J Food Sci Nutr
Vol 16, p 184~188 (2011)
DOI: 10.3746/jfn.2011.16.2.184

J Food Science and Nutrition

Melatonin and Polyphenol Contents in Some Edible Sprouts (Alfalfa, Chicory, Rape, Red Kale and Sunflower)

- Research Note -

Seok Joong Kim[1†] and Moo Ho Cho[2]

[1]*Department of Food and Nutrition, Dongduk Women's University, Seoul 136-714, Korea*
[2]*Department of Food Science and Technology, Catholic University of Daegu, Gyeongbuk 713-702, Korea*

Abstract

The melatonin, total polyphenol contents, and DPPH radical scavenging activity were determined in alfalfa, chicory, rape, red kale and sunflower after germination for four days at 24±0.1°C. Compared with seeds, melatonin content was increased in all sprouts, at the highest level in red kale (2,502.9 pg/g, 5.6 times higher than seed) followed by rape (2,430.1 pg/g), chicory (2,037.7 pg/g), alfalfa (1,160.8 pg/g) and sunflower (768.2 pg/g) sprout, however, the addition of tryptophan (0.5 mM), the precursor of melatonin synthesis, did not show any desirable effect. Both polyphenol content and DPPH radical scavenging activity were substantially increased in chicory (8.7 mg/g, 66%), rape (10.7 mg/g, 51%) and red kale (11.0 mg/g, 53%) sprouts, but not in alfalfa and sunflower sprouts. Melatonin content per gram polyphenol (ng/g) was also increased in all sprouts through germination. Germination was effective in increasing melatonin in all seeds tested, while its effect on polyphenol content and DPPH radical scavenging activity was species dependent.

Key words: germination, sprout, melatonin, polyphenol, antioxidant

INTRODUCTION

Sprouts have received a lot of attention recently as functional foods because of the high nutritional values obtained during the germination of seeds. In addition to the nutritional compounds, such as free amino acids, reducing and non-reducing sugars, dietary fiber, and vitamins (1,2), sprouts also contain high levels of some bioactive compounds, including sulforaphane in broccoli (3), isoflavone and flavonoid in legumes (4), γ-amino butyric acid and β-sitosterol in brown rice (5), and resveratrol in peanut kernels (6). Antioxidant compounds, such as polyphenols, are regarded as the most desirable bioactive compounds from sprouts (7-10). Their increases during germination are presumed to be for the protection of the developing embryo against the reactive oxygen species (ROS), which are extensively produced by the vigorous aerobic respiration and biochemical metabolism of germination stage (11,12). The removal of anti-nutrients such as trypsin inhibitor and phytates in seeds is an additional benefit of sprout intake as well (13).

Melatonin (*N*-acetyl-5-methoxytryptamine) is a substance with a broad spectrum of functions, such as regulation of the circadian and seasonal rhythms, immunomodulation, anti-inflammation, and antitumor activity (14). Another important functional aspect is melatonin's predominant antioxidant potential (15). Melatonin acts not only as a direct antioxidant to scavenge a variety of ROS and nitrogen species, including hydroxyl radical, hydrogen peroxide, singlet oxygen, nitric oxide, and peroxynitrite anion, but also as an indirect antioxidant to up-regulate the antioxidant enzymes (superoxide dismutase, glutathione peroxidase) and down-regulate the prooxidant enzymes (nitric oxide synthases, lipoxygenases). In addition, due to its amphiphilic structure, this antioxidant can cross physiological barriers to prevent oxidative damage in both lipid and aqueous environments, which is something most other antioxidants can't do (16). While originally isolated from the pineal gland in vertebrates (17), melatonin's wide distribution has since been well documented (14). Even though knowledge of melatonin's actual role in plants is still fragmentary, a greater melatonin content has been reported in plants sources, such as vegetables, seeds, herbs and fruits, than in the blood of animals (18). Plant melatonin behaves similarly to melatonin isolated from vertebrates and can be absorbed into gastrointestinal tract of vertebrates and incorporated in the blood stream (19).

