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Quantitative Determination of Sesaminol Glucosides in Sesame Seed

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ABSTRACT

The sesaminol glucosides in 80% EtOH extract from sesame seeds were separated by high performance liquid chromatography (HPLC). A HPLC system using a Develosil ODS-5 column and gradient elution system from 30% to 80% methanol was selected for separation and quantitative determination of sesaminol triglucoside, sesaminol diglucoside, and sesaminol monoglucoside. Quantitative analyses for these sesaminol glucosides, sesaminol triglucoside, sesaminol diglucoside, and sesaminol monoglucoside were determined on the basis of standard curve of sesaminol glucosides. Sesaminol triglucoside, sesaminol diglucoside and sesaminol monoglucoside contents of the seed of one Korean sesame cultivar, Danbaekggae, were 56.4 mg/100g, 9.6 mg/100g, and 7.5 mg/100g, respectively. The most abundant aglycon of lignan glucosides in sesame seed was sesaminol triglucoside

Key words : sesame, sesaminol glucosides, quantitative analysis, *Sesamum indicum*

Lignans, which are abundant in the sesame oil, have been known to exert diverse physiological functions in addition to their strong antioxidant activity. Antioxidative activities in food have been extensively investigated in sesame seed (Budowski, 1964; Fukuda et al., 1985; Katsuzaki et al., 1992, 1993, 1994b; Osawa et al., 1985, 1992; Ryu et al., 1994).

Recently, Katsuzaki et al. (1993 and 1994b) reported a large-scale isolation and identification of type of antioxidants, lignan type antioxidants, and lipid-soluble antioxidants. Two novel lipid-soluble lignan type antioxidants, sesamolinol, and sesaminol were found to be present only in sesame seeds, together with other lipid-soluble lignan derivatives and water-soluble lignan glucosides. Katsuzaki et al. (1994) have succeeded in isolating precursors of antioxidants which were determined to be novel sesaminol mono-, di-, and tri-glucoside. These compounds, especially, sesaminol mono- and diglucoside were resistant to hydrolysis by β-glucosidase.

Shimizu et al. (1989) have reported that sesamin and sesaminol interfered with the metabolism of linoleic acid at the step catalyzed by $\triangle$5-desaturase in the microorganisms. Sesaminol has been already been reported to show strong antioxidative activity both *in vitro* and *in vivo* system, and to show synergistic effects in raising antioxidative activity both in food and biological model system.

Until now, most lipid-soluble lignans sesamolinol and sesaminol have been isolated from sesame seed as the antioxidative components. However, we have little information on the quantitative analysis of sesaminol glucosides in sesame seed. More over, in order to develop a rapid and simple evaluation method for practical purpose of sample analysis, we studied quantitative analysis of sesaminol glucoside components.

MATERIAL AND METHOD

Materials

Danbaekggae was used, which were grown in National Crop Experiment Station, Rural Development Administration (RDA), Suwon, Korea in 1996.

Solvent and regent

Water was purified by the Milli-Q (Millipore, Bedford, MA) system. All other solvent used for extraction and analyses were of HPLC grade (Fisher Scientific Fairlawn, N.J). Solvent for the HPLC analyses was filtered through sep-pak C18 cartridge and purged with helium prior to use.

Extraction and identification

The sesaminol mono- and di-glucosides of sesame seed were ground and defatted with n-hexane, and extracted with 80% ethanol.

The extract was incubated at 37℃ for 8 hrs with β-glucosides (5 unit ml^{-1}) in 50 mM acetate buffer (pH 5). The reaction mixture was extracted with ethylacetate (EtOAc). The EtOAc extract was fractionated in S1~S6 using Prep. HPLC under the following conditions : column, Develosil ODS-10 (250×20 mm id), mobile phase, methanol (MeOH)-water (H_2O) (3:2), flow rate, 6 ml/min.

