



Research article

Effects of glucose on growth and health-promoting compounds in sunflower (*Helianthus annuus* L.) sprouts

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Abstract

The increasing demand for healthy food products is increasing the pressure to enhance the beneficial phytochemicals in plants. The effects were investigated of glucose treatment on the growth, bioactive compounds and antioxidant capacity in sunflower sprouts. Application of 1,000 mM glucose reduced the fresh weight and increased the relative water content in sprouts but other concentrations (10 mM and 100 mM) had no effect on sprout biomass. Treatment with 100 mM and 1,000 mM resulted in an increase in the amount of flavonoid and radical scavenging activity. γ -Aminobutyric acid in sprouts treated with glucose increased in a dose-dependent manner. Treatment with 1,000 mM increased phenylalanine ammonia-lyase activity resulting in an increase in the phenolic compound content (61.24% higher than in the control sprouts). These results suggested that glucose solution could improve the functional composition and antioxidant property of sunflower sprouts.

Introduction

Plant products with high potential health benefits have been consumed continuously (Rouphael et al., 2018). Edible sprouts have been consumed as a dietary food due to their high nutritional quality (Martinez-Villaluenga et al., 2010; Ebert et al., 2017). Based on this evidence, research interests have focused on strategies to increase the content of beneficial bioactive compounds in sprouts without breeding or genetic manipulation. A basic and efficient method is to use a plant stress response system. Environmental stresses, such as chemical stress, salt stresses and osmotic stress, have been reported to exert a valuable effect on the secondary metabolites content in many sprouts (Guo et al., 2011a; 2011b; 2014; Mendoza-Sánchez et al., 2016).

Glucose is an important carbon source for cellular nutrient and also acts as effective signaling molecules throughout plant life, modulating gene expression related to plant metabolism, stress resistance, growth and development (Bolouri-Moghaddam et al., 2010). Glucose is involved in response pathways allowing plants to adapt to environmental stresses (Rolland et al., 2001). Glucose can affect the osmotic pressure of the medium, leading to osmotic stresses that increase the amounts of phytochemicals and antioxidant in the plant; for example, broccoli sprouts grown in agar medium containing glucose increased their anthocyanin, glucosinolate and antioxidant capacity (Guo et al., 2011a).

Sunflower (*Helianthus annuus* L.), a member of the Asteraceae family, is a global commercial oilseed crop. Sunflower seeds are a rich

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source of polyunsaturated fatty acids (Fernández-Moya et al., 2003). In addition, some secondary metabolites such as phenolic compounds, flavonoid and antioxidant compounds have also been observed in the seeds (Cevallos-Casals and Cisneros-Zevallos, 2010). However, germination of sunflower seeds to produce sprouts increases their nutritional value as well as health-promoting compounds (Cevallos-Casals and Cisneros-Zevallos, 2010; Pająk et al., 2014). Sunflower sprouts are sources of valuable natural substances containing amino acids, phenolic compounds, melatonin, isoflavone, flavonoids and antioxidant property (Cho et al., 2008; Cevallos-Casals and Cisneros-Zevallos, 2010; Pająk et al., 2014). In view of the increased interest in naturally sourced healthy foods, increasing utilization of sunflower sprouts in human foods appears realistic. However, limited data are available on the impact of glucose on the health-promoting metabolites and the nutritional value of sunflower sprouts.

The objective of the present study was to investigate the effect of glucose on the growth, the content of phenolic compounds, flavonoids, γ -aminobutyric acid (GABA) and antioxidant capacity as well as the activity of phenylalanine ammonia-lyase (PAL) in sunflower sprouts.

