Available online at www.sciencedirect.com

ScienceDirect

Food Chemistry 102 (2007) 1071–1075

Food Chemistry

www.elsevier.com/locate/foodchem

Effects of genetic variability and planting location on the phytosterol content and composition in soybean seeds

Akihiko Yamaya [a], Yasushi Endo [a,*], Kenshiro Fujimoto [a,c], Keisuke Kitamura [b]

[a] *Laboratory of Food and Biomolecular Science, Graduate School of Agricultural Science, Tohoku University, 1-1 Tsutsumidori-Amamiyamachi, Aoba-ku, Sendai, Miyagi 981-8555, Japan*
[b] *Laboratory of Plant Genetics and Evolution, Graduate School of Agriculture, Hokkaido University, Kita-ku, Sapporo, Hokkaido 060-8589, Japan*
[c] *Koriyama Womens' University, Koriyama, Fukushima 963-8503, Japan*

Abstract

Phytosterols present in soybean seeds have specific physiological activities related to serum cholesterol reduction. Therefore, soybean seeds containing high levels of phytosterols are required for soybean products as functional foods. However, little is known about effects of genetic variability and planting location on the phytosterol content and composition in soybean seeds. In this study, we analyzed phytosterol composition of 510 germplasm accessions of cultivated soybean seeds by gas liquid chromatography (GLC) as well as oil content. Phytosterol contents were in the range of 202 and 843 μg/g seed, and they depended on varieties of soybean seeds, but there was no significant difference between the phytosterol content in Japanese and non-Japanese seeds. The phytosterol content was higher in high oil soybean seeds. β-Sitosterol was the major phytosterol (43–67%) in soybean seeds, followed by campesterol (17–34%) and stigmasterol (10–30%). The phytosterol proportions were almost the same in all soybean seeds and it was not influenced by genetic variability and planting location.

Keywords: Campesterol; Phytosterol; β-Sitosterol; Soybean; Stigmasterol

---

1. Introduction

Phytosterols are isoprenoids and are widely distributed in plants. The content and composition of phytosterols that are enzymatically synthesized from acetyl-CoA are different among plant species. Especially, phytosterols are rich in oilseeds such as sesame, soybean and rapeseed. Recently, the nutritional function of the phytosterol has been attractive because of effects on human health. It was reported that phytosterols such as β-sitosterol offer anticancer effect (Award & Fink, 2000), prostatic hypeplasia-lowering effect (Berges, Wineler, & Trampisch, 1995; Klippel, Hiltl, & Schipp, 1997), and stimulation of a plasminogen-activating factor (Kojima, Soga, Hagiwara, Shimonaka, & Inada, 1986). In particular, there are many reviews on cholesterol-lowering activity of phytosterols (Jones, MacDougall, Ntanios, & Vanstone, 1997; Ostlund, 2004; Satou, 2003; Wong, 2001). It has been demonstrated that soybean phytosterols are able to reduce serum cholesterol by inhibiting the absorption of cholesterol (Ikeda & Sugano, 1983). Therefore, phytosterols are often supplemented into functional foods to improve human health.

Soybean, one of most important oil crops, is widely utilized for production of many foods (Miso, Soy source, Tofu and etc.) in Japan and other Asian countries. Soybean seeds contain 0.2–0.3% phytosterols (Kajimoto, Shibahara, & Yamashoji, 1982; Nagao & Yamazaki, 1988). Phytosterols are especially rich in the germ. Soybean phytosterols consist mainly of β-sitosterol, campesterol and stigmasterol; β-sitosterol content being highest among them. Recently soybean seeds with high levels of phytosterols are sought, but little is known about the genetic variability and planting location on their content and profile of phytosterols. In this study, we analyzed the phytosterol composition of 510 soybean seeds (263 Japanese seeds and 247 non-Japanese seeds) cultivated in the world, and discuss the effect of genetic variability and planting location.

---

* Corresponding author. Tel.: +81 22 717 8799; fax: +81 22 717 8802.
E-mail address: endo@biochem.tohoku.ac.jp (Y. Endo).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.07.001

2. Materials and methods

2.1. Seed materials

Five hundred and ten cultivars of soybean [*Glycine max* (L.) Merr.] seeds were obtained from the Graduate School of Agriculture, Hokkaido University (Sapporo, Japan) and the gene bank of National Institute of Agrobiological Sciences in Tsukuba, Japan in 2003. Soybean seeds were mainly harvested from 1981 to 1996, although the harvest year of some non-Japanese seeds was unidentified. As for some cultivars including *Kuromame* and *Shirodaizu*, 2–8 varieties with different harvest year or planting location were sampled for the same cultivar. More than 10 beans were pooled at least for each variety.

