 15 mL of 85% ethyl alcohol and then centrifuged at 4,000 g for 20 min. The supernatant was used to analyze the DPPH radical scavenging ability, explained by the method of Kraboun (2019) with a slight modification. Two mL of the supernatant was mixed with 5 mL of 0.5 mM DPPH and then kept in darkness for 45 min at ambient temperature. The ability was obtained the absorbance at 515 nm and calculated following the below formula.

$$\text{DPPH radical scavenging ability (\%)} = (100 - (\frac{\text{Abs sample}}{\text{Abs control}})) \times 100.$$

5. Vitamin C content

The extraction of vitamin C from the sample was obtained from 2.0 g of the sprouts transferred into 10 mL of 4.5% phosphoric acid. Then the mixture was centrifuged at 10,000 g for 12 min. The supernatant was read at 525 nm (Kampfenkel et al., 1995). Vitamin C was used as a standard. Vitamin C content is expressed as mg 100 g^{-1} FW.

6. L-galactono-1, 4-lactone dehydrogenase (GalLDH, EC 1.3.2.3) activity

To examine the GalLDH activity, 1.0 g of the sample was extracted with 5 mL of 200 mM potassium phosphate buffer (pH 7.0) and then centrifuged at 6,000 g for 15 min. The supernatant was again centrifuged at 10,000 g for 15 min. The collected precipitate was transferred into 5 mL of the potassium phosphate buffer. GalLDH activity was described by Tabata et al. (2001) with some modifications. 0.50 mL of the extract was put into 5.0 mL of 1.50 mg mL^{-1} cytochrome C and then incubated at ambient temperature for 5 min. To react t = 0, 0.5 mL of 60 mM L-galactono-1, 4-lactone (GalL) was added to the mixture. GalLDH activity was read at 560 nm. 1 unit of the activity is the enzyme content oxidizing 3 nmol of GalL (3 nmol of reduced Cytochrome C) per min.

7. Antioxidant enzyme activities

The activities of SOD, CAT and APX were described according to He et al. (2001) with some modifications. 500 mg of the sample was mixed with 5 mL of 200 mM potassium phosphate buffer (pH 7.5) and then centrifuged at 5,000 g for 25 min. The supernatant was obtained to measure the enzyme activities.

SOD (EC1.15.1.1) activity was explained by the procedure of Giannopolitis and Ries (1977) with a slight modification. The reaction reagent (5 mL) contained 60 mM phosphate buffer (pH 7.0), 70 mM riboflavin (7,8-dimethyl-10-ribitylisoalloxazine), 200 mM methionine [2-amino-4-(methyl-thio)-butyric acid], 5 mM EDTA and 2.0 mM nitro blue tetrazolium [NBT; 2,2′-di-p-nitrophenyl-5,5′-diphenyl-(3,3′-dimethoxy-4,4′- diphenylene) ditetrazolium chloride] and was mixed with 200 mL of the supernatant. The mixture without the enzyme solution was defined as the control. The mixture was exposured to fluorescent lights at 200 mM m^2 s for 10 min and then kept in darkness for 15 min. Subsequently, it was read at 560 nm, where 1 unit of the enzyme activity in which the enzyme content could destroy 50% of NBT photoreduction.

CAT (CAT, EC1.11.1.6) activity was defined as the study on H2O2 oxidation, described by Shao et al. (2013)

with some modifications. The reaction reagent (5 mL) was the mixture composing of 100 mM phosphate buffer (pH 7.5) and 50 mM H_2O_2 and mixed with 200 mL of the supernatant. The absorbance of sample was read at 240 nm in every 10 sec. intervals for 70 sec. 1 unit of CAT activity was the absorbance change of 0.02 per min.

APX (APX, EC1.11.1.11) activity was the study on the ascorbate oxidation, explained according to the method of Nakano & Assada (1981) with a slight modification. The reaction reagent (5 mL) was as the following: 200 mM sodium acetate buffer (pH 6.0), 5 mM EDTA and 6 mM H_2O_2 and transferred into 200 mL of the supernatant. The absorbance of sample was read at 290 nm in every 10 sec intervals for 70 sec. 1 unit of APX activity was the absorbance change of 0.02 per min.

