

## Variation of $\beta$ -carotene Concentration in Soybean Seed and Sprout

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**ABSTRACT** In this study,  $\beta$ -carotene concentrations was determined in soybean cultivar according to seed size, usage, seed coat color and cotyledon color as well as the process of seed germination. The total average concentration of  $\beta$ -carotene was 6.6  $\mu\text{g/g}$  in soybean seed, 33.3  $\mu\text{g/g}$  in soybean sprout. According to seed size, the total  $\beta$ -carotene concentration of soybean was 6.9  $\mu\text{g/g}$  in large soybean seed, 6.7  $\mu\text{g/g}$  in medium soybean seed, and 6.31  $\mu\text{g/g}$  in small soybean seed. In soybean sprout, the total  $\beta$ -carotene concentration was 21.4  $\mu\text{g/g}$  in large soybean sprout, 30.5  $\mu\text{g/g}$  in medium soybean sprout, and 43.5  $\mu\text{g/g}$  in small soybean sprout. According to the utilization of seed, the total  $\beta$ -carotene concentration of soybean seed was 7.2  $\mu\text{g/g}$  in cooked with rice soybean seed, 6.1  $\mu\text{g/g}$  in paste and curd soybean seed, and 6.3  $\mu\text{g/g}$  in sprout soybean seed. In soybean sprout, the total  $\beta$ -carotene concentration was 25.9  $\mu\text{g/g}$  in cooked with rice soybean sprout, 32.4  $\mu\text{g/g}$  in paste and curd soybean sprout, and 41.9  $\mu\text{g/g}$  in sprout soybean sprout. When comparison with seed coat color, the total  $\beta$ -carotene concentration of soybean with brown seed coat (8.8  $\mu\text{g/g}$ ) was slightly higher than those of soybean with yellow (6.1  $\mu\text{g/g}$ ). In soybean sprout, the total  $\beta$ -carotene concentration was 21.8  $\mu\text{g/g}$  in black seed coat sprout, 38.7  $\mu\text{g/g}$  in brown seed coat sprout, 34.1  $\mu\text{g/g}$  in green seed coat sprout, 39.5  $\mu\text{g/g}$  in yellow seed coat sprout, and 30.5  $\mu\text{g/g}$  in mottle seed coat sprout. The results of this study suggested the functional characteristics of soybean through quantitative analysis of  $\beta$ -carotene.

**Keywords :**  $\beta$ -carotene, soybean, seed, sprout

**Approximately** 100 million tons per year or more carotenoids are produced in the natural ecosystem (Kim *et al.*, 1997). And, the carotenoids content in plant affected by genetics and their external environment (Bauernfeind, 1973). The carotenoids belong to tetra-terpenoid ( $\text{C}_{40}$ ) group and

shows orange color, usually fat-soluble (Kim, 2001; Simpson and Chichester, 1981). In addition, that is very unstable substance, so oxidation or isomerization takes place easily (Kim, 2001).

Nutritional importance of carotenoids are the provitamin A activity, food colorants, antioxidant activity, anticarcinogenic substance, oxygen transporters, immune activation, absorbers of light energy. Of these,  $\beta$ -carotene has  $\beta$ -rings at both ends, the most powerful active oxygen remover and the highest vitamin A activity (Kim, 2001 ; Jo and Jung, 2000 ; Stahelin *et al.*, 1991 ; Lee and Kim, 1997 ; Kim *et al.*, 1997). Also, it reduces the risk of prostatic cancer, related to the arteriosclerosis and cardiovascular disease, and inhibition of LDL oxidation (Kirsh *et al.*, 2006 ; Oliver *et al.*, 1998 ; Mascio *et al.*, 1991). And, it has been known low carotene was related to a bronchus cancer (Stahelin *et al.*, 1991).

Because the human body cannot synthesize vitamin A itself, human must be obtained from the diet. In other words, no side effects as  $\beta$ -carotene intake is recommended, this is converted into vitamin A in the body (Provitamin A).

