[Chem. Pharm. Bull. 33(9)3834—3837(1985)]

Histochemistry. VI.[1] Tocopherols and Isoflavones in Soybeans (*Glycine max* MERRILL, Seeds)

TADATO TANI,*,[a] TADAHISA KATSUKI,[a] MICHINORI KUBO,[a] SHIGERU ARICHI,[a] and ISAO KITAGAWA[b]

The Research Institute of Oriental Medicine, Kinki University,[a] 380 Nishiyama, Sayama, Minamikawachi, Osaka 589, Japan and Faculty of Pharmaceutical Sciences, Osaka University,[b] 1-6, Yamada-oka, Suita, Osaka 565, Japan

(Received January 10, 1985)

The possible relationship between some morphological characteristics of soybeans and the contents of their bioactive components (tocopherols and isoflavones) was histochemically examined. It was found that tocopherols were distributed at high concentration in the cotyledon and that isoflavones were mainly localized in the plumule, hypocotyl, and radicle, which subsequently grow to form the adult plant. It was also found that the isoflavone content in soybeans with green-colored seed coats might be higher than that in soybeans with yellow seed coats.

Keywords——*Glycine max*; Leguminosae; soybean; histochemistry; seed organ; HPLC; TLC; tocopherol; isoflavone; daidzin

For the purpose of qualitative evaluation of crude drugs, the authors have been histochemically investigating the bioactive components in crude drugs by a combination of morphological and chemical analyses. The previous reports[1] revealed that soyasaponins were distributed predominantly in specific organs of soybeans (plumule, hypocotyl and radicle) which subsequently grow to form the adult plant.

In addition to soyasaponins, soybeans contain tocopherols and isoflavones as bioactive constituents. In this report, we wish to describe a histochemical investigation on the distribution of tocopherols and isoflavones in various organs of soybeans.

Results

Tocopherols and Isoflavones in Three Organs of Soybeans

Eleven (A—K) kinds of soybeans were each divided into organ I [cotyledon (cot)], organ II [seed coat (sc) and hilum (hi)], and organ III [plumule (plm), hypocotyl (hyp) and radicle (rad)] (Fig. 1), and each organ was extracted as described previously.[1]

The content and composition of tocopherols and isoflavones in the three organs of soybeans were analyzed by a high-performance liquid chromatographic (HPLC) method. Tocopherols were well separated into α, β, γ and δ-isomers under the conditions used in this experiment.

It was found that tocopherols (mainly consisting of γ- and δ-tocopherol) were predominantly distributed in organ I in sample C. The content and the composition of tocopherols in whole seeds essentially coincided with the value obtained from organ I, which accounted for about 90% of the weight of the whole grain.[1]

In organ III, which subsequently grows to form the adult plant, the content ratio of δ-tocopherol was lower than that in organ I. Tocopherols were hardly detected in organ II.

Further analysis of the distribution of tocopherols in other commercially available soybeans (A—K) was performed. The content of total tocopherols in organ I [0.014—0.020%, $0.017 \pm 0.0016\%$ (average $\pm$ standard deviation (S.D.)), coefficient of variation (C.V.) $= 0.09$]

NII-Electronic Library Service

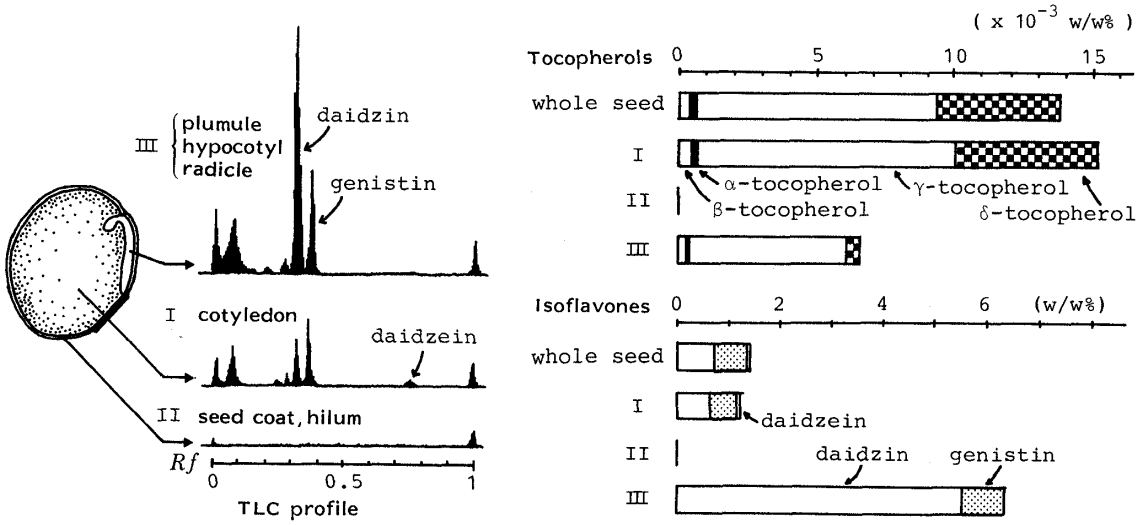

Fig. 1. Tocopherols and Isoflavones in Organ I, II and III of Soybeans (Sample C)

