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Effects of gibberellic acid on the antioxidant activity of soybean seeds (*Glycine max L. Merr*.) during germination

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Abstract

Changes in antioxidant compounds and antioxidant activity were studied in soybean seeds during germination applied gibberellic acid (GA_3) in soaking water. GA_3 concentrations in soaking water were set up as 0; 0.001; 0.01; 0.1; 1 and 10mg/L. The soaking process was carried out at ambient temperature for 12 hours prior germination. Soaked soybeans were germinated in dark condition, at 25ºC for 0, 12, 23, 36, 48, 60 and 72 hours. The total phenolic content (TPC), total flavonoid content (TFC), vitamin C and α-tocopherol contents as well as antioxidant activity assayed by DPPH radical-scavenging activity in terms of IC50 of germinated soybean were determined. It was found that, the increase of germination time caused an increase the TPC, TFC, vitamin C, α-tocopherol as well as antioxidant activity and most of them tend to reach the maximum values after a period of 60 hours of germination. In addition, GA_3 has a profound effect upon the antioxidant potentials and it caused a significant enhancement in the production of antioxidant compounds when compared to control. GA_3 1mg/L in soaking solution was proposed as optimum concentration to apply for soaking process.

Keywords: Gibberellic acid, germination, phenolics, flavonoids, antioxidant compounds

1. Introduction

Soybeans (*Glycine max L. Merr.*) are contributed to the human diet and widely used in the food industry for centuries because of their excellent source of protein, oil and other bioactive compounds such as polyphenols, isoflavones [1]. Soybean bioactive compounds act as antioxidants that play a role in the human health prevention [2] by removing reactive oxygen species and thereby prevent oxidative damage in living tissue [3]. However, soybeans and soy products contain antinutritional factors such as trypsin inhibitors, oligosaccharides and phytic acid which can negatively affect their nutritional value [4]. For this reason, soybeans have to be processed prior to their consumption [5].

Germination has been identified as an inexpensive and effective technology for improving the nutritional quality of soybeans [6, 7]. During germination of seeds, the reserve materials of the seeds are degraded and used partly for respiration and partly for synthesis of new cell constituents of the developing embryo, this process causes important changes in the biochemical, nutritional and sensory characteristics of legumes. Numerous studies have reported the significant changes in biochemical and nutritional characteristics, for detail, germination increases nutritional (protein, acid amin, soluble sugars, various minerals, vitamins and dietary fibers) [8, 9] and antioxidant compounds (phenolics, vitamins E, vitamin C) [10, 11]. Beside, this process reduces antinutritional factors in soybean seeds [12].

The effect of germination on the seed compositions is known to differ greatly with specie, variety, germination conditions such as temperature, humidity, possibly light or darkness and time [12, 13]. In addition, a significant factor that control seed germination include plant hormones such as gibberellic acid also impress to germination process. Gibberellic acid (GA_3) was known to be one of the endogenous growth regulators that has the capability of regulation plant growth through increasing cell division and cell elongation, biosynthesis of enzymes, protein, carbohydrates and photosynthetic pigments by affecting the DNA and RNA levels [14]. Gibberellic acid is used as a food additive for barley malting. Firstly, it was used in 1959 for commercial brewing in the UK, and was used in over 70% of all malt produced there until 1973 [15].

There are some studies have indicated the influence of gibberellic acid on germination rate of seeds [16-18], while information on the effect of gibberellic acid on antioxidant compounds in seeds (such as phenolic, flavonoids, vitamin C, vitamin E) is scanty. Therefore, this research investigate the effect of gibberellic acid concentrations on antioxidant compounds and antioxidant activity of soybean seed during germination.

2. Materials and methods

2.1 Soybean and germination process

Soybeans (*Glycine max L.*, MTD 760 variety) were supplied from Department of Agricultural Genetic, College of Agricultural and Applied Biology, Cantho University.

