Korean J Crop Sci 49(5) 410~414(2004)

Changes in Postharvest Respiration, Growth, and Vitamin C Content of Soybean Sprouts under Different Storage Temperature Conditions

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Abstract : To understand the postharvest characteristics of soybean sprouts, 5-day-old sprouts were harvested, packed in PE film, and stored at 4, 12, and 20 °C for up to 4 days. In addition, the sprout respiration rate was measured after storage at 4, 8, 12, 16, 20, and 24 °C for up to 20 h. During the first day of storage at 20 °C, the sprouts maintained temperature-dependent longitudinal growth, especially of hypocotyl length; hypocotyl and root grew 0.8 cm and 0.2 cm, respectively. The hypocotyl thickness decreased by 11, 13, and 18 % after 4 days of storage at 4, 12, and 20 °C, respectively. No temperature-dependent differences in fresh weight, dry weight, or water content were found, despite decreases of 3 % over the 4 days of storage. A significant postharvest decrease of 50 % in vitamin C content was observed in the sprouts stored at 20 °C for 3 days. Based on the CO_2 production rate, the soybean sprouts exhibited an increase in respiration in proportion to the storage temperature; sprouts stored at 8, 12, 16, 20, and 24 °C showed approximately 2, 5, 6, 11, and 17 times, respectively, than the respiration rate of sprouts stored at 4 °C. These results indicate the importance of low temperature storage during market circulation for minimizing the postharvest morphological and nutritional degradation of soybean sprouts.

Keywords: *Additional Keywords. Glycine max* L., postharvest handling, nutritional quality

Soybean sprouts are an important traditional food in Korea; they are low cost, of high nutritional quality, and relatively easy to produce (Lee *et al.*, 1999). Today soybean sprouts are an important agricultural business in Korea owing to their popularity and the recent industrialization of their cultivation (e.g., Bae *et al.*, 2004b). Many studies have examined cultivation techniques, nutritional value, breeding, prevention of sprout rot, etc., in soybean sprout production (Kim *et al.*, 1993; Lee, 2000; Lee *et al.*, 1999; Yang *et al.*, 1979; Shin & Choi, 1996). Little emphasis, however, has been placed on the postharvest physiology of soybean sprouts, and consequently no standard methods for postharvest handling of soybean sprouts have been suggested. Soybean sprouts are very perishable, and their shelf life rarely exceeds 5 to 7 days, in some cases, film-packed soybean sprouts are discarded within 1 to 2 days of display in the market (Bae *et al.*, 2004a; Lee & Lee, 1996). Traditionally, soybean sprouts have been distributed to end users in the same cultivation vessel used for sprout production. The recent preferences of customers and the proliferation of cold-chain systems, however, have led to the circulation of soybean sprouts packed in transparent polyethylene or polypropylene films. Such film-packed distribution prompts various postharvest concerns. The greening of cotyledons as a result of light-dependent chlorophyll formation (Kasemir, 1983) in sprouts packed in transparent film decreases the visual quality of the sprouts, as customers prefer yellow-colored cotyledons. Furthermore, the off-flavors produced under conditions of low oxygen inside the packing film, the browning of sprout roots, and the loss of reserved nutrients used as substrates for sprout respiration, among other factors, may readily result in lower marketability and subsequent economic loss for both the producing farmers and the customers. Nevertheless, the distribution of film-packed soybean sprouts will probably increase in popularity in the future. The objective of the present study is to develop a basic understanding of the postharvest growth and nutritional changes in soybean sprouts under different storage temperature conditions, as a prerequisite for the development of postharvest handling techniques for soybean sprouts.

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MATERIALS AND METHODS

Soybean sprout preparation

Soybean (*Glycine max* L. cv. Junjory) seeds were purchased from the Korean Bean Sprout Association and stored at 5 °C prior to experimental use. For sprout cultivation, 1 kg of soybean seeds was soaked in drinking-quality tap water for 3 h, transferred into a commercial cultivation vessel of 25 × 25 × 25 cm, and cultivated for 5 days. Top-watering irrigation was performed for 3 min every 4 h with drinking-quality well water. The temperature inside the cultivation room during sprout production was 20 to 25 °C.

Film packing and storage

The harvested sprouts were washed briefly in tap water for about 30 s. After the removal of excess water from the sprout surfaces, homogeneous soybean sprouts of marketable quality were selected and packed per 300 g in a PE film (thickness 0.03 mm) envelope commonly used for commercial packing and circulation in the Korean market. The packed sprouts were stored for up to 4 days at 4, 12, and 20 °C, representing the temperatures of a refrigerator, a display shelf in the market, and room temperature, respectively.

