J Sci Food Agric 85:943–947 (2005)
DOI: 10.1002/jsfa.2050

Effects of germination conditions on ascorbic acid level and yield of soybean sprouts

Mao-Jun Xu,[1,2] Ju-Fang Dong[2] and Mu-Yuan Zhu[1]*

[1]State Key Laboratory of Plant Physiology and Biochemistry, College of Life Sciences, Zhejiang University, Hangzhou 310012, China
[2]College of Food Science and Biotechnology and Environmental Engineering, Hangzhou University of Commerce, Hangzhou 310035, China

Abstract: The effects of light illumination on the ascorbic acid content and growth of soybean sprouts were investigated. Among the six light qualities studied, ultraviolet light had the highest promoting effect on the ascorbic acid content in soybean sprouts, increasing it by 77.0% compared with the darkness control, while red light had the highest promoting effect on the growth of soybean sprouts, increasing the total fresh weight by 16.6% compared with the darkness control. Experiments with different durations of ultraviolet and red light illumination in a day showed that 12 h ultraviolet (500 Lx) and 12 h red (1000 Lx) light diurnal cycles had the highest promoting effects on both the ascorbic acid level and fresh weight of soybean sprouts, increasing the ascorbic acid content and total fresh weight by 78.7 and 17.4% respectively compared with the darkness control. The results indicated that germination of soybeans under 12 h ultraviolet and 12 h red light diurnal cycles was an effective process for increasing the yield and enhancing the nutritional quality of soybean sprouts.

Keywords: ascorbic acid; soybean sprout; germination; light quality

INTRODUCTION

Soybeans have been consumed by Asians, including the Chinese, for centuries and are often advocated in Western diets nowadays because of their beneficial nutritional effect.[1,2] However, soybean seeds contain many antinutritional factors such as lectins and enzyme inhibitors.[3] Therefore various processes have been investigated to improve the nutritional quality of soybeans.[4] Germination has been identified as an inexpensive and effective technology for improving the nutritional quality of soybeans. Numerous studies have been done to investigate the effect of germination on antinutritional factors,[3,5,6] protein and amino acid levels[7] and the digestibility of soybeans.[7–9] However, there is a paucity of literature about the effect of such treatment on the ascorbic acid content in soybeans. In previous studies we found that ascorbic acid, not detected in soybean seeds, significantly increased in the germinated products of soybeans, eg soybean sprouts,[10] which indicated that soybeans were able to accumulate this vitamin during germination.

Ascorbic acid has many important biological functions in both plants and animals.[11–13] Humans are unable to synthesise ascorbic acid and are thus entirely dependent on dietary sources to meet their needs.[14] Plant foods are the main dietary sources of ascorbic acid in China and most other developing countries. Although ascorbic acid is a universal constituent of fruits and vegetables,[15] the seasonal availability of fruits and vegetables and the significant losses of the vitamin which occur during storage, preparation and cooking mean that ascorbic acid remains one of the few nutrients in which the diet can be deficient.[16] Therefore enhancing ascorbic acid contents in vegetables and fruits is beneficial for human health.

Soybean sprouts are highly digestible and a good source of protein and minerals[17] and have been eaten for a long time in China and other Asian countries as a kind of 'vegetable' to complement a low intake of fruits and vegetables, especially in rural areas of China where seasonal fruits and vegetables are not available all-year-round. Several problems are encountered during the production of soybean sprouts, including yield reduction, quality deterioration and rot occurrence. Much attention has been given to developing new techniques to overcome these problems, including the use of plant hormones,[18] food additives,[19] antimicrobial agents[20] and calcium.[21] However, to date, none of these methods have been effectively employed to increase the ascorbic acid content in soybean sprouts. In order to enhance the nutritive value and increase the yield of soybean sprouts, the effects of light illumination, including

* Correspondence to: Mu-Yuan Zhu, State Key Laboratory of Plant Physiology and Biochemistry, College of Life Sciences, Zhejiang University, Hangzhou 310012, China
E-mail: myzhu@zju.edu.cn
Contract/grant sponsor: Natural Scientific Foundation of Zhejiang Province; contract/grant number: 302785
(*Received 26 November 2003; revised version received 13 August 2004; accepted 18 August 2004*)
Published online 26 January 2005

various light qualities and alternate illumination with different lights, on the ascorbic acid content and yield of soybean sprouts were investigated in this study.