Soybean sprout is short growing time and short relatively easy to grow, low price, and also occupies a large proportion in the daily diet (Oh *et al.*, 2007). Mainly, the soybean sprouts grown to 100-seed weight range was 7 -15 g, and smaller seed has the higher the yield of soybean sprouts, good vitality, excellent hypocotyls growth (Lee *et al.*, 2002 ; Bates and Matthews, 1975). Seed nutrition was changed in germination process, while the fat content was reduced, fiber and crude protein were increased and especially, remarkable increase in the content of vitamins (Kim, 1981). In particular, vitamin C is not found in soybean seed, but it is synthesized in the germination process (Kim *et al.*, 1993 ; Kim *et al.*, 2007). Shimoyamada *et al.* (1991) reported that

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after germination, fresh weight was increased an 8-fold, and dry weight was not changed. Kim *et al.* (2003) reported that isoflavone content was increased about 1 times in the process of germination. Among the soybean sprouts, green sprout had a high nutritional value, specially.

In our study,  $\beta$ -carotene contents of soybeans examined according to the size, usage, seed coat color and cotyledon color as well as the process of seed germination.

## MATERIALS & METHODS

### Preparation of samples

The 68 Korean soybean varieties were cultivated at Yesan of Chungnam in 2006, according to standard cultivation method. Soybean seed were harvested at triplicate replications in each cultivar and stored at 4°C for analysis of  $\beta$ -carotene. The varieties were classified according to the seed coat color, cotyledon color, 100-seed weight and usage.

In this study, soybean was classified into three types, large (above 25 g, 22 varieties), medium (15-25 g, 27 varieties), and small (below 15 g, 30 varieties) by 100-seed weight, respectively. Soybean sprout was prepared with following procedure. 4 g of soybean seed was washed and submerged in tap water at  $20 \pm 2^\circ\text{C}$  for 12 h. Seed was spread uniformly in conical tube with holes and gave enough water more than 6 times a day, and cultivated five days in the dark chamber. After all soybean and soybean sprout was freeze-dried at  $47^\circ\text{C}$ , and then, crushed and stored in dessicator for analysis. When the soybean sprout was grown by absorbing water, its weight was increased the four times, compared to the seed fresh-weight. But, freeze-dried to completely remove the water, weight was reduced 22%, compared to the seed fresh-weight. Similarly, Shimoyamada *et al.* (1991) reported that during seed germination, seed fresh weight was increased about 8 times, while seed dry weight did not changed.

### Analysis of $\beta$ -carotene

The concentration of  $\beta$ -carotene was analyzed by the modified method of Jo and Jung (2000). The 1 g of finely ground soybean seed and sprout powder were extracted with 40 mL of acetone : petroleum ether = 1 : 1 solution and stirred 30 min at room temperature. The suspension

was filtered through Whatman filter paper (No. 4). This process repeated twice in each sample. The extract was transferred to a separatory-funnel and added 50 mL of saturated sodium chloride solution, then mixed. To obtain a supernatant, wait until the layer was separated, and then was added 100 mL of distilled water, then mixed. The following process was expressed as saponification. But Oliver *et al.* (1998) reported that the photo-diode array detection enables the spectra of each individual peak of the chromatogram, it could allow carotenoid separation without sample saponification. And Khachik *et al.* (1986) reported that saponification of carotenoid extracts underestimated the individual carotenoid content (Individual carotenoid contents were reduced by saponification). For this reasons, saponification was omitted in this experiment.

After obtaining a supernatant, residual moisture was removed by sodium sulfate anhydrous. Supernatant was transferred to a 100 mL round flask and evaporated to dryness under reduced pressure using a rotary vacuum evaporator at below  $35^\circ\text{C}$ , and the remaining residue was reconstituted with 4 mL of 0.01% BHT (Butylated Hydroxy Toluene) in chloroform. The aliquot sample was filtered through a  $0.2 \mu\text{m}$  nylon filter (TITAN, USA) and analyzed by HPLC. For all of the experimental process was conducted in dark condition. All of solvents were used as HPLC grade (J. T. Baker, USA) without further purification and all of reagents were used as special grade (Junsei, Japan).