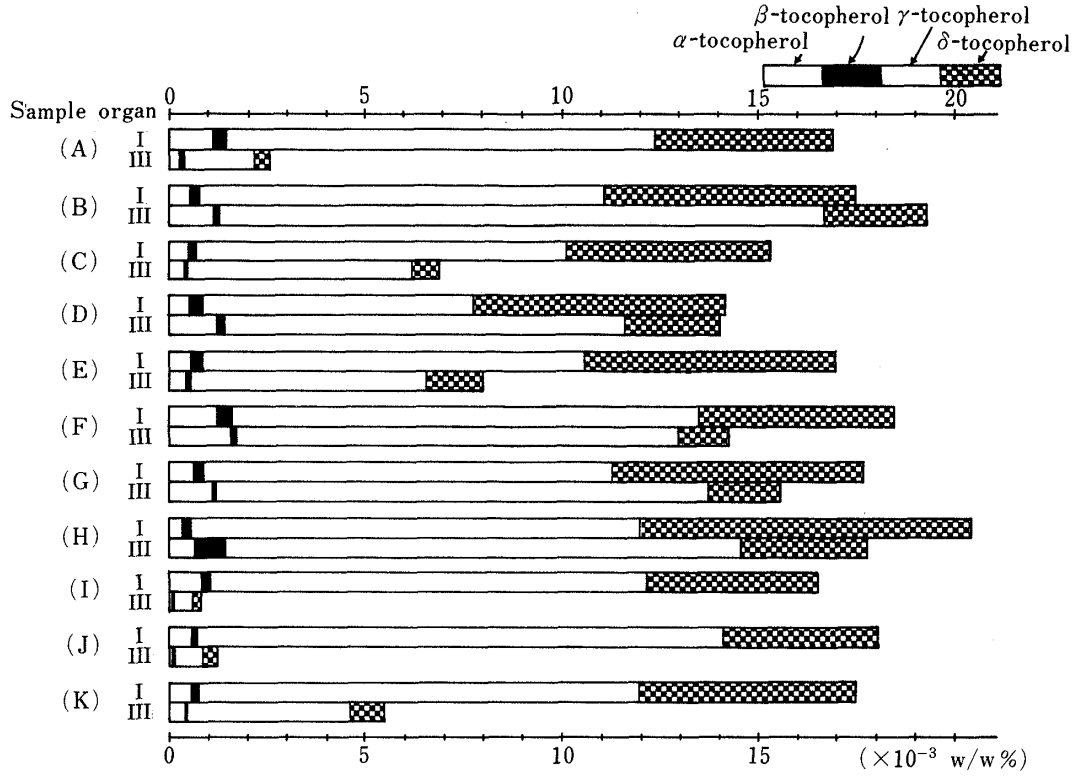

Fig. 2. Contents of Tocopherols in Organs I and III of Soybeans

Samples A—K, see Experimental; organs I and III, see Fig. 1.

did not vary markedly among the kinds of soybeans. As regards composition, more than 90% of total tocopherols was accounted for by γ-tocopherol (48.8—73.9%, 61.4±6.6%) and δ-tocopherol (22.1—45.3%, 33.2±7.1%). The contents of tocopherols in organ III (0.0007—0.019%, 0.0096±0.0068%) were found to differ significantly from those in organ I (0.014—0.020%, 0.017±0.0016%). It was also found that the composition ratio of δ-tocopherol to total tocopherols in organ III (17.6±7.7%) was only about half of the ratio in organ I (33.1±7.1%).