2.2. Phytosterol analysis

Based on a standard method [2.4.9.1-1996] of the Japan Oil Chemists' Society (JOCS, 1996), oils were extracted from 1 g of ground seeds (3–6 beans) each by the Soxhlet extraction method and were weighed. Cholesterol (500 μg/mL ethanol), as an internal standard, was introduced into 10 mL- screw-capped test tubes together with extracted oils for quantification of individual phytosterols and total phytosterols. Four milliliters of potassium hydroxide (1 M) in ethanol solution was added into the test tube, and samples were heated at 80 °C for 90 min. After saponification, unsaponifiables were extracted with petroleum ether, and then were supposed to gas liquid chromatography (GLC) after acetylation with acetic anhydride and pyridine (1:2) at room temperature overnight. A Shimadzu gas chromatograph with a flame-ionization detector and a DB-1 capillary column (0.25 mm × 30 m, J&W Scientific, Folsom, CA) were used. The column temperature was 280 °C, and the injection and detector temperature was 300 °C. Individual sterols were mainly identified by the retention time of corresponding standard phytosterols and their contents were determined from the ratio of each peak area compared to the internal standard, although phytosterols in several soybean seeds were also identified using a Shimadzu GC–MS QP1000EX gas chromatograph–mass spectrometer. Total phytosterol content was calculated by summing up the contents of individual phytosterols.

All analytical experiments were performed in duplicate; that is sample preparation to measure phytosterol and oil contents. The results were averaged for analytical data of two samples.

3. Results and discussion

3.1. Effects of genetic variability on the phytosterol content and composition

The phytosterols in 510 cultivars of soybean seeds (263 Japanese varieties and 247 non-Japanese varieties) were determined by GLC. Non-Japanese soybean seeds included varieties from 22 countries in USA, South America, Europe and Asia. Phytosterol content in oils was 0.17–0.91%, and the average was 0.38%. The average was almost the same as that in a previous report (Abe, 1988). Total phytosterol content in soybean seeds is shown in Fig. 1. Japanese seeds contained 202–694 μg/g seed of phytosterols (the average was 414 μg/g seed), while non-Japanese seeds contained 223–843 μg/g seed of phytosterols (the average was 427 μg/g seed). There was no significant difference between the phytosterol content in Japanese and non-Japanese seeds, although the phytosterol content was influenced by varieties of soybean seeds. Moreover, there was no correlation between the phytosterol content in soybean seeds examined and the size and weight of beans (data not shown). Harvest year did not apparently affect the phytosterol content in soybean seeds.

Figs. 2–4 show the contents of β-sitosterol, campesterol and stigmasterol in 510 seeds, respectively. The content of each phytosterol in soybean seeds was different by genotypic variation, but no seeds containing extremely high levels of sterols were found. The β-sitosterol contents in Japanese and non-Japanese seeds were 102–455 and 126–512 μg/g seed, respectively, as shown in Fig. 2. Japanese and non-Japanese seeds contained 230 and 244 μg/g seed of β-sitosterol as an average, respectively. No significant

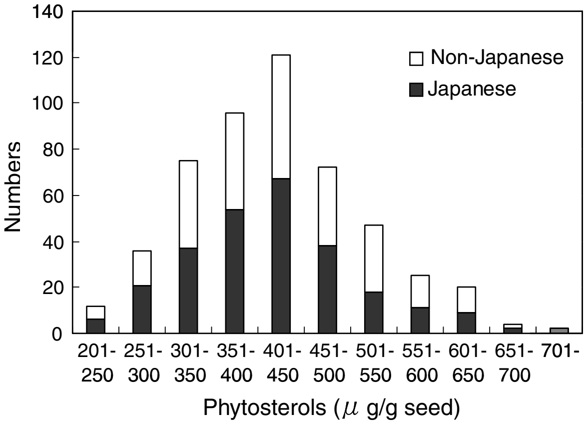

Fig. 1. Total phytosterol content in 510 cultivars of soybean seeds.