MATERIALS AND METHODS

Germination

The soybean utilised in this study was a native Chinese cultivar, Ludou 2, provided by the National Soybean Center of China (Nanjing, China). Soybeans (1 kg) of identical size and weight were selected, rinsed in distilled water and drained. They were then placed in distilled water (25 ± 3 °C) and soaked overnight in the dark for 8 h at 25 ± 3 °C. After pouring off the soaking water, the seeds were rinsed in water (20 ± 3 °C) for about 5 min, spread evenly on a tray lined with absorbent paper and then placed in a controlled environment chamber at 28 °C and ambient humidity (*ca* 90–95% relative humidity). The seeds were rinsed with water (20 ± 3 °C) once a day in the morning. The seeds were showered with distilled water (25 ± 3 °C) for about 5 min and left to drain completely. The sprouts were harvested at the end of treatment.

Light exposure

The selected soybeans were divided evenly into six groups, each group consisting of two trays of soybeans, one of which was illuminated by light (white, blue, ultraviolet, orange, red and far-red light respectively) distributed so as to give a uniform flux density at tray level. The absorbent paper lining the trays was watered twice daily to prevent any drying-out of the sprouts during their period of illumination. The other trays of soybeans were inserted into black polyethylene light-proof bags and transferred into the same illuminated controlled environment chamber to serve as non-illuminated darkness controls, except for the treatment with ultraviolet light, where the dark control was transferred into another chamber without ultraviolet light.

Fluorescent lamps (40 W, GB 10 682-89, Huaxiing, Shanghai, China) were used as white light source. Far-red and blue light were obtained by filtering white light through a Schott RG filter (cut-on 690 nm) for far-red light or through a blue filter (350–530 nm, max 450 nm, 195 filter, LEE Filters, Andover, UK) for blue light. Orange light was obtained from white light filtered through two filters: an orange filter (cut-on 580 nm, 021 filter, LEE Filters) and a cut-off 650 nm filter (Oriel, Courtaboeuf, France). Red light was obtained by filtering white light through a red filter (610–690 nm, max 660 nm, PG 501/3 filter, Philips, Eindhoven, The Netherlands). UV lamps (40 W, GB 10 682-79, Huaxiing) were used as ultraviolet light source.

Different flux densities were obtained by turning on different numbers of lamps and by adjusting the distance between the lamps and the test materials. Flux densities were measured using a quantum sensor (ZDZ, Xuelian, Shanghai, China).

Sampling

Whole soybean sprouts (about 5000 soybean sprouts per replicate) were collected for various analyses after 1–9 days of sprouting. The samples from each treatment were weighed for fresh weight and then divided into two portions. One was used for ascorbic acid analyses, while the other was stored at −20 °C for enzyme analyses after snap-freezing.

Ascorbic acid determination

Ascorbic acid was measured as described by Stasalloa and Yeung.[22] Soybean sprouts (50 mg) were homogenised in 0.5 ml of 1 M $HClO_4$ with a mortar and pestle in an ice bath and centrifuged at $13\,000 \times g$ for 5 min at 4 °C. Then 0.1 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)/KOH buffer, pH 7.0, was added to the supernatant at a ratio of 1:5 (buffer/extract), neutralised with 1 M K_2CO_3 to pH 5.6 and centrifuged again as above. The supernatant was divided into two tubes, one of which was treated with 1.5 ml of ascorbic acid oxidase (100 U mL^{-1}, Sigma, St Louis, MO, USA) and the other received the same volume of distilled water. Ascorbic acid was measured as the difference in A_{265} between the two tubes after 30 min.