Considering the functionalities and bioavailability of melatonin, plants that can produce high levels of it are a matter of great interest. In this study, we considered germination as a process to improve melatonin content in plant. For this, melatonin contents of five edible sprouts were compared with those of their corresponding

†Corresponding author. E-mail: skim@dongduk.ac.kr
Phone: +82-2-940-4466, Fax: +82-2-940-4466

seeds, and the effect of tryptophan supplementation, a precursor of melatonin synthesis, was investigated. The changes in polyphenol content were also monitored as a source of antioxidant activity.

MATERIALS AND METHODS

Materials

Alfalfa, chicory, rape, red kale and sunflower seeds were obtained from Asia Seed Company (Seoul, Korea) and stored at 4°C until used. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu's phenol reagent, and gallic acid were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). ELISA kit for melatonin analysis was purchased from IBL (Hamburg, Germany). All other chemicals used were of analytical grade available commercially.

Germination for sprout cultivation

For sprout cultivation, seeds were soaked in sterilized tap water for 4 hr at room temperature, drained, and germinated in the dark at 24 ± 0.1°C for 4 days using a commercial sprout cultivator (Asia Seed Company) with a daily change of the sterilized tap water. The tryptophan-treated sprouts were prepared by supplying the sterilized tap water containing 0.5 mM tryptophan. After washing sufficiently with distilled water (DW), the lengths of sprouts were measured with ruler. The washed sprouts were freeze-dried (Labconco Co., Kansas, MI, USA) and grounded into powder with a blender for the further analysis. The control seed powder was also prepared by the same way for comparison.

Determination of melatonin content

The powdered seeds and sprouts (0.5 g) were extracted with 10 mL of 50% ethanol at room temperature for 40 min. The extract was centrifuged at $10,000 \times g$ for 4 min, filtered through 0.2 μm PVDF membrane filter, and 1.5 mL of the supernatant was evaporated to near dryness using a CentriVap concentrator (Labconco Co., Kansas, MI, USA). After dissolving the residue in 0.6 mL of DW by vortexing for 30 sec, further purification and analysis for melatonin were performed using an ELISA kit according to the manufacturer's instructions. That is, 0.5 mL of the reconstituted aliquot was applied on the activated Sep-Pak C_{18} column, centrifuged at $200 \times g$, centrifuged again at $500 \times g$ for 1 min after adding 1 mL of 10% methanol (two times), and eluted with 1 mL methanol to obtain melatonin faction. This fraction was evaporated to dryness, dissolved in 0.15 mL of DW, and pipetted in 50 μL into 96 well microtiter plate coated with goat-anti-rabbit antibody. Fifty μL of melatonin-biotin and 50 μL of antiserum were added to the well in that order, incubated at 4°C for 15 hr, and washed three times with 0.25 mL of diluted assay buffer. After 0.15 mL of anti-biotin-alkaline phosphatase solution was added and incubated at room temperature for 2 hr, the wells were washed three times with 0.25 mL of diluted assay buffer, 0.2 mL of *p*-nitrophenyl phosphate solution was added, and the samples incubated for 20 min on an orbital shaker at 500 rpm. Absorbance at 405 nm (reference wavelength; 605 nm) was measured after stopping the reaction by adding 50 μL of 1 N NaOH to estimate melatonin content which was calculated using a standard curve prepared with standard melatonin solution of 0 to 300 pg/mL content. Melatonin content of sample was expressed as pg melatonin/g powder.