8. Statistical analysis

Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA) for Windows. The difference of data was separated by Duncan's multiple range tests (DMRT). Values are defined as mean± standard deviation (SD). Differences were investigated at a significant level of 0.05.

Results and discussion

1. Impact of exogenous sucrose on L*, a* and b* of soybean sprouts

As shown in Table 1, the higher concentration of sucrose significantly exhibited a decrease in the L* values of soybean sprouts (p<0.05). The L* values of soybean sprouts obtained from 1 mM sucrose-treated seeds and the control (untreated) were highest, which ranged from 72.88 to 75.78 (p>0.05). On the other hand, all the soaking sucrose solutions did not affect the a* and b* values of soybean sprouts (p>0.05). This may be a high concentration of sucrose causing osmotic stress affecting the moisture removal from the plant cells, which resulted in retarding the plant growth and then affecting a less amount of chlorophyll accumulation (Sharma et al., 2019). Thus, the chlorophyll was formed slowly during the growth of the soybean sprouts, affecting higher L* value as well (Price et al., 2003). Moreover, this germination period of soybean sprouts is very short (5 days), indicating the small amount of accumulated chlorophyll. This result was in agreement with Pertiwi et al. (2013), who noted that a short germination period of the legume seeds showed the higher L* values of the sprouts. While, this finding was in disagreement with Murugkar (2014), who claimed that the germination period did not impact on the L* value of soybean sprouts.

Table 1 L*, a* and b* of soybean sprouts from 0-100 mM sucrose-treated seeds

Sucrose (mM)	L*	a*^{ns}	b*^{ns}
0	75.78±0.08^a	-2.54±0.03	-1.46±0.02
1	72.88±0.01^a	-1.60±0.03	-2.47±0.08
10	50.61±0.05^b	-1.96±0.09	-2.61±0.07
100	45.61±0.06^c	-2.61±0.01	-1.32±0.08

Remark: Different letters behind means within a column are significantly different (p<0.05). ns is not significantly different (p>0.05).

2. Impact of exogenous sucrose on DPPH free radical scavenging ability and total phenolic content of soybean sprouts

DPPH free radical method is an antioxidant assay based on both electron transfer (SET) and hydrogen atom transfer (HAT) reactions. This free radical, stable at room temperature, is reduced when appearing as an antioxidant molecule, giving colorless ethanol solution (Guo et al., 2012; Liang & Kitts, 2014). The DPPH free radical scavenging ability of soybean sprouts is displayed in Fig. 2a. The higher sucrose concentrations improved the DPPH radical scavenging ability of soybean sprouts. The soybean sprouts from 100 mM sucrose-treated seeds indicated the highest DPPH radical scavenging ability by 85%. However, the DPPH radical scavenging abilities of soybean sprouts from 1 mM sucrose-treated seeds and the control (untreated) were lowest and showing not different significantly (p>0.05). This was in agreement with Xu et al. (2016), who reported that the sucrose-treated broccoli had higher DPPH radical scavenging activity and total phenolic content as compared with the control (untreated).

Phenolic compounds are the products of secondary metabolism in plants and promote health benefits due to reducing the risk of chronic diseases (Li et al., 2019). The total phenolic content of soybean sprouts is illustrated in Fig. 2b. The patterns of total phenolic content and DPPH free radical scavenging ability were the same. The highest total phenolic content obtained from the soybean sprouts from 100 mM sucrose-treated seeds was 164.95 mg GAE/g FW. The solution of soaking sucrose may play the significant role in accumulating phenolic compounds during growing sprouts due to stimulate gene expression related with phenolic metabolism (Guo et al., 2011; Nishikawa et al., 2005; Xu et al., 2016).

