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Determination of Sesamin and Sesamolin in Sesame (*Sesamum indicum* L.) Seeds Using UV Spectrophotometer and HPLC

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ABSTRACT: Sesamin and sesamolin, antioxidant lipid-soluble lignan compounds, are abundant in sesame (*Sesamum indicum* L.) seed oil and provide oxidative stability of oil related to sesame quality. The sesamin and sesamolin contents of 403 sesame landraces of Korea were determined by HPLC analysis of methanol extract (HPLC value), and their total lignan content was compared with those by using UV-Vis spectrophotometric analysis (UV method) of methanol (UV-MeOH value) and hexane (UV-Hexane value) extracts. HPLC values of total lignan content were strongly associated with UV-Hexane (r = 0.705) and UV-MeOH (r = 0.811**) values. The UV values from both the extracts were 3.8-4.7 times higher than those of HPLC values. Lignan content was overestimated by UV method because total compounds in the mixture solution were quantified by absorbing at the same ultraviolet wavelength as in HPLC method. UV method could more rapidly analyze small amount of sample with higher sensitivity of detection than HPLC method. Average contents of lignans in sesame germplasm evaluated in this study were 2.09 ± 1.02 mg/g of sesamin, and 1.65 ± 0.61 mg/g of sesamolin, respectively, showing significant variation for lignan components. The results showed that UV method for the determination of sesamin and sesamolin could be practically used as a faster and easier method than HPLC by using the regression equations developed in this study.**

Keywords: sesame (*Sesamum indicum* L.), sesamin, sesamolin, HPLC, UV spectrophotometer

Sesame (*Sesamum indicum* L.) is one of the most important oil crops and has been remarkably used as a traditional health food in Korea. The sesame oil and seeds have excellent nutritional value (McKevith, 2005) and are mainly used for the commercial products. An advantage of both sesame seed and oil is their resistance to oxidative deterioration resulting in oxidative stability during storage and processing (Fukuda *et al.*, 1985; Fukuda *et al.*, 1986). Sesame seed contains a number of antioxidants including lipid-soluble lignans like sesamin and sesamolin, water-soluble lignan glycosides like sesaminol triglucoside and sesaminol diglucoside (Katsuzaki *et al.*, 1994; Moazzami *et al.*, 2006). Antioxidant compounds and their antioxidative activities were also investigated in defatted cake and seed coat as well as in seed and oil (Chang *et al.*, 2002; Shyu & Hwang *et al.*, 2002; Suja *et al.*, 2005). Average contents of sesamin and sesamolin of 27 Korean sesame varieties including 7 black sesame varieties cultivated in 2003, were 4.08 mg/g (1.34-6.27 mg/g) and 2.48 mg/g (0.96-3.21 mg/g), respectively (Kim *et al.*, 2004). Lee *et al.* (1999) also reported the average contents of sesamin and sesamolin as 3.06 mg/g (0.51-6.08 mg/g of range) and 2.42 mg/g (0.30-3.67 mg/g of range), respectively, in 132 accessions of Korean sesame landraces. They also found that lignan contents of sesame varieties having white colored seed coat were higher than those of black colored seed coat varieties.

Recently, more emphasis has been given on the evaluation of qualitative traits in food processing and plant breeding, especially increasing bioactive lignan content. For qualitative determinations of sesame oil and seeds, the analytical methods of high performance liquid chromatography (HPLC), gas chromatography (GC) and thin layer chromatography (TLC) were compared (Kamal-Eldin, *et al.*, 1994). Recently, lignan was generally determined by HPLC and ultraviolet-visible detector (UV-Vis) system equipped with a reversed phase column (Kim *et al.*, 2004; Moazzami *et al.*, 2006). However, the analytical method using HPLC is time-consuming, costly and labor-intensive to select the superior lines in the breeding program. Thus, development of a more rapid and simpler method is highly required. UV-Vis spectrophotometer has been continuously used in analyzing bioactive compounds, and now has a potential to be applied as a faster and cheaper method without using an analytical column and elution solvents for HPLC analysis. UV method is practically useful for massive screening in the quality breeding of sesame. The results of analyses using both UV and HPLC methods were reported (Ryu *et al.*, 2003; Busaranon *et al.*, 2006).