Sprout growth and measurement of vitamin C content

The postharvest growth and vitamin C content were measured daily, following storage. On each measurement day, two replications of soybean sprout packs were unpacked and 20 to 40 representative sprouts were selected for the measurement of growth parameters, such as fresh weight, hypocotyl thickness, hypocotyl length, and root length. The dry weight and water content were measured after drying the sprouts at 80 °C for 3 days.

For the vitamin C content analysis, the unpacked sprouts were briefly washed with flowing tap water, excess water was removed from the sprout surfaces by blotting on clean paper, and representative sprouts were separated into cotyledon, hypocotyl, and root sections. Five-gram samples of each sprout section were soaked in 5 mL of 10 % metaphosphoric acid for 10 min, homogenized with a mortar and pestle, and diluted to 50 mL with 5 % metaphosphoric acid. After centrifugation at 3,000 rpm for 15 min, the supernatants were filtered through nylon syringe filters (0.4 μm) and analyzed by high-performance liquid chromatography (HPLC; Model S1211, Sykam, Germany). The HPLC analysis conditions were: mobile phase, 30 % MeOH with 0.14 % hexanesulfonic acid and 1 % acetic acid; column, 250 × 4.6 mm (5 μm) Luna C18, flow rate, 1.0 mL min^{-1}, and detection, UV absorbance at 254 nm. The vitamin C content analysis was conducted at least three times per single treatment, and an authentic ascorbic acid standard was purchased from Sigma for vitamin C quantification.

Measurement of sprout respiration

To measure the respiration of soybean sprouts stored under different temperature conditions, harvested sprouts were briefly washed with flowing tap water. After the removal of excess water, sprouts were pre-stored for 4 h at 4, 8, 12, 16, 20, and 24 °C for temperature adjustment. For the respiration measurements, gas-tight, pre-cleaned glass jars (1.5 L) equipped with Teflon-PTFE septa (Wheaton, USA) were each packed with 200 g of soybean sprouts and then stored at 4, 8, 12, 16, 20, and 24 °C. The respiration jars were pre-cooled to the corresponding storage temperature prior to experimental use. Following storage, the head space gas inside each respiration jar was sampled with a gas-tight syringe, and 1 mL of the gas sample was injected into a gas chromatograph (GC; Varian 3800, USA). The GC conditions for CO_2 and O_2 analysis were as followings: column, CTR1 (Altech, 3m); injector temperature, 120 °C; detector (TCD) temperature, 120 °C; oven temperature, 35 °C; carrier gas, He; flow rate, 30 mL min^{-1}. Authentic standard CO_2 and O_2 gases were purchased from Supelco Co (USA) for quantification. To compare the relative CO_2 production rates of sprouts stored at different temperature conditions, linear regression equations relating storage duration (X) and headspace CO_2 concentration (Y) were calculated with SPSS (ver. 10.0, SPSS Institute, 2000) The slopes of the regression lines, representing the CO_2 concentration increment per unit time, were used for comparisons.

RESULTS AND DISCUSSION

Postharvest soybean sprout growth

Soybean sprouts packed in PE film exhibited longitudinal growth during storage, especially of hypocotyl length (Fig. 1A) The longitudinal growth was dependent on storage temperature, in that the hypocotyl length of sprouts stored at 12 and 20 °C for 4 days grew 7.2 cm and 7.8 cm, respectively, corresponding to increases of 6 and 13 %, respectively, as compared with pre-storage lengths (6.8 cm). At 4 °C, however, no significant increase in hypocotyl length could be observed after 4 days of storage. In the case of sprouts stored at 20 °C, most of the postharvest hypocotyl growth occurred during the first day of storage, while the hypocotyl length of sprouts stored at 12 °C increased during the first 1 to 2 days of storage.

Unlike the hypocotyl length, no significant increase was observed in the root length under any of the experimental temperature conditions (Fig. 1B). The reason for postharvest growth in the hypocotyl length rather than in the root length might be related to the fact that preharvest soybean sprouts also show relatively higher growth rates in hypocotyl length than in root length (Suh *et al.*, 1995).