The HPLC system was consists of SPD-M10A Photo Diode Array (PDA) Detector (Shimazu, Japan), Midas auto-injector (Younglin, Korea) and a 20  $\mu\text{L}$  sample loop and LC-10AD VP pump. The separation of  $\beta$ -carotene was performed by ODS  $\text{C}_{18}$  column ( $250 \times 4.6 \text{ mm I.D.}$ ). The maximum absorption wavelength of  $\beta$ -carotene was measured at 452 nm. The mobile phase was followed, methanol (MeOH) : tetrahydrofuran (THF) = 8 : 2, isocratic. In selecting the appropriate solvent ratio for analysis, several solvents combinations were done. Because a  $\beta$ -carotene is a non-polar substance, the percentage of THF (non-polar solvents) increased in mobile phase. Retention time (RT) was detected at 13.7 min. Running time was 25 min and flow rate was 1 mL/min. The  $\beta$ -carotene standard, which was purchased from Sigma-Aldrich (USA), was used to make the calibration curve. The  $\beta$ -carotene standard was dissolved in 0.01% BHT in

4 mL chloroform, the plotting standards were made at four concentrations of 25, 50, 100 and 250  $\mu\text{g}$ , and a high linearity ( $R^2=0.996$ ) was obtained. The interpretation of the  $\beta$ -carotene was supported its retention time ( $\beta$ -carotene : 13.7 min) and adsorption spectra in HPLC.

### Statistical analysis

The analysis of statistical was undertaken using the general linear model procedure (GLM) of the statistical analysis program (SAS, 2000). All experiments were determined as duplicates. The least significant difference (LSD) test was based on the 0.05 probability level.

## RESULTS AND DISCUSSION

The total average concentration of  $\beta$ -carotene was 6.6  $\mu\text{g/g}$  in soybean seed, 33.3  $\mu\text{g/g}$  in soybean sprout. In comparison with soybean seed, soybean sprout increased about 5 times for total average of  $\beta$ -carotene concentration. And the average concentration of  $\beta$ -carotene in soybean seed and sprout was significantly different ( $p < 0.05$ ) (Fig. 1). That is,  $\beta$ -carotene content was increased significantly in the germination process. These results is similar to those of previous studies by Kim *et al.* (1993) and Liu (1997), who demonstrated that  $\beta$ -carotene content increased 17-fold during seed germination process on the dry weight. Also,  $\beta$ -carotene content in the soybean sprout was 6 times higher than that of soybean seed. In soybean seed,  $\beta$ -carotene concentration of Cheongbang-kong (12.9  $\mu\text{g/g}$ ) was the highest

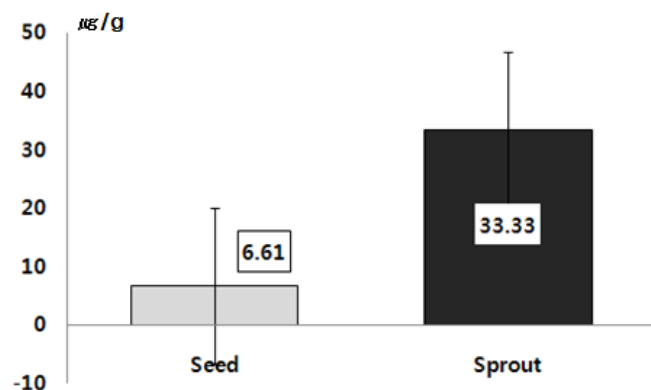


Fig. 1. Comparison of  $\beta$ -carotene concentration between soybean seed and sprout.

\*Each vertical bar indicates the standard error values.

content, while, Sojin-kong (5.8  $\mu\text{g/g}$ ) was the lowest content. In soybean sprout, total  $\beta$ -carotene concentration of Buakdari-kong (75.8  $\mu\text{g/g}$ ) was the highest content, while, Ogapi-kong (6.5  $\mu\text{g/g}$ ) was the lowest content.

The result of this study may provide plant breeders with information for selecting soybean varieties that contain high concentrations of  $\beta$ -carotene. Also, this results will be helpful as basic information for food industries.