Qualitative and Quantitative Analyses of Isoflavones

Figure 1 shows thin-layer chromatographic (TLC) profiles of the MeOH extractives obtained from the defatted organs. It is clear that daidzin was present in organ III at a

NII-Electronic Library Service

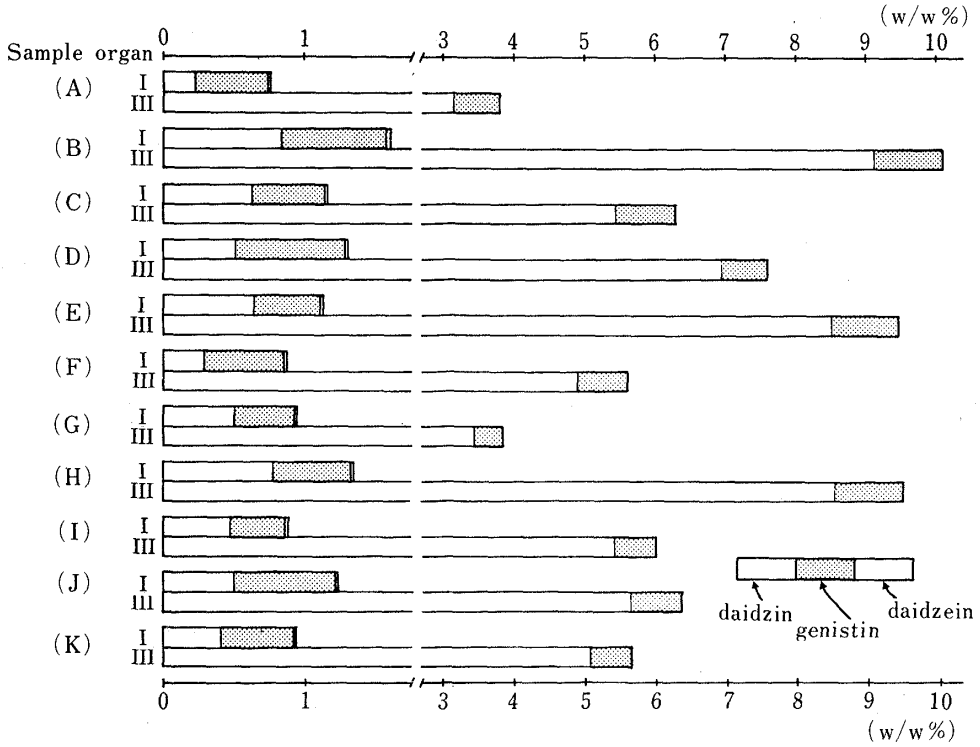

Fig. 3. Contents of Isoflavones in Organs I and III of Soybeans

remarkably high concentration. Genistin was also found in organ III and organ I, but daidzein was detected only in organ I.

Gradient elution HPLC assay was applied to separate genistin, daidzein and daidzin. As is apparent from Fig. 3, the content of daidzin in organ III (3.16—8.76%, $6.00 \pm 1.97\%$) was 7 to 17 times that in organ I (0.23—0.84%, $0.50 \pm 0.20\%$). Genistin was almost equally distributed in organ I (0.39—0.78%, $0.53 \pm 0.13\%$) and organ III (0.39—0.97%, $0.73 \pm 0.19\%$), but not in organ II. Daidzein was detected only in organ I (0.013—0.023%, $0.016 \pm 0.006\%$).

The content and the composition of isoflavones in whole grains of soybeans mostly coincided with those in organ I, as was found for tocopherols. The content to total isoflavones ranged from 0.82 to 1.62% ($1.20 \pm 0.26\%$). Individual isoflavone contents were as follows: daidzin, 0.32—0.98% ($0.63 \pm 0.20\%$); genistin, 0.36—0.73% ($0.53 \pm 0.10\%$); and daidzein, 0.012—0.025% ($0.016 \pm 0.004\%$).

Discussion

The contents and compositions of tocopherols and isoflavones in various organs of several commercial samples of soybeans were histochemically investigated. It was found that tocopherols in soybeans were distributed in organs I and III. The contents of tocopherols in whole grains were mostly accounted for by those in organ I, cotyledon, whose weight corresponds to about 90% of that of the whole seed. It was observed that γ- and δ-tocopherol represented more than 90% of the total content of tocopherols, and that the contents and compositions of tocopherols varied depending upon the kinds of soybeans tested. It was reported that, during the maturation of soybeans, δ-tocopherol content increased remarkably, while γ-tocopherol content remained constant.[2,3] Therefore, the variations of tocopherols contents observed in the present study may be ascribable to differences in the degree of maturity of the specimens.

NII-Electronic Library Service

It was reported that the contents of tocopherols in soybeans varied depending upon the color of the seed coats.[4] However, in the present study, no noticeable relationship was observed between the contents and compositions of tocopherols in 11 commercial specimens and their morphological characteristics, such as weight, width, length, and thickness, as well as the color of the seed coat and hilum.