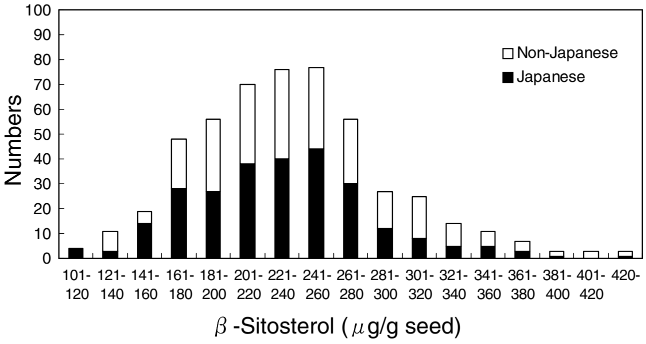

Fig. 2. β-Sitosterol content in 510 cultivars of soybean seeds.

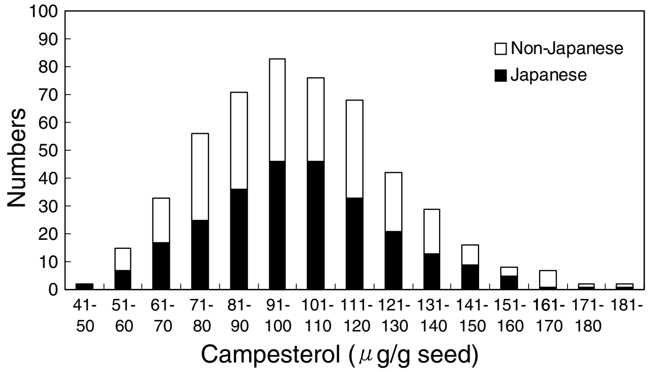

Fig. 3. Campesterol content in 510 cultivars of soybean seeds.

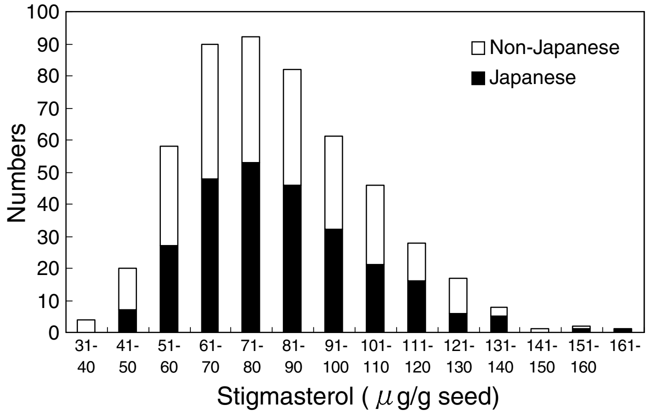

Fig. 4. Stigmasterol content in 510 cultivars of soybean seeds.

difference was observed between the β-sitosterol content in Japanese and non-Japanese seeds, although the β-sitosterol content depended on varieties of soybean tested. The corresponding campesterol contents in Japanese and non-Japanese seeds were 45–181 (the average was 101 μg/g seed) and 51–199 μg/g seed (the average was 102 μg/g seed), respectively (Fig. 3). The stigmasterol contents in Japanese and non-Japanese seeds were 45–177 (the average was 83 μg/g seed) and 34–152 μg/g seed (average was 81 μg/g seed), respectively (Fig. 4). There were no significant differences between the campesterol and stigmasterol contents in Japanese and non-Japanese seeds, although they were also affected by genotypes of soybean.

Soybean seeds showed almost the same phytosterol composition regardless of different total phytosterol contents. The major phytosterol was β-sitosterol (43–67%), followed by campesterol (17–34%) and stigmasterol (10–30%). β-Sitosterol level was highest among phytosterols in all soybean seeds. No seeds containing higher amounts of campesterol or stigmasterol than β-sitosterol were found. These observations show that the phytosterol composition was almost constant and independent of genotypic variation. Moreover, the size and weight of beans and harvest year did not influence the phytosterol composition. Mohamed and Rangappa (1992) analyzed the sterol content and composition of 17 soybean seed genotypes in USA. They found that the phytosterol content was different in varieties, but that the phytosterol composition was almost the same for 17 genotypes. When Maestri, Meriles, and Guzman (1998) measured the phytosterol composition in 19 cultivars of soybean seeds in Argentina, they observed no significant differences. Our results are consistent with the observations of these two research groups.

