# A Rapid Method to Analyze Saponin Precursors in Soybean (*Glycine max* (L.) Merrill)

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**Abstract**

**A rapid and efficient analytical method was developed to distinguish and to quantify two major saponin precursors in soybeans; soyasapogenol A, contributing to the undesirable sensory characteristics, and soyasapogenol B, representing group B and E saponins, which have chemoprotective properties. Aqueous ethanolic extraction (80 % v/v) yielded the highest recovery of saponin from finely ground soybean powder. Acid hydrolysis of extracted saponin in 8 % HCl in anhydrous methanol at 75 C° for 3 hrs cleaved the attached sugar moietie(s) completely from the triterpene aglycones and produced no artifacts. Resultant soyasapogenols were isolated using solid phase extraction and chromatograms of high resolution were obtained by HPLC equipped with evaporative light scattering detector (ELSD). Histochemical analysis revealed that soyasapogenols are mainly concentrated in the axis of seed compared with cotyledon and seed coat. To determine the distribution of soyasapogenol A and B in soybean, 10 advanced food-grade soybean lines were grown in 1999 at four locations in Ontario. In general, total soyasapogenol content in soybeans was 0.2±0.02 %. Soyasapogenol B content (1480±200 μg/g) was 2.5 to 4.5-fold higher than soyasapogenol A content (479±72 μg/g). A significant variation in soyasapogenol content was observed among the cultivars tested.**

## INTRODUCTION

The presence of saponins in soybean has attracted considerable interest owing to both health benefits and adverse sensory characteristics. Soybean saponins are heat-stable glycosides and comprise a hydrophobic aglycone (triterpenoid sapogenin) linked to one or more hydrophilic mono- or oligosaccharide moiety (Lasztity et al., 1998). Soyasaponins are classified into three groups (A, B, and E) based on their respective aglycone moieties (Tsukamoto et al., 1993). Group A acetylated saponins present in soybean, are implicated as the compounds mostly responsible for the undesirable taste (Okubo et al., 1992) while group B and E saponins have health benefits. Group B and E saponins are indicated to possess inhibitory activity against the infection of human immunodeficiency virus (HIV) (Nakashima et al., 1989) and the activation of the Epstein-Barr virus early antigen (Konoshima and Kozuka, 1991). Recent in vitro studies suggest that saponin B and E have hypocholesterolemic, immuno-stimulatory, anticarcinogenic, antioxidative, anti-tumour, anti-virus, anti-hepatitic, anti-diabetic, and hepatoprotective effects (Fournier et al., 1998).

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Six kinds of group A saponins, designated as Aa, Ab, Ac, Ad, Ae, and Af have been identified (Shiraiwa et al., 1991). The oligosaccharide chain attached to the C-22 position of the soyasapogenol A is acetylated. Acid hydrolysis of all six saponin A compounds has yielded the common aglycone, soyasapogenol A (Shiraiwa et al., 1991). The other soyasaponins, which have been isolated (designated as I, II, III, and IV) all contain soyasapogenol B as the common aglycone (Ireland and Dziedzic, 1987). It has been suggested that soyasapogenol C and E are formed as artifacts during acid hydrolysis (Ireland and Dziedzic, 1987). In contrast, Tsukamoto et al. (1993) recognized that soyasapogenol E is the aglycone of the third type of saponin, namely saponins Bd and Be. However, Bd and Be saponins are heat labile (Kudou et al., 1992), thus are presumed to be transformed into soyasapogenol B during acid hydrolysis. The total soyasaponin content is approximately twice the total soyasapogenol content (Duhan et al., 2001).

The objective of this study was to develop a rapid analytical technique to isolate soyasapogenol A and B (Fig. 1) and to estimate total saponin content of soybeans.

## MATERIALS AND METHODS

### Analysis of Soyasapogenols A and B

The isolation of saponins from soybean was accomplished by a modification of the method described by Daveby et al. (1998). Finely ground soybean powder (0.2 g) was dissolved in 30 mL of 80 % ethanol in a round-bottom flask with consistent mixing by a horizontal shaker for 3 h at 50 °C. The residue was removed by centrifuging the extract at 2,000 rpm for 10 min and decanting the clear supernatant. Half (15 mL) of the supernatant was dried under reduced pressure, and the residue was dissolved in 8 % HCl in anhydrous methanol and was subjected to acid hydrolysis at 75 °C for 3 h to release the aglycones (soyasapogenols) from saponins. The soyasaponogels produced were isolated by solid-phase extraction using a $C_{18}$ cartridge. They were then eluted with 100 % methanol, filtered using 0.2 micron nylon filters. The quantification of soyasapogenol A and B was carried out using reverse-phase high performance liquid chromatography (RP-HPLC) with external standards. The two soyasapogenols were well resolved using an ODS $C_{18}$ (250 × 4.6 mm internal diameter) column at a flow rate of 0.9 mL/min with a mobile phase consisting of acetonitrile:1-propanol:water:0.1 % acetic acid whose proportions were 80:6:13.9:0.1, respectively. Owing to a lack of chromophores for these analytes, detection by UV spectrophotometry was inefficient. Evaporative light scattering detection (ELSD) was much more successfully employed to characterize and quantitate soyasapogenol A and B with very high resolution and high sensitivity.