Determination of total polyphenol content

Total polyphenol content of samples was measured as gallic acid equivalents (mg gallic acid/g powder) according to the method of Singleton et al. (20) with some modification. Briefly, the powdered seeds and sprouts (0.1 g) were suspended in 80% methanol (1 mL) and extracted with vortexing for 2 hr at room temperature. The extract was centrifuged (Model 5810R, Eppendorf, Hamburg, Germany) at $10,000 \times g$ for 10 min, filtered through 0.2 μm PVDF membrane filter (Alltech, Deerfield, IL, USA). An aliquot (50 μL) of the extract was diluted with DW (1.95 mL) and added to 0.2 mL of 2 N Folin–Ciocalteu's phenol reagent. After standing for 3 min at room temperature, 0.4 mL of saturated Na_2CO_3 solution and 1.4 mL of DW was added in order, mixed well, and kept in the dark at room temperature for 1 hr. Absorbance was measured at 765 nm using a spectrophotometer (Genesys 10UV, Thermo Electron Co., Madison, WI, USA).

Determination of DPPH radical scavenging activity

The antioxidant activity of samples was evaluated with a reducing power assay using DPPH as free radical substrate and expressed as free radical scavenging activity (21). An aliquot (50 μL) of the extract prepared in the determination of total polyphenol content was added to 3 mL of 0.1 mM DPPH radical methanol solution of which absorbance at 525 nm reached 0.95 to 0.99. The reaction mixture was shaken vigorously, stored in the dark at room temperature for 30 min, and the absorbance at 525 nm was measured. The DPPH radical scavenging activity was calculated as follows: DPPH radical scavenging activity (%) = $[1 - (\text{absorbance}_{\text{sample}} / \text{absorbance}_{\text{control}})] \times 100$. Control used 50 μL of 80% methanol instead of the extract.

Statistical analysis

All determinations were performed in at least triplicate

except for melatonin determined in duplicate, and means ±standard deviations were reported. Data were analyzed for significance by one-way ANOVA in combination with the Duncan's multiple range test ($p<0.05$) using SPSS software package (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Melatonin content

Seeds are rich source of plant melatonin (18). Because seeds are in a dormant state in which enzyme activities are poor and not up-regulated, and because seeds are generally rich in lipids, melatonin is presumed to act in a protective role in seeds against the oxidative damage induced by ultraviolet light, temperature, toxicants, and drought (22,23). Among seeds tested by ELISA, alfalfa seed had the highest melatonin content (703.4 pg/g) followed by chicory (580.2 pg/g), rape (561.5 pg/g), red kale (444.1 pg/g), and sunflower (204.3 pg/g), as shown in Fig. 1. Such amounts, however, were comparatively lower than those reported by Manchester et al. (22) which were 20 ng/g in alfalfa seed and 16 ng/g in sunflower seed. Recent studies (18,23) indicated that melatonin content in plants had greater variation than melatonin content in animals. The disparity of plant melatonin content could be explained by the following reasons: first, plants require relatively harsh destruction and extraction processes for melatonin analysis, which may result in varying amounts of melatonin being isolated, second, melatonin in plant extracts is rather unstable, which could cause variations in melatonin quantification, and third, even with popular methods used for melatonin quantification, structurally similar compounds present in plant could be co-eluted in HPLC analysis and show cross-reactivity in immunoassays like ELISA. Therefore, data disparity between our study and previous studies could stem from the differences of the extraction and analytical methods, as well as the species differences. While we adapted ELISA after 50% ethanol extraction followed by the further purification through C_{18} column, Manchester et al. (22) used the radioimmunoassay (RIA) and HPLC-electrochemical detection assay after 100% ethanol extraction.