3.2. Effects of planting location on the phytosterol content and composition

The effect of planting location on the phytosterol content and composition in soybean seed was investigated using two typical soybean cultivars, *Kuromame* and *Shirodaizu*. The phytosterol content in 5 *Kuromame* and 4 *Shirodaizu* seeds grown in different districts was measured. As shown in Fig. 5, total phytosterol contents in *Kuoromame* and *Shirodaizu* seeds were somewhat dependent on the planting location. *Kuromame* seeds grown in Nara contained 498 μg/g seed of phytosterols, whereas those in Fukushima contained 304 μg/g seed. *Shirodaizu* seeds grown in Okayama contained 385 μg/g seed of phytosterols, whereas those in Shimane contained 272 μg/g seed. Soybean seeds grown in the warm area tended to contain more phytosterols. These observations suggest that planting location may be a factor in determining the phytosterol content in soybean seeds. Different phytosterol contents in soybean seeds may be due to their different state maturity based on planting temperature. On the other hand, the phytosterol composition was almost same in all soybean seed samples, and it was not affected by planting location. The relative percentages of β-sitosterol, campesterol and stigmasterol in *Kuromame* seeds were 51–59, 21–28 and 19–22%, while those in *Shirodaizu* seeds were 51–58, 22–28 and 20–24%, respectively. From these results, it is clear that plantation location and genetic variability could affect the phytosterol content in soybean seeds but not the phytosterol composition.

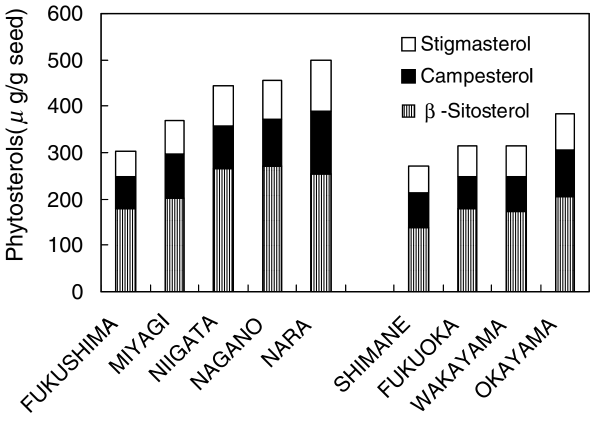

Fig. 5. Phytosterol contents in *Kuromame* and *Shirodaizu* soybean seeds.

Although the phytosterol content in soybean seed might be controlled by gene modification and planting condition, the phytosterol composition could not be modified by them.

3.3. Relationship between phytosterol content and oil content

The oil content in soybean seeds was also measured, in order to explore the relationship between the oil and phytosterol contents. The oil content was in the range of 4 and 21%, and the average was 11.5%. The oil content depended on genotypic variation and planting location. When the phytosterol content in seeds was plotted against oil content in seeds, a positive correlation ($r = 0.537$) was observed (Fig. 6). The phytosterol content was higher in high oil seeds.

The phytosterol is considered to relate to fluidity and permeability of cell membranes in plants because of its physicochemical properties such as melting point, hydrophobicity and crystallization (Piironen, Kindsay, Miettinen, Toivo, & Lampi, 2000). Soybean accumulates the oil as lipid body in its seeds. Probably phytosterol could be necessary for structuring the lipid body in soybean seed. Thus, the phytosterol content might be higher with increased oil content in soybean seed.

In a previous paper (Ujiie, Yamada, Fujimoto, Endo, & Kitamura, 2005), we determined the content of tocopherol homologues, being isoprenoid compounds, in soybean seeds. We found α-tocopherol-rich seeds, although γ-tocopherol was the major tocopherol in most of the soybean seeds tested. Tocopherol content and composition were influenced by the genotypic varieties of soybean seeds. In this study, we measured phytosterols in 510 kinds of soybean seeds, but no drastic variations in the phytosterol content and composition were observed. Actually, no correlation ($r = -0.142$) existed between tocopherol and phytosterol contents in soybean seeds. These results suggest that the biosynthesis of phytosterol may be controlled by systems different from that of tocopherols in soybean seeds, although both phytosterols and tocopherols are isoprenoid compounds. The key enzymes to produce phytosterols always maintain a constant activity in all soybean seeds, and it may not be influenced by genotypes and environment. This study would be helpful for breeding soybean with high bioactive content.

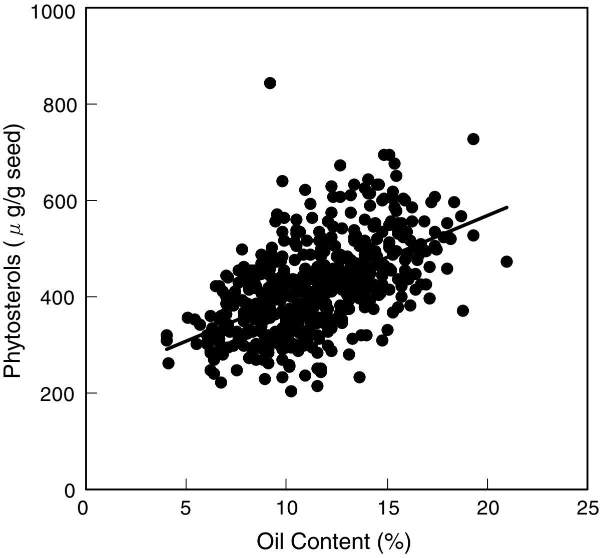

Fig. 6. The relationship between total phytosterol content and oil content in 510 cultivars of soybean seeds.