Materials and Methods

Plant material and cultivation of sunflower sprouts

Sunflower seeds were obtained commercially and were of a quality ready-for-sprouting (> 95% germination). Seeds were washed with water, drained and soaked in warm water (approximately 40°C) for 8 hr. Then seeds were transferred to an incubation chamber for 12 hr. After that approximately 350 seeds were sown in trays (19.5 cm \times 22.0 cm) containing 50% soil mixed with commercial growing medium and 50% coconut coir. Seed germination was conducted at room temperature (30 \pm 5°C) for 3 d under dark conditions to increase the sprout hypocotyl elongation. Then, the sprouts were transferred to a controlled condition with a 12 hr light and 12 hr dark cycle and an average temperature of 30 \pm 5°C. Light (500 μ mol/m²/s) was provided using a fluorescent tube (FL36W/T8/D; Toshiba; Japan). The trays were irrigated twice daily with 100 mL of distilled water.

Treatments with glucose

The experiment was conducted in a completely randomized design with three replications (one tray equaled one replication). There were three treatments of glucose solutions (10 mM, 100 mM and 1,000 mM), while distilled water alone served as the control. These concentrations were used in the experiment based on the results of preliminary work which showed that glucose had little effect on growth (data not shown). After 6 d of germination, glucose was applied as an exogenous spray on the aerial part with 50 mL of test solution per tray. Sprout samples were collected gently 24 hr after treatment. The plant samples were provided for determination of growth, phytochemical content and enzyme activity. The sprout samples were randomly picked for each analysis measurement.

Measurement of growth

The sunflower sprouts were randomly sampled from each tray at 100 plants per replication and their fresh and dry weights were measured using a balance. For a sample of 30 sprouts per replication, root and shoot lengths were measured using a ruler, and stem thickness was determined using a set of calipers.

Determination of photosynthetic pigment content

Chlorophyll a, b, total chlorophyll and carotenoids were extracted from the shoots of sprouts (1 g) using 5 mL of dimethyl sulfoxide for 48 hr under dark conditions (Hiscox and Israelstam, 1978). The absorbance of the extracts was determined at 440 nm, 645 nm and 663 nm using a spectrophotometer (UV-1800; Shimadzu; Japan). The photosynthetic pigment contents were calculated according to equations given by Arnon (1949).

Determination of relative water content

The relative water content (RWC) was determined as previously described (Jones and Turner, 1978). Sprout shoot samples (1 g) were cut and weighed quickly to determine their fresh weight (FW). Then, the samples were immediately soaked in distilled water to saturate them with water. After soaking for 6 hr, the turgid weight (TW) was recorded. The dry weight (DW) of the samples was measured after dehydrating them at 60°C for 24 hr. The RWC was calculated using the equation $RWC (\%) = [(FW - DW) / (TW - DW)] \times 100$.

Determination of total phenolic compound content

The total phenolic compound content was determined using the Folin Ciocalteu (FC) method (Singleton et al., 1999) with minor modifications. Fresh sunflower sprout shoots (1 g) were extracted using 80% methanol (10 mL). The sample was centrifuged at 5,000 revolutions per minute (rpm) for 15 min at room temperature. A sample of 50 μ L of supernatant was mixed with 250 μ L of FC reagent and kept at room temperature for 8 min. Then, 20% Na₂CO₃ (750 μ L) and distilled water (950 μ L) were added, respectively, mixed and incubated at 25°C for 30 min. After incubation, the absorbance was taken using the spectrophotometer at 765 nm. Gallic acid was used as a standard, and the results were expressed as milligrams of gallic acid equivalent (GAE)/g FW.

Determination of flavonoid content

A total flavonoid content assay was performed using the AlCl₃ colorimetric method (Zhishen et al., 1999). Sunflower sprout shoots were extracted using the same method described in the total phenolic compounds assay above. The supernatant (500 μ L) was mixed with distilled water (2 mL) and 5% NaNO₂ (15 μ L). After incubation at room temperature for 6 min, 10% AlCl₃ (150 μ L), 2 M NaOH (2 mL)

and distilled water (200 μ L) were added, respectively. The solution was mixed and incubated at 25°C for 30 min. The supernatants were collected and the absorbance was determined at 415 nm using the spectrophotometer. Quercetin was used as the standard and the results were expressed as milligrams of quercetin equivalent (QE)/g FW.