The sesaminol triglucoside of sesame seed was extracted with 80% ethanol. The 80% ethanol extract was charged into an Amberlite XAD-2 column and eluted with H_2O, 20% MeOH, 40% MeOH, 60% MeOH, 80% MeOH, MeOH and Me_2CO. The 60% MeOH fr. was then purified by Prep. HPLC under the following conditions : column, Develosil ODS-10 (250×20 mm id), mobile phase, MeOH-H_2O (2 : 3), flow rate, 4 ml/min.

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The sesaminol glucosides was identified according to the procedure of Katsuzaki et al. (1993) and Osawa et al. (1985).

RESULTS AND DISCUSSION

Extraction and isolation of sesaminol glucosides

A flow diagram for the extraction and isolation procedure of sesaminol glucosides from sesame seed is shown Fig 1. Sesame seed (250 g) was ground and defatted with n-hexane and extracted with 80% ethanol. The 80% ethanol extract was dissolved in 50 mM acetate buffer pH 5.4 and hydrolysed overnight with β-glucosidase.

Compound S_1-S_6 were identified as pinoresinol, sesaminol diglucoside, sesaminol monoglucoside, sesamolinol, sesaminol, and sesaminol triglucoside by comparison of spectra analytical data with those of authentic samples. Compound S_7 was isolated from the 80% ethanol extract of sesame seed using an XAD-2 column and preparative HPLC (ODS).

As shown is Fig 2., Katsuzaki et al. (1993 & 1994) reported the structure of novel sesaminol glucosides isolated from sesame seed were identified to be sesaminol, seseminol monoglucoside, sesaminol diglucoside, and sesaminol triglucoside.

Finally, 13.1 mg pinoresinol, 8.9 mg sesaminol diglucoside, 113.8 mg sesaminol monoglucoside, 6.9 mg P1, 12.1 mg sesamolinol, 6.7 mg sesaminol and 19.0 mg sesaminol triglucoside were obtained.