### Concentration of $\beta$ -carotene, according to the seed size

In this study, soybean varieties were classified as large (above 25 g, 16 varieties), medium (15 ~ 25 g, 26 varieties), and small (below 15 g, 26 varieties) based on 100-seed weight. The total  $\beta$ -carotene concentration of soybean seed was ranged from 5.9 to 11.8  $\mu\text{g/g}$  in large soybean seed, from 5.8 to 12.9  $\mu\text{g/g}$  in medium soybean seed, and from 5.8 to 7.6  $\mu\text{g/g}$  in small soybean seed. In soybean sprout, the total  $\beta$ -carotene concentration was ranged from 6.5 to 41.9  $\mu\text{g/g}$  in large soybean sprout, from 7.7 to 75.8  $\mu\text{g/g}$  in medium soybean sprout, and from 11.4 to 73.6  $\mu\text{g/g}$  in small soybean sprout (Table 1). According to the seed size,

Table 1. Comparison of  $\beta$ -carotene concentration between soybean seed and sprout on seed size difference.

Seed size		Seed	Sprout
		----- $\mu\text{g/g}$ -----	----- $\mu\text{g/g}$ -----
Large seed (16 varieties)	Maximum	11.8	41.92
	Minimum	5.9	6.45
	Mean	6.88	21.44
	CV (%)	8.99	9.95
	LSD (0.05)	1.31	4.52
Medium seed (26 varieties)	Maximum	12.85	75.77
	Minimum	5.82	7.68
	Mean	6.74	30.52
	CV (%)	8.92	15.77
	LSD (0.05)	1.24	9.90
Small seed (26 varieties)	Maximum	7.61	73.55
	Minimum	5.78	11.35
	Mean	6.31	43.46
	CV (%)	5.07	10.44
	LSD (0.05)	0.66	9.32

the total  $\beta$ -carotene in soybean seed was not significant different ( $p < 0.05$ ). Whereas, the soybean sprout concentration according to different seed size was significant difference ( $p < 0.05$ ).

In the  $\beta$ -carotene concentration change during germination process, large soybean seed was 14.6  $\mu\text{g/g}$  (312%), medium soybean seed was 23.8  $\mu\text{g/g}$  (453%), and small soybean seed showed 37.2  $\mu\text{g/g}$  (689%). The total  $\beta$ -carotene was significantly increased in small soybean seed. From this study,  $\beta$ -carotene concentrations of soybean sprouts higher than soybean seeds and showed difference by seed size.

#### Concentration of $\beta$ -carotene, according to the seed usage

According to seed usage, soybeans divided into three types : cooked with rice (27 varieties), paste and curd (16 varieties), and sprout (25 varieties). The total  $\beta$ -carotene concentration of soybean seed was ranged from 5.8 to 12.9  $\mu\text{g/g}$  in cooked with rice soybean seed, from 5.8 to 6.5  $\mu\text{g/g}$  in paste and curd soybean seed, and from 5.8 to 7.6  $\mu\text{g/g}$  in sprout soybean seed. In soybean sprout, the total  $\beta$ -carotene concentration was ranged from 6.5 to 71.1  $\mu\text{g/g}$

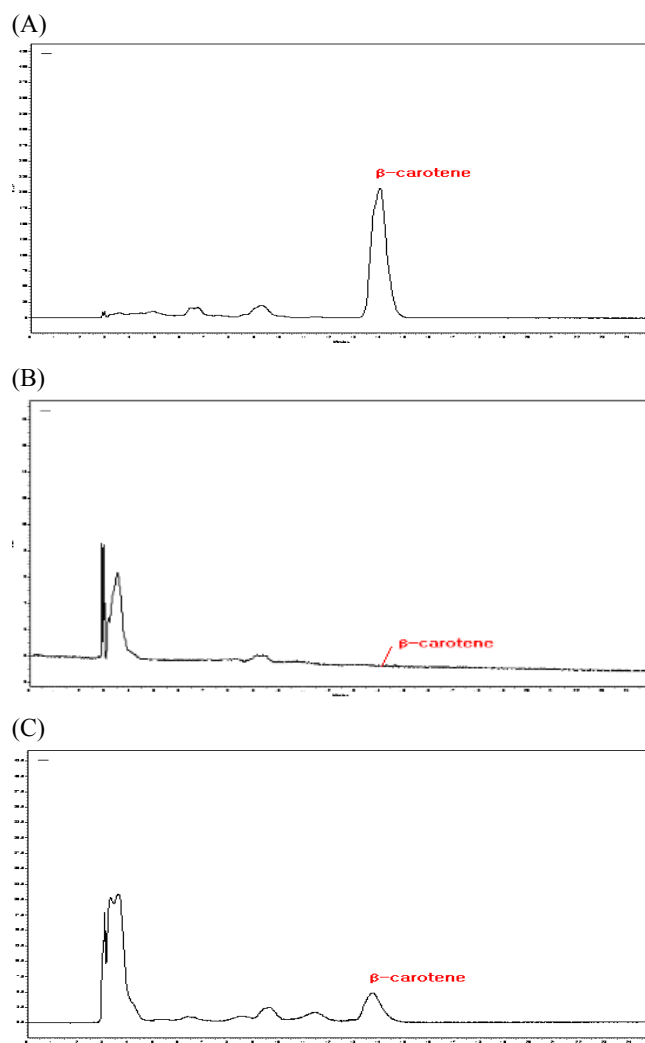
**Table 2.** Comparison of  $\beta$ -carotene concentration between soybean seed and sprout on seed usage.

