Phytochemistry, 1966, Vol. 5, pp. 803 to 806. Pergamon Press Ltd. Printed in England

SOYA BEAN SAPONINS—VII*

A METHOD FOR THE DETERMINATION OF SAPOGENIN AND SAPONIN CONTENTS IN SOYA BEANS†

B. GESTETNER, YEHUDITH BIRK, A. BONDI and Y. TENCER

Hebrew University, Faculty of Agriculture, Rehovot, Israel

(*Received* 18 *August* 1965)

Abstract—A quantitative method has been devised for the determination of the saponin content in acid hydrolysates of soya beans, based on the use of a modified Lieberman–Burchard reagent and employing certain essential steps of purification from accompanying interfering materials. All five known soya sapogenins (soya sapogenols A, B, C, D and E) have been found to give the same colour yield per unit weight with this reagent. A 1:1 sapogenin:sugar ratio has been found to be typical for various soya bean saponin extracts prepared from different soya bean varieties, and has been used as a conversion factor of sapogenin into saponin content. The saponin content determined in six different soya bean varieties has been found to amount to ~ 0·60 per cent of the defatted soya bean meal.

INTRODUCTION

THE determination of the saponin content in soya beans and in other leguminous saponin-containing plants has been based, up to now, on the yield resulting after a lengthy isolation procedure.[1–3] The growing interest in saponins, which are present in many nutritionally important plant feeds, such as soya bean and alfalfa, suggested the need for an easier quantitative analytical method for these constituents.

This paper describes a quantitative method for sapogenins and its application to the determination of saponin content in soya beans.

EXPERIMENTAL AND RESULTS

Preparation of Soya Bean Saponins

Soya bean saponin extract (SBSE) was prepared from ether-extracted soya bean flour, Lincoln variety, as described by Birk *et al.*[4] It was separated by a combination of column (Al_2O_3) and preparative paper chromatography[3] into four fractions, designated *a*, *b*, *c* and *e*.

Quantitative Method for Determination of Soya Bean Sapogenins

The soya sapogenins were isolated by hydrolyzing a sample of SBSE (10 mg/10 ml) or its fractions in 1 N H_2SO_4 in dioxane–water (1:3) for four hours under reflux.[5] The solution was cooled to room temperature, diluted with (20 ml) water and the sapogenins extracted

* Part VI. *Phytochem.* **5**, 799 (1966).

† Supported by Grant No. FG-Is-112 from the U.S. Department of Agriculture.

[1] C. B. COULSON, *J. Sci. Food Agric.* **9**, 281 (1958).
[2] G. R. VAN ATTA, J. GUGGOLZ and C. R. THOMPSON, *J. Agric. Food Chem.* **9**, 77 (1961).
[3] B. GESTETNER, I. ISHAAYA, Y. BIRK and A. BONDI, *Israel J. Chem.* **1**, 461 (1963).
[4] Y. BIRK, A. BONDI, B. GESTETNER and I. ISHAAYA, *Nature* **197**, 1089 (1963).
[5] B. GESTETNER, Y. BIRK and A. BONDI, *Phytochem.* **5**, 799 (1966).

with three successive portions of ether. The combined ether extracts were washed with water, dried (anhydrous Na_2SO_4) and the ether removed. The residue, containing the soya sapogenins, taken up in a small amount of benzene and purified on a column of Al_2O_3 (10 g freshly activated Al_2O_3 (ALCOA F-20) suspended in benzene and 0·4 ml of a 10% aq. acetic acid[6] was stirred vigorously for 30 min, poured into a column of 15 mm dia. and washed with 0·5 l. of benzene). Various impurities were removed by washing the column with 200 ml benzene, and the sapogenins were then eluted with 500 ml of a 3% solution of methanol in benzene. This solution was concentrated nearly to dryness and the residue taken up in chloroform (25 ml). Sapogenin determinations were carried out in glass stoppered tubes on 0·5–1·0 ml aliquots which were evaporated to dryness, and after cooling 3 ml glacial acetic acid followed by 2 ml conc. H_2SO_4 were added. The tubes were stoppered and shaken vigorously. A yellowish colour developed immediately which after a few seconds changed to violet. The tubes were again cooled to room temperature, and their absorbancies were read at 530 mμ against a suitable blank.

One of the difficulties generally encountered when using the Lieberman–Burchard reagent has been the instability of the colour.[7] However, with the modified Lieberman–Burchard reagent the colour intensity of the reaction mixture was stable for at least 3 hr and very good agreement has been found between replicates. The absorptivity was found to be proportional to the amount over the range 10–400 μg soya sapogenins.

Standard reference curves were prepared for each of the individual soya sapogenins[8] and mixtures of them. It was found that equal amounts, either of each of the individual soya sapogenins or of their mixture, yield the same colour intensity with the modified Lieberman–Burchard reagent (100 μg gave an absorbancy of 0·20 ± 0·01 in a 1 cm cell at 530 mμ). Tests were performed on known amounts of soya sapogenins, which had been added to SBSE and then submitted to the various steps of the determination procedure, such as acid hydrolysis, column chromatography and showed that the method gave 100 per cent recoveries.