The objectives of this study were to investigate the varia-

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tion of lignans such as sesamin and sesamolin contents among Korean sesame landraces using HPLC and UV methods, and to evaluate their efficiencies for a massive screening of sesame seeds.

MATERIALS AND METHODS

Plant materials

A total of 403 accessions of sesame landraces were obtained from National Genebank, National Institute of Agricultural Biotechnology, Rural Development Administration (RDA), Suwon, Korea in 2004. The seeds of each accession were stored in desiccators prior to UV-Vis spectrophotometric analysis (UV method) and HPLC analysis (HPLC method).

Extraction of lignans

About 2 g of each seed sample was homogenized using a homogenizer, extracted in 30 mL of n-hexane for 1 day by shaking at 100 rpm, and filtered with a filter paper (Whatman No. 2, USA). The residues were extracted two more times, and the final volume of each extract solution was exactly adjusted to 100 mL. Both UV and HPLC methods used the hexane extract for lignan analysis.

HPLC and UV analyses

The 10 mL of n-hexane extract was completely vacuum-dried, and then 3 mL of methanol was added and was shaken at warm condition (under 60 °C). The MeOH extract was stored for 1 day at -20 °C, and the upper layer was transferred to a 2 mL autosampler vial before HPLC injection for the determination of lignans, sesamin and sesamolin. The 10 μL of MeOH extract was injected without any dilution. The HPLC instrument (Agilent 1100 Series, Agilent Technologies Co., USA) equipped with an ultraviolet-visible detector (Diode-Array Detector) at 290 nm, which also provided the UV spectra of the peaks, and a reversed-phase column, Capcell Pak C18 UG 120, 5 μm, 4.6 × 150 mm (Shiseido Co., Japan) was used. The mobile phase was a mixture of methanol: water (75 : 25, v/v) as an isocratic condition and flow rate was set at 1.0 mL/min. Running time was 20 minute for each sample. Each peak of lignan compounds was identified for further analysis with LC-MS (HCTultra, Bruker Daltonics Inc., USA), and comparing HPLC retention time and UV spectra with standard compounds of sesamin and sesamolin.

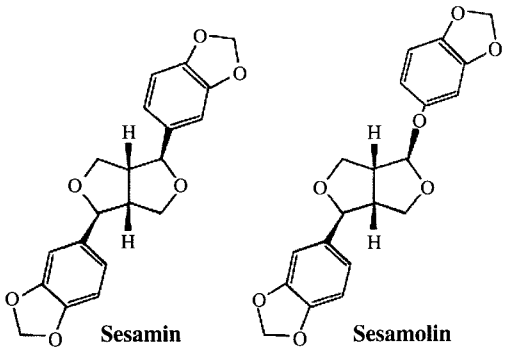

Fig. 1. Chemical structures of sesamin and sesamolin in sesame seeds.

