Chitosan Treatments Affect Growth and Selected Quality of Sunflower Sprouts

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ABSTRACT: The effects of chitosan molecular weights, solvent types, and concentrations of chitosan solution, and seed soaking times on growth and selected quality of sunflower sprouts were investigated. Among 5 chitosans tested (746, 444, 223, 67, and 28 kDa), 28 kDa chitosan exhibited the highest DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity both at 0.1% and 1.0% concentrations. Optimal conditions selected for cultivation of sunflower sprouts involved soaking seeds in 0.5% chitosan with 28 kDa (dissolved in 0.5% lactic acid) for 18 h. After cultivation for 6 d at 20 °C, sunflower seeds soaked in chitosan solution for 18 h under the optimal conditions yielded sprouts with 12.9% higher total weight and 16.0% higher germination rate, compared with those of seeds soaked in water for 18 h (control). Furthermore, the total amino acid content of the former sprouts (12098 mg/100 g) was slightly higher than that of the latter (12057 mg/100 g). Sprouting of sunflower seeds improved DPPH radical scavenging activity, probably due to the increased total phenolic, melatonin, and total isoflavone contents. Similarly, chitosan-treated sprouts exhibited slightly improved DPPH radical scavenging activity, probably due to slightly increased total phenolic and melatonin contents, and moderately increased total isoflavone content compared with those of the control. Chitosan treatment increased the total isoflavone content of sprouts by 11.8%, due mainly to the increased daidzein content, compared with that of the control.

Keywords: antioxidant activity, chitosan, isoflavone, sprouts, sunflower

Introduction

In recent years, increasing attention has been paid to the role of diet in human health. Several epidemiological studies have indicated that a high intake of plant products is associated with a reduced risk of a number of chronic diseases, such as atherosclerosis and cancer (Hashimoto and others 2002; Kris-Etherton and others 2002; Gosslau and Chen 2004). These beneficial effects have been partly attributed to the compounds that possess antioxidant activity (Podsędek 2007). The major antioxidants of vegetables are vitamins C and E, carotenoids, and phenolic compounds, especially flavonoids (Podsędek 2007).

Sunflower (*Helianthus annuus* L.) is one of the most widely cultivated oil crops and ranks second as a source of vegetable oil in the world (Balasaraswathi and Sadasivam 1997; Canibe and others 1999). Sunflower seeds are an excellent source of vitamin E and polyunsaturated fatty acids (Sankar and others 2005). These natural antioxidants and polyunsaturated fatty acids show protective function against hypertension and cardiovascular disease (Binkoski and others 2005; Sankar and others 2005). In preliminary studies from our laboratory, we found that sunflower sprouts showed higher antioxidant activity than sunflower seeds, due mainly to the increased antioxidant compounds during sprouting. In view of increased interests in healthy foods from a natural origin, increasing utilization of sunflower sprouts in human foods appears realistic. Earlier, Balasaraswathi and Sadasivam (1997) investigated changes in oil, sugars, and nitrogenous components during germination of sunflower seeds. Lee (1999) analyzed proximate composition, vitamins, and minerals in commercially available sunflower sprouts. To date, however, no further reported information is available on the cultivation of sunflower sprouts.

Chitosan is a natural biopolymer derived by deacetylation of chitin, a major component of the shells of crustaceans such as crab, shrimp, and crawfish. Recently, chitosan and its oligomers have attracted notable interest due to their biological activities, including antimicrobial (Kendra and Hadwiger 1984; Sudarshan and others 1992; No and others 2002), antitumor (Suzuki and others 1986; Tokoro and others 1988), antioxidative (Youn and others 2001; Park and others 2004), and hypocholesterolemic functions (Sugano and others 1992). In addition, chitosan treatment has been shown to stimulate plant growth (Kim 1998) and to improve storability of postharvest fruits and vegetables (El Ghaouth and others 1991, 1992).

Recent studies have revealed that chitosan treatment is effective in enhancement of yield and quality of soybean sprouts. For example, Lee and others (1999) and Choi and others (2000) found that chitosan treatment increased growth rate and decreased the rot of soybean sprouts. More recently, No and others (2003) demonstrated that chitosan treatment increased the total weight of soybean sprouts by 13% and vitamin C content by about 10% compared with those of control. Therefore, it was realized that chitosan may be employed for cultivation of sunflower sprouts for enhancement of yield and quality. However, to effectively utilize chitosan for such application, basic information is needed on how different molecular-weight chitosans and various cultivation conditions affect growth and quality of sunflower sprouts.

The objective of the study was to examine the effect of different chitosan treatments on growth and selected quality of sunflower sprouts. For this, 4 separate cultivation treatments were sequentially conducted to establish optimal conditions for molecular weights, concentrations, and solvents of chitosan to be used as well

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as seed soaking times. For quality evaluation, proximate and amino acid composition and antioxidant activity of sunflower sprouts cultivated under optimal conditions were determined.