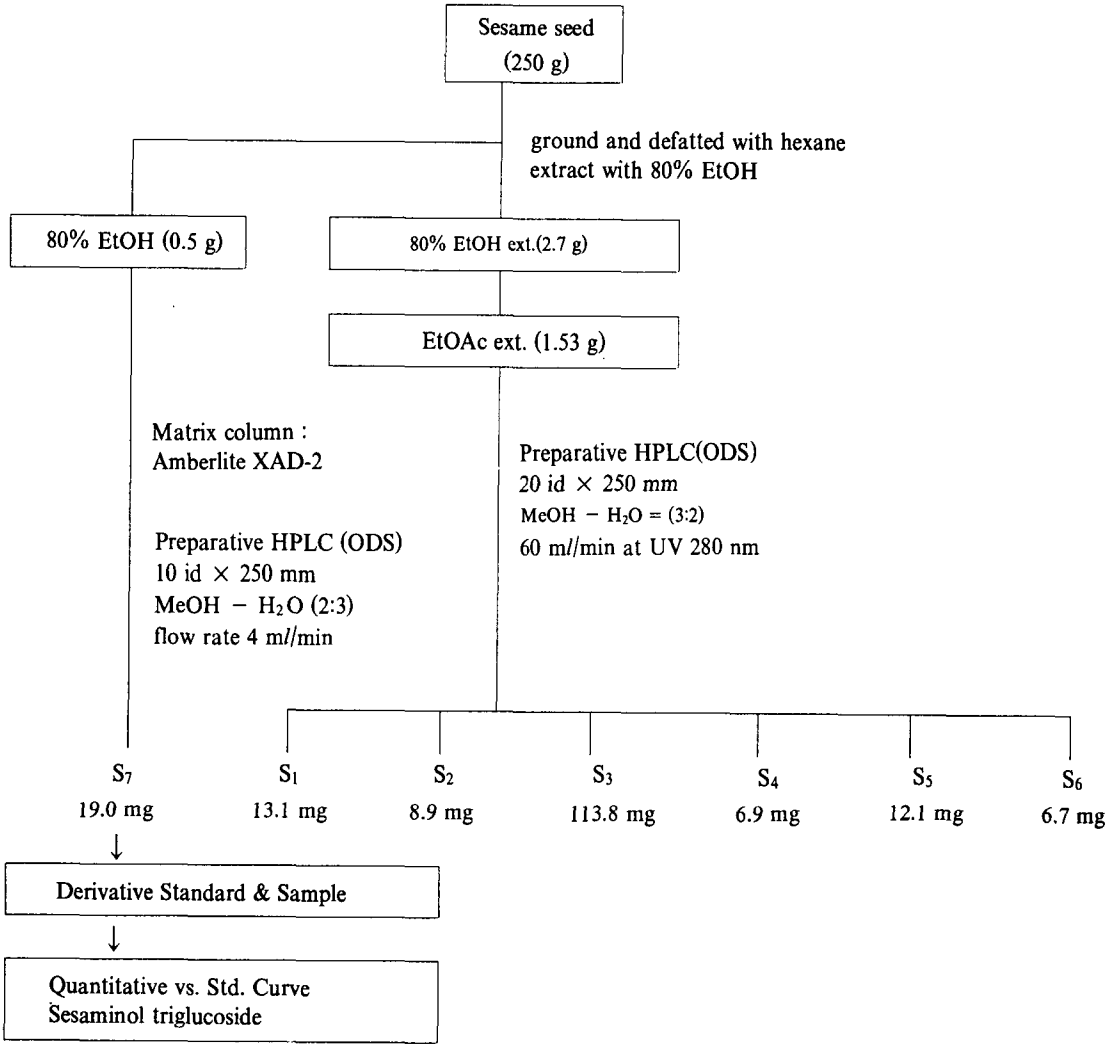

Fig. 1. Flow diagram for the extraction and isolation of lignan components and matrix column with Amberlite XAD-2.
S1 : Pinoresinol, S2 : Sesaminol diglucoside, S3 : Sesaminol monoglucoside, S4 : P1,
S5 : Sesamolinol, S6 : Sesaminol, S7 : Sesaminol triglucoside.