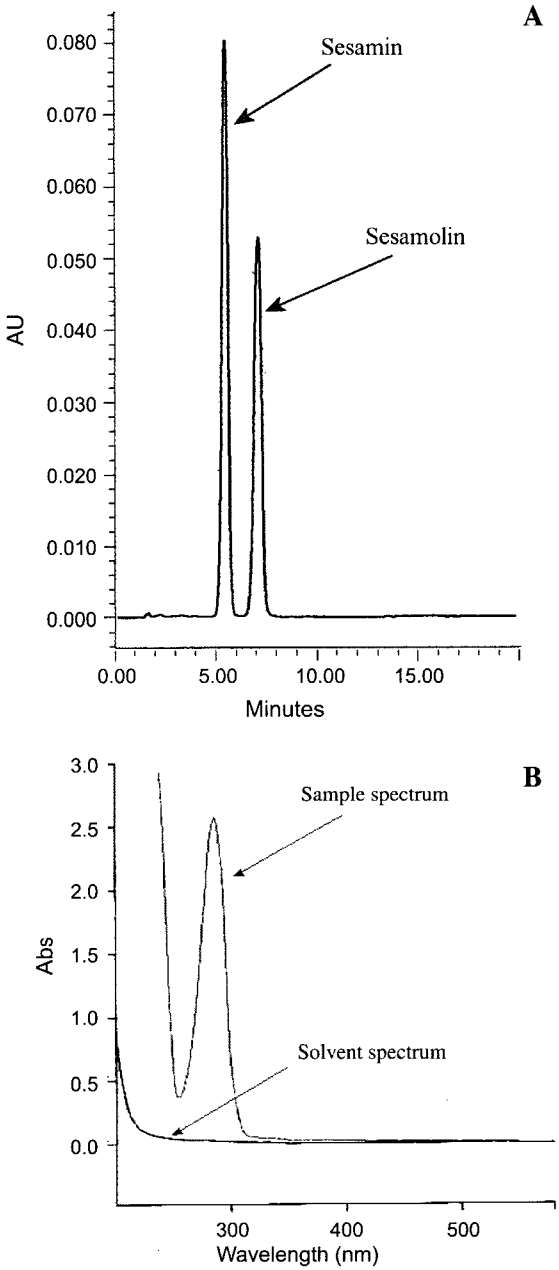

Fig. 2. HPLC chromatogram (A) of MeOH extract and UV-Vis absorption spectra (B) of hexane extract from sesame seeds.

Chemical structures of sesamin and sesamolin, antioxidant lignans are shown in Fig. 1.

For UV-Hexane analysis, 0.5 mL of n-hexane extract was diluted 9 times in a final volume of 4.5 mL using n-hexane solvent, and analyzed by UV–Vis spectrophotometer (Cary 100, Varian Inc., USA) at 290 nm, which is the same UV wavelength as in HPLC. The 0.2 mL of MeOH extract was also diluted 21 times in a final volume of 2.2 mL using MeOH solvent for UV-MeOH analysis.

RESULTS AND DISCUSSION

Calibration curves for determination of lignans in UV and HPLC methods

The HPLC analysis was done for evaluation of the linearity of calibration curves to determine total lignans, and then its results were compared with those obtained by using the UV spectrophotometric method (UV method). The UV absorption spectra and HPLC chromatogram are shown in Fig. 2. Peaks of sesamin and sesamolin were well separated in the chromatographic condition and appeared at 5.1 min and 6.5 min, respectively. Spectrum of sample solution showed high absorption peak at 290 nm of ultraviolet range as compared with spectra of the solvents (MeOH and hexane).

In order to compare the linearity of calibration curves by using both UV and HPLC methods, triplicate calibration curves for sesamin and sesamolin in HPLC method and total lignans in UV method were obtained. Five levels of standard concentrations were measured in the ranges between 3.2-51.0 mg/100 mL for HPLC and 0.36-5.67 mg/mL for UV method. The standard solution for total lignans was the mixture of sesamin and sesamolin. In UV method, this standard solution was diluted 9 times due to higher sensitivity to UV absorption. UV method needed small amount (below 100 mg) of sample as compared to that (1-2 g) of HPLC method. Total lignans for calibration in HPLC method were calculated by summing the peak areas of sesamin and sesamolin, and then linearity of calibration by HPLC was compared with that of the UV method. Linearity of calibration curves was obtained as shown in Fig. 3, and was confirmed by regression statistics. The coefficients of correlation were above 0.999 with statistical significance at 0.01 level of probability in both methods. The results showed that UV and HPLC analysis could be sufficiently performed to determine the total lignan contents of sesame seeds with good accuracy.