Materials and Methods

Materials

Three acid-soluble chitosans [746, 444, and 223 kDa molecular weight (MW)] were purchased from Kitto Life (Seoul, Korea), and 2 water-soluble chitosans (MW = 67 and 28 kDa) from Keumho Chemical (Seoul, Korea). All chitosans were in a powder form and prepared from crab leg shell. Sunflower seeds were obtained from Dongbu Hannong Chemicals (Hayang, Korea) and stored at 4 °C until used. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu's phenol reagent, and gallic acid were purchased from Sigma-Aldrich Co. (St. Louis, Mo., U.S.A.). Melatonin ELISA was purchased from IBL (Hamburg, Germany). Isoflavone standards were purchased from LC Laboratories (Woburn, Mass., U.S.A.) and Wako Pure Chemical Ind. (Osaka, Japan). All other chemicals used were of analytical or HPLC grade available commercially.

Preparation of chitosan solutions

Chitosan solution was prepared in 1% (v/v) organic acid (acetic, lactic, or ascorbic acid) at 1% (w/v) concentration and diluted with water to give a final chitosan concentration of 0.01%, 0.05%, 0.1%, or 0.5%. All solutions were freshly prepared before applied to sunflower seeds.

Cultivation of sunflower sprouts

Four separate treatments were sequentially conducted at different time intervals. In treatment 1 (selection of the most effective MW of chitosan), sunflower seeds (48 g, approximately 740 seeds) were washed with water, drained, and soaked in 240 mL of different MW chitosan solutions (0.1% in 0.1% acetic acid) for 18 h in the incubator at 25 °C. Sunflower seeds soaked in 240 mL of water (control 1) and in 240 mL of 0.1% acetic acid (control 2) for 18 h at 25 °C were used as the control groups. Following this step, sunflower seeds were drained, divided into 4 equal parts on a wet weight basis, and placed in a commercial soybean sprout cultivator (Daechun Anypass, Gyunggi-do, Korea) consisting of 4 separate trays, and cultivated in the dark at room temperature (20 ± 2 °C) for 6 d. Water was automatically sprayed for 4 min on the sunflower seeds/sprouts every 20 min.

In treatment 2 (selection of the most effective soaking time), sunflower seeds (48 g) were soaked in 240 mL of chitosan (28 kDa) solutions (0.1% in 0.1% acetic acid) for 0, 6, 12, 18, or 24 h at 25 °C and then cultivated as mentioned previously.

In treatment 3 (selection of the most effective chitosan solvent), sunflower seeds (48 g) were soaked in 240 mL of chitosan (28 kDa) solutions (0.1% in 0.1% acetic, lactic or ascorbic acid, or in water) and/or in 240 mL of water (control) for 18 h at 25 °C and then cultivated as mentioned previously.

In treatment 4 (selection of the most effective chitosan concentration), sunflower seeds (48 g) were soaked in 240 mL of different concentrations of chitosan (28 kDa) solutions (0.01%, 0.05%, 0.1%, or 0.5% in corresponding lactic acid concentration) and/or in 240 mL of water (control) for 18 h at 25 °C, and then cultivated as mentioned previously. From these 4 cultivation treatments, the optimal conditions for cultivating sunflower sprouts were selected.

Measurement of growth

Total weight of sunflower sprouts in each of the 4 trays was measured with a balance after 6 d of cultivation. For measurement of length and thickness of hypocotyl, 40 sunflower sprouts were randomly sampled from each tray. Length was measured with a ruler and thickness with a caliper (CD-20B, Mitutoyo, Kawasaki, Japan). Germination rate (%) was calculated as (number of germinated sunflower sprouts [> 1 cm]/total number of sunflower seeds) × 100.

Proximate analysis of sunflower sprouts

Moisture and ash contents were determined by standard AOAC methods 930.15 and 942.05, respectively (AOAC 1990). Fat was determined using a Soxhlet extractor (Sox 416, Gerhardt, Germany). Nitrogen was determined using an elemental analyzer (EA 1110, CE Instruments, Rodano-Milan, Italy). Crude protein was calculated by multiplying nitrogen content of the sample by 6.25.

Amino acid analysis of sunflower sprouts

Freeze-dried sunflower sprouts (0.1 g) were hydrolyzed with 6 N HCl for 24 h at 110 °C in sealed tubes. The hydrolysates were filtered, evaporated to near dryness, and dissolved in 20 mL of sodium citrate buffer of pH 2.2. An aliquot was filtered through a cartridge filter (0.2 μm, Sartorius) and analyzed by an amino acid analyzer (Biochrom 30, Pharmacia Biotech Ltd., Cambridge, U.K.).

Determination of DPPH radical scavenging activity

DPPH free radical scavenging activity of sunflower seeds/sprouts and chitosan was determined by the method of Blois (1958) with some modifications. Sunflower seeds or sprouts were washed with distilled water, freeze-dried (Labconco Co., Kansas, Mich., U.S.A.), and ground with a blender (MCH600SI, Dong Yang Magic, Seoul, Korea). The ground powder sample (0.1 g) was extracted with 1 mL of 80% methanol using a vortex mixer at room temperature for 2 h. The extract was centrifuged (Model 5810 R, Eppendorf, Hamburg, Germany) at 10000 × *g* for 10 min, filtered through a 0.2 μm PTFE membrane filter paper (Alltech, Deerfield, Ill., U.S.A.), and then diluted 100 times with methanol. An aliquot (0.2 mL) of the extract was added to 3 mL of 0.1 mM DPPH radical methanolic solution. In the case of chitosan, 0.2 mL of chitosan solution (1.0% and 0.1% in 1.0% and 0.1% acetic acid, respectively) was added to 3 mL of 0.1 mM DPPH radical methanolic solution. The reaction mixture was shaken vigorously, stored in the dark at room temperature for 30 min, and the absorbance measured at 517 nm using a spectrophotometer (Ultraspec® 1000, Pharmacia Biotech Co.). The free radical scavenging activity was calculated by the following equation: scavenging activity (%) = [1 – (absorbance$_{sample}$/absorbance$_{control}$)] × 100.