Seed usage		Seed	Sprout
		$\mu\text{g/g}$	
Cooked with rice (27 varieties)	Maximum	12.85	71.13
	Minimum	5.81	6.45
	Mean	7.21	25.91
	CV (%)	9.90	10.87
	LSD (0.05)	1.46	5.78
Paste and curd (16 varieties)	Maximum	6.47	70.43
	Minimum	5.82	7.68
	Mean	6.06	32.42
	CV (%)	5.49	18.68
	LSD (0.05)	0.71	12.84
Sprout (25 varieties)	Maximum	7.61	75.77
	Minimum	5.78	11.35
	Mean	6.31	41.94
	CV (%)	5.16	9.65
	LSD (0.05)	0.67	8.34

in cooked with rice soybean sprout, from 7.7 to 70.4  $\mu\text{g/g}$  in paste and curd soybean sprout, and from 11.4 to 75.8  $\mu\text{g/g}$  in sprout soybean sprout (Table 2). The total  $\beta$ -carotene in soybean seed and sprout as based on their seed usage was significantly different ( $p < 0.05$ ) (Fig. 3).

$\beta$ -carotene concentration increased during germination process. Cooked with rice soybean seed was 18.7  $\mu\text{g/g}$  (359%), paste and curd soybean seed was 26.4  $\mu\text{g/g}$  (535%), sprout soybean seed was 35.6  $\mu\text{g/g}$  (664%). The total  $\beta$ -carotene significantly increased in sprout soybean seed.

As a result, the concentration of total  $\beta$ -carotene was increased with germination process, and depends on the



**Fig. 2.** The chromatogram of  $\beta$ -carotene in Akjongdari-kong soybean seed and sprout.  
(A)  $\beta$ -carotene standard; (B) Akjongdarikong soybean; (C) Akjongdari-kong soybean sprout.