TABLE 1. THE SAPOGENIN AND SUGAR CONTENT OF SBSE AND OF ITS FRACTIONS

Saponin preparation assayed	Total amount of		Sapogenin/sugar ratio
	Sapogenins (μg/mg SBSE)	Sugars*	
SBSE	502	477	1·05:1
Fractions $a+b$	520	433	1·2 :1
Fraction c	500	505	0·99:1
Fraction e	528	173	3 :1

* See preceding paper.

The total amount of soya sapogenins in SBSE and in its fractions has been determined by the above described colorimetric method and the corresponding sapogenin/sugar ratios have been calculated (Table 1). The sapogenin/sugar ratios in SBSE and in saponin fraction c are 1 : 1, and a similar ratio was also found for fractions $a+b$ (Table 1). However, in fraction e,

[6] E. D. WALTER, G. R. VAN ATTA, C. R. THOMPSON and W. D. MACLAY, *J. Am. Chem. Soc.* 76, 2271 (1953).
[7] P. BLADON, In *Cholesterol* (Edited by R. P. COOK) p. 84. Academic Press, New York (1958).
[8] B. GESTETNER, *Isolation and Characterization of Soya Bean Saponins*, Ph.D. Thesis, The Hebrew University, Jerusalem (1965).

which amounts only to ~ 7 per cent of the total SBSE and differs from the other fractions in its comparatively high haemolytic activity as well as in other respects,[3] a sapogenin/sugar ratio of 3:1 was found.

The Quantitative Determination of Saponins in Soya Beans

The 1:1 sapogenin/sugar ratio found in SBSE, prepared from soya beans of Lincoln variety, could be used for the determination of the saponin content in soya beans provided that the same ratio held also for saponins of other varieties. SBSE has therefore been isolated from defatted soya beans of Harosoy and Lee varieties[4] and subjected to acid hydrolysis in 1 N H_2SO_4 in dioxane–water (1:3).[5] The total amounts of reducing sugars were determined in 1 ml aliquots of the hydrolysates using the 3,5-dinitrosalicylic acid reagent,[9] and the quantitative determination of the sapogenins was carried out as described above. The results of these determinations are given in Table 2 and clearly indicate that the sapogenin/sugar ratio is 1:1 valid for the preparations from all three varieties although they are of different degrees of purity. It may therefore be concluded that the saponin content of soya beans can be estimated as twice that of the amount of sapogenins.

TABLE 2. SAPOGENIN AND SUGAR CONTENTS OF SBSE PREPARED FROM THREE DIFFERENT SOYA BEAN VARIETIES

SBSE from soya beans	Total amount of sapogenins (in mg/g SBSE*)	Total amount of reducing sugars (in mg/g SBSE*)	Sapogenin/sugar ratio
Lincoln variety	502 ± 14	477 ± 5	1·05:1
Harosoy variety	382 ± 16	385 ± 8	1·00:1
Lee variety	325 ± 22	331 ± 11	0·99:1

* Average of determinations carried out on four SBSE preparations.

The following method has been devised for the quantitative determination of soya bean saponins. A sample of defatted soya bean flour (2 g/200 ml) is dispersed in a solution of 1 N H_2SO_4 in dioxane–water (1:3) and hydrolysed under reflux for 8 hr, cooled, diluted with water (200 ml) and extracted with 100 ml and then with three portions of 50 ml ethyl ether. The combined ether extracts are washed with water, dried (Na_2SO_4) and after taking to dryness the residue is taken up in the minimal amount of benzene. The chromatographic

TABLE 3. THE SAPONIN CONTENT OF SIX SOYA BEAN VARIETIES

Soya bean variety	Saponin content of defatted soya bean flour (%)	Saponin content of whole soya bean (%)
Lincoln	0·58	0·49
Harosoy	0·57	0·47
Lee	0·57	0·48
Adams	0·60	0·50
Shelby	0·55	0·46
Norchief	0·60	0·50

[9] G. NOELTING and P. BERNFELD, *Helv. Chim. Acta* **31**, 286 (1948).

purification and the colorimetric determination of the sapogenins in the residue are carried out as described above. The sapogenin content of six soya bean varieties (Lincoln, Harosoy, Lee, Adams, Shelby and Norchief) has been determined and is given in Table 3. No significant difference was found in the saponin content of these six soya bean varieties.

DISCUSSION

The quantitative method for determination of soya sapogenins described in this report is based on the use of a modified Liebermann–Burchard reagent. The use of this reagent has certain limitations since it is not specific for sapogenins but may react with accompanying sterols and related substances, and also the presence of highly concentrated sulphuric acid in the reaction mixture may lead to interfering colour reactions with other organic substances. These difficulties have been overcome by introducing a step of column chromatographic purification of the soya sapogenins. This step, which did not result in any loss of sapogenins, has been found to be essential not only for determining the sapogenin content in acid hydrolysates of soya bean flour, but also for the relatively pure SBSE (Table 2). Since soya bean saponins contain five different sapogenins, the reliability of the method might have been affected if either the same quantitative ratio between the sapogenins did not exist in all soya bean varieties or each of the five sapogenins yielded a different colour intensity with the Lieberman–Burchard reagent. Fortunately this was not found to be the case and either one of the purified sapogenins or their mixture may be used as a reference.

The 1:1 sapogenin/sugar ratio was found to be typical for various SBSE preparations from several varieties (Table 2), and can be used as a conversion factor of the sapogenin content to saponin. It is hoped that the method for the determination of the saponin content in soya beans will also be applicable to other leguminous saponins.