Soaking of Soybean Seeds in Milled Chitosan Solution and Its Effect on Growth and Quality of Soybean Sprouts

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ABSTRACT

The effects of milled chitosan solutions on growth and selected quality of soybean sprouts were evaluated. Chitosan solution (1% in 1% acetic acid) was milled for 0, 0.5, 1 and 2 h, and diluted with distilled water to 0.05% to use as soaking solutions. Soybean seeds were soaked in water (control), 0.05% acetic acid and milled chitosan solutions for 8 h, and then cultivated for 3 days at 25°C. Results indicated that chitosan treatment increased total weight by 7.8-10.0% and hypocotyl length by 6.2-11.1% compared with the control and that the effectiveness of chitosan treatment was not affected by milling times of chitosan solution. Chitosan treatment, irrespective of milling times, did not affect the quality of soybean sprouts. No significant differences in moisture content, hardness and DPPH radical scavenging activity were observed among six treatment groups. The total free amino acid contents of soybean sprouts treated with 0.5 and 1 h-milled chitosan solutions were comparable to that of the control soybean sprouts.

Keywords: Chitosan, Growth, Quality, Soybean sprouts, Milling

INTRODUCTION

Chitosan has been approved as a food additive in Korea and Japan since 1995 and 1983, respectively (1,2) because it is biocompatible, non-antigenic, non-toxic, and biofunctional (3,4). During the past several decades, the effectiveness of chitosan for its ability to enhance quality and shelf life of foods has been reported by numerous workers (5). Soybean sprouts have long been consumed as a traditional food in Korea and other East Asian countries, due mainly to their high nutritional value, relatively low price, and easy cultivation in a short period (6,7). However, several problems are encountered during cultivation of soybean sprouts, including yield reduction, quality deterioration and rot occurrence (8,9). Considerable attention has been given to development of adequate methods to overcome these problems. Earlier studies on the cultivation of soybean sprouts have revealed that chitosan treatment in seed soaking solution was effective in increasing the growth rate and decreasing the rot of soybean sprouts (8-11).

The growth of soybean sprouts may be affected by specific characteristics of chitosan such as molecular weight or viscosity. No et al. (6) demonstrated that the effectiveness of chitosan treatment in increasing the total weight of soybean sprouts differed with molecular weight of chitosan. In general, viscosity of chitosan solution is affected by various physicochemical treatments (heating, autoclaving, ultrasonication and ozone) and decreases with an increase in treatment time and temperature, due mainly to depolymerization of chitosan (12). Viscosity of chitosan solution may also be affected by wet-milling and decreases with an increase in milling time. The physicochemical characteristics of chitosan influence its final functional properties (12). Thus, effectiveness of chitosan treatment on growth of soybean sprouts may differ with milling times of chitosan solution. However, very few attempts have been made to study the effect of chitosan solution with milling times on growth and quality of soybean sprouts.

The objective of the present research was to examine the effects of chitosan solutions milled for various times (0, 0.5, 1 and 2 h) on growth and selected quality of soybean sprouts.

MATERIALS AND METHODS

Materials

Chitosan (molecular weight = 496 kDa), acid-soluble and white-colored powder prepared from crab shell, was purchased from Keumho Chemical (Seoul, Korea). Soybeans (Orialte cultivar, cultivated in Korea), with an average weight of 10.95 g/100 seeds, were purchased from Orialte Co. (Gyeonggi-do, Korea) and stored at 4°C until used. 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

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Milling of chitosan solution

Chitosan was dissolved in 1% (v/v) acetic acid at 1% (w/v) concentration and milled using a rotate ring mill (Armstec Ind. Co., Seoul, Korea) (vessel, 150 rpm; agitator, 1000-1100 rpm) for 0, 0.5, 1 and 2 h at 25°C. After milling, viscosity (mPa s) of each chitosan solution was determined in triplicate with a Brookfield viscometer (model LVDV-II+, Brookfield Engineering Labs., Stoughton, MA, USA), equipped with a small sample adapter and operated at a shear rate of 132 s⁻¹ in the 8 mL solution at 25±3°C. Each milled chitosan solution was diluted with distilled water to give a final chitosan concentration of 0.05% and used as a soaking solution of soybean seeds. To simplify the text, chitosan solution was designated as CH-0, CH-0.5, CH-1 and CH-2, respectively.