Determination of PAL activity

The PAL activity was determined with minor modifications using the method described by Wei et al. (2011). For preparing the crude enzyme, 0.2 g of sprout shoot was extracted using 1.7 mL of 1 mM sodium-borate buffer (pH 8.8) containing 1 mM ethylenediaminetetraacetic acid and 20 mM β -mercaptoethanol in an ice bath. After centrifugation at 10,000 rpm at 4°C for 30 min, the supernatant (0.2 mL) was mixed with 3 mL of 0.5 mM L-phenylalanine dissolved in 1 mM sodium-borate buffer (pH 8.8). After incubation at 37°C for 1 hr, the amount of cinnamic acid (the product of the reaction) was measured at 290 nm using the spectrophotometer. One unit of enzyme activity was defined as an increase in the absorbance of 0.1 per hour per milliliter of the enzyme solution (Zhu et al., 2015). The PAL activity was expressed as enzyme units (U)/g FW.

Determination of γ -aminobutyric acid content

The GABA content was determined according to Karladee and Suriyong (2012) with some modifications. Fresh sprout shoots (0.5 g) were ground with liquid N₂ and dissolved with 2.5 mL of 95% ethanol and filtered using Whatman no. 1 filter paper. The filtrate was boiled in a water bath (90°C) to evaporate the ethanol. Then, 0.5 mL of distilled water was added and centrifuged at 10,000 rpm for 10 min. The supernatant was collected followed by the addition of 0.2 mL of 0.2 M sodium borate buffer (pH 8.8) and 1.0 mL of 6% phenol. The solution was mixed thoroughly and cooled in a cooling bath for 5 min. Next, 0.4 mL of 14.5% NaOCl was added and the solution was shaken vigorously for 1 min and again cooled in the cooling bath for 5 min. Finally, the solution was boiled in a water bath (100°C) for 10 min and allowed to cool. The absorbance was determined using the spectrophotometer at 630 nm. GABA was used as the standard for the calibration curve.

Determination of antioxidant activity

The radical scavenging activity was determined spectrophotometrically using the 2,2-diphenyl-1-picrylhydrazyl (DPPH)

method (Brand-Williams et al., 1995). Shoot of sprouts (1.0 g) were extracted with ethanol (10 mL) and then the mixture was centrifuged at 5,000 rpm for 15 min. A fixed volume (1.9 mL) of the supernatant was mixed to 100 μ L with 1 mM DPPH and stored in the dark at room temperature for 30 min. Absorbance was measured at 517 nm using the spectrophotometer. Controls were prepared as for the tested group except that the plant solution was replaced with the extraction solvent. The DPPH free radical scavenging activity was calculated using the following equation: DPPH scavenging activity (%) = $[(A_0 - A_1)/A_0] \times 100$, where A₀ is the absorbance of the control and A₁ is the absorbance of the sample.

Statistical analysis

All the data were expressed as mean \pm SD of three replications. Data were subjected to analysis of variance followed by Duncan's multiple-range test ($p < 0.05$ was considered as a statistically significant difference) using the R program (R Core Team, 2015). Correlations between the parameters were evaluated using Pearson's correlation coefficient.

Results

Effect of glucose on sunflower sprouts growth

The effects of glucose on sunflower sprout growth are shown in Table 1. The stem diameter tended to decrease compared with the control but only the treatment of 100 mM glucose was significantly different. The treatments of 10 mM and 100 mM glucose did not affect shoot and root lengths whereas 1,000 mM glucose significantly decreased the shoot length but significantly increased the root length of the sprouts. The fresh weight of sprouts was essentially unaffected by glucose except in the treatment of 1,000 mM glucose which had a significantly decrease in fresh weight (17.59% lower than the control). However, a significant increment in the dry weight of the sprouts was recorded following the treatments of 100 mM and 1,000 mM glucose.