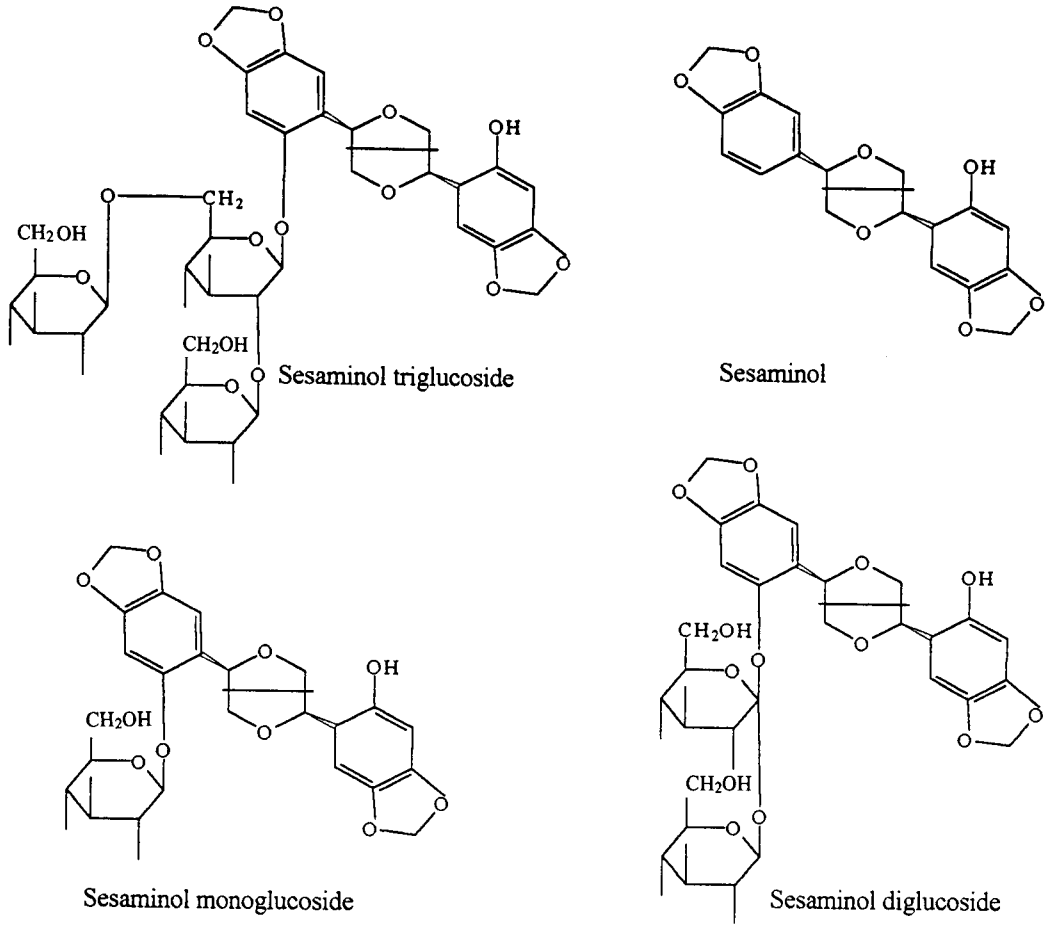

Fig. 2. Structure of sesaminol glucosides isolated from sesame seed.

On the other hand, Katsuzaki et al. (1993 & 1994) reported the significant effects of sesaminol glucosides on the oxidative stability of sesame seed. The sesaminol content of sesame oil was found to be dramatically increased during the manufacturing process, in particular bleaching process (Fukuda et al., 1986). Sesaminol was also found in high concentration in unroasted sesame oil, due to the high yield conversion of sesamolin to sesaminol by intermolecular group transfer catalyzed by the acid clay used for decolorization (Fukuda, 1994).

Quantitative analysis of sesaminol glucosides

HPLC analyses were performed with Gulliver Jasco DG-980 3 line Degasser, PU-980 intelligent HPLC pump, a model MD-910 multi wavelength spectrophotometric detector set at 290 nm. About 2 g of sample was accurately weighed and shaken with 20 ml of 80% ethanol for 10 hrs at room temperature. The slurry was centrifuged at 12,000 rpm for 15 min. The supernatant was separated by decantation and filtrated by using C_{18} sep-pak SPE cartridges attached to 10 ml syringes.

The sesaminol glucoside content of this filtrate was determinated by HPLC system. This system was equipped with a Develosil ODS-5 column (4.6×150 mm), precolumn Develosil ODS-5 column (4×10 mm, Nomura Chemical Co, Ltd, Japan) and a spectrophotometric detector (Tosoh UV-8000) with UV 290 nm, and used was linear gradient system from 30% methanol to 80% as solvent for 60 min at a flow rate of 1.0 ml/min.

The standards solutions of 3.1, 4.1, 6.9, and 10 μg sesaminol glucosides were injected on the HPLC, respectively. Standard calibration curve was drawn by average of peak area for the triplicate determinations of sesaminol glucoside standards. Fig. 3 show the typical chromatogram of standard solution (A) and sesame seed extract (B) using solvent system. Twenty μl portions of both sample and the standard solution were injected. Identification of the peaks was performed with authentic standards based on their retention times and UV-VIS spectra. As shown in Fig. 3B, some peak appeared was corresponded to the retention time of sesaminol glucoside standard of Fig. 3A. Thus, these peaks were regarded as an sesaminol triglucoside (C), sesaminol diglucoside (D),