Determination of total phenolic content

Total phenolic content in sunflower seeds/sprouts was estimated as gallic acid equivalents following the method described by Singleton and others (1965). The extract of sunflower seeds or sprouts prepared, as described in the determination of DPPH radical scavenging activity, was centrifuged at 10000 × *g* for 10 min, filtered through a 0.2 μm PTFE membrane filter paper, and then diluted 100 times with water. An aliquot (1.0 mL) of the extract was added to 1.0 mL of 0.2 N Folin–Ciocalteu's phenol reagent. After standing for 3 min at room temperature, 1.0 mL of saturated Na_2CO_3 (75 g/L) was added and kept in the dark for 1 h at room temperature. Absorbance was measured at 765 nm using a spectrophotometer (Ultraspec® 1000, Pharmacia Biotech Co.) and the results expressed as mg of gallic acid/mL.

Determination of melatonin

Sunflower seeds or sprouts were washed with distilled water, freeze-dried, and ground with a blender. The ground powder sample (0.5 g) was extracted with 10 mL of 50% ethanol in a water bath at 30 °C for 40 min. The extract was centrifuged at 10000 × *g* for 4 min and 1.5 mL of the supernatant were evaporated to near dryness using a CentriVap concentrator (Labconco Co.). The residue was dissolved in 0.6 mL of deionized water by vortexing for 30 s. A 0.5 mL aliquot was used for analysis using a melatonin ELISA kit (IBL, Hamburg, Germany), following the manufacturer's instructions.

Determination of isoflavone

The HPLC analysis method (Song and others 1998; Murphy and others 1999) was used to separate and quantify the individual isoflavone moieties. The extract of sunflower seeds or sprouts prepared as described in the determination of DPPH radical scavenging activity was centrifuged at 10000 × g for 10 min and then filtered through a 0.2 μm PTFE membrane filter paper. Isoflavones were separated using an Inertsil® ODS-3 LC-18 column (5 μm, 4.6 × 250 mm; GL Sciences Inc., Tokyo, Japan) on Waters HPLC (Model 510, Milford, Mass., USA) with an UV/VIS detector (UV-2077 Plus, JASCO Co., Tokyo, Japan). Absorbance maximums at 249 and 261 nm were monitored for the quantification of 6″-*O*-acetyldaidzin, 6″-*O*-malonyldaidzin, daidzin, daidzein (249 nm), 6″-*O*-acetylgenistin, 6″-*O*-malonylgenistin, genistin, genistein, 6″-*O*-acetylglycitin, 6″-*O*-malonylglycitin, glycitin, and glycitein (261 nm).

Statistical analysis

Experiments on cultivation of sunflower sprouts were carried out in quadruplicate and means ± standard deviations (or standard errors of mean) were reported. Chemical analyses were determined in triplicate and means ± standard deviations were reported, except for amino acids determined in duplicate. Data were analyzed using analysis of variance (ANOVA), followed by the Duncan's multiple range test using the SPSS (SPSS Inc., Chicago, Ill., U.S.A.) software package.

Results and Discussion

DPPH radical scavenging activity of different molecular-weight chitosans

For the relative comparison purpose, all chitosans were dissolved in acetic acid. The DPPH radical scavenging activity of chitosans was not proportional to their molecular weight (MW) as shown in Figure 1. However, the scavenging activity of chitosan generally increased with decreasing MW and increasing concentration from 0.1% to 1.0%. Among 5 chitosans tested, 28 kDa chitosan exhibited the highest scavenging activity at both concentrations. At 1% concentration, the scavenging activity of the 28 kDa chitosan was about 20 times higher than that of the 746 kDa chitosan. Thus, the radical scavenging activity of 28 kDa chitosan may play a role in the antioxidant activity of sunflower sprouts.

The scavenging activity of chitosan may be due to the reaction between the free radicals and the hydrogen ion from the ammonium ions (NH_3^+) to form a stable molecule. The NH_3^+ ions are formed by the amino groups of chitosan absorbing a hydrogen ion from the solution (Xie and others 2001). Park and others (2004) suggested that the scavenging activity of chitosan depended on its degree of deacetylation (DD). Chitosan with a higher DD has better scavenging activity, thereby suggesting the action of nitrogen at the C-2 position in elimination of free radicals.