Effect of glucose on photosynthetic pigment and relative water content

The treatments of glucose had no effect on the chlorophyll and carotenoid contents (Table 2). The RWC tended to increase after treatment with glucose (Fig. 1). The highest RWC value was observed in the treatment of 1,000 mM glucose (20.99% higher than the control).

Table 1 Effects of different glucose concentrations on growth of sunflower sprouts.

Concentration (mM)	Stem diameter (mm)	Shoot length (cm)	Root length (cm)	Fresh weight (g/100 plants)	Dry weight (g/100 plants)
0	1.82 \pm 0.04 ^{a*}	9.78 \pm 1.21 ^a	10.30 \pm 1.30 ^b	44.97 \pm 4.91 ^a	3.00 \pm 0.18 ^b
10	1.78 \pm 0.06 ^{ab}	8.99 \pm 0.55 ^{ab}	10.72 \pm 2.34 ^b	41.26 \pm 3.24 ^{ab}	2.93 \pm 0.10 ^b
100	1.73 \pm 0.05 ^b	9.15 \pm 0.21 ^{ab}	11.26 \pm 1.97 ^{ab}	44.43 \pm 4.31 ^a	3.29 \pm 0.17 ^a
1000	1.76 \pm 0.04 ^{ab}	8.21 \pm 0.89 ^b	13.99 \pm 1.22 ^a	37.06 \pm 5.98 ^b	3.37 \pm 0.10 ^a

* Values represent mean \pm SD; different lowercase superscript letters in the same column are significantly different ($p < 0.05$).

Table 2 Effects of different glucose concentrations on photosynthetic pigment contents of sunflower sprouts.

Concentration (mM)	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Total chlorophyll (mg/g FW)	Carotenoid (mg/g FW)
0	0.0191±0.001	0.0072±0.001	0.0263±0.001	0.0127±0.002
10	0.0171±0.004	0.0069±0.001	0.0240±0.005	0.0118±0.003
100	0.0174±0.005	0.0062±0.001	0.0236±0.006	0.0107±0.002
1000	0.0162±0.002	0.0064±0.001	0.0226±0.003	0.0123±0.002

FW = Fresh weight.

* Values represent mean ± SD; all means in the same column are not significant difference ($p \geq 0.05$).

Effect of glucose on total phenolic compound, flavonoid and phenylalanine ammonia-lyase activity

The amount of total phenolic compound was affected differently by the different treatments. The treatments of 10 mM and 100 mM glucose increased the amount of phenolic compound (6.98% and 25.52% higher than the control, respectively) but were not significantly different from the control. Compared to the control, 1,000 mM glucose induced a significant increase in the phenolic content with an increment of 61.24% (Fig. 2A). The flavonoid content increased in a concentration-dependent manner (Fig. 2B). High amounts of flavonoid were found in 100 mM and 1,000 mM glucose (81.48% and 95.67% higher than the control, respectively). The PAL activity was significantly affected only by 1,000 mM glucose, with an increment of 45.20% compared to the control. The other treatments also induced increases in the PAL activity but they were not significantly different from the control (Fig. 2C).

Effect of glucose on γ -aminobutyric acid and radical scavenging activity

All treatments produced a significant increase in the GABA concentration of the sprouts (Fig. 3). The highest amount of GABA was found in the 1,000 mM glucose treatment (77.32% higher than the control). Free radical scavenging activity was expressed as a percentage of the DPPH[•] decrease. The 100 mM and 1,000 mM glucose treatments were associated with significant increases in the DPPH radical scavenging activity with respect to the control, with increments of 9.25% and 11.00%, respectively (Fig. 4).