In contrast to longitudinal growth, horizontal growth, i.e., the hypocotyl thickness, exhibited a significant postharvest decrease (Fig. 1C), which was accelerated by high temperatures. Soybean sprouts stored for 2 days at 4, 12, and 20 °C exhibited 8, 14, and 17 % reductions, respectively, in hypocotyl thickness as compared with pre-storage thickness. The speed of hypocotyl thinning was also temperature-depen-

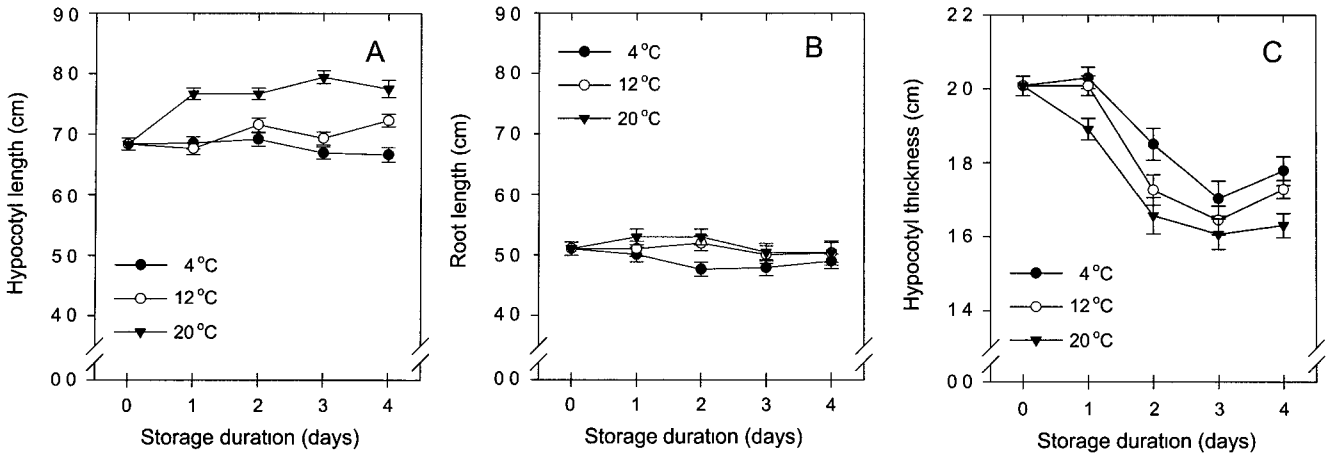

Fig. 1. The postharvest growth of soybean sprouts as measured by (A) hypocotyl length, (B) root length, and (C) hypocotyl thickness under different storage temperature conditions The harvested sprouts were packed in PE film and stored at 4, 12, and 20 °C. The data shown are the mean ± standard deviation of at least 20 representative sprout measurement replications

Table 1. Postharvest changes in fresh weight, dry weight, and water content of soybean sprouts packed in PE-film and stored at 4, 10, and 20°C conditions. The data shown are the mean ± standard deviation of 10 replications

Growth parameter	Storage temperature (°C)	Storage duration (days)				
		0	1	2	3	4
Fresh weight (g / 10 plants)	4	4 63 ± 0 41 b[z]	4.61 ± 0.22 a	4.45 ± 0.25 ab	4 46 ± 0 12 ab	4.30 ± 0.23 a
	10	4.63 ± 0.41 ab	4 46 ± 0 16 b	4.56 ± 0.23 b	4 38 ± 0.18 ab	4.42 ± 0.18 a
	20	4.63 ± 0.41 a	4 68 ± 0 12 a	4.41 ± 0.18 a	4.48 ± 0.15 a	4 38 ± 0 27 a
Dry weight (g / 10 plants)	4	1.89 ± 0.05 a	1 90 ± 0 04 a	1.94 ± 0.05 a	1.89 ± 0.03 a	1 85 ± 0 06 a
	10	1 89 ± 0 05 a	1.84 ± 0.05 a	1.86 ± 0.05 a	1.88 ± 0.03 a	1 88 ± 0.02 a
	20	1 89 ± 0 05 a	1.87 ± 0.02 a	1 84 ± 0 03 a	1.87 ± 0.04 a	1 83 ± 0 09 a
Water content (%)	4	58 9 ± 3 8 a	58.7 ± 1.3 a	57.0 ± 3 2 a	57 6 ± 1 4 a	57.1 ± 1.5 a
	10	58 9 ± 3.8 b	58.7 ± 1.1 ab	59.2 ± 1 3 ab	57 0 ± 1 5 ab	57.5 ± 2.0 a
	20	58 9 ± 3.8.1 a	60.1 ± 1 0 a	58.3 ± 1.6 a	58 3 ± 0.9 a	58.2 ± 1.8 a

[z] Mean separation within rows by Duncans multiple range test, P≤0 05

dent; a significant thickness reduction could be observed within 1 day of storage at 20 °C, while sprouts stored at 4 and 12 °C exhibited no reduction by the first day, but significant reduction after 1 to 3 days of storage. Hypocotyl thickness might decrease owing to water loss during storage, as water plays an import role in maintaining the turgor pressure of hypocotyl cells. Considering the preference of customers for thick hypocotyls, the decrease in hypocotyl thickness during storage may reduce the marketability of sprouts.