Previous workers have also revealed that the DPPH radical scavenging activity of chitosan increased with decreasing MW and increasing concentration (Rao and others 2005; Chien and others 2007; Kim and Thomas 2007; Yen and others 2007). For example, Kim and Thomas (2007) found that the scavenging activity (40% to 100%) of 30 kDa chitosan was superior to those (9% to 37%) of 90 and 120 kDa chitosans in the concentration range of 0.2% to 1.0%. For this, these authors explained that the 120 kDa chitosan would have lower mobility than the lower MW chitosans. Consequently, this would increase the possibility of inter- and intramolecular bonding of the high MW chitosan molecules, and thus the chance of exposure of their amine groups might be restricted. This may explain our findings in that scavenging activity of chitosans

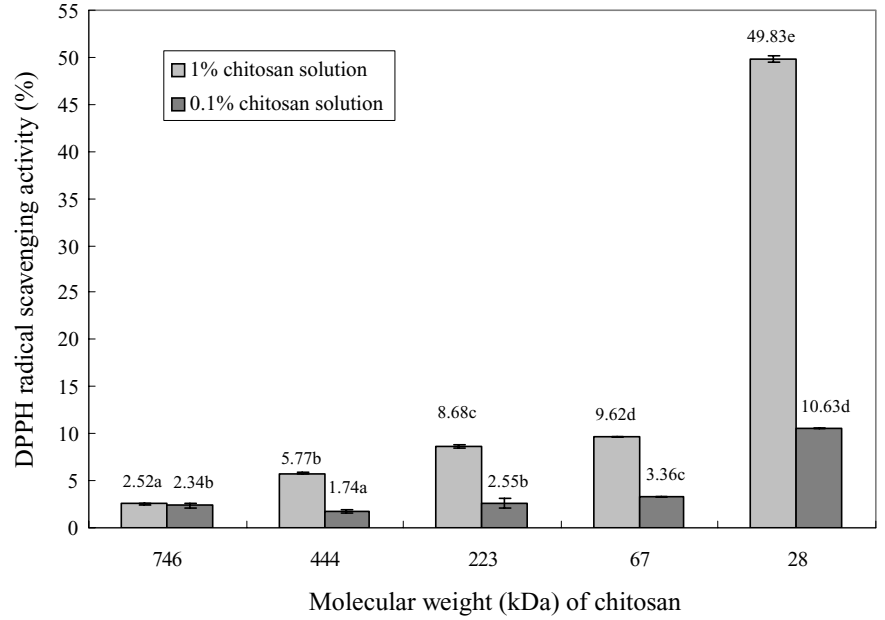

Figure 1—DPPH radical scavenging activity of different molecular-weight chitosans. For each chitosan concentration, bars/means (of triplicate determinations) with different letters indicate significant differences ($P < 0.05$).

generally increased with decreased MW (Figure 1). Furthermore, Kim and Thomas (2007) reported that the increased scavenging activity of 30 kDa chitosan with increasing concentration was attributed to the increased total amine groups responsible for scavenging more radicals. In the study of Kim and Thomas (2007), however, the 120 kDa chitosan showed no significant differences in scavenging activity at various concentrations.

Effects of different molecular-weight chitosans on growth of sunflower sprouts (treatment 1)

The effects of different molecular-weight (MW) chitosans on growth of sunflower sprouts cultivated for 6 d at room temperature (20 ± 2 °C) are shown in Table 1. The results indicated that chitosan treatment, irrespective of MW, increased the total weight (by 4.6% to 7.1% compared with that of the control 1), germination rate, and length and thickness of hypocotyl of sunflower sprouts, compared with the control 1 (water) and control 2 (acetic). Among 5 chitosans tested, no significant differences in total weight and germination rate were observed. These results generally demonstrate that chitosan treatment is effective in increasing the total weight and germination rate of sunflower sprouts and that the effectiveness of treatment was not affected by MW of chitosan.

Treatment with 0.1% acetic acid (control 2) resulted in a lower total weight and germination rate than treatment with water (control 1). As shown in Table 1, the acetic acid treatment (control 2) did not affect the length and thickness, and therefore the decreased total weight was due mainly to the decreased germination rate. The length and thickness of hypocotyl among 5 chitosan treatments were comparable, with a few exceptions. Thus, insignificant differences in the total weight among 5 chitosan treatments were probably due to their comparable germination rate, and length and thickness of hypocotyl (Table 1).

There is little information available on the effect of chitosan treatment on the growth of sunflower sprouts. In studies with soybean sprouts, No and others (2003) observed that chitosan (0.1% in 0.1% acetic acid) treatment, irrespective of MW, increased the total weight by 11% to 29% compared with the control (water). Among 5 chitosans (22, 59, 224, 493, and 746 kDa) tested, however, the most effective increase in total weight was achieved by treatment with 493 kDa chitosan. Other previous researchers also observed increase in weight (Lee and others 1999; Choi and others 2000) and hypocotyl length (Suh and others 1996; No and others 2003) of soybean sprouts by chitosan treatment.

Data from Figure 1 and Table 1 suggest that among the 5 different MW chitosans tested, use of 28 kDa chitosan may be advantageous in cultivating sunflower sprouts with higher DPPH radical scavenging activity than other MW chitosans. Based on these results, all subsequent studies were conducted with 28 kDa chitosan.

Effects of seed soaking time on growth of sunflower sprouts (treatment 2)

The effects of seed soaking times in chitosan solution on growth of sunflower sprouts cultivated for 6 d at 20 ± 2 °C are shown in Table 2. Soaking in chitosan solution, irrespective of soaking time (6, 12, 18, and 24 h), increased the total weight by 6.4% to 17.1% compared with that of no soaking (0 h). The total weight and germination rate gradually increased with soaking time, and reached the highest values at 18 h. Further increase in soaking time from 18 to 24 h rather resulted in decreased total weight, due mainly to the decreased hypocotyl length and partly to slightly decreased germination rate. The increased percentage (17.1%) of total weight of sunflower sprouts at 18 h in the present study is higher than the values reported by Lee and others (1999), Choi and others (2000), and No and others (2003), who observed an increase (8.5%, 6.9%, and 13.1%) in weight of soybean sprouts by chitosan treatment for 1 h, 20 min, and 8 h of seed soaking time, respectively.