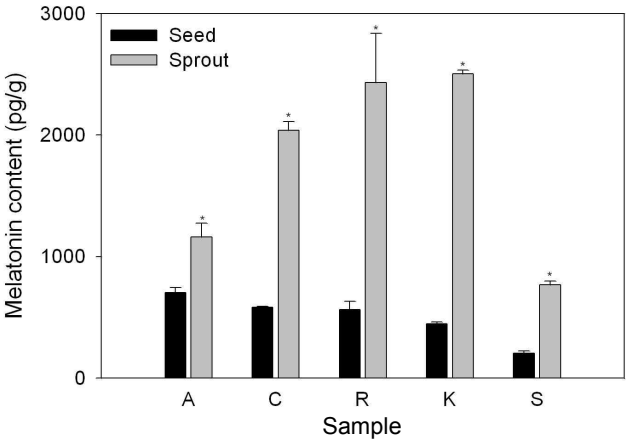

Fig. 1. Melatonin content of seeds and sprouts. A, alfalfa; C, chicory; R, rape; K, red kale; S, sunflower. Melatonin content was expressed as pg melatonin per g powder of sample. Each value represents the mean±standard deviation, and * indicates that the mean values of sprouts are significantly different at $p<0.05$ from those of the corresponding seeds.

When sprouts were prepared by 4 days germination at 24±0.1°C, the usual condition for edible sprout preparation, melatonin content was substantially increased in all sprouts compared to the corresponding seeds at the highest level in red kale sprouts (2,502.9 pg/g, 5.6 times than seed) followed by rape (2,430.1 pg/g), chicory (2,037.7 pg/g), alfalfa (1,160.8 pg/g) and sunflower (768.2 pg/g) sprouts as shown in Fig. 1. The higher melatonin content in sunflower sprouts was reported in our previous study, too (24). Based on the five kinds of seeds used in this study, we carefully conclude that germination could be a valuable process to enrich melatonin.

As a possible means to increase melatonin levels in sprouts, tryptophan supplement during germination was considered because tryptophan is known as a precursor for melatonin formation in plants, just like in animals (25). Treatment of 0.5 mM tryptophan, however, showed no significant effect (Table 1).

Table 1. Effect of tryptophan treatment on length and melatonin content in sprouts

Sample[1)]	Sprout length (mm)		Melatonin content (pg/g)	
	Con[2)]	Trp[3)]	Con	Trp
A	54.5±12.1	49.8±7.6	1160.8±113.5	1281.2±75.9
C	39.3±5.8	40.6±4.8	2037.7±73.5	1662.2±85.6*
R	42.2±9.4	50.3±8.3	2430.2±405.1	2522.3±110.4
K	44.1±8.3	58.2±10.4	2502.9±32.2	2403.6±185.6
S	168.5±61.5	94.4±40.6	768.2±28.8	610.6±74.0*

[1)]A, alfalfa; C, chicory; R, rape; K, red kale; S, sunflower.
[2)]Con, sprout without tryptophan treatment. [3)]Trp, sprout with tryptophan treatment at 0.5 mM.
Each value represents the mean±standard deviation, and * indicates that the mean values of sprouts treated with 0.5 mM tryptophan are significantly different at $p<0.05$ from those of the corresponding control.

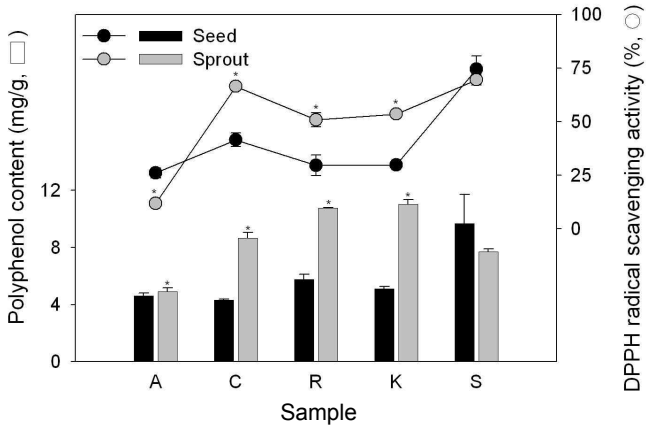

Fig. 2. Total polyphenol content and DPPH radical scavenging activity of seeds and sprouts. A, alfalfa; C, chicory; R, rape; K, red kale; S, sunflower. Total polyphenol content was expressed as mg gallic acid equivalents per g powder of sample. Each value represents the mean±standard deviation, and * indicates that the mean values of sprouts are significantly different at $p<0.05$ from those of the corresponding seeds.