Extraction and assay for GLDH

L-Galactono-γ-lactone dehydrogenase (GLDH, EC 1.3.2.3) was extracted and assayed as described by Oba *et al*.[23] Soybean sprouts (10 g) were ground in 50 ml of extraction buffer (30 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS), pH 7.5, 2 g l^{-1} bovine serum albumin (BSA), 4 mM Cys, 0.35 M mannitol, 10 g l^{-1} PVP-40, 25 mM $Na_4P_2O_7$ and 2 mM ethylene diamine tetraacetic acid (EDTA)), filtered through four layers of cheesecloth and centrifuged at $2200 \times g$ for 5 min. $(NH_4)_2SO_4$ (200 g l^{-1}, 5 ml) was added to the supernatant, incubated at 4 °C for 30 min under continuous stirring and centrifuged at $10\,000 \times g$ for 20 min. The pellet containing membrane-bound proteins was resuspended in 1 ml of washing medium without BSA. GLDH activity was measured in the resuspended pellet after a semi-purification step through Sephadex G-25. GLDH activity was assayed by the reduction of Cyt *c* at 550 nm ($\varepsilon = 21$ mM^{-1} cm^{-1}) in a medium consisting of 50 mM Tris-HCl, pH 8.0, 60 μM Cyt *c*, 1.5 g l^{-1} Triton X-100 and about 25–50 μg of sample protein. The activity of GLDH was determined as μmol Cyt min^{-1} mg^{-1} soybean sprouts (fresh weight).

Statistical analysis

The analysis of variance employed an SAS program for PCs (SAS Institute, Inc, Cary, NC, USA). Duncan's multiple range test was also applied to determine whether there were significant differences between individual treatments.

RESULTS AND DISCUSSION

Accumulation of ascorbic acid in soybean sprouts during germination

Ascorbic acid has been directly implicated in the modulation of plant growth, including the early stage of germination of embryos.[24,25] The effects of germination treatments on the ascorbic acid level in soybeans are summarised in Table 1. The results show that ascorbic acid, not detected in unsprouted soybeans, increased significantly during germination. This is in agreement with observations on *Pinus pinea* L., which showed that ascorbic acid was not found in dry seeds but increased during germination owing to the reactivation of its biosynthesis.[25]

In order to confirm the reactivation of the biosynthesis of ascorbic acid in soybeans during germination, the activity of the key enzyme in ascorbic acid biosynthesis was investigated (Table 1). GLDH, which catalyses the oxidation of L-galactono-1,4-lactone to ascorbic acid, the last step of the ascorbic acid biosynthetic pathway,[26] has been proved to be one of the key enzymes in ascorbic acid biosynthesis.[27] Table 1 indicates that the activity of GLDH in soybean sprouts increased significantly during germination, accompanied by increases in ascorbic acid level. This confirms that the accumulation of ascorbic acid in soybeans during germination was due to the reactivation of its biosynthesis.

Effects of light quality on ascorbic acid level and growth of soybean sprouts

As might be expected from its antioxidant-based functions in plant metabolism, the levels of ascorbic acid in plants are highly responsive to a wide variety of environmental factors.[16] Several groups have reported that plants increase their ascorbic acid levels in response to high light intensity,[28,29] but they did not provide information about the effects of different light qualities on ascorbic acid levels. The effects of red, far-red, white, blue, orange and ultraviolet light on the ascorbic acid level and growth of soybean sprouts were examined here (Table 2). The results show that ultraviolet light had the highest promoting effect on the ascorbic acid content in soybean sprouts, increasing it by 77.0% compared with the darkness control, while red light had the highest promoting effect on the yield of soybean sprouts, increasing the total fresh weight by 16.6% compared with the darkness control. This is in agreement with observations on callus of *Saussurea medusa* Maxim which showed that ultraviolet light had a promoting effect on the contents of secondary products and red light had a promoting effect on cell growth.[30]

The changes in the activity of GLDH in soybean sprouts germinated under different lights exhibited the same trend as the changes in ascorbic acid level (Table 2). This indicates that the activity of the key enzyme in ascorbic acid biosynthesis was regulated by light and that increases in the activity of GLDH resulted in the accumulation of ascorbic acid in soybean sprouts germinated under ultraviolet illumination.