As shown in Table 2, soaking the seeds in chitosan solution for 12 or 18 h resulted in an increase in hypocotyl length of sprouts without affecting the hypocotyl thickness compared with those of no soaking. Increased length of soybean sprouts by chitosan treatment has also been reported by Lee and others (1999), Choi and others (2000), and No and others (2003).

Table 1—Effects of different molecular-weight chitosans on total weight, germination rate, length, and thickness of sunflower sprouts cultivated for 6 d at 20 °C (treatment 1).

Molecular weight[A]	Total weight (g)[B]	Germination rate (%)[B]	Length (cm)[C]	Thickness (mm)[C]
Control 1 (water)	35.94 ± 2.37b (100.0%)	53.05 ± 3.99b	6.16 ± 0.46a	1.87 ± 0.03a
Control 2 (acetic)	31.28 ± 1.07a (85.1%)	44.66 ± 2.81a	5.70 ± 0.48a	1.88 ± 0.06a
Chitosan treatments				
746 kDa	38.49 ± 2.00b (107.1%)	66.14 ± 3.48c	9.22 ± 0.46c	2.01 ± 0.03c
444 kDa	37.72 ± 1.56b (105.0%)	66.04 ± 4.84c	8.75 ± 1.12bc	1.95 ± 0.04b
223 kDa	37.59 ± 1.45b (104.6%)	62.33 ± 6.10c	8.21 ± 0.31b	2.01 ± 0.01c
67 kDa	38.42 ± 1.89b (106.9%)	66.59 ± 4.94c	8.77 ± 0.42bc	2.04 ± 0.06c
28 kDa	37.75 ± 1.21b (105.1%)	65.90 ± 2.57c	8.14 ± 0.55b	2.05 ± 0.01c

[A]For control 1, sunflower seeds were soaked in water for 18 h; for control 2, sunflower seeds were soaked in 0.1% acetic acid for 18 h; for all chitosan treatments, sunflower seeds were soaked in chitosan solution (0.1% in 0.1% acetic acid) for 18 h.
[B]Mean ± standard deviation of 4 batches. For total weight, numbers in parentheses are percent total weight and calculated based on the control 1.
[C]Mean ± standard error of mean (SEM) of 4 batches. 40 samples/batch.
[a–c]Means with different letters within a column indicate significant differences ($P < 0.05$).

Table 2—Effects of seed soaking times on total weight, germination rate, length, and thickness of sunflower sprouts cultivated for 6 d at 20 °C (treatment 2).

Soaking time (h)[A]	Total weight (g)[B]	Germination rate (%)[B]	Length (cm)[C]	Thickness (mm)[C]
0	37.70 ± 1.65a (100.0%)	48.14 ± 2.90a	4.34 ± 0.31b	1.96 ± 0.03a
6	41.38 ± 1.99bc (109.8%)	58.20 ± 2.96b	4.64 ± 0.14bc	1.97 ± 0.01a
12	42.67 ± 0.59cd (113.2%)	58.31 ± 3.06b	4.95 ± 0.23cd	1.98 ± 0.05a
18	44.13 ± 0.81d (117.1%)	64.97 ± 4.46c	5.00 ± 0.20d	2.12 ± 0.20a
24	40.11 ± 1.40b (106.4%)	60.11 ± 3.44bc	3.89 ± 0.05a	1.99 ± 0.02a

[A]Sunflower seeds were soaked in chitosan (28 kDa) solution (0.1% in 0.1% acetic acid) for 0, 6, 12, 18, and 24 h.
[B]Mean ± standard deviation of 4 batches. For total weight, numbers in parentheses are percent total weight and calculated based on the value at 0 h soaking time.
[C]Mean ± SEM of 4 batches. 40 samples/batch.
[a–d]Means with different letters within a column indicate significant differences ($P < 0.05$).

The reported soaking time of soybeans ranged from 20 min (Choi and others 2000) to 12 h (Lee and others 1999). Sunflower seeds may need longer soaking time to absorb enough water than do soybeans. The above results (Table 2) indicate that among soaking times tested, soaking time of 18 h was the most effective in increasing total weight and germination rate. Thus, treatment 3 was subsequently conducted with soaking time of 18 h.

Effects of chitosan solvent on growth of sunflower sprouts (treatment 3)

The effects of chitosan solvents on growth of sunflower sprouts cultivated for 6 d at 20 ± 2 °C are noted in Table 3. Besides 3 organic acids, water as a chitosan solvent was tested because 28 kDa chitosan is water-soluble. The results indicated that chitosan treatment, irrespective of solvent types, yielded a higher total weight of sprouts than that of the control. The increased total weight by chitosan treatment was mainly due to increased germination rate and, to a lesser extent, to length and/or thickness of sunflower sprouts. Among 4 solvent types, no significant differences in total weight were observed. However, the germination rate of sunflower sprouts treated with chitosan in water was lower than those treated with chitosan in acetic, lactic, and ascorbic acids. The length of hypocotyl treated with chitosan in water and lactic acid was longer than that of control, while the thickness of hypocotyl treated with chitosan in water and all 3 organic acids was greater than that of the control.

