*Carbohydrate Research*, 138 (1985) 83–89
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

# EXTRACTION AND QUANTIFICATION OF THE RAFFINOSACCHARIDES IN SOYA BEAN

IBOLYA MOLNÁR-PERL*, MARGIT SZAKÁCS-PINTÉR,
*Institute of Inorganic and Analytical Chemistry, L. Eötvös University, Muzeum krt. 4/B, 1088 Budapest (Hungary)*

ÁGNES KOVÁGÓ, AND JÁNOS PETRÓCZY
*Hajduság Agrarian-Industrial Association, Hajduság, 4181 Nádudvar (Hungary)*

(Received January 21st, 1984; accepted for publication, September 17th, 1984)

ABSTRACT

The raffinosaccharides and their possible metabolites can be rapidly and quantitatively extracted from soya bean with aqueous 80% methanol, and quantified by g.l.c. after trimethylsilylation or oximation and trimethylsilylation. The procedure has been applied variously to crude, defatted, untreated, heat-treated, and acid-treated soya bean without deproteinisation. L-Arabinose, L-rhamnose, D-fructose, D-glucose, D-galactose, D-mannose, sucrose, cellobiose, galactobiose, melibiose, raffinose, cellotriose, galactotriose, manninotriose, stachyose, verbascotetraose, and verbascose have been identified and quantified.

## INTRODUCTION

The two problems in the investigation of the saccharides of soya bean involve isolation from the plant matrix and analysis of the extract. These problems are important because of the world-wide utilisation of soya-bean products and the fact

TABLE I

STRUCTURAL RELATIONSHIPS OF THE RAFFINOSE FAMILY OF OLIGOSACCHARIDES

| | |
|---|---|
| α-D-Gal*p*-(1→6)-α-D-Gal*p* | Galactobiose |
| α-D-Gal*p*-(1→6)-α-D-Gal*p*-(1→6)-α-D-Gal*p* | Galactotriose |
| α-D-Gal*p*-(1→6)-α-D-Gal*p*-(1→6)-α-D-Gal*p*-(1→6)-α-D-Glc*p* | Verbascotetraose |
| α-D-Gal*p*-(1→6)-α-D-Gal*p*-(1→6)-α-D-Glc*p* | Manninotriose |
| α-D-Gal*p*-(1→6)-α-D-Glc*p* | Melibiose |
| α-D-Glc*p*-(1→2)-β-D-Fru*f* | Sucrose |
| α-D-Gal*p*-(1→6)-α-D-Glc*p*-(1→2)-β-D-Fru*f* | Raffinose |
| α-D-Gal*p*-(1→6)-α-D-Gal*p*-(1→6)-α-D-Glc*p*-(1→2)-β-D-Fru*f* | Stachyose |
| α-D-Gal*p*-(1→6)-α-D-Gal*p*-(1→6)-α-D-Gal*p*-(1→6)-α-D-Glc*p*-(1→2)-β-D-Fru*f* | Verbascose |

*To whom correspondence should be addressed.

0008-6215/85/$ 03.30 

that no procedure suitable for the rapid and complete analysis of the saccharides in soya bean has been reported. Such analytical data are important since, in the commercial preparation of digestible and palatable soya-bean products (the so-called denatured products), reducing species which participate in the undesirable Maillard reactions[1] are formed.

The saccharides of the raffinose family, shown in Table I, have bean discussed in various monographs[2-5]. Soya-bean saccharides have been extracted with alcohols[6-8], water[9-10], and aqueous alcohols[11-13], and paper[12,14,15], carbon column[16], centrifugal[17], thin-layer[8-13], liquid[7,8], and gas chromatographic[6,10,11,18,19] methods have been applied in qualitative and quantitative analysis. The extraction procedures proposed earlier[6-13] are laborious and time-consuming (3–20 h), because of the low ratio of extracting agent to soya-bean sample (10–20:1) which necessitated repeated extractions, the high content of protein in the material extracted by water[9,11] or highly aqueous alcohols[6-8,10,12,13], and the need to remove these proteins by precipitation[9,11,12], ion-exchange[8], filtration through an adsorbent[6], or t.l.c.[7].