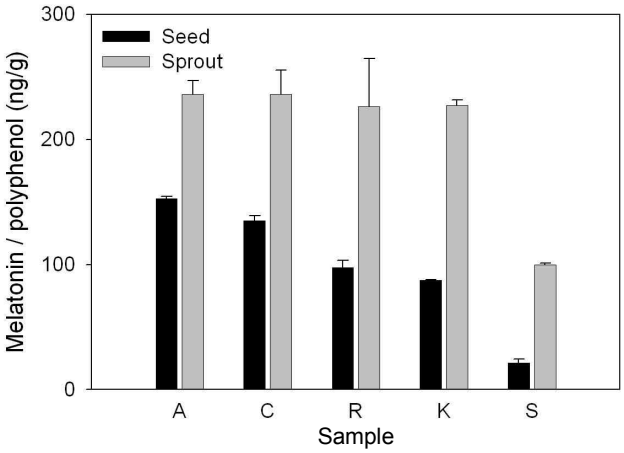

Fig. 3. Melatonin/polyphenol ratio in seeds and sprouts. A, alfalfa; C, chicory; R, rape; K, red kale; S, sunflower. Content rate was expressed as ng melatonin per g polyphenol. Each value represents the mean±standard deviation.

Total polyphenol content and DPPH radical scavenging activity

The total polyphenol contents of seeds and sprouts are shown in Fig. 2. Among the seeds, the highest polyphenol content (9.9 mg/g) was found in sunflower while other seeds were in the range of 4.4 to 5.8 mg/g. When seeds were germinated for 4 days, considerable increases of total polyphenol content were noticed in chicory (8.7 mg/g), rape (10.8 mg/g) and red kale (11.0 mg/g) sprouts, however, alfalfa and sunflower sprouts showed only trivial changes. According to our DPPH radical-scavenging activity results, the antioxidant ability of the seed extracts did not necessarily correlate with total polyphenol content (Fig. 2), even though their close correlation has been generally recognized elsewhere (7). In other words, even with greater total polyphenol content, rape and red kale sprouts showed the lower DPPH radical scavenging activities (51% and 53% inhibition, respectively) than chicory and sunflower sprouts (66% and 70% inhibition, respectively). This could be due to the diversity of polyphenol composition and/or the presence of other antioxidants exhibiting DPPH radical scavenging activity depending on species. Lin and Lai (9) reported flavonoid content rather than polyphenol content had close correlation with DPPH scavenging activity in legumes. Kim et al. (10) suggested ascorbic acid could contribute to overall antioxidant activity together with polyphenol in buckwheat sprout. Sattler et al. (26) have also reported that vitamin E is an essential antioxidant during germination.

Seeds often contain the highest lipid content among plant tissues, with high levels of polyunsaturated fatty acids (27). During germination, this storage lipid could be metabolized by two extremely activated processes, β-oxidation and gluconeogenesis for energy and fixed carbon, which are likely sources of the elevated ROS (11,28). As excessive ROS can cause the oxidative damages leading to cell and/or organism injury (11), an increase of melatonin and/or polyphenol are presumed to control ROS overproduced during germination (11,12). However, a low level of ROS is necessary for regulating plant growth and development, programmed cell death, and stress response (29). Notably, the relative increase in melatonin compared to polyphenol (as measured by melatonin content per gram polyphenol) was seen during the germination process of all the sprouts (Fig. 3). This phenomenon could be explained by the rather diverse roles of melatonin in plant kingdom. Besides a possible role for melatonin as an antioxidant during germination, the recent prospective studies (18,23) have suggested that it might act as a night signal, coordinating responses to diurnal and photoperiodic environmental cues in plants similarly as in animals, and an independent growth regulator analogous to indole acetic acid.