Moreover, Wei et al. (2019) suggested that the sucrose treatment increased the levels of ascorbic acid, phenolic compounds and antioxidant activities of mung bean sprouts. Furthermore, López-Amorós et al. (2006) noted that sucrose could affect a decrease in anti-nutrient phenomenon, but an increase in contents of amino acids,

sugars, dietary fibers and antioxidants during the germination period of legumes. The stimulation of synthesis of polyphenols found in plants, e.g. phenolic compounds and flavonoids was from the abiotic stress, which promotes the plants to handle with the unsuitable environmental conditions (Li et al., 2016). Therefore, phenylpropanoid biosynthetic pathway is generated from the abiotic stress (drought, heavy metals, high content of salt, inappropriate temperatures, high content of carbohydrates and UV radiation) leading to increase phenolic compounds (Aghdam et al., 2020). Winarsi et al. (2020), who confirmed that an increase in phenolic content during the germination is present due to the induction of phenylalanine ammonia-lyase (PAL) activity synthesized biogenetically through a shikimate/phenylpropanoid pathway (Koodkaew, 2019).

C (L-ascorbate) biosynthesis in plants (Wheeler et al., 1998). As shown in Fig. 3, the higher sucrose concentrations increased the levels of vitamin C and GalLDH activity of soybean sprouts. The vitamin C content and GalLDH activity of soybean sprouts from 100 mM sucrose-treated seeds increased 1,065.70% and 1,925.60%, respectively as compared with the control (untreated). Obviously, the increased concentrations of sucrose played an important role on the synthesis of vitamin C and GalLDH activity. This was in agreement with Guo et al. (2011), who noted that the treatment of 88 mM sucrose affected the increment of ascorbic acid in broccoli sprouts by 41% versus the control. Moreover, Cao et al. (2015) also found that the application of sucrose solution for soaking cucumber seeds contributed to the increased content of vitamin C in cucumber seedlings.

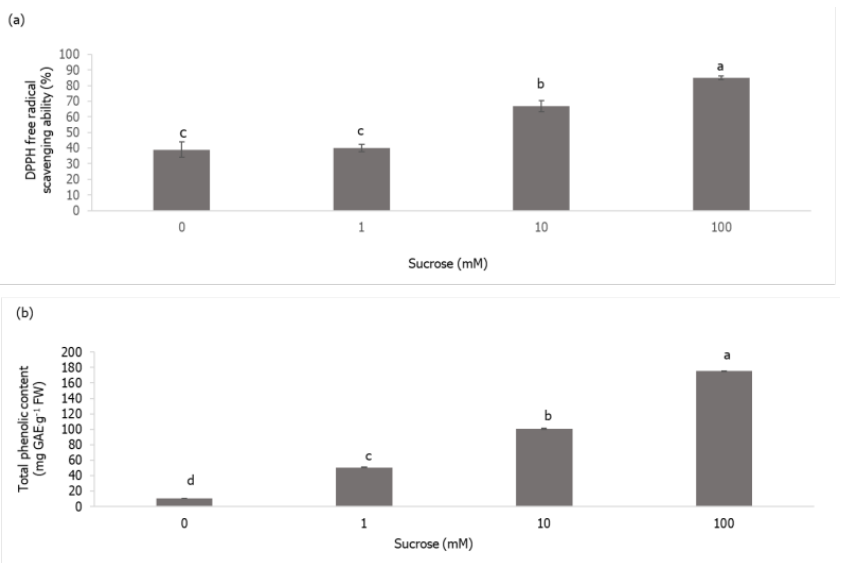

Fig. 2 DPPH free radical scavenging ability (a) and total phenolic content (b) in soybean sprouts on day 5 from 0-100 mM sucrose-treated seeds
Remark: Different letters are significantly different (p<0.05)

3. Impact of exogenous sucrose on vitamin C content and L-galactono-1,4-lactone dehydrogenase (GalLDH) activity of soybean sprouts