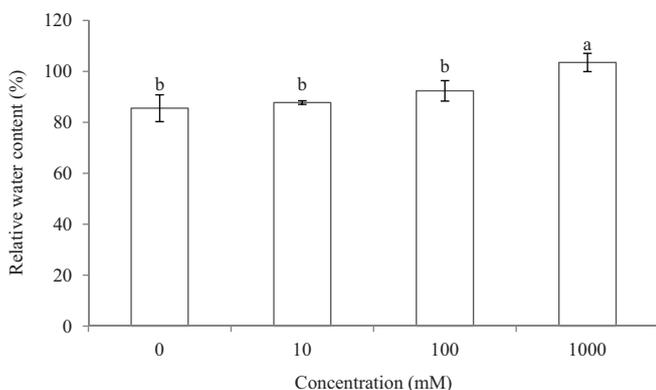


Fig. 1 Effects of different glucose concentrations on relative water content of sunflower sprouts, where data are expressed as the mean ± SD and bars with different lowercase letters are significantly different ($p < 0.05$).

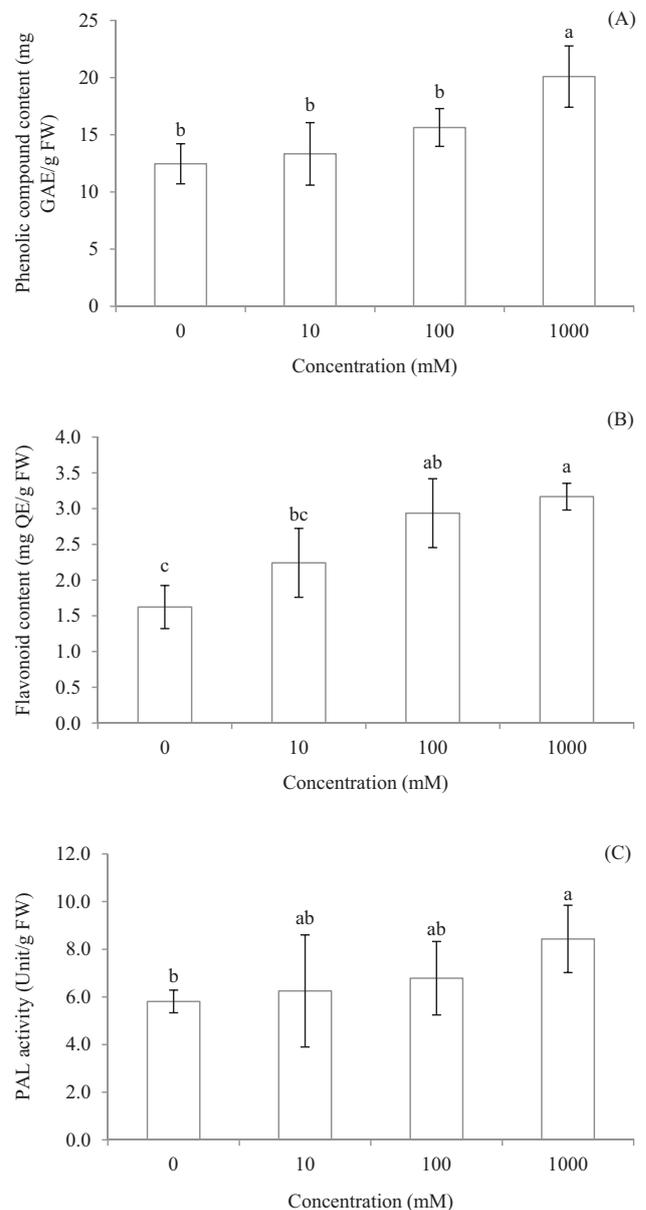


Fig. 2 Effects of different glucose concentrations on: (A) phenolic compound content; (B) flavonoid content; (C) phenylalanine ammonia-lyase (PAL) activity of sunflower sprouts, where data are expressed as the mean ± SD, bars with different lowercase letters are significantly different ($p < 0.05$); FW = fresh weight, GAE = gallic acid equivalent, QE = quercetin.