No and others (2003) observed that the total weight, length, and thickness of soybean sprouts treated with chitosan in acetic and lactic acids were all comparable. In the present study, however, the hypocotyl thickness (1.94 mm) of sunflower sprouts treated with chitosan in lactic acid was greater than that (1.89 mm) treated with chitosan in acetic acid. In our study, the total weight of sunflower sprouts treated with chitosan in acetic and lactic acids increased by 8.7% and 10.5%, respectively, compared with that of control (water). These increased total weight percentages are different from the corresponding values (21.8% and 18.8%, respectively) reported by No and others (2003) with soybean sprouts. These differences between sunflower sprouts and soybean sprouts are due to differences in seed cultivars, seed storage periods, cultivation conditions (soaking time, watering frequency, temperature, and so forth), and chitosan products applied (Kim and others 1990; Suh and others 1996).

As shown in Table 3, the total weight, germination rate, and hypocotyl length were comparable among 3 organic acids. However, use of lactic acid as a chitosan solvent may be advantageous in obtaining higher total weight and germination rate, and longer and thicker of hypocotyl than those of control. Avoidance of strong acid smell by using lactic acid instead of acetic acid may be another advantage. Therefore, lactic acid was used as a chitosan solvent in treatment 4.

Effects of chitosan concentrations on growth of sunflower sprouts (treatment 4)

The effects of chitosan concentrations (in lactic acid) on growth of sunflower sprouts cultivated for 6 d at 20 ± 2 °C are shown in Table 4. Chitosan treatment at above 0.01% concentration increased the total weight by 3.9% to 12.9% compared with the control. Total weight generally increased with increasing chitosan concentration. At 0.01% concentration, however, the total weight, germination rate, and length and thickness of hypocotyl were comparable with those of the control. Among 4 chitosan concentrations, the highest total weight and germination rate were achieved with 0.50% concentration; at this concentration, total weight was increased by 12.9% and germination rate by 16.0% compared with those of the control.

No and others (2003) observed that total weight of soybean sprouts increased with increasing chitosan concentration from 0.01% to 0.05%, but was comparable at concentration ranges of 0.05% to 0.50%. Choi and others (2000) found that 0.1% chitosan treatment was more effective in increasing the ratio of germination/rot of soybean sprouts than 1.0% chitosan treatment. In the present study, 0.50% chitosan treatment was the most effective in

Table 3—Effects of chitosan solvents on total weight, germination rate, length, and thickness of sunflower sprouts cultivated for 6 d at 20 °C (treatment 3).

Solvent[A]	Total weight (g)[B]	Germination rate (%)[B]	Length (cm)[C]	Thickness (mm)[C]
Control (water)	66.75 ± 0.68a (100.0%)	73.91 ± 3.30a	5.52 ± 0.24a	1.78 ± 0.03a
Chitosan treatments				
water	72.21 ± 1.52b (108.2%)	76.02 ± 2.49a	6.59 ± 0.14b	1.90 ± 0.04bc
acetic acid	72.58 ± 1.28b (108.7%)	83.17 ± 4.62b	6.07 ± 0.35ab	1.89 ± 0.04b
lactic acid	73.75 ± 3.57b (110.5%)	83.43 ± 6.42b	6.56 ± 0.84b	1.94 ± 0.01cd
ascorbic acid	71.65 ± 2.32b (107.3%)	83.18 ± 4.57b	5.94 ± 0.35ab	1.96 ± 0.03d

[A]For control, sunflower were seeds soaked in water for 18 h; for all chitosan treatments, sunflower seeds were soaked in chitosan (28 kDa) solution (0.1% in water or 0.1% organic acids) for 18 h.
[B]Mean ± standard deviation of 4 batches. For total weight, numbers in parentheses are percent total weight and calculated based on the control (water).
[C]Mean ± SEM of 4 batches. 40 samples/batch.
[a–d]Means with different letters within a column indicate significant differences ($P < 0.05$).

Table 4—Effects of chitosan concentrations on total weight, germination rate, length, and thickness of sunflower sprouts cultivated for 6 d at 20 °C (treatment 4).

Chitosan concentration (%)[A]	Total weight (g)[B]	Germination rate (%)[B]	Length (cm)[C]	Thickness (mm)[C]
Control (water)	65.85 ± 0.82a (100.0%)	76.68 ± 1.31a	5.57 ± 0.19a	1.86 ± 0.04a
0.01	67.43 ± 2.91ab (102.4%)	74.97 ± 1.92a	5.82 ± 0.20ab	1.88 ± 0.04ab
0.05	68.42 ± 1.12bc (103.9%)	77.57 ± 4.05a	5.91 ± 0.03bc	1.92 ± 0.02bc
0.10	70.70 ± 0.60c (107.4%)	82.72 ± 2.48b	6.11 ± 0.24c	1.93 ± 0.02c
0.50	74.36 ± 1.00d (112.9%)	88.99 ± 3.09c	5.89 ± 0.06bc	1.96 ± 0.02c

[A]For control, sunflower seeds were soaked in water for 18 h; for all chitosan treatments, sunflower seeds were soaked in different concentrations of chitosan (28 kDa in lactic acid) solution for 18 h.
[B]Mean ± standard deviation of 4 batches. For total weight, numbers in parentheses are percent total weight and calculated based on the control (water).
[C]Mean ± SEM of 4 batches. 40 samples/batch.
[a–d]Means with different letters within a column indicate significant differences ($P < 0.05$).