### Comparison of Seed Parts and Soybean Cultivars

Twenty grams of samples (replicates) of seeds were soaked in distilled water for 10 hours with aeration before separating the axis, seed coat, and cotyledons. The seed parts were freeze-dried and were ground into a fine powder.

Ten food-grade soybean varieties (S20-F8, CL970321, 7025308, 9910, 2004, AC X790P, Harovinton, AC Vin-Pro, AC Hime, and 9305) were selected and were grown at four locations (Chatham, Malden, Tilbury, and Woodslee) in Ontario, Canada during 1999.

## RESULTS AND DISCUSSION

The primary goal of the research was to develop analytical technique to determine the two major saponin components in soybeans; one (soyasapogenol A) contribute to the adverse sensory characteristics and other (soyasapogenol B) produces saponins with health benefits. In literature, quantification of saponin precursors (soyasapogenols) was contradictory because of the formation of artifacts (soyasapogenols C, D and F) during the acid hydrolysis. As well, commonly used UV-detection at 204nm is inefficient due to the lack of chromophores for soyasapogenols. As well, methods to quantify total saponins are limited due to the complexity of available procedures. The present method is developed and validated to avoid the above constraints and to quantify soyasapogenols

rapidly and efficiently. The recoveries of soyasapogenol A and B were over 95 % and the limits of quantitation (LOQ) for soyasapogenol A and B were 0.06 and 0.08 mg/g, respectively.

The ratio of concentration of soyasapogenol A in axis:cotyledon was 40:1, whereas that of soyasapogenol B was 9:1. The total soyasapogenols concentration is 14-fold greater in the axis (15.6 mg/g DW) compared with that in cotyledons (1.1 mg/g DW) (Table 1). However, once dry weight ratios of seed part to total seed weight is taken into the consideration, the percentage distributions of total soyasapogenols in axis, cotyledons and seed coat were 62, 0.8, and 37.2 %, respectively.

The concentrations of soyasapogenol A and B were determined in 10 selected food grade soybean cultivars (Fig. 2) In general, the concentration of soyasapogenol B was 2.5 to 4.5-fold higher than the concentration of soyasapogenol A in all the soybean cultivars. The concentrations of soyasapogenols A and B were influenced by both the different variety of soybeans ($p>0.01$) and by the different locations of growth ($p>0.5$). However, significant differences in total soyasapogenol content among cultivars were observed (Fig. 2).

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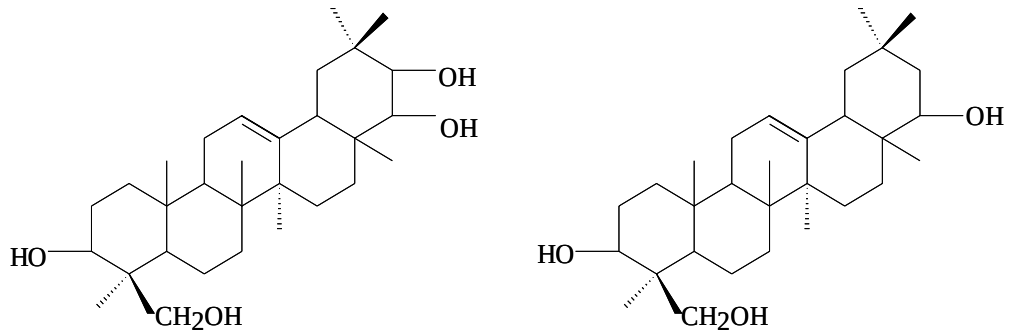
## Tables

Table 1. Content of soyasapogenols in seed parts of three selected cultivars.