Cultivation of soybeans

Soybean seeds (120 g) were washed with water, drained and soaked in 600 mL milled chitosan solutions (0.05% in 0.05% acetic acid) for 8 h. Soybean seeds (120 g) soaked in 600 mL water (CO) and 600 mL 0.05% acetic acid (AC) for 8 h were used as control groups. Following this step, soybean seeds were drained, equally divided into 4 parts on a wet basis, and placed in the commercial soybean sprouts cultivator (Daechun Anypass Co., Gyeonggi-do, Korea) consisting of 4 separate trays, and cultivated in the dark at 25°C for 3 days. Water was automatically sprayed for 5 min on the soybean seeds/sprouts every 20 min.

Measurement of growth

After 3 days of cultivation, soybean sprouts in each of the four trays were washed with water to remove bean hulls and drained for 10 min. Total fresh weight of soybean sprouts in each tray was recorded. For measurement of length and thickness of hypocotyl, 20 soybean sprouts were randomly sampled from each tray and separated into cotyledon and hypocotyl. Length (including root) was measured with a ruler, thickness with a caliper (CD-20B, Mitutoyo Co., Kawasaki, Japan) and weight with a balance (GT 480, Ohaus, Florham Park, NJ, USA).

Determination of moisture of soybean sprouts

Moisture content was determined in triplicate by a standard method (13).

Determination of hardness of hypocotyl

Hardness was measured individually for 10 samples by a rheometer (COMPAC-100 II, Sun Scientific Co., Japan) under the following operational conditions: test type, mastication; adaptor type, circle; adaptor area, 0.20 cm²; and table speed, 60 mm/min (6).

Determination of free amino acids

Freeze-dried (Labconco Co., Kansas, MI, USA) soybean sprouts (0.2 g) were extracted with 10 mL of 70% ethanol for 24 h with gentle shaking. The extract was centrifuged at 3,000 rpm for 10 min and the supernatant was collected. The solvent was removed from the supernatant at 45°C by a vacuum evaporator. The residue was dissolved in 5 mL of lithium citrate loading buffer (pH 2.2) and filtered through a 0.22 µm membrane filter. Free amino acid was analyzed by an amino acid analyzer (Biochrom 30, Pharmacia Biotech Ltd., Cambridge, England).

Determination of DPPH radical scavenging activity

DPPH free radical scavenging activity of soybean sprouts was determined in triplicate by the method of Blois (14) with some modifications. Freeze-dried soybean sprouts (1 g) were extracted with 9 mL of 80% ethanol using a shaker (200 rpm) at room temperature for 24 h. The extract was centrifuged (Model 5810 R, Eppendorf, Hamburg, Germany) at 10,000 rpm for 10 min, filtered through a 0.2 µm PTFE membrane filter paper (Alltech, Deerfield, IL, USA) and then diluted 10 times with ethanol. An aliquot (0.4 mL) of the extract was added to 0.8 mL of 0.4 mM DPPH radical ethanolic solution. The reaction mixture was shaken vigorously, stored in the dark at room temperature for 10 min and the absorbance measured at 517 nm using a spectrophotometer (Ultraspec® 1000, Pharmacia Biotech Co., Cambridge, England). The DPPH free radical scavenging activity was calculated by the following equation:

\[ \text{Scavenging activity (\%)} = \left[ 1 - \left( \frac{\text{absorbance}_{\text{sample}}}{\text{absorbance}_{\text{control}}} \right) \right] \times 100. \]

Statistical analysis

Experiments on cultivation of soybean sprouts were carried out in quadruplicate and means ± standard deviations (or standard errors of mean) were reported. Quality of soybean sprouts were determined in triplicate, except for hardness (n = 10), and means ± standard deviations or average values were reported. Data were analyzed using analysis of variance (ANOVA), followed by the Duncan’s multiple-range test using the SPSS (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA) software package.

RESULTS AND DISCUSSION

Viscosity of milled chitosan solutions

Effects of milling times on viscosity of chitosan solution are given in Fig. 1. Viscosity decreased from initial 25.2 mPa s to 21.2, 20.9 and 20.4 mPa s after milling for 0.5, 1 and 2 h at 25°C, respectively. Slight reduction of chitosan viscosity with milling times is probably due to the low viscosity of ini-