Soybeans were cleaned and rinsed with cleaned water before being soaked for 12 hours to reach the equilibrium moisture content at ambient temperature (30±2ºC). Soaking process was carried out in drinkable water with the ratio of soybean seeds and water as 1: 5 and the concentration of gibberellic acid in soaking water as 0 (control), 0.001, 0.01, 0.1, 1 and 10mg/L. The soaked beans were drained, rinsed and placed in a germination chamber in dark condition. Watering the seeds was set up two minutes for every 4 hours with cleaned water automatically. The germination process was carried out at the 25ºC for 0, 12, 24, 36, 48, 60 and 72 hours. Germinated soybeans were freeze dried for analyzing the total phenolic content (TPC), total flavonoid content (TFC), vitamin C, vitamin E (α–tocopherol) and IC50 value. The extraction procedure for analysis of the antioxidant compounds followed the study results of Duong *et al.* (2015) [19], α-tocopherol content was detected in soybean oil.

2.2 Determination of the TPC, TFC, vitamin C, α-tocopherol and antioxidant capacity (IC50)

The TPC was estimated by Folin-Ciocalteu method [20]. The TPC of samples was expressed as milligrams garlic acid equivalents per gram of dry matter (mgGAE/g).

The TFC was determined by the Dowd method with slight modification [21] and using a standard curve of quercetin. Thus, the results were expressed as milligrams of quercetin equivalents (QE) per gram of dry matter sample (mgQE/g).

Ascorbic acid (vitamin C) content was determined by redox titration with iodine [22].

Vitamin E (α–tocopherol) content was determined by Emmerie-Emmerie Engel reaction which is based on the reduction by tocopherol of ferric to ferrous ions which is then form a red complex with α,α-dipyridyl that can reading at optimum wave length of 520nm [23].

Antioxidant activity of the phytochemicals extracted from soybean was assessed by measuring their radical scavenging activity that was measured by the bleaching of the purple-coloured methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH). This spectrophotometric assay uses stable DPPH radical as a reagent. The DPPH radical scavenging activity was evaluated from the difference in peak area decrease of the DPPH radical detected at 517nm between a blank and a sample [24]. Percentage of radical scavenging activity was plotted against the corresponding concentration of the extract (μg/ml) to obtain IC50 value in mg/mL. IC50 is defined as the amount of antioxidant material required to scavenge 50% of free radical in the assay system. The IC50 values are inversely proportional to the antioxidant activity.

2.3 Statistical analysis

All evaluations of total phenolic content, total flavonoid content, antioxidant activity were performed thrice. The data were submitted to analysis of variance (ANOVA) by Portable Statgraphics Centurion 15.2.11.0 and were expressed as mean values and standard deviation. The graphs were plotted by Microsoft Excel 2007 and Portable Statgraphics Centurion 15.2.11.0.

3. Results & Discussion

3.1 Effect of soaking process on antioxidant compounds and antioxidant activity of soybean seeds

The changes of antioxidant compounds such as TPC, TFC, vitamin C, α–tocopherol and antioxidant activity expressed in IC50 value during soaking 12 hours in different gibberellic acid solutions were shown in Table 1.

The results from Table 1 showed that the TPC, TFC and vitamin C in soybean seeds decreased significantly after soaking without gibberellic acid leading to increase in IC50 value, but the α-tocopherol content increased significantly after soaking. Seasonal changes in TPC, TFC and vitamin C after soaking of soybean seeds due to the water-soluble phenolics and vitamin C leaching into the soaking water [25, 26], while insoluble α-tocopherol remained in seeds with higher proportion.

Table 1: Effects of gibberellic acid concentration on antioxidant compounds and antioxidant activity during soaking process of soybean seeds