Isoflavones, especially daidzin, were found not to be distributed equally in whole grains, but were present at high concentration in organ III, which subsequently grows to form the adult plant. In contrast genistin was found to be distributed almost equally in the organs except the seed coat.

Possible relationships between the variations of contents and compositions of isoflavones in 11 samples of soybeans and the morphological characteristics were investigated. It was found that the daidzin content in soybeans (B, C, and E—I) with a green-colored seed coat ($0.64 \pm 0.15\%$) was higher ($t = 3.27$, $p < 0.005$) than that in soybeans (A, D, J and K) with a yellow coat ($0.35 \pm 0.13\%$). However, the genistin contents in soybeans of differently colored seed coats were similar (green-colored grains, $0.52 \pm 0.13\%$, yellow ones, $0.55 \pm 0.17\%$).

The variations of isoflavones contents among the soybeans may also be dependent on the degree of maturity of the samples. Further work is in progress.

The relationship between isoflavones contents and morphological parameters (width/length and thickness/width) was investigated, but no significant relationship was found.

Experimental

Materials——Eleven commercial samples of soybeans (A, Tamanishiki daizu; B, Hokkai 2-go edamame; C, Tokachi-wase edamame; D, Daizu (Hokkaido); E, Yuzufuri-wase edamame; F, Chitose-wase edamame; G, Shiratori-wase edamame; H, Wasemidori edamame; I, dàdòu (China, Shan-tung); J, soybean (South America); and K. dàdòu (China, Peking)) were used. These specimens were the same as those used for analysis of soyasaponins in the previous work.[1]

The division of each grain into three organs (I, II, and III as shown in Fig. 1), and the extraction of tocopherols and isoflavones were carried out by the same procedures as described in the previous paper.[1]

Quantitative Analysis of Tocopherols——Tocopherols in soybeans were quantitatively analyzed in the *n*-hexane extractive, which was obtained by maceration of 30 grains of soybeans or 100 organs. The extractive was injected directly into the HPLC instrument to identify tocopherols (α-, β-, γ- and δ-tocopherol). As an internal standard, tocol synthesized from hydroquinone and phytol[5] was used.

Liquid chromatograph, LC-2 (Shimadzu); fluorescence detector, RF-530 (Shimadzu); column, ZORBAX NH_2 4.6 mm × 25 cm (Shimadzu); mobile phase, *n*-hexane–PrOH (100 : 3); flow rate, 1 ml/min; $\lambda_{EX} = 296$ nm, $\lambda_{EM} = 320$ nm. The peak areas were measured with a Shimadzu C-R1A computing integrator.

Qualitative Profile Analysis of Isoflavones——Qualitative analysis of isoflavones in the MeOH extractive obtained from defatted samples was carried out by means of TLC on a precoated silica gel plate (60 F-254, Merck) using $CHCl_3$–MeOH–H_2O–AcOEt (2 : 2 : 1 : 4, lower phase) as a developing solvent. Detection was done with a Shimadzu CS-910 dual-wavelength TLC scanner (zig-zag scan (reflection); 1.25 × 1.25 mm slit; $\lambda_S = 254$ nm; $\lambda_R = 400$ nm).

Quantitative Analysis of Isoflavones——The MeOH extractives obtained from defatted soybeans or the organs were injected directly into the HPLC instrument to analyze quantitatively daidzin, genistin, and daidzein. Liquid chromatograph, LC-3 (Shimadzu); ultraviolet (UV) detector, SPD-1 (Shimadzu); column, ZORBAX ODS 4.6 mm × 25 cm (Shimadzu); mobile phase, 20% aq. CH_3CN---(2%/min)---35% aq. CH_3CN---(4%/min)---100% CH_3CN; flow rate, 1 ml/min; wavelength, 254 nm. The peak areas were measured with a Shimadzu C-R1A computing integrator.

References

1) Part V: T. Tani, T. Katsuki, M. Kubo, S. Arichi, and I. Kitagawa, *Chem. Pharm. Bull.*, **33**, 3829 (1985).
2) G. Kajimoto, A. Shibahara, and S. Yamashoji, *J. Jpn. Soc. Nutr. Food Sci.*, **35**, 345 (1982).
3) G. Kajimoto and A. Hasebe, *J. Jpn. Soc. Nutr. Food Sci.*, **35**, 291 (1982).
4) K. Oikawa, *J. Home Econ.*, **33**, 628 (1982).
5) H. K. Pendse and P. Karrer, *Helv. Chim. Acta*, **40**, 1837 (1957).

NII-Electronic Library Service