Table 1. Changes in ascorbic acid content, GLDH activity and total fresh weight of soybean sprouts during germination[a]

Germination time (days)	Ascorbic acid (mg kg^{-1} fresh weight)	GLDH (μmol min^{-1} mg^{-1})	Total fresh weight (g)
0	ND	ND	1000 ± 37i
1	26.3 ± 3.6e	1.92 ± 0.17d	1895 ± 58h
2	76.8 ± 8.9d	5.32 ± 0.36c	1978 ± 69h
3	123.1 ± 12.1c	8.87 ± 0.98b	2824 ± 87g
4	217.9 ± 28.6a	11.43 ± 1.42a	4758 ± 93f
5	199.4 ± 20.2a	12.18 ± 2.15a	5462 ± 97e
6	163.2 ± 21.3b	11.43 ± 2.06a	5964 ± 103d
7	158.3 ± 13.4b	12.22 ± 1.97a	6462 ± 127c
8	126.5 ± 14.5c	11.86 ± 1.69a	7088 ± 153b
9	81.9 ± 10.7d	7.89 ± 0.45b	7490 ± 186a

[a] Each value is an average of three determinations. The values were obtained from soybeans germinated in darkness at 28 °C. ND, not detected. Means followed by different letters within the same column are significantly different from each other ($P < 0.05$).

Table 2. Effects of light quality on ascorbic acid content, GLDH activity and total fresh weight of soybean sprouts[a]

Light quality	Ascorbic acid (mg kg^{-1} fresh weight)	GLDH (μmol min^{-1} mg^{-1})	Total fresh weight (g)
Darkness	208.3 ± 23.4c	13.82 ± 1.97c	5560 ± 106b
Red light	113.6 ± 6.3d	9.79 ± 0.57d	6482 ± 132a
Far-red light	89.4 ± 9.8e	7.48 ± 0.96e	5876 ± 115b
Orange light	95.8 ± 11.7e	6.83 ± 0.84e	5604 ± 108b
Blue light	237.9 ± 26.7b	19.73 ± 2.16b	5174 ± 106c
White light	233.8 ± 22.1b	20.36 ± 2.52b	5586 ± 117b
Ultraviolet	368.7 ± 41.3a	29.67 ± 3.38a	5220 ± 97c

[a] Each value is an average of three determinations. The values were obtained from soybeans germinated for 5 days in continuous darkness or with illumination at 28 °C. The illumination intensity of different light qualities was 1000 Lx. Means followed by different letters within the same column are significantly different from each other ($P < 0.05$).

Effects of intensity of ultraviolet and red light on ascorbic acid level and fresh weight of soybean sprouts

It has been reported that plants increase their ascorbic acid levels in response to high light intensity.[29] Our experiments showed that the ascorbic acid level and total fresh weight of soybean sprouts increased significantly when the intensities of ultraviolet and red light were higher than 500 and 1000 Lx respectively (data not shown). Therefore 500 Lx ultraviolet and 1000 Lx red light were selected for the alternate illumination treatments.

Effects of alternate illumination with ultraviolet and red light on ascorbic acid level and fresh weight of soybean sprouts

Ultraviolet light had the highest promoting effect on the ascorbic acid level in soybean sprouts but significantly inhibited their growth (Table 2). In

Table 3. Effects of alternate illumination with ultraviolet (UV) and red (RL) light on soybean growth, GLDH activity and ascorbic acid content[a]

UV/RL diurnal cycle (h/h)	Ascorbic acid (mg kg^{-1} fresh weight)	GLDH (μmol min^{-1} mg^{-1})	Total fresh weight (g)
0/24	114.5 ± 11.9c	11.26 ± 1.79c	6476 ± 131a
4/20	283.9 ± 31.3b	24.35 ± 4.81b	6556 ± 133a
8/16	304.2 ± 32.4b	28.93 ± 4.37a	6552 ± 138a
12/12	372.4 ± 38.3a	30.87 ± 4.18a	6526 ± 141a
16/8	368.6 ± 32.3a	31.17 ± 3.76a	6130 ± 132b
20/4	375.7 ± 31.7a	30.38 ± 4.17a	5672 ± 129c
24/0	372.4 ± 41.3a	29.17 ± 4.26a	5231 ± 114d

[a] Each value is an average of three determinations. The values were obtained from soybeans germinated for 5 days at 28 °C. The illumination intensities of ultraviolet and red light were 500 and 1000 Lx respectively. Means followed by different letters within the same column are significantly different from each other ($P < 0.05$).