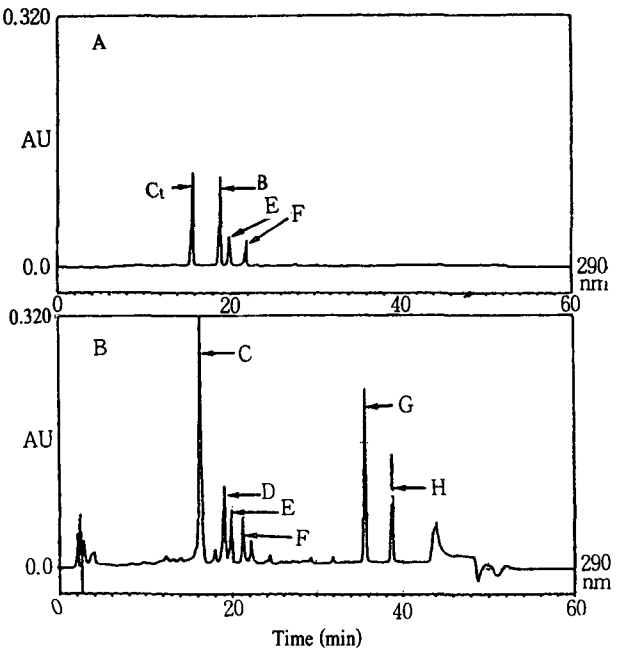

Fig. 3. HPLC chromatogram of sesaminol glucosides and sesaminol.

A : standard solution, B : sesame seed extract, C : sesaminol triglucoside, D : sesaminol diglucoside, E : sesaminol monoglucoside, F : sesaminol, G : sesamin, H : sesamolin.

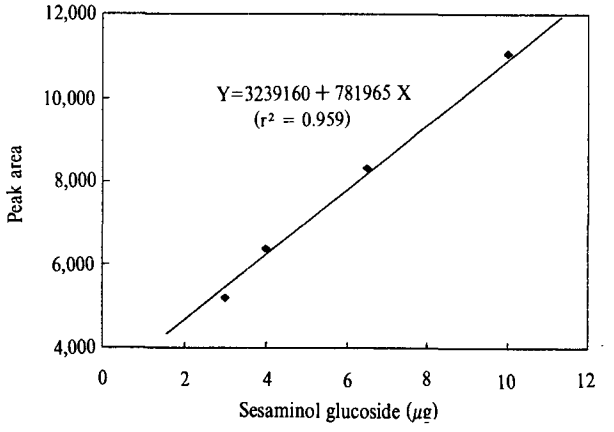

Fig. 4. Calibration curve in the quantification of sesaminol glucosides by HPLC.

sesaminol monoglucoside (E), and sesaminol (F). These components were identified by method of Katsuzaki et al. (1994). On the other hand, the peak of (G) and (H) were identified as an sesamin and sesamolin by method of Ryu et al. (1992).

In quantitative analysis, sesame sample was shaken with 20 ml of 80% ethanol for 10 hrs. at room temperature. The slurry was centrifuged at 12,000 rpm for 15 min. The supernatant was separated by decantation and filtered through a 0.45 μm filter to protect the column prior to HPLC analysis.

Fig. 4. shows the standard calibration of sesaminol glucosides. Sesaminol glucosides standard calibration was Y = 3239160 + 781965X (X; standard concentration, Y; peak area). This HPLC technique permitted rapid quantification of sesaminol glucosides in sesame at 100 μg level on analytical Develosil ODS-5 column (4.6 X 150 mm i. d). When the amounts of sesaminol were quantified by HPLC in commercially available sesame oils, the total amounts of sesaminol isomers was about four times higher than that of γ-tocopherol in the most commercially available sesame seed oils (Osawa et al., 1992), but content of sesaminol glucosides in sesame seed has not been determined. Sesaminol triglucoside, sesaminol diglucoside, and sesaminol monoglucoside of Danbaekggae were 56.4 mg/100g, 9.6 mg/100g, and 7.5 mg/100g, respectively. The most abundant aglycon of lignan glucosides in sesame seed was sesaminol triglucoside

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