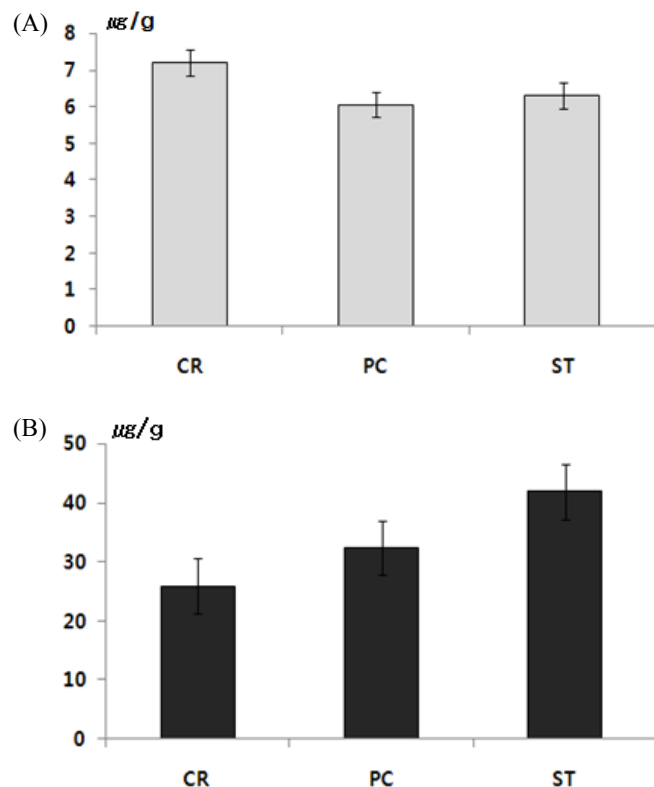


Fig. 3. Comparison of  $\beta$ -carotene concentration in soybean seed and sprout on seed usage.

\*Each vertical bar indicates the standard error values.  
A, soybean seed; B, soybean sprout; CR, cooked with rice ; PC, paste and curd ; ST, sprout.

usage of soybean seed. The large and medium soybean has been mainly used as a cooked with rice soybean, and paste and curd soybean, small soybean seed has been used as sprout seed. In this point, it could be connected with the result of on the seed size.

#### Concentration of $\beta$ -carotene, according to the seed coat color

Soybean seeds were divided into five types according to seed coat colors : black (17 varieties), brown (3 varieties), green (13 varieties), yellow (30 varieties), and mottle (5 varieties). The total  $\beta$ -carotene concentration of soybean with brown seed coat (8.8  $\mu\text{g/g}$ ) was slightly higher than those of soybean with yellow (6.1  $\mu\text{g/g}$ ) (Fig. 4). In soybean sprout, the total  $\beta$ -carotene concentration was 21.8  $\mu\text{g/g}$  in black seed coat sprout, 38.7  $\mu\text{g/g}$  in brown seed coat sprout, 34.1  $\mu\text{g/g}$  in green seed coat sprout, 39.5  $\mu\text{g/g}$  in yellow

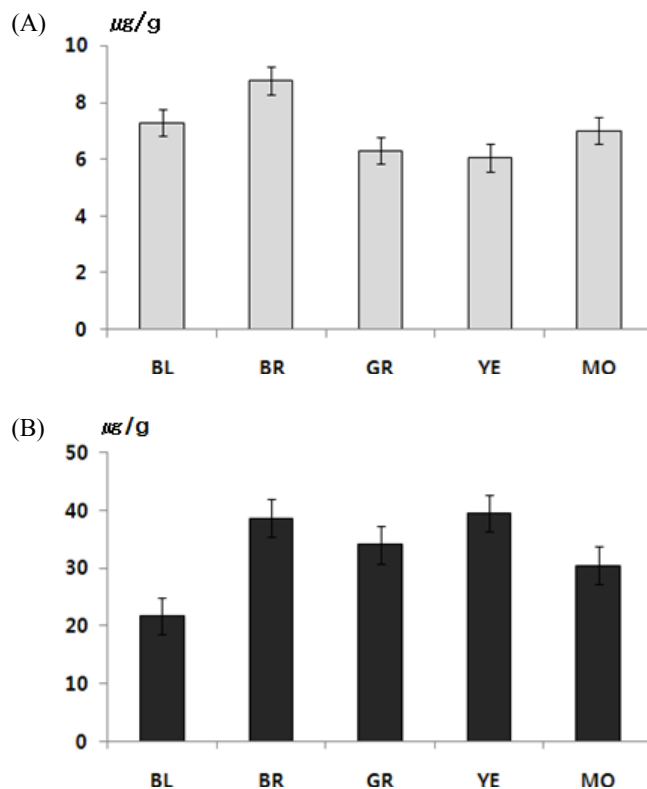


Fig. 4. Comparison of  $\beta$ -carotene concentration in soybean seed and sprout on seed coat color.

\*Each vertical bar indicates the standard error values.  
(A) soybean seed; (B) soybean sprout; BL, black; BR, brown ; GR, green; YE, yellow; MO, mottle.

seed coat sprout, and 30.5  $\mu\text{g/g}$  in mottle seed coat sprout.