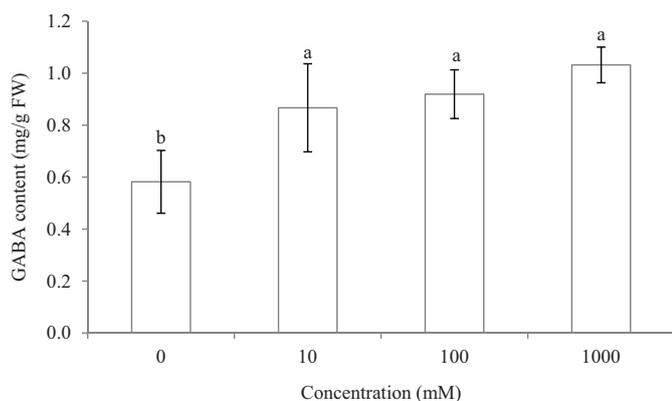


Fig. 3 Effects of different glucose concentrations on γ -aminobutyric acid (GABA) contents of sunflower sprouts where data are expressed as the mean \pm SD, bars with different lowercase letters are significantly different ($p < 0.05$); FW = fresh weight.

Discussion

Application of a low glucose concentration (10 mM and 100 mM) to sunflower sprouts had no significant effects on sprout length and fresh weight. However, the shoot growth and fresh weight were significantly inhibited by 1,000 mM glucose. Such a discrepancy could have been due to the seed reserve being sufficient to permit some osmotic adjustment to maintain a high cellular turgor, allowing sprout growth only under a low glucose-induced stress. Lower growth and yield of the sprouts in the high concentration of glucose treatments can be explained by the increasing osmotic stress conditions which caused an artificial drought during plant growth. This can cause a removal of water from the cytoplasm resulting in a reduction of the cytosolic and vacuolar volumes (Ramakrishna and Ravishankar, 2011). Photosynthetic pigments were monitored as an indicator of stress toxicity. The results showed that the glucose treatments tended to decrease the photosynthetic pigment of the sprouts but were not significantly different from the control. This implied that the concentration of glucose up to 1,000 mM did not induce severe stress to the sprout.

Regulating of water balance is fundamental to cell survival under osmotic stress conditions and the leaf RWC has been proposed as a plant water status indicator more than other water potential parameters under drought stress conditions (Lugojan and Ciulca, 2011). The present results indicated out that the RWC did not decrease after treatment with glucose. This suggested that maintaining the water status was a common adaptation of the sprout under osmotic stress to sustain sprout growth. However, glucose at a concentration of 1,000 mM significantly increased the RWC of the sprouts. The increase in RWC might have been due to the adaptation of the sprout to maintain a water potential differential between the cell and its environment with 1,000 mM glucose-induced mild osmotic stress.

The analyzed sprouts showed significant increases in the total phenolic compounds and flavonoids content as well as the PAL activity. Glucose was used as an elicitor of enhanced phenolic compounds and

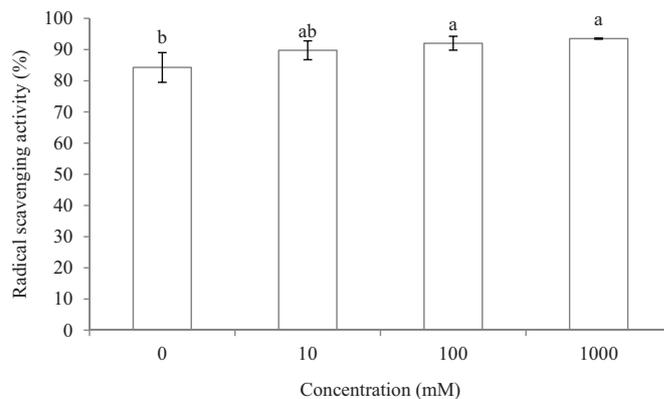


Fig. 4 Effects of different glucose concentrations on 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity of sunflower sprouts, where data are expressed as the mean \pm SD, and bars with different lowercase letters are significantly different ($p < 0.05$).