4. Conclusions

Phytosterol content and composition were measured for 510 kinds of soybean seeds. Soybean phytosterols consisted of β-sitosterol, campesterol and stigmasterol; β-sitosterol was the major phytosterol. There was no significant difference in phytosterol content between Japanese and non-Japanese seeds. Genetic variability and planting location affected the phytosterol content in seeds, but not the phytosterol composition.

References

Abe, Y. (Ed.). (1988). *Handbook of fats and oil seeds*. Tokyo: Saiwaishobo.

Award, A. B., & Fink, C. S. (2000). Phytosterols as anticancer dietary components: evidence and mechanism of action. *Journal of Nutrition, 130*, 2127–2130.

Berges, R. R., Wineler, J., & Trampisch, H. J. (1995). Randomised, placebo-controlled, double-blind clinical trial of beta-sitosterol in patients with benign prostatic hyperplasia. Beta-Sitosterol Study Group. *Lancet, 345*, 1529–1532.

Ikeda, I., & Sugano, M. (1983). Some aspects of mechanism of inhibition of cholesterol absorption by β-sitosterol. *Biochimica et Biophysica Acta, 732*, 651–658.

Japan Oil Chemist' Society (Ed.). (1996). *Sterol in standard methods for the analysis of fats, oils and related materials*. Tokyo: JOCS, 2.4.9.1-1996.

Jones, P. J., MacDougall, D. E., Ntanios, F., & Vanstone, C. A. (1997). Dietary phytosterols as cholesterol-lowering agents in humans. *Canadian Journal of Physiology and Pharmacology, 75*, 217–227.

Kajimoto, G., Shibahara, A., & Yamashoji, S. (1982). Changes in the contents and compositions of lipids, fatty acids, tocopherols and sterols in soybean seed during maturation. *Journal of the Japanese Society for Nutrition and Food Science, 35*, 345–350.

Klippel, K. F., Hiltl, D. M., & Schipp, B. (1997). A multicentric, placebo-controlled, double-blind clinical trial of beta-sitosterol (phytosterol) for the treatment of benign prostatic hyperplasia. German BPH-Phyto Study Group. *British Journal of Urology, 80*, 427–432.

Kojima, S., Soga, W., Hagiwara, H., Shimonaka, M., & Inada, Y. (1986). Visible fibrinolysis by endothelial cells: effect of vitamins and sterols. *Bioscience Reports, 6*, 1029–1033.

Maestri, D., Meriles, J. M., & Guzman, C. A. (1998). Correlation of maturity groups with seed composition in soybeans, as influenced by genotypic variation. *Grssas y Aceites, 49*, 395–399.

Mohamed, A. I., & Rangappa, M. (1992). Nutrient composition and anti-nutritional factors in vegetable soybean: II. Oil, fatty acids, sterols, and lipoxygenase activity. *Food Chemistry, 44*, 277–282.

Nagao, A., & Yamazaki, M. (1988). Changes in lipid composition of soybean seeds during maturation. *Journal of the Japanese Oil Chemist Society, 37*, 991–999.

Ostlund, R. E. Jr., (2004). Phytosterols and cholesterol metabolism. *Current Opinion Lipidology, 15*, 37–41.

Piironen, V., Kindsay, D. G., Miettinen, T. A., Toivo, J., & Lampi, A.-M. (2000). Plant sterols: biosynthesis, biological function and their importance to human nutrition. *Journal of Science and Food Agriculture, 80*, 939–966.

Satou, H. (2003). Physiological effects and safety of plant sterols. *Oleoscience, 3*, 395–401.

Ujiie, A., Yamada, T., Fujimoto, K., Endo, Y., & Kitamura, K. (2005). Identification of soybean varieties with high levels of α-tocopherol content. *Breeding Science, 55*, 123–125.

Wong, N. C. (2001). The beneficial effects of plant sterols on serum cholesterol. *Canadian Journal of Cardiology, 17*, 715–721.