In germination process,  $\beta$ -carotene concentration change of yellow seed coat soybean seed was 33.5  $\mu\text{g/g}$  (652%), brown was 29.9  $\mu\text{g/g}$  (440%), green was 27.8  $\mu\text{g/g}$  (539%), mottle was 23.5  $\mu\text{g/g}$  (434%), and black was 14.5  $\mu\text{g/g}$  (298%). The total  $\beta$ -carotene content significantly increased in yellow seed coat soybean.

Consequently, the total  $\beta$ -carotene accumulation of soybeans showed difference according to their seed coat color.

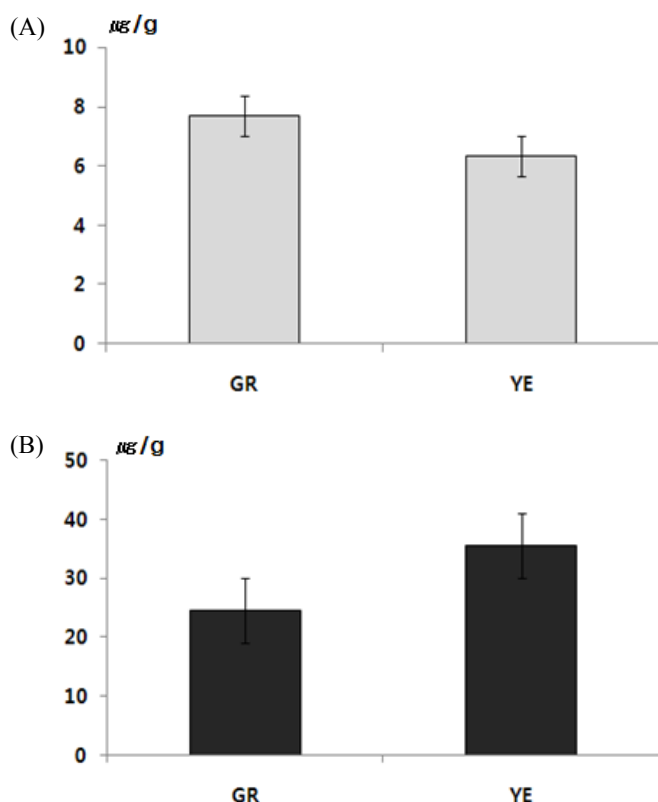
#### Concentration of $\beta$ -carotene, according to the seed cotyledon color

The soybean samples divided into two types according to cotyledon color, that green (14 varieties) and yellow (54 varieties). The total  $\beta$ -carotene concentration of soybean with green cotyledon (7.7  $\mu\text{g/g}$ ) was higher than those of soybean with yellow (6.3  $\mu\text{g/g}$ ), and content difference was

significant ( $p < 0.05$ ). In soybean sprout, the total  $\beta$ -carotene concentration was 24.5  $\mu\text{g/g}$  in green cotyledon sprout, and

**Table 3.** Comparison of  $\beta$ -carotene concentration between soybean seed and sprout on seed cotyledon color.

Seed cotyledon color		Seed	Sprout
		----- $\mu\text{g/g}$ -----	
Green (14 varieties)	Maximum	12.85	71.13
	Minimum	5.80	6.86
	Mean	7.68	24.55
	CV (%)	12.45	9.30
	LSD (0.05)	2.05	4.90
Yellow (54 varieties)	Maximum	8.29	75.77
	Minimum	5.78	6.45
	Mean	6.33	35.61
	CV (%)	4.98	12.89
	LSD (0.05)	0.63	9.20



**Fig. 5.** Comparison of  $\beta$ -carotene concentration in soybean seed and sprout on seed cotyledon color.

\*Each vertical bar indicates the standard error values. (A) soybean seed; (B) soybean sprout; GR, green; YE, yellow.

35.6  $\mu\text{g/g}$  in yellow cotyledon sprout, however, content difference was not significant ( $p < 0.05$ ) (Table 3, Fig. 5). During germination, the content of total  $\beta$ -carotene increased in green (16.9  $\mu\text{g/g}$ , 319%), yellow (29.3  $\mu\text{g/g}$ , 562%). The total  $\beta$ -carotene was significantly increased in yellow seed coat soybean.

From this study, the average total  $\beta$ -carotene concentrations affected by seed cotyledone color. And  $\beta$ -carotene concentrations increased during germination process.

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