The reason that tryptophan supply didn't affect the melatonin content might be due to the fact that the seeds required no further physiological requirement of melatonin under our germination conditions; however this observation requires further study.

In spite of the recent availability of exogenous melatonin as a supplement for treating sleep disorders and as an antioxidant, a whole diet containing natural melatonin is expected to be still worthwhile for human health, judging from the scientific consensus that the whole diet has a larger impact on health than one single food component; with a whole diet, there are additive and synergic

effects of a variety of phytochemicals (30). Together with their known nutritional values, sprouts with high amounts of natural melatonin and polyphenols could prove to be a valuable functional food, and germination could be the potential process for even greater health benefits.

ACKNOWEDGEMENTS

This study was supported by the Technology Development Program for Agriculture and Forestry, the Ministry of Agriculture and Forestry (204010-03-3-HD110), Korea.

REFERENCES

1. Chavan JK, Kadam SS. 1989. Nutritional improvement of cereals by sprouting. *Crit Rev Food Sci Nutr* 28: 401-437.
2. Sattar A, Shah A, Zeb A. 1995. Biosynthesis of ascorbic acid in germinating rapeseed cultivars. *Plant Food Human Nutr* 47: 63-70.
3. Fahey JW, Zhang Y, Talalay P. 1997. Broccoli sprouts: An exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proc Natl Acad Sci USA* 94: 10367-10372.
4. Lv Q, Yang Y, Zhao Y, Gu D, He D, Yili A, Ma Q, Cheng Z, Gao Y, Aisa HA, Ito Y. 2009. Comparative study on separation and purification of isoflavones from the seeds and sprouts of chickpea by HSCCC. *J Liq Chromatogr Relat Technol* 32: 2879-2892.
5. Kum JS, Choi BK, Lee HY, Park JD. 2004. Physicochemical properties of germinated brown rice. *Korean J Food Preserv* 11: 182-188.
6. Wang KH, Lai YH, Chang JC, Ko TF, Shyu SL, Chiou RYY. 2005. Germination of peanut kernels to enhance resveratrol biosynthesis and prepare sprouts as a functional vegetable. *J Agric Food Chem* 53: 242-246.
7. Paśko P, Bartoń H, Zagrodzki P, Gorinstein S, Fołta M, Zachwieja Z. 2009. Anthocyanins, total polyphenols and antioxidant activity in amaranth and quinoa seeds and sprouts during their growth. *Food Chem* 115: 994-998.
8. Randhir R, Lin YT, Shetty K. 2004. Stimulation of phenolics, antioxidant and antimicrobial activities in dark germinated mung bean sprouts in response to peptide and phytochemical elicitors. *Process Biochem* 39: 637-647.
9. Lin PY, Lai HM. 2006. Bioactive compounds in legumes and their germinated products. *J Agric Food Chem* 54: 3807-3814.
10. Kim SJ, Zaidul ISM, Suzuki T, Mukasa Y, Hashimoto N, Takigawa S, Noda T, Matsuura-Endo C, Yamauchi H. 2008. Comparison of phenolic compositions between common and tartary buckwheat (*Fagopyrum*) sprouts. *Food Chem* 110: 814-820.
11. Perl-Treves R, Perl A. 2002. Oxidative stress: an introduction. In *Oxidative Stress in Plants*. Inzé D, van Montagu M, eds. Taylor & Francis Inc., London, UK. p 1-32.
12. Wojtyla Ł, Garnczarska M, Zalewski T, Bednarsk W, Ratajczak L, Jurga S. 2006. A comparative study of water distribution, free radical production and activation of antioxidative metabolism in germinating pea seeds. *J Plant Physiol* 163: 1207-1220.
