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Simultaneous quantification of differently glycosylated, acetylated, and 2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one-conjugated soyasaponins using reversed-phase high-performance liquid chromatography with evaporative light scattering detection

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Abstract

A novel method utilizing high-performance liquid chromatography (HPLC) with evaporative light scattering detection (ELSD) and electrospray ionisation mass spectrometry (ESI-MS) was developed for the analysis of soyasaponins, a divers group of triterpenic compounds with one or two sugar side chains, occurring in soy. Group A soyasaponins in different degrees of acetylation, as well as group B soyasaponins in both their 2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP)-conjugated and non-conjugated forms could be separated and quantified using authentic soyasaponin standards, in one single run. The method was tested by the determination of the soyasaponin content and composition of eight soygerm samples of different origin. Differences in the composition and the degree of acetylation of the group A soyasaponins were observed among these samples. The group B soyasaponins showed much less variability and they were mainly present in their DDMP-conjugated form.

Keywords: Soy; Soygerm; Soyasaponin; Reversed phase HPLC; Evaporative light scattering detection; DDHP

1. Introduction

Saponins form a diverse group of amphiphilic molecules and are found widespread over the plant kingdom. Soybean (*Glycine max* L. Merill) and soy-based food products are major dietary sources of saponins [1,2]. Soyasaponins, which are triterpenoid glycosides, are divided into three major groups, based on differences in substitution of the C-22 and C-23 position of the aglycone (or soyasapogenol): group A, B and E soyasaponins (Table 1). Group A soyasaponins have a glycosyl chain attached to the C-3 and the C-22 position of the aglycone. Group B soyasaponins carry only one glycosyl chain (connected to the C-3 position), and they can be conjugated to 2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP) at C-22 [3]. According to Kudou et al.'s findings [4], the latter is the genuine form of group B saponins in soybeans, but conditions applied during processing can easily result in loss of DDMP, and formation of the non-DDMP counterparts. Group E soyasaponins are the least abundant of the three, and they are considered to be photo-oxidation products of group B soyasaponins [5]. The C-3 sugar chain is similar for group A, B, and E soyasaponins; it starts with a glucuronyl residue, followed by a galactosyl or arabinosyl residue, and in most cases followed by a glucosyl or rhamnosyl residue [6,7]. The C-22 side chain of group A soyasaponins consists of two sugar residues, starting with an arabinosyl. The terminal sugar moiety is a xylosyl or glucosyl residue, which can be acetylated at three or four positions, respectively. It is believed that in soybean group A soyasaponins are fully acetylated [5,8].

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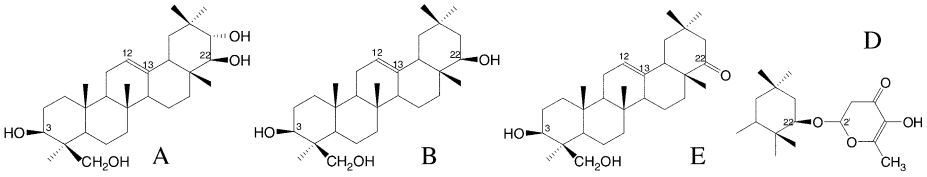
Table 1
Structures of known soyasaponins and their calculated molecular weight; the retention times of the various soyasaponins in the chromatograms obtained with the method applied in this study are indicated as well



A: soyasapogenol A; **B**: soyasapogenol B; **E**: soyasapogenol E; D: DDMP conjugated to soyasapogenol B