PAL activity in this study, in accordance with a study on Chinese kale and pak choi sprouts (Wei et al., 2011). PAL is a key regulatory enzyme of the phenylpropanoid pathway which plays a major role in controlling the amount of the phenolic compounds including flavonoids (Vogt, 2010). Flavonoid metabolism is down-regulated by enzyme PAL (Winkel-Shirley, 1999). Therefore, the activity of PAL was investigated in order to provide better comprehending the process involved in the sprouts response to glucose. The amounts of phenolic compounds and flavonoids were positively correlated with the PAL activity. The results revealed that exogenous glucose treatments induced the accumulation of phenolic compounds and flavonoids in sprouts, likely through the phenylpropanoid pathway. It has been reported that PAL activity in sprouts can be modulated by elicitors including radish sprout treatment with methyl jasmonate (Kim et al., 2006) and broccoli sprouts treatment with sucrose and mannitol (Guo et al., 2011b). According to the current results, it was hypothesized that the phenylpropanoid pathway associated with the production of sunflower sprout metabolites was up-regulated by glucose.

Beside the increment in phenolic compounds, an increase in GABA was also observed after treatment with glucose. GABA is a non-protein amino acid existing in the wild in both plants and animals; it functions as a major inhibitory neurotransmitter in the mammalian central nervous system (Bowery and Smart, 2006). Rapid increases in the GABA concentration occur in plants in response to biotic and abiotic stresses such as high temperature, dehydration, salinity and oxidative stress (Kinnersley and Turano, 2000; Bouché and Fromm, 2004). Exogenous glucose increased the GABA content in sunflower sprouts indicating that its physiological metabolism was enhanced. In the current study, a positive correlation between the increment of RWC value and GABA content was observed, suggesting that they could be involved in the osmotic adjustment under low water potential. Due to GABA being one of the osmolytes in plants, GABA accumulation in the cell could balance the decrease in water potential that occurs during cellular dehydration (Kinnersley and Turano, 2000).

A significant increase in the level of antioxidant activity was observed in the glucose treatment of sunflower sprouts compared to the control. Glucose improved the radical scavenging activity, probably due to the increased phenolic compounds and flavonoids contents because these compounds are known as antioxidant molecules (Sharma et al., 2018). This was consistent with Wei et al. (2011) who reported that the antioxidant activity of Chinese kale and pak choi sprouts increased significantly after glucose (5%) treatment. In comparison to Wei et al. (2011), the glucose level induced antioxidant activity of sunflower sprouts in the present study (1,000 mM) was higher than those reported for Chinese kale and pak choi sprouts (278 mM), perhaps because of the duration the sprouts were exposed to glucose, since glucose was added to the growth medium of Chinese kale and pak choi sprouts 3 d after sowing, whereas the sunflower sprouts were sprayed with glucose 6 d after sowing.

The formation of secondary metabolites after glucose treatment could be attributed to a certain level of osmotic stress, which initiated the signal perception through a receptor in the cell membrane to activate the biosynthesis of target metabolites via the signal transduction network (Zhao et al., 2005). In addition, glucose is the original precursor for the synthesis of phenolic compounds and several important molecular signaling pathways (Gan et al., 2017).

The current results indicated that glucose could enhance the nutritional value of sunflower sprouts and suggested that glucose could play a role in the induction of health-promoting compounds as a cause of osmotic stress or as a signal molecule. The application of a sufficient concentration of glucose on sprouts could benefit the human diet by producing more health-promoting compounds. The beneficial aspects of glucose, as demonstrated in this study, clearly warrant scale-up trials using large volumes typical of commercial conditions.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

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