Relationship between UV and HPLC values for lignans content

Plots of HPLC versus UV values are shown in Fig. 4. The

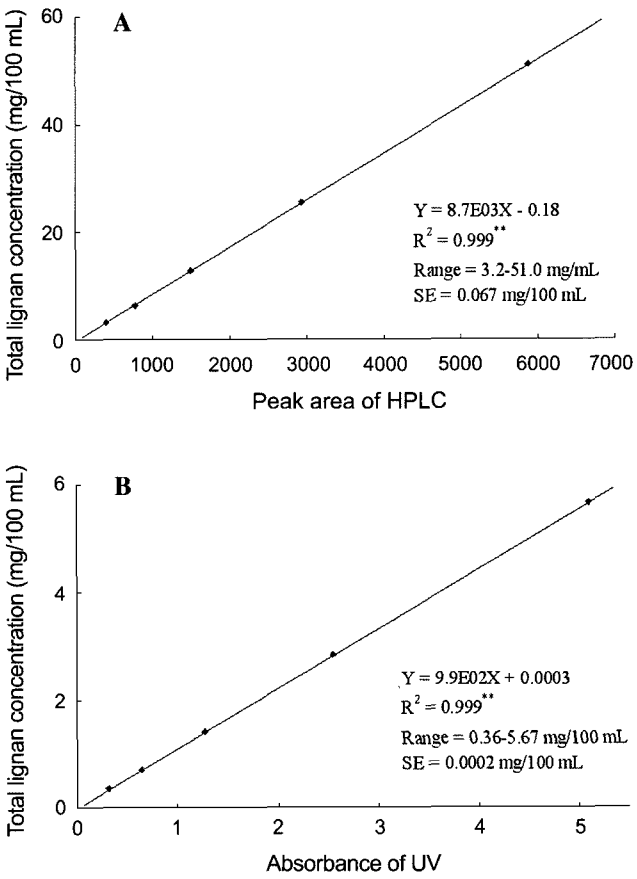

Fig. 3. Calibration curves for total lignan of methanol extract in HPLC (A) and UV (B) methods. R^2, coefficient of determination in calibration; SE, standard error of calibration. Calibration in HPLC method was calculated by summing the peak areas of sesamin and sesamolin per each concentration step.

HPLC values were strongly correlated with UV-MeOH values ($r = 0.706^{**}$) and UV-Hexane values ($r = 0.811^{**}$). Coefficient of determination of regression ($R^2 = 0.657^{**}$) between UV-Hexane and HPLC values was higher and standard error (SE) was lower than those ($R^2 = 0.498^{**}$) between UV-MeOH and HPLC values. More accurate detection in UV method could be obtained from UV-Hexane method using hexane extract solution, indicating that UV method was easier due to the simple extraction procedure with one step of hexane extracting. The linear regression equations (Fig. 4) were $y = 2.79x + 0.40$ between UV-MeOH and HPLC values, and $y = 3.44x + 4.98$ between UV-Hexane and HPLC values, indicating both UV values were in good agreements with HPLC values as the reference. Correlation between UV-MeOH and UV-Hexane values were strong ($r = 0.735^{**}$), showing similarity of both methods.