| Seed part | Cultivar | Concentration of soyasapogenol A (mg/g DW) | Concentration of soyasapogenol B (mg/g DW) | Concentration of total soyasapogenol (mg/g DW) | Distribution among seed parts* (%) |
|---|---|---|---|---|---|
| | | | | | |
| Axis | Cultivar 1 | 8.0 | 8.4 | 16.4 | 59.9 |
| | Cultivar 2 | 7.3 | 5.3 | 12.6 | 63.1 |
| | Cultivar 3 | 7.6 | 10.2 | 17.8 | 62.9 |
| | Mean | 7.6 | 7.96 | 15.6 | 62 |
| | | | | | |
| Seed coat | Cultivar 1 | 0.046 | 0.083 | 0.13 | 1.5 |
| | Cultivar 2 | 0.015 | 0.047 | 0.06 | 0.8 |
| | Cultivar 3 | 0.016 | 0.013 | 0.03 | 0.3 |
| | Mean | 0.026 | 0.047 | 0.07 | 0.8 |
| | | | | | |
| Cotyledons | Cultivar 1 | 0.14 | 0.9 | 1.0 | 38.7 |
| | Cultivar 2 | 0.12 | 0.68 | 0.79 | 36.1 |
| | Cultivar 3 | 0.32 | 1.10 | 1.42 | 36.8 |
| | Mean | 0.19 | 0.89 | 1.07 | 37.2 |

*The % distribution of total soyasapogenols among 3 seed parts is based on the dry weight ratios between seed parts and whole seed. The data represent mean of 3 replicates.

## Figures




Soyasapogenol A Soyasapogenol B

Fig. 1. Chemical structures of saponin precursors; soyasapogenol A and B.

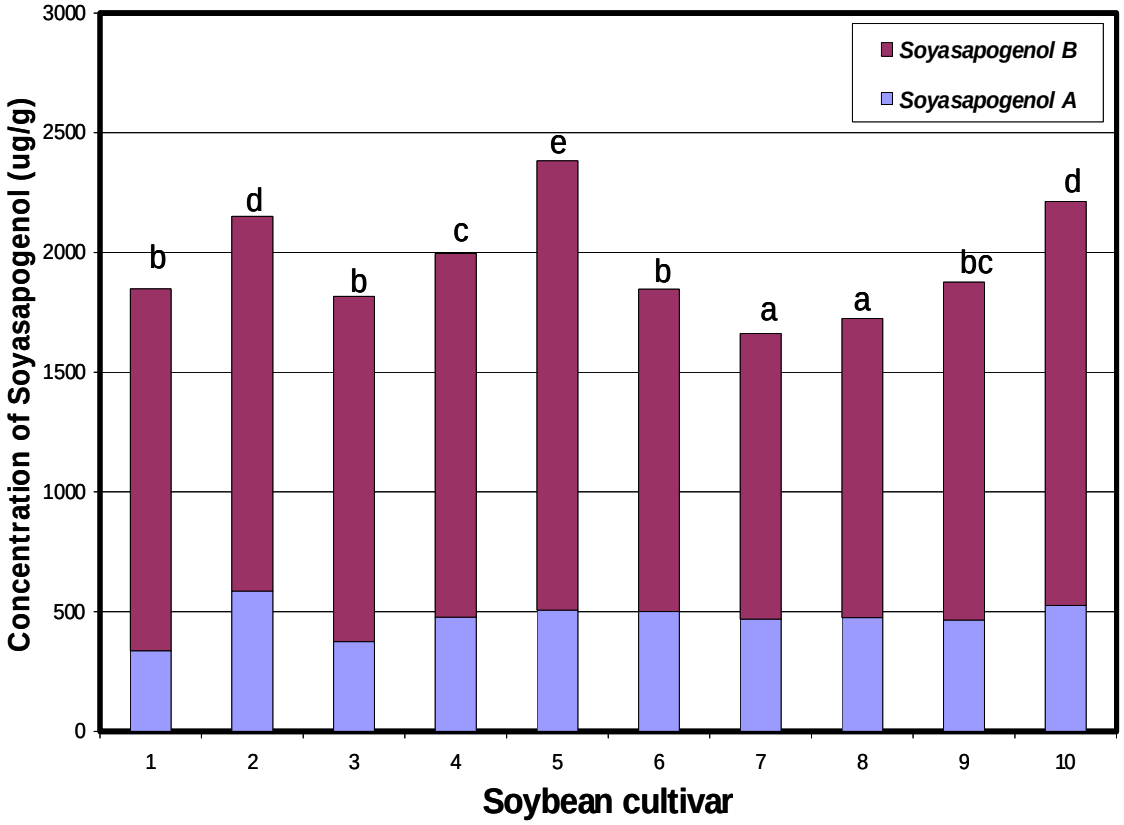



Fig. 2. Distribution of soyasapogenol A and B in ten food-grade soybean cultivars. Bars of total soyasapogenols with a letter in common are not significantly different at $p \leq 0.05$.