Samples	**TPC (mgGAE/g)**	**TFC (mgQE/g)**	**Vitamin C (mg/g)**	**α-tocopherol (mg/g)**	**IC50 (mg/mL)**
Soybean seeds	3.02^{a}±0.03	2.14^{ab}±0.01	6.23^{e}±0.20	0.066^{e}±0.006	9.20^{d}±0.03
Soaked (0 mg/L, GA_3)	2.46^{c}±0.10	1.92^{c}±0.02	5.07^{f}±0.03	0.085^{d}±0.005	10.40^{a}±0.26
Soaked (0.001 mg/L, GA_3)	2.49^{c}±0.05	1.99^{c}±0.03	6.78^{d}±0.20	0.109^{c}±0.010	9.77^{b}±0.10
Soaked (0.01 mg/L, GA_3)	2.67^{b}±0.12	1.99^{c}±0.06	7.59^{c}±0.19	0.117^{c}±0.005	9.47^{c}±0.18
Soaked (0.1 mg/L, GA_3)	2.77^{b}±0.06	2.08^{b}±0.04	7.70^{c}±0.16	0.144^{b}±0.005	9.23^{ab}±0.01
Soaked (1 mg/L, GA_3)	3.00^{a}±0.03	2.13^{ab}±0.07	8.17^{b}±0.13	0.172^{a}±0.005	9.16^{d}±0.05
Soaked (10 mg/L, GA_3)	3.05^{a}±0.09	2.20^{a}±0.02	8.57^{a}±0.28	0.171^{a}±0.012	9.10^{d}±0.17

(Means±SD, The values showing different superscripts within a column are significant different at p = 0.05)

However, the increasing of gibberellic acid concentration in soaking water resulted in the increasing in TPC, TFC, vitamin C as well as α-tocopherol content in soybean seeds after soaking. With 1mg/l gibberellic acid in soaking water these parameters tend to reach the maximum values (Table 1), in which, the TPC and TFC was not significant different with these content in raw soybean seeds, but the vitamin C and α-tocopherol content were significant higher than that of raw soybeans. The higher level of gibberellic acid (10mg/l) these parameter seem to be not significant different. These results permitted to consider that gibberellic acid has a role in enhancement the antioxidant compound of soybean seed during the soaking stage.

3.2 Effect of gibberellic acid on antioxidant compounds and antioxidant activity of soybean seeds during germination process

During germination, the TPC, TFC, vitamin C, α–tocopherol contents and IC50 value of soaked soybean seeds were significantly changed and displayed on Figure 1, 2, 3, 4 and 5. These changes depended on not only germination time but also on gibberellic acid content in soaking water.

Effects of GA_3 soaking solution on TPC and TFC during germination

The TPC and TFC increased significantly during germination and tend to reach the maximum values at the germination time of 60 hours. In addition, the gibberellic acid in soaking water effected significantly on the increasing of TPC and TFC.

The production of secondary metabolite in plants is related to its growing condition [27] and a high content of total phenols was recorded in certain crops grown organically than the crops grown by conventional farming [28]. In the study of Saikia and Upadhyaya (2011) [27], the jamun (*Syzygium cumini L. Skeels*) seeds were either soaked in distilled water or in different doses of gibberellic acid (100 ppm and 200 ppm) for 24 hours. They found that total phenols and total antioxidants in sample soaked in GA_3 200ppm after 30 days sowing increased 42.70 and 77.17% respectively, comparing to control sample. The increasing trend of total phenols and total antioxidants with increased dose of GA_3 was also reported by Sardoei *et al.* (2014) when observation the effects of gibberellic acid on phenolic compounds of marigold (*Calendula officinalis*) extract [29]. In this study, TPC and TFC contents tended to get the maximum values corresponding to the content of gibberellic acid in soaking solution as 1mg/L. Increasing the GA_3 content in soaking solution (10mg/L) the accumulation of TPC and TFC should be decreased (Figure 1 and 2). These findings are similar to those of previous study of Hameed *et al.* (2015) [30], who reported that GA_3 (40 ppm) application increased TPC (274.07±8.22 mg GAE/100g DW) and TFC (154.37 ± 2.04 mg CE/100g DW) in broccoli (*Brassica oleracea L.*) as compared to control (220.92±6.62 mg GAE/100g DW and 77.95±2.46 mg CE/100g DW), whereas the GA_3 (60 ppm) application significantly decreased the TPC (256.36 ± 7.69 mg GAE/100g DW) and TFC (90.21 ± 1.13 mg CE/100g DW) [30]. So, the TPC and TFC of vegetables can be enhanced only when the suitable plant growth regulators were used [31].