Name	Structure[a]	MW[b]	RT[c]
Aa	glc(1→2)gal(1→2)glcUA(1→3)**A**(22←1)ara(3←1)xyl(2,3,4-tri-*O*-acetyl)	1364	52.0
Ab	glc(1→2)gal(1→2)glcUA(1→3)**A**(22←1)ara(3←1)glc(2,3,4,6-tetra-*O*-acetyl)	1436	54.0
Ac	rha(1→2)gal(1→2)glcUA(1→3)**A**(22←1)ara(3←1)glc(2,3,4,6-tetra-*O*-acetyl)	1420	55.3
Ad	glc(1→2)ara(1→2)glcUA(1→3)**A**(22←1)ara(3←1)glc(2,3,4,6-tetra-*O*-acetyl)	1390	56.1
Ae	gal(1→2)glcUA(1→3)**A**(22←1)ara(3←1)xyl(2,3,4-tri-*O*-acetyl)	1202	56.7
Af	gal(1→2)glcUA(1→3)**A**(22←1)ara(3←1)glc(2,3,4,6-tetra-*O*-acetyl)	1274	59.5
Ag	ara(1→2)glcUA(1→3)**A**(22←1)ara(3←1)xyl(2,3,4-tri-*O*-acetyl)	1172	64.5
Ah	ara(1→2)glcUA(1→3)**A**(22←1)ara(3←1)glc(2,3,4,6-tetra-*O*-acetyl)	1244	65.1
Ba	glc(1→2)gal(1→2)glcUA(1→3)**B**	958	57.0
Bb	rha(1→2)gal(1→2)glcUA(1→3)**B**	942	58.1
Bc	rha(1→2)ara(1→2)glcUA(1→3)**B**	912	61.1
Bb′	gal(1→2)glcUA(1→3)**B**	796	60.7
Bc′	ara(1→2)glcUA(1→3)**B**	766	n.d.[d]
Bd	glc(1→2)gal(1→2)glcUA(1→3)**E**	956	60.1
Be	rha(1→2)gal(1→2)glcUA(1→3)**E**	940	61.5
αg	glc(1→2)gal(1→2)glcUA(1→3)**B**(22→2′)DDMP	1084	68.5
βg	rha(1→2)gal(1→2)glcUA(1→3)**B**(22→2′)DDMP	1068	71.0
βa	rha(1→2)ara(1→2)glcUA(1→3)**B**(22→2′)DDMP	1038	n.d.
γg	gal(1→2)glcUA(1→3)**B**(22→2′)DDMP	922	n.d.
γa	ara(1→2)glcUA(1→3)**B**(22→2′)DDMP	892	n.d.

[a] glc, β-D-glucopyranosyl; gal, β-D-galactopyranosyl; glcUA, β-D-glucoronopyranosyl; ara, α-L-arabinopyranosyl; rha, α-L-rhamnopyranosyl; xyl, β-D-xylopyranosyl; for **A**, **B** and **E**, see above.
[b] MW: molecular weight.
[c] RT: retention time (min).
[d] n.d.: not detected.

Soyasaponins are considered as important bioactive components contributing to the beneficial health effects of soy consumption. Biological effects of especially group B soyasaponins have been reported extensively, and include immunostimulatory, anti-viral, hypocholesterolaemic, hepatoprotective, haemolytic and antitumourigenic activities [9–16]. The group A soyasaponins are considered to be responsible for the astringent and bitter taste of soy-based food products, mainly because of the presence of the acetyl groups [17]. The exact mechanisms behind the biological properties of soyasaponins are yet to be revealed, due to the absence of purified test compounds and limited information on the contents and the compositions of soyasaponins in soybean and soy-based products.

Quantification of individual soyasaponins has always been a difficult issue, partly due to the difficulties in isolating authentic standards and the structural complexity of this group of phytochemicals. Moreover, the covalent bonds connecting the acetyl, and particularly the DDMP groups, to the saponin molecule are relatively weak, even under relatively mild extraction conditions, which makes it difficult to obtain the saponins in their native form [12,18]. Historically, the total saponin content has been determined with very low accuracy by colorimetric [19] and biological methods (such as the haemolytic activity of the saponins towards erythrocytes [20]). Recently, Gurfunkel and Rao [21] developed a rapid and accurate densitometric method using TLC for the quantification of total soyasaponins.

Many of the methods developed so far include a treatment prior to quantitative analysis to reduce the complexity of the soyasaponin mixture originally present in soybeans, and hence to simplify the separation of the individual components. Acid hydrolysis converts the saponins into their corresponding sapogenols, which are subsequently quantified by liquid chromatography [22] or gas chromatography after derivatisation to trimethylsilyl ethers [23]. Gu et al. proposed an alkaline treatment, which converts the group A soyasaponins into their non-acetylated forms and deconjugates DDMP saponins, facilitating separation by liquid chromatography. Other authors include a pre-separation in group A and B saponins [24,25], or focus only on one of the two groups [26]. All these methods, although accurate, are often laborious and do not reflect the exact composition of the native soyasaponin mixture. Until now, no methods have been