contrast, red light promoted the growth of soybean sprouts but had a significant inhibiting effect on their ascorbic acid level (Table 2). In order to obtain the highest ascorbic acid level and highest fresh weight of soybean sprouts, the effects of alternate illumination with ultraviolet and red light were investigated (Table 3). The results shows that 12 h ultraviolet and 12 h red light diurnal cycles had the highest promoting effects on both the ascorbic acid level and fresh weight of soybean sprouts. The ascorbic acid content and total fresh weight of soybean sprouts germinated under such conditions were 372.4 mg kg^{-1} fresh weight and 6526 g, which are 78.7 and 17.4% higher than the darkness control values respectively.

CONCLUSION

This study has demonstrated that the germination process, in particular under ultraviolet illumination, has a significant promoting effect on the ascorbic acid content in soybeans. In the germination process the increases in ascorbic acid level were considered to be a consequence of the reactivation of ascorbic acid biosynthesis undergone in the seeds during germination. Germination of soybeans under ultraviolet and red light diurnal cycles appears to be an effective process for increasing the yield and enhancing the nutritional quality of soybean sprouts.

ACKNOWLEDGEMENT

This work was supported by grant 302785 from the Natural Scientific Foundation of Zhejiang Province.

REFERENCES

1 Sgarbieri VC, Composition and nutritive value of beans (*Phaseolus vulgaris* L.), in *Nutritional Value of Cereal Products, Beans and Starches*, Vol 60, ed by Bourne GH. World Nutrition Diet, Basel, pp 132–198 (1989).
2 Doughty J and Walker A, *Etude FAO: Alimentation et Nutrition*. FAO, Rome (1982).
3 Bau HM, Villaume C, Nicolas JP and Mejean L, Effects of germination on chemical composition, biochemical constituents and antinutritional factors of soya bean seeds. *J Sci Food Agric* **73**:1–9 (1997).
4 Johnson LA, Deyoe CW, Hoover WJ and Schwenke JR, Inactivation of trypsin inhibitors in aqueous soybean extracts by direct steam infusion. *Cereal Chem* **57**:376–379 (1980).
5 Frias J, Diaz-Pollan C, Hedley CL and Vidal-Valverde C, Evolution of trypsin inhibitor activity during germination of lentils. *J Agric Food Chem* **43**:2231–2234 (1995).
6 Vidal-Valverde C, Frias J, Estrella I, Gorospe MJ, Ruiz R and Bacon J, Effect of processing on some antinutritional factors of lentils. *J Agric Food Chem* **42**:2291–2295 (1994).
7 Chang KC and Harrold RL, Changes in selected biochemical components *in vitro* protein digestibility and amino acids in two bean cultivars during germination. *J Food Sci* **53**:783–787 (1988).
8 Schhulze H, Savelkoul FH, Verstegen MW, van der Poel AF, Tamminga S and Groot Nibbelin S, Nutritional evaluation of biological treated white kidney beans (*Phaseolus vulgaris* L.) in pigs: ileal and amino acid digestibility. *J Anim Sci* **75**:3187–3194 (1997).
9 Reddy NR, Sathe SK and Salunkhe DK, Carbohydrates, in *CRC Handbook of World Legumes, Nutritional Chemistry, Processing Technology and Utilization*, Vol 1, ed by Salunkhe DK and Kadam SS. CRC Press, Boca Raton, FL, pp 65–131 (1989).
10 Xu MJ, Zhu MY and Gu Q, Studies on the GLDH activity and synthesis of ascorbic acid in soybean products induced by light. *Acta Nutr Sinica* **2**:212–215 (2002).
11 Ginter E, Ascorbic acid in cholesterol metabolism and in detoxification of xenobiotic substances: problem of optimum vitamin C intake. *Nutrition* **5**:369–374 (1989).
12 Ghosh MK, Mukhopadhyay M and Chatterjee JB, NADPH-initiated cytochrome P450-dependent free iron-independent microsomal lipid peroxidation: specific prevention by ascorbic acid. *Mol Cell Biochem* **166**:457–462 (1997).
13 Jimenez A, Hernandez JA, Ros Barcelo Am Sandalio LM, del Rio LA and Sevilla F, Evidence for the presence of the ascorbate–glutathione cycle in mitochondria and peroxisomes of pea leaves. *Physiol Plant* **104**:689–692 (1997).
14 Nishikimi M and Yagi K, Biochemistry and molecular biology of ascorbic acid biosynthesis, in *Ascorbic Acid: Biochemistry and Biomedical cell Biology*, ed by Harris RJ. Plenum, New York, pp 17–38 (1996).
15 Loewus FA and Loewus MW, Biosynthesis and metabolism of ascorbic acid in plants. *CRC Crit Rev Plant Sci* **5**:101–119 (1987).
16 Davey MW, Montagu MV, Inze D, Sanmartin M, Kanellis A, Smirnoff N, Benzie IJJ, Strain JJ and Flavell D, Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *J Sci Food Agric* **80**:825–850 (2000).
17 Lee YS, Park RD and Rhee CO, Effect of chitosan treatment on growing characteristics of soybean sprouts. *Kor J Food Sci Technol* **31**:153–157 (1999).
18 Kang CK, Yun DW, Kim YK and Choe HT, Determination of minimum concentration and dipping time for inhibition of lateral root and growth stimulation in soybean sprouts as influenced by benzyladenine. *J Kor Soc Hort Sci* **37**:773–776 (1996).
19 Choi YS and Park EH, Effects of food additives application on the decay reduction and growth of soybean sprouts. *Kor Soybean Dig* **13**:1–6 (1996).
20 Park WP and Cho SH, Effect of grapefruit seed extract and ascorbic acid on the spoilage microorganisms and keeping quality of soybean sprouts. *J Kor Soc Food Sci Nutr* **27**:1086–1093 (1998).
21 Choi HD, Kim SS, Kim KT, Lee YJ and Park WM, Effect of presoaking treatments on the growth and rot of soybean sprouts. *Kor J Food Sci Technol* **32**:584–589 (2000).
22 Stasalloa S and Yueng EC, Ascorbic acid metabolism during white spruce somatic embryo maturation and germination. *Physiol Plant* **108**:405–412 (2000).