Plant hormone such as gibberellic acid plays an important role in flavonoid formation by speeding growth and reducing chalcone synthase activity resulted in flavonoid biosynthesis [32]. Gibberellic acid has also known to promote flavonoid synthesis by promotion levels of flavonoid specific mRNAs [33]. Kim *et al.* (2009) [31] observed an increase in flavonoid content approximately 30% in seeds of *Taraxacum officinale* after one week of gibberellic acid (0.5mM) application. Their result confirmed the significance of these gibberellic acid in the biosynthesis of flavonoids [34].

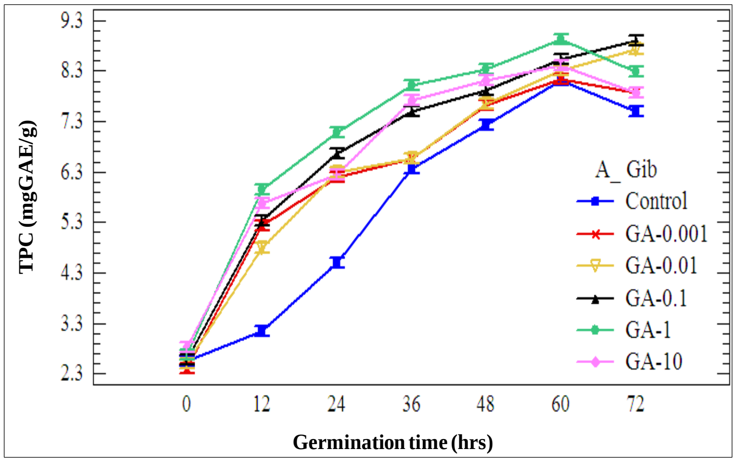

Fig 1: Effect of GA_3 concentration in soaking water on TPC of soybean seeds during germination (Mean±LSD)

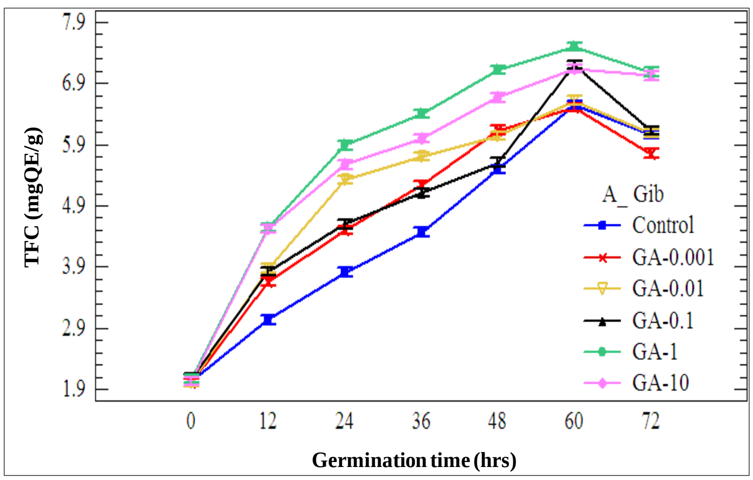

Fig 2: Effect of GA_3 concentration in soaking water on TFC of soybean seeds during germination (Mean±LSD)

Effects of GA_3 soaking solution on vitamin C content during germination

Vitamin C contents in soybean seeds increased fastly during germination and depended strongly to gibberellic acid content in soaking water (Figure 3). In this study, the higher gibberellic acid concentration in soaking water and the longer germination time resulted in the higher in vitamin C content in germinated soybean seeds. So, the maximum value of vitamin C in germinated soybean seed was 15.40±0.09 mg/g in case of soaking soybean seed in gibberellic acid solution 10mg/L and for 72 hours germination. The augmentation of ascorbic acid content in the presence of gibberellic acid might be due to either increased ascorbic acid biosynthesis or to protection of synthesized ascorbic acid from oxidation through ascorbic acid oxidase [35]. Ascorbic acid content was reported significant increasing with the application of GA_3 for the chilli fruit [36] and peppers [35].