Although the fresh weight and water content of stored sprouts tended to decrease with the duration of the storage period (Table 1), the postharvest changes were relatively negligible (i.e., less than 10 %) when compared to pre-storage values, and consequently no statistically significant differences among storage temperatures or durations were observed. The sprouts apparently maintained their fresh weight by compensating for decreased hypocotyl thickness with increased hypocotyl length during storage, as shown in Fig. 1. Similarly, no significant changes in sprout dry weight were observed during storage (Table 1). Therefore, storing soybean sprouts at low temperatures seems to be important for maintaining high visual quality of soybean sprouts during circulation.

Measurement of Vitamin C content

Vitamin C is an important nutritional quality factor of soybean sprouts. Soybean sprouts packed in PE film exhibited a rapid and significant decrease in vitamin C content during storage (Fig. 3). The decrease in vitamin C could be observed in all sprout parts, cotyledons, hypocotyls, and roots, although the hypocotyl exhibited a relatively smaller

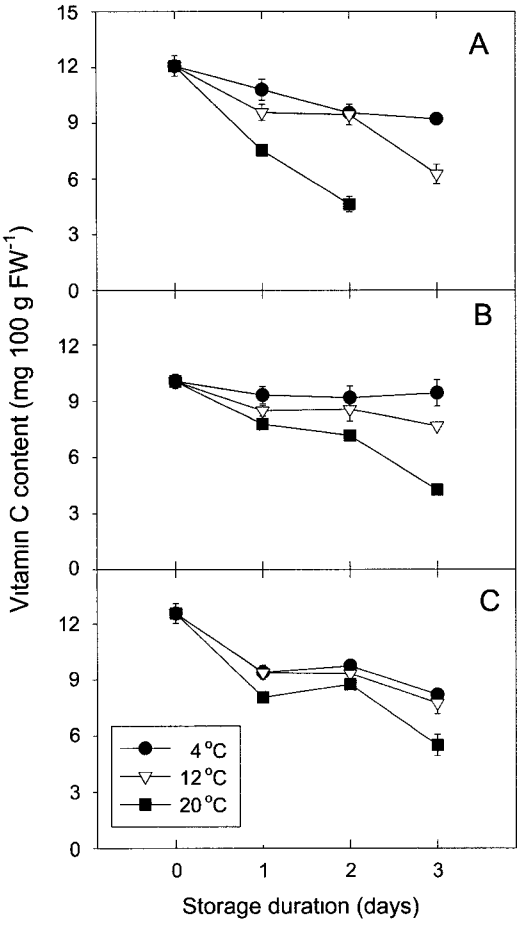

Fig. 2. The changes in vitamin C content of (A) cotyledons, (B) hypocotyls, and (C) roots of soybean sprouts packed in PE film and stored at different temperature conditions The data shown are the mean ± standard deviation of at least three independent replications.

decrease than did the other sprout parts. The storage temperature greatly affected the vitamin C loss in sprouts. Storage at 20 °C resulted in a reduction of more than 50 % during the first 3 days of storage. Even sprouts stored at 4 °C for 3 days showed vitamin C losses of 24, 6, and 35 % in the cotyledons, hypocotyls, and roots, respectively. Considering that sprouts stored at 4 °C for 3 days showed no significant visible storage-related symptoms, such as shrivel, these results indicate that the nutritional quality of soybean sprouts, such as the vitamin C content, decreases quickly and without apparent visible symptoms. Therefore, soybean sprouts should be used quickly after purchase, even if they are stored at 4 °C.

Sprout respiration

Respiration is one of the most important factors in postharvest technology, because agricultural commodities are in a living state during storage (Kader, 1992). Respiration inevitably requires substrates such as carbohydrates and consequently is followed by the reduction of the nutritional quality of the food item. The headspace gas in a gas-tight respiration jar containing soybean sprouts exhibited significant O_2 reduction and CO_2 increase in proportion to the storage temperature (Fig. 3). Even at 4 °C, the CO_2 concentration in a respiration jar increased by as much as 5 and 10 % when measured after 20 h and 48 h of storage, respectively. Considering that 4 °C is the lowest practical temperature to which sprouts can be exposed during postharvest circulation, it can be concluded that significant respiration and subsequent substrate loss in soybean sprouts occur during storage. Higher storage temperatures dramatically enhanced the relative CO_2 production rates. The slopes of the linear regression equations relating storage duration (X) to headspace CO_2 concentration (Y) were 1.0, 1.8, 4.8, 5.8, 11.0, and 16.7 for 4, 8, 12, 16, 20, and 24 °C, respectively.