Table 5—Amino acid composition[a] of sunflower sprouts cultivated for 6 d at 20 °C without and with chitosan treatment.

	Content (mg/100 g)	
Amino acid	Control[b]	Chitosan[b]
Aspartic acid	1313.5	1355.8
Threonine	532.9	521.1
Serine	633.8	588.4
Glutamic acid	1979.5	2751.1
Proline	499.7	441.5
Glycine	595.6	581.4
Alanine	628.7	628.4
Cystine	95.3	67.0
Valine	1168.4	753.3
Methionine	61.6	21.1
Isoleucine	670.5	590.8
Leucine	881.7	834.3
Tyrosine	238.8	259.5
Phenylalanine	629.3	604.5
Histidine	355.7	348.3
Lysine	565.6	563.0
Arginine	1206.9	1188.8
Total amino acid	12057.5	12098.3

[a]Average of duplicate determinations, on a dry basis.
[b]For control, sunflower seeds were soaked in water for 18 h; for the chitosan treatment, sunflower seeds were soaked in chitosan (28 kDa) solution (0.5% in 0.5% lactic acid) for 18 h.

increasing the total weight and germination rate among 4 concentrations. This chitosan concentration (0.50%) is higher than the optimum chitosan concentration (0.05%) reported by No and others (2003) for soybean sprouts. To date, the mechanism by which chitosans act as a growth stimulator for sunflower sprouts as well as soybean sprouts is unknown and, therefore, has great merit for further investigations.

From results for treatments 1 to 4 (Table 1 to 4), optimal conditions for cultivation of sunflower sprouts can be summarized as follows: chitosan with MW 28 kDa; chitosan concentration, 0.5%; chitosan solvent, 0.5% lactic acid; soaking time, 18 h.

Comparison of selected quality between control and chitosan-treated sunflower sprouts

The selected quality of sunflower sprouts cultivated for 6 d at 20 ± 2 °C after soaking seeds in chitosan solution under the aforementioned optimal conditions (designated chitosan sunflower sprouts) was evaluated and compared with that of sunflower sprouts cultivated for 6 d at 20 ± 2 °C after soaking seeds in water for 18 h (designated control sunflower sprouts). The results are reported in Table 5 and 6.

Proximate composition. The moisture contents were comparable between control (89.80 ± 0.71%) and chitosan sunflower sprouts (89.72 ± 0.81%). On a dry basis, chitosan sunflower sprouts contained slightly lower fat (33.50 ± 2.12%) and ash (4.73 ± 0.31%) contents than those (36.00 ± 1.41% and 5.07 ± 0.46%, respectively) of the control. The protein content was comparable for both (34.18 ± 0.06% and 34.14 ± 0.01%, respectively). Earlier, Lee (1999) reported that the moisture, protein, fat, and ash contents of commercially available sunflower sprouts (hypocotyl length of 6 cm) were 94.7%, 1.3%, 0.3%, and 1.4%, respectively, on a wet basis. The proximate compositions reported by Lee (1999) are different from those observed with control sunflower sprouts in our present study. These differences are probably due to differences in sunflower seed cultivars, seed storage periods, and cultivation conditions (soaking time, watering frequency, temperature, and so forth) (Kim and others 1990; Suh and others 1996). The fat content (33.50%) of chitosan sunflower sprouts is comparable to that (33.30%) of cotyledons of sunflower sprouts cultivated for 5 d at room temperature (Balasaraswathi and Sadasivam 1997).

Amino acid composition. Table 5 shows the amino acid composition of control and chitosan sunflower sprouts, on a dry basis. A common notable feature of both sprouts is their very high contents of glutamic acid, aspartic acid, and arginine. Significantly, these 3 amino acids accounted for 37.3% of the total amino acid in the control sunflower sprouts and 43.8% of the total amino acid in the chitosan sunflower sprouts. The amino acid profiles of the 2 sunflower sprouts were similar, except for glutamic acid and valine contents. Chitosan treatment notably increased the glutamic acid content, but decreased valine content, compared with control sunflower sprouts. The total amino acid content per 100 g of chitosan sunflower sprouts on a dry weight basis was slightly higher than the control sprouts, probably due to the increased glutamic acid. However, chitosan treatment increased the total weight of sunflower sprouts by 12.9% (Table 4) compared with control sunflower sprouts. Thus, when calculated based on sunflower seeds, overall amino acid contents obtainable from chitosan sunflower sprouts are higher than those from control sunflower sprouts.

Sunflower proteins have a good nutritional quality except that lysine content is very low and insufficient (Balasaraswathi and Sadasivam 1997). Balasaraswathi and Sadasivam (1997) observed that sprouted sunflower seeds had a 20% increase in lysine content with no significant decrease in the protein content. This indicates that the sprouted sunflower seeds have a higher nutritional quality than the raw seeds.