Effects of GA_3 soaking solution on α–tocopherol content during germination

Gibberellic acid treatments resulted in an increase in α-tocopherol content in soybean seeds during germination. The α-tocopherol content tended to reach the maximum value when gibberellic acid 1–10mg/L were applied in soaking water and took about 36 hours of germination (Figure 4). Literature sources indicate that both α-tocopherol and chlorophyll contain a phytol moiety as part of their molecule and the substrate used for their biosynthesis may be derived from a common pool [37]. In dark condition, chlorophyll was not priority synthesized. However, GA_3 treatment stimulated the biosynthesis in seed, it is possible that without increasing in chlorophyll biosynthesis caused an increase in the substrate accumulation and therefore α-tocopherol biosynthesis [37]. The increase in α-tocopherol level was also found in the seed of *Cannabis sativa L.* treated with GA_3 100μM [38], and in the GA_3 (5 μM) treated *Catharanthus roseus* [39].

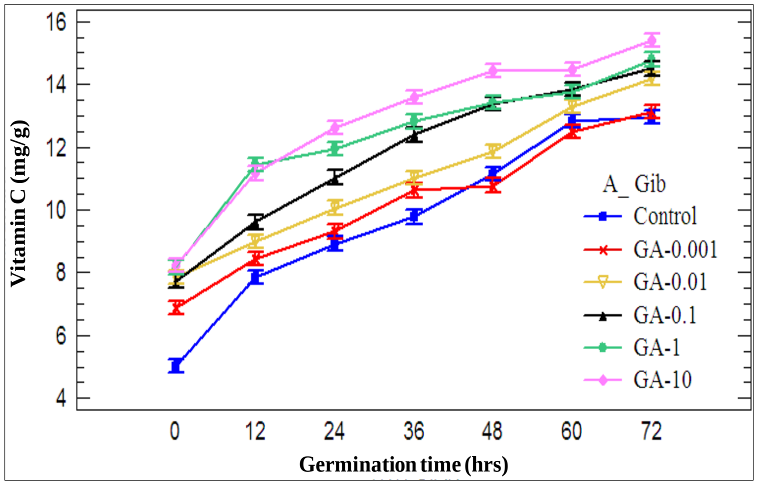

Fig 3: Effect of GA_3 concentration in soaking water on vitamin C content of soybean seeds during germination (Mean±LSD)

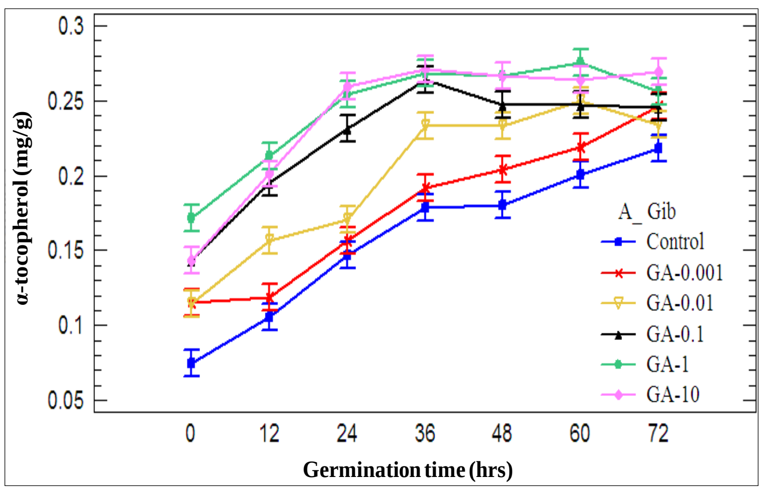

Fig 4: Effect of GA_3 concentration in soaking water on α–tocopherol content of soybean seeds during germination (Mean±LSD)