23 Oba K, Ishikawa S and Nogami K, L-Galactono-γ-lactone dehydrogenase: partial purification, induction of activity and role in the synthesis of ascorbic acid in wounded white potato tuber tissue. *Plant Cell Physiol* **35**:473–478 (1994).
24 Citterio S, Sgorbati S, Scippa S and Sparvoli E, Ascorbic acid effect on the onset of cell proliferation in pea root. *Physiol Plant* **92**:601–607 (1994).
25 Tommasi F, Paciolla C, de Pinto MC and De Gara L, A comparative study of glutathione and ascorbate metabolism during germination of *Pinus pinea* L. seeds. *J Exp Bot* **361**:1647–1654 (2001).
26 Wheeler GL, Jones MA and Smirnoff N, The biosynthetic pathway of vitamin C in higher plants. *Nature* **393**:365–369 (1998).
27 Oba K, Washikawa S, Nwashikawa M, Mizuno H and Yamamoto T, Purification and properties of L-galactono-γ-lactone dehydrogenase, a key enzyme for ascorbic acid biosynthesis, from sweet potato. *J Biochem* **117**:120–124 (1995).
28 Foyer CH, Lelandais M, Edwards EA and Mullineaux P, The role of ascorbate in plants, interactions with photosynthesis, and regulatory significance, in *Active Oxygen/Oxidative Stress and Plant Metabolism*, ed by Pell E and Steffen K. American Society of Plant Physiologists, Rockville, MD, pp 131–144 (1991).
29 Logan BA, Barker DH, Demmig-Adams B and Adams WW, Acclimation of leaf carotenoid composition and ascorbate levels to gradients in the light environment within an Australian rainforest. *Plant Cell Environ* **19**:1083–1090 (1996).
30 Zhao DX, Li MY, Xing JM and Tong Z, Effects of light on cell growth and flavonoids biosynthesis in callus of *Saussurea medusa* Maxim. *Acta Phytophysiol Sinica* **25**:127–132 (1999).