13. Mwikya SM, Camp JV, Rodrigez R, Huyghebaet A. 2001. Effects of sprouting on nutrient and antinutrient composition of kidney beans (*Phaseolus vulgaris* var. Rose coco). *Eur Food Res Technol* 212: 188-191.
14. Hardeland R, Pandi-Perumal SR, Cardinali DP. 2006. Melatonin. *Int J Biochem Cell Biol* 38: 313-316.
15. Bonnefont-Rousselot D, Collin F. 2010. Melatonin: Action as antioxidant and potential applications in human disease and aging. *Toxicology* 278: 55-67.
16. Reiter RJ, Tan DX, Gitto E, Sainz RM, Mayo JC, Leon J, Manchester LC, Kilic VE, Kilic U. 2004. Pharmacological utility of melatonin in reducing oxidative cellular and molecular damage. *Pol J Pharmacol* 56: 159-170.
17. Lerner AB, Case JD, Takahashi Y, Lee TY, Mori W. 1958. Isolation of melatonin, the pineal gland factor that lightens melanocytes. *J Am Chem Soc* 80: 2587.
18. Paredes SD, Korkmaz A, Manchester LC, Tan DX, Reiter RJ. 2009. Phytomelatonin: a review. *J Exp Botany* 60: 57-69.
19. Hattori A, Migitaka H, Iigo M, Itho M, Yamamoto K, Ohtani-Kaneko R, Hara M, Suzuki T, Reiter RJ. 1995. Identification of melatonin in plants and its effects on plasma melatonin levels and binding to melatonin receptors in vertebrates. *Biochem Mol Biol Int* 35: 627-634.
20. Singleton VL, Joseph A, Rossi J. 1965. Colorimetry of total phenolics with phosphomolibidic-phosphotungstic acid reagent. *Am J Enol Vitic* 16: 144-158.
21. Blois MS. 1958. Antioxidant determination by the use of a stable free radical. *Nature* 181: 1199-1201.
22. Manchester LC, Tan DX, Reiter RJ, Park W, Monis K, Qi W. 2000. High levels of melatonin in the seeds of edible plants: possible function in germ tissue protection. *Life Sci* 67: 3023-3029.
23. Posmyk MM, Janas KM. 2009. Melatonin in plants. *Acta Physiol Plant* 31: 1-11.
24. Cho MH, No HK, Prinyawiwatkul W. 2008. Chitosan treatments affect growth and selected quality of sunflower sprouts. *J Food Sci* 73: S70-S77.
25. Murch SJ, KrishnaRaj S, Saxena PK. 2000. Tryptophan is a precursor for melatonin and serotonin biosynthesis in *in vitro* regenerated St. John's wort (*Hypericum perforatum* L. cv. Anthos) plants. *Plant Cell Rep* 19: 698-704.
26. Sattler SE, Gilliland LU, Magallanes-Lundback M, Pollard M, DellaPenna D. 2004. Vitamin E is essential for seed longevity and for preventing lipid peroxidation during germination. *Plant Cell* 16: 1419-1432.
27. Mansfield SG, Briarty LG. 1992. Cotyledon cell-development in *Arabidopsis thaliana* during reserve deposition. *Can J Bot* 70: 151-164.
28. Schopfer P, Plachy C, Frahry G. 2001. Release of reactive oxygen intermediates (superoxide radicals, hydrogen peroxide, and hydroxyl radicals) and peroxidase in germinating radish seeds controlled by light, gibberellin, and abscisic acid. *Plant Physiol* 125: 1591-1602.
29. Gechev TS, van Breusegem F, Stone JM, Denev I, Laloi C. 2006. Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. *Bioessays* 28: 1091-1101.
30. Jacobs DR Jr, Steffen LM. 2003. Nutrients, foods, and dietary patterns as exposures in research: a framework for food synergy. *Am J Clin Nutr* 78: 508S-513S.

(Received May 4, 2011; Accepted June 2, 2011)