Determination of total lignan contents by HPLC method

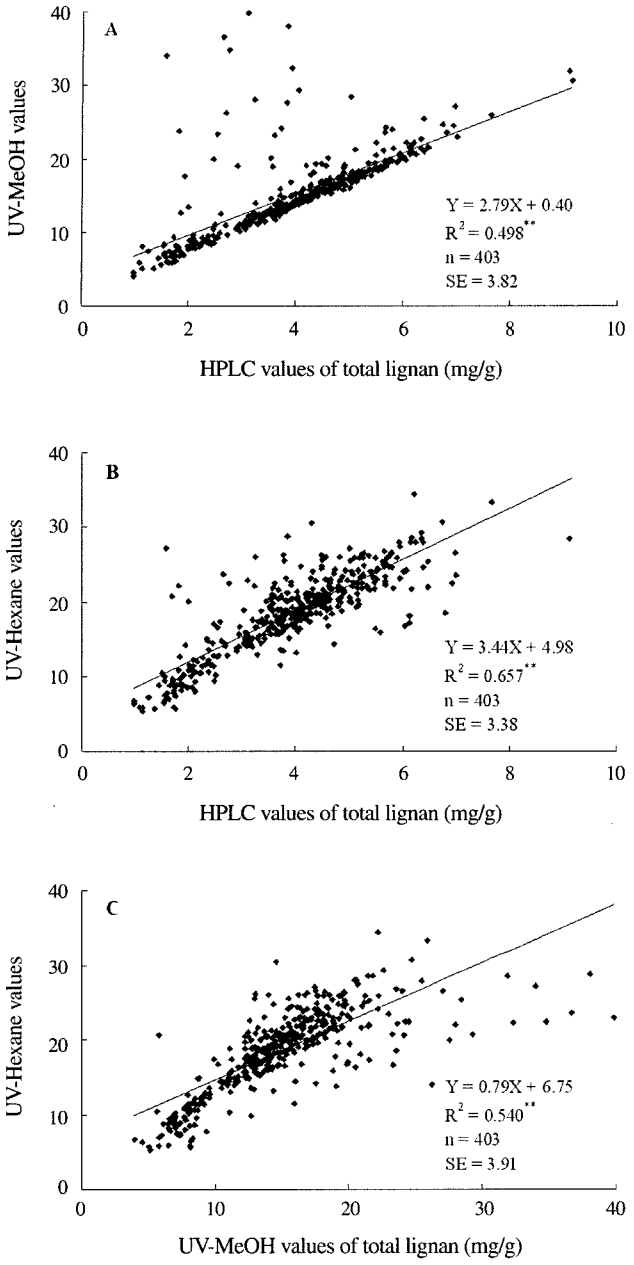

Fig. 4. Relationship between HPLC and UV-MeOH values (A), HPLC and UV-Hexane values (B), and UV-MeOH and UV-Hexane values (C) for lignan contents in sesame. HPLC values are the sum of sesamin and sesaminol contents, and UV-MeOH and UV-Hexane values are the values calculated by UV absorbance of MeOH and Hexane extracts, respectively. R^2, coefficient of determination; SE, standard error of each regression.

was compared with those by UV methods (Table 1). Based on the HPLC values as a reference, the mean values were 4.7 times higher in UV-Hexane method and 3.8 times in UV-MeOH method. These three methods had similar 30.5-35.7 % of coefficients of variation (CV). As shown in Table 1, the accuracy of UV method was very poor because the absorbance at 290 nm of UV range was not only due to sesamin and sesamolin, but also to other lipid-soluble lignans including sesamolinol, sesaminol, P1 and pinoresinol whose maximum wavelengths were overlapped ranging 280-295 nm (Fukuda *et al.*, 1985). The quantification of total lignans using UV methods gave the overestimation of 3.8 and 4.7 times as much as HPLC values. However, small amount of sample is required along with short duration of analysis and high sensitivity of detection for UV method. Therefore, it is suggested that UV method can be used as a rapid and inexpensive technique with a reliable accuracy for massive screening of sesame breeding lines, provided that the regression equations developed in this study is applied for the determination of lignans content. Sesame breeding lines selected by UV method would need further confirmation by HPLC and then should be continuously evaluated through progeny test in breeding program of sesame.