Unlike the linear accumulation of headspace CO_2, the O_2 concentration displayed different time-series patterns; it exhibited a linear reduction to approximately 1 %, below which no further significant reduction was observed. These results were consistent with those of Bae *et al.* (2004a); they reported a temperature-dependent, linear increase in CO_2 and the rapid consumption of O_2 in soybean sprouts packed in PE film, but with differences in the rates of CO_2 production and O_2 consumption, which may in part have been a result of the experimental methods and conditions, such as the gas measurement methods and the gas-penetrable packing materials. The 1 % O_2 concentration was reached more quickly at higher temperatures, requiring approximately 300 min and 900 min with storage at 20 and 12 °C, respectively, whereas the 10 % headspace O_2 was maintained after 1,200 min with storage at 4 °C (Fig. 3). These results indicate the importance of low temperature storage for maintaining soybean sprouts of the highest possible quality during market circulation The fact that continuous CO_2 production could be observed without further oxygen consumption below the 1 % level suggests that the sprouts nevertheless maintained respiration. Such anaerobic respiration might be an important cause of off-flavor in film-packed soybean sprouts, because many by-products of anaerobic respiration, such as acetaldehyde, ethanol, and methanol, are widely accepted as the source of off-odors from various vegetables exposed to low-oxygen conditions (Hansen *et al.*, 2001; Forney and Jordan, 1999). More research is needed to identify the sources of soybean sprout off-flavors and their relationship to oxygen levels in order to establish optimal PE-packing methods for soybean sprouts. The results of this study highlight the importance of low temperature storage during circulation for minimizing temperature-dependent respiration

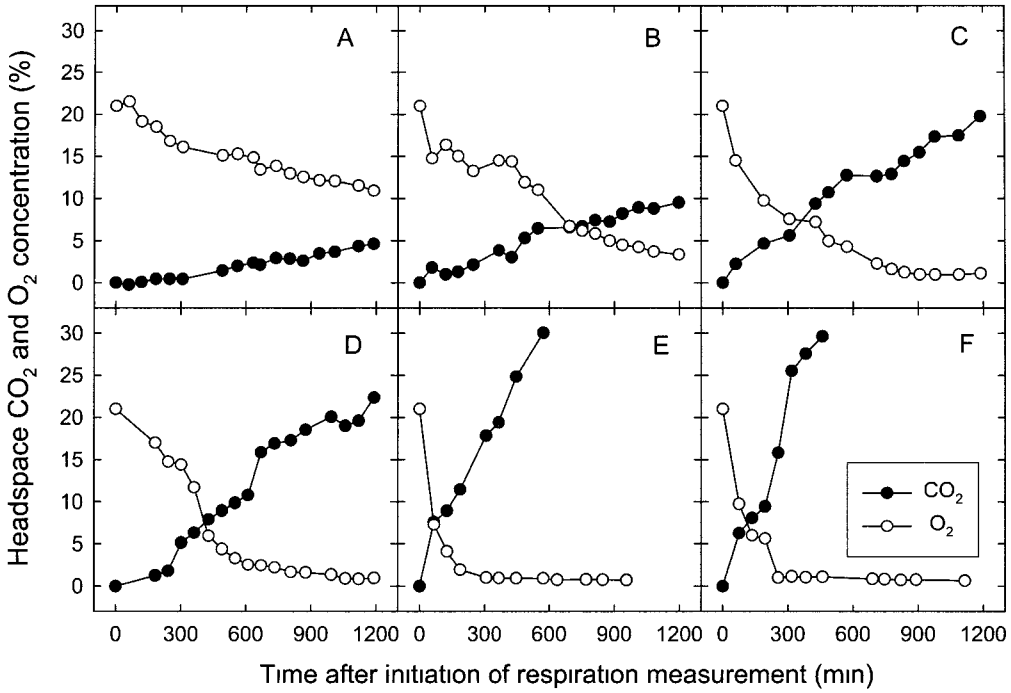

Fig. 3. Time-series changes in headspace CO_2 and O_2 concentrations inside the respiration jar Harvested sprouts (200 g each) were placed in gas-tight, glass respiration jars (1.5 L) equipped with Teflon-PTFE septa and stored at (A) 4 °C, (B) 8 °C, (C) 12 °C, (D) 16 °C, (E) 20 °C, and (F) 24 °C

and the resultant decrease in marketability and nutritional quality of soybean sprouts.

ACKWLEDGEMENT

This work was supported by the special grant research program from Ministry of Agriculture (Project No. 299023-3, 2001).

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