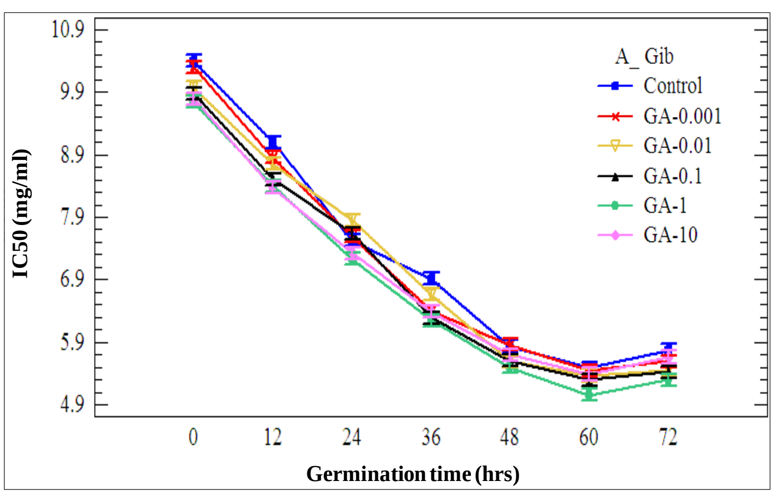

Fig 5: Effect of GA_3 concentration in soaking water on IC50 of soybean seeds during germination (Mean±LSD)

Effects of GA_3 soaking solution on antioxidant ability during germination

A high correlation between free radical scavenging and the phenolic contents in seeds and seedlings has been reported by many authors [40-42]. Polyphenols exert their beneficial health effects mainly through their antioxidant activity by capacity in removing free radicals, chelating metal catalysts, activating antioxidant enzymes, and inhibiting oxidases [43]. In this study, the rule of antioxidant activity changes (expressed by IC50) was similar to TPC and TFC changes. The IC50 value reached to minimum (maximum antioxidant activity, 5.06±0.12 mg/mL) for soybean seeds soaked 12 hours in gibberellic acid 1mg/L solution and germinated for 60 hours (Figure 5). The enhanced total antioxidant capacity could be associated with enhanced phenolics content in seeds and seedlings [44]. In this study, the TPC was significantly correlated (R^2=0.92) to their IC50 (Figure 6). Giannakoula *et al.* (2012) [41] found that in lentil seeds treated with GA_3, the (TPC) was significantly correlated (R^2= 0.99) to their total antioxidant capacity. The high TPC and antioxidant activity in germinated soybean seeds treated by soaking in GA_3 solution prior germination could be of interest and make them useful for daily inclusion in the human diet.

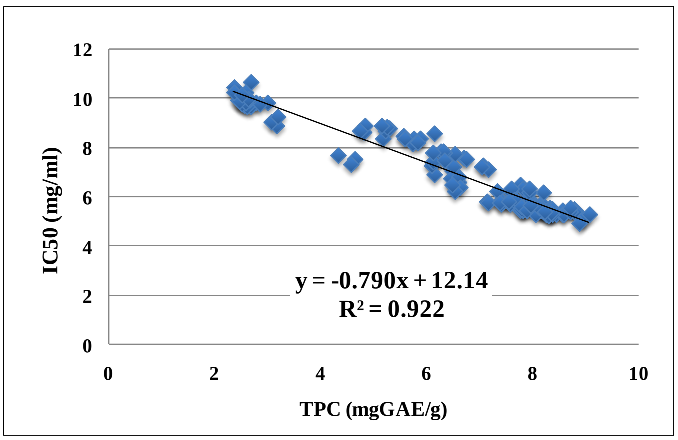

Fig 6: Correlation between TPC and IC50 of germinated soybean seeds

4. Conclusions

The results of the study clearly indicated that the application of gibberellic acid 1mg/L in the soaking water is suitable for high production of antioxidant compounds as well as antioxidant activity of soybean seeds during germination process. Gibberellic acid have key role in different physiological processes related to enhance the antioxidant capacity for soybean seeds. The combination of gibberellic acid and germination process could provide germinated soybean with high antioxidant properties. It might be used as a source of raw materials naturally occurring antioxidants for functional food processing.

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