Variation of lignan contents in Korean landraces of sesame

A total of 403 sesame accessions were evaluated for sesamin and sesamolin contents using HPLC. Fig. 5 shows the distribution plots of sesamin, sesamolin and total lignan contents of sesame samples (n = 403). Mean values of sesamin, sesamolin and total lignan contents were 2.23 mg/g (0.38-5.12 mg/g of range) based on the seed weight, 1.74 mg/g (0.46-4.41 mg/g of range) and 3.98 mg/g (0.98-9.49 mg/g of range), and standard deviation (SD) were 0.92 mg/g, 0.53 mg/g and 1.36 mg/g, respectively. Variation (40.8 % of CV) and ranges of sesamin were higher than those of sesamolin (30.5 % of CV). Thus total lignan content would be mainly influenced by sesamin content while sesamin and sesamolin contents were highly correlated (r = 0.762**). The accessions displayed significant variation for lignan content and were selected as superior lines for sesame breeding. Those germplasms with high content of lignans, sesamin and sesamolin, should be further studied to confirm whether they are genetically useful for the quality breeding of sesame, in addition to testing variations of lignan contents according to different environment factors.

In conclusion, although UV method has a shortcoming of imprecision and overestimation for measured values, it will be a beneficial and practical technique when the regression model has good agreement with HPLC values to adjust UV values. This method would be practically useful, especially in breeding program of sesame because the relative evaluation of chemical compounds in large population was very important for rapid selection.

Table 1. Comparison of mean and standard deviation of total lignan contents determined by HPLC, UV-MeOH and UV-Hexane methods.

Method	Mean (mg/g)	SD (mg/g)	Range	CV (%)	Bias	Ratio to reference
HPLC (Reference)	3.98	1.36	0.98-9.19	34.2	-	1.00
UV-Hexane	18.69	5.70	5.34-46.04	30.5	14.71	4.70
UV-MeOH	15.09	5.38	4.02-39.82	35.7	11.11	3.79

SD, standard deviation; CV, coefficient of variation; Bias, average difference between UV and HPLC values; Ratio to reference, the ratio of the values of each UV method to HPLC values as reference. HPLC values for total lignan were the sum of sesamin and sesamolin contents.

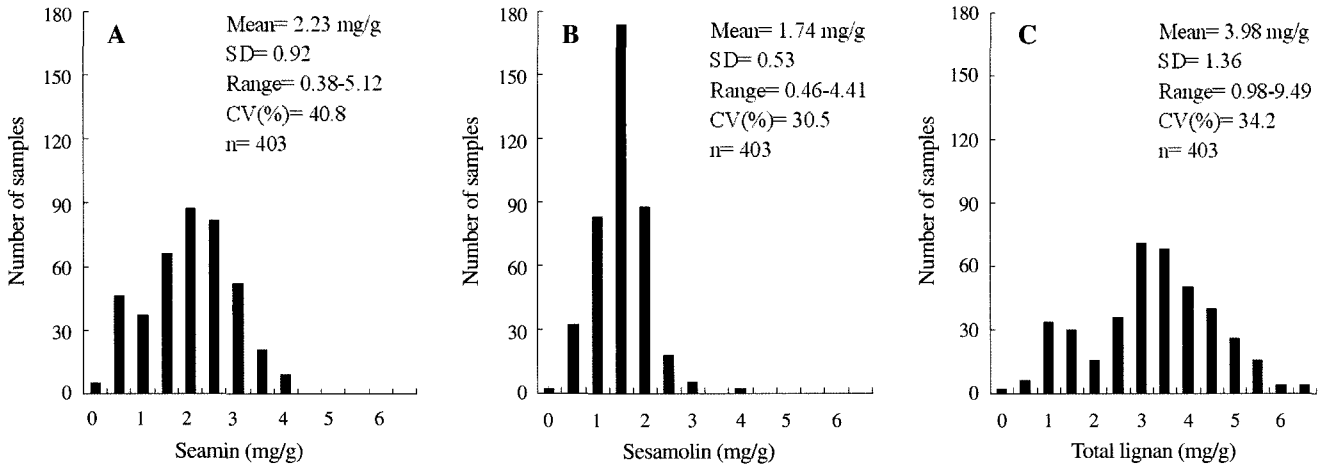

Fig. 5. Frequency distribution plots for contents of sesamin (A), sesamolin (B) and total lignan (C) in seeds of sesame accessions. Total lignan is the sum of sesamin and semolina contents.

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