Vitamin C or ascorbic acid, possessing a strong antioxidant property, is a cofactor for a lot of the enzymes and neutralizes the effect of reactive oxygen species (ROS) (Aghdam et al., 2022; Smirnoff et al., 2001). L-galactono-1,4-lactone dehydrogenase (GalLDH) is a FAD-containing oxidoreductase that catalyzes the terminal step of Smirnoff–Wheeler pathway of vitamin

Our results indicated the higher GalLDH activity and vitamin C biosynthesis would generate together since our experiment condition may be under the optimal condition for soybean seed germination. This phenomenon was explained because of the appropriate condition for sprouting cereal seeds enhancing the levels of both L-galactose and the enzyme GalLDH in the sprouts; therefore, this enzyme GalLDH can hydrolyze L-galactose to transform ascorbic acid with a high concentration (Guo et al., 2011).

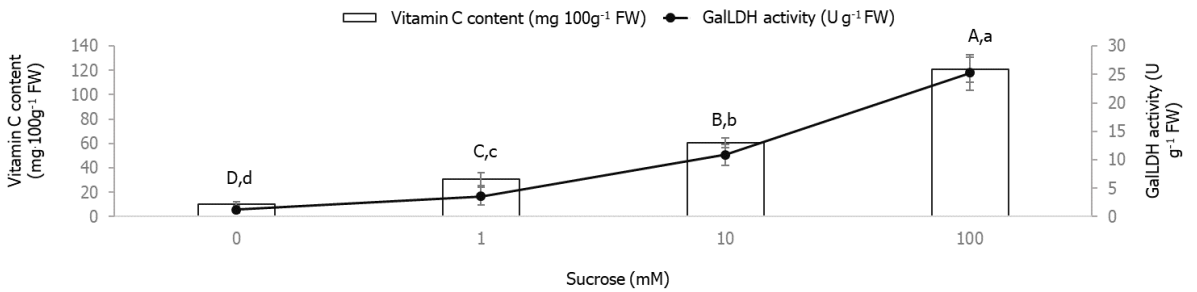

Fig. 3 Vitamin C content and GalLDH activity of soybean sprouts from 0-100 mM sucrose-treated seeds
Remark: Capital and small letters indicate significantly different ($p<0.05$) of vitamin C content and GalLDH, respectively

4. Impact of exogenous sucrose on the activities of antioxidant enzymes of soybean sprouts

SOD catalyses the disproportionation of superoxide ($O_2^{\cdot-}$) radicals to become H_2O_2 and O_2 (McCord & Fridovich, 1969), which plays a significant role in order to protect the biological cells (Xu et al., 2019). Plant SODs are the metalloenzymes consisting of Fe, Mn or Cu/Zn (a prosthetic group). The number, type, and distribution of SOD isoenzymes may be changed due to the species, developmental stage and environmental conditions (Bridges & Salin, 1981). Catalase (CAT) is an enzyme-containing homotetramer ferriheme, with Fe as a cofactor that catalyzing hydrogen peroxide (H_2O_2) into water and oxygen. CAT activity plays an important role in detoxifying H_2O_2, which is increased with age (Lobo et al., 2010). Ascorbate peroxidase (APX) isoenzymes are important in changing H_2O_2 into H_2O, especially found in the chloroplast (Chen et al., 2012).

The antioxidant enzymes, i.e. SOD, CAT and APX are depicted in Fig. 4. The SOD, CAT and APX activities of soybean sprouts of all treatments had the same pattern. The higher SOD, CAT and APX activities were found