DPPH radical scavenging activity and phytochemicals. The DPPH radical scavenging activity and phytochemicals in sunflower

Table 6—DPPH radical scavenging activity and phytochemicals[a] in sunflower seeds and sunflower sprouts cultivated for 6 d at 20 °C without and with chitosan treatment.

		Sprout	
Property	Seed	Control[b]	Chitosan[b]
DPPH radical scavenging activity (%)	43.30 ± 4.03	60.07 ± 0.47	63.19 ± 0.94
Total phenolic content (mg/g)	1.06 ± 0.18	3.60 ± 0.07	3.75 ± 0.06
Melatonin content (ng/g)	ND	1.44 ± 0.04	1.53 ± 0.04
Total isoflavone content (ng/g)	534.0 ± 18.1	613.7 ± 88.0	685.9 ± 18.9
Daidzin	n.d.	566.7 ± 69.3	529.1 ± 17.9
6″-*O*-Acetyldaidzin	n.d.	27.6 ± 15.1	ND
Daidzein	n.d.	19.3 ± 16.8	156.7 ± 10.9

[a]Mean ± standard deviation of triplicate determinations, on a dry basis.
[b]For control, sunflower seeds were soaked in water for 18 h; for the chitosan treatment, sunflower seeds were soaked in chitosan (28 kDa) solution (0.5% in 0.5% lactic acid) for 18 h.
ND = not detectable.
n.d. = not determined.

seeds were evaluated and compared with those of control and chitosan sunflower sprouts (Table 6). Sprouting of sunflower seeds improved the DPPH radical scavenging activity, probably due to the increased total phenolic, melatonin, and total isoflavone contents. Total phenolic compounds are the major source of natural antioxidants in foods from plant origin. During sprouting, the total phenolic content of sunflower seeds increased from 1.06 to 3.60 (control) and 3.75 (chitosan treated) mg/g (Table 6), indicating a change in the composition of antioxidants in the sprouts (Zhu and others 2005). Recently, melatonin has been proved to be a potent free radical scavenger and a broad spectrum antioxidant (Tan and others 2002). In the present study, melatonin was not detected in seeds, but detected at 1.44 and 1.53 ng/g (Table 6) in the control and chitosan sunflower sprouts, respectively. However, Manchester and others (2000) reported the concentration of melatonin in sunflower seeds to be 29 ng/g dry seed. Isoflavone is a known phytoestrogen and has been reported to have various health beneficial roles such as antioxidation (Murphy and others 1999), an inhibitor for low-density lipoprotein (LDL) oxidation, and a scavenger for DPPH radical activity (Kerry and Abbey 1998; Lee and others 2005). As shown in Table 6, the total isoflavone content increased from 534 ng/g in seeds to 613.7 (control) and 685.9 (chitosan) ng/g after sprouting. These data (Table 6) indicate that sunflower sprouts may offer a better functional food source than the raw sunflower seeds.

Chitosan treatment slightly improved the DPPH radical scavenging activity of sprouts, probably due to slightly increased total phenolic and melatonin contents and moderately increased total isoflavone content compared with those of the control. Chitosan treatment increased the total isoflavone content of sprouts by 11.8% compared with that of the control. The increased total isoflavone content by chitosan treatment was due mainly to the increased daidzein content (Table 6). As shown in Table 6, chitosan treatment considerably increased the daidzein content but slightly decreased daidzin content compared with those of the control. The 6″-*O*-acetyldaidzin observed in control sunflower sprouts was not detected in chitosan sunflower sprouts. It is known that isoflavone is composed of 12 isomers (Xie and others 2003). In this study, the aglycone forms (glycitein and genistein), glycoside forms conjugated with sugar only (glycitin and genistin), malonylglycoside derivatives (6″-*O*-malonyldaidzin, 6″-*O*-malonylglycitin and 6″-*O*-malonylgenistin), and acetylglycoside derivatives (6″-*O*-acetylglycitin and 6″-*O*-acetylgenistin) were, however, not detected in both control and chitosan sunflower sprouts.

Conclusions

This study demonstrated that chitosan treatment was effective in increasing both growth and selected quality of sunflower sprouts. Chitosan treatment increased total weight by 12.9% and germination rate by 16.0% compared with that of the control (water). Sprouting of sunflower seeds improved the DPPH radical scavenging activity, probably due to the increased total phenolic, melatonin, and total isoflavone contents. Similarly, chitosan treatment slightly improved the DPPH radical scavenging activity, probably due to the increased total phenolic, melatonin, and total isoflavone contents compared with those of the control. Chitosan treatment increased the total isoflavone content by 11.8% compared with those of control. Nevertheless, the proposed soaking conditions for optimal sunflower sprouts cultivation need to be examined within actual sunflower sprouts commercial facilities, because conditions used in a large-scale commercial sunflower sprouts cultivation, in all likelihood, will vary from those based on laboratory trials. Furthermore, investigations are needed with various sunflower seed cultivars, since different cultivars of sunflower seeds may influence yield and quality of sunflower sprouts, due largely to differences in size and weight of seed and in chemical composition. The beneficial aspects of chitosan-treated sunflower sprouts, as demonstrated in this study, clearly warrant scale-up trials under a large volume typical of commercial conditions.

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