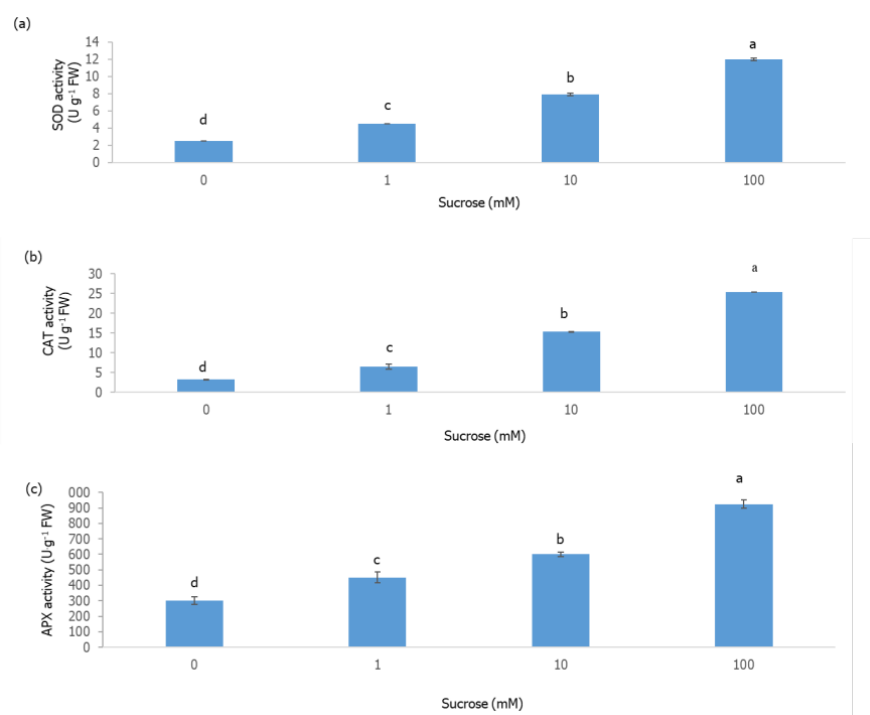

Fig. 4 SOD (a), CAT (b) and APX (c) activties in soybean sprouts from 0-100 mM sucrose-treated seeds
Remark: Different letters are significantly different ($p<0.05$)

in the soybean sprouts produced from the sucrose-treated seeds soakedand/or sprayed using the higher sucrose concentrations. The maximum activities of SOD, CAT and APX obtained from the soybean sprouts produced from 100 mM sucrose-treated seeds were observed, which increased 380, 692.5 and 208%, respectively as compared with the control (untreated). Obviously, the incremental accumulation of secondary methabolites such as the total phenolic content, vitamin C and GalLDH (Fig. 2b and Fig. 3) of soybean sprouts was related to SOD, CAT and APX activities (Fig. 4). These results indicated that the sucrose treatment could serve more effectiveness of SOD, CAT and APX activities of soybean sprouts. This suggested that the osmotic substances, i.e. a high concentration of sucrose could stimulate the development of an anti-oxidative defence system, thus the accumulation of antioxidant enzymes was observed (Xue-Feng et al., 2019). This was in agreement with Cao et al. (2014), who reported that the higher sucrose concentration activated the SOD and APX activities in cucumber seedlings. Wei et al. (2019) and Xu et al. (2016) further reported that the activities of SOD, APX and CAT in sucrose-treated broccoli florets and mung bean sprouts were higher than those in the untreated control. In this study, the results exposed that the changes of DPPH free radical scavenging ability and total phenolic content (Fig. 2) that exactly corresponded to the activities of SOD, CAT and APX (Fig. 4). Thus, exogenous sucrose treatment is an effective way to rise the DPPH free radical ability, total phenolic content and the antioxidant enzymes of soybean sprouts.

Conclusion

Soybean sprouts are an important dietary source containing both antioxidant activities and enzymes. The soybean sprouts from 100 mM sucrose-treated seeds resulted in significantly increased levels of total phenolics, DPPH free radical scavenging ability, vitamin C and GalLDH, SOD, CAT and APX activities. However, the L* values of soybean sprouts were lower when the soybean seeds were treated with the higher sucrose concentration; however, the a* and b* values of all treatments were not significantly different (p>0.05). Therefore, the sucrose treatment at 100 mM should be an alternative way for the production of soybean sprouts composing of good antioxidant properties and physicochemical qualities.

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