Most data have been obtained using a semi-quantitative, p.c. method[12] for the analysis of extracts. Thus, sucrose, raffinose, and stachyose were identified as the main components of soluble soya-bean saccharides in average proportions of 6.5%, 1.4%, and 5.3%, respectively, with arabinose, fructose, glucose, and verbascose being present in traces.

We now report a rapid method for the extraction and quantification of the raffinosaccharides and their possible metabolites, which does not necessitate the removal of proteins.

## EXPERIMENTAL

*Soya-bean samples.* — $S_t$ and $S_1$ were fat-containing, $S_2$ was defatted, and all three were untreated soya beans (glycine max.: variety N.K.S. 1346). Samples $S_{1a}$ and $S_{1b}$ were obtained from $S_1$ by acidification[20] to pH ~1.9 and ~2.1, respectively, with phosphoric acid, and then neutralisation immediately with sodium carbonate and calcium oxide; $S_{1c}$ and $S_{2c}$ were obtained from $S_2$ by acidification[20] to Ph ~2 with hydrochloric acid without neutralisation; $S_{2d}$ was heated to 100° for 5 min by applying microwaves[21].

*G.l.c.* — A Chromatron Model G.C.H.F. 18.3 instrument Heim Electric (Berlin), equipped with a flame-ionisation detector, was used together with stainless-steel columns (50 cm × 3 mm i.d.) packed with 3% of SP-2250 on Supelcoport 80/100. Peak areas were measured using a Chinoin Model Digint-34$\mu$ computing integrator.

The injection and detector ports were at 410° and 430°, respectively, and the temperature-programme was 80 → 380° at 16°/min. The carrier gas was nitrogen at 60 mL/min. Elution of trimethylsilylated oxime derivatives required 20 min.

*Extraction and derivatization of soya samples.* — Untreated or treated

TABLE II

CONDITIONS AND RESULTS OF MODEL EXTRACTIONS

| *Model* | *Sample (g)* | *Alcohol (mL)* | *Extractions* | | | | |
|---|---|---|---|---|---|---|---|
| | | | *Time (min)* | *Temp. (°)* | *Yield (%)*[a] | | |
| | | | | | *S* | *R* | *St* |
| a | 2 | 20 EtOH | 60 | 80 ±0.2 | 3.16 | 0.96 | 2.08 |
| b | | | 120 | | 4.33 | 1.06 | 2.38 |
| c | | | 120 | | 4.48 | 1.09 | 2.33 |
| d | 1 | 50 | 10 | | 4.80 | 1.06 | 3.85 |
| e | | | 30 | | 5.40 | 1.21 | 4.05 |
| f | | | 60 | | 5.30 | 1.23 | 4.22 |
| g | | | 300 | | 4.98 | 1.21 | 3.95 |
| h | 0.5 | 50 | 300 | | 4.75 | 1.21 | 4.12 |
| i | 1 | 50 | 120 | 20–22 | 5.05 | 1.27 | 3.60 |
| j | | | 300 | | 5.20 | 1.36 | 4.15 |
| k | 1 | 50 MeOH | 10 | 74.2 ±0.2 | 4.87 | 1.27 | 4.36 |
| l | | | 30 | | 5.18 | 1.31 | 4.47 |
| m | | | 60 | | 5.04 | 1.29 | 4.25 |
| n | | | 300 | | 5.12 | 1.30 | 4.20 |
| o | 0.5 | 50 | 300 | | 5.25 | 1.34 | 4.44 |
| p | 1 | 50 | 120 | 20–22 | 3.58 | 1.27 | 3.40 |
| r | | | 300 | | 4.62 | 1.20 | 4.03 |
| Mean of d–h, j, and k, o | | | | | 5.08 | 1.25 | 4.19 |
| | | | | s.e. | ±0.21 | ±0.083 | ±0.19 |
| | | | | s.e. % | 4.16 | 6.5 | 4.6 |

[a]S, sucrose; R, raffinose; and St, stachyose; expressed relative to the dry weight of the sample.

samples (1 g; fat-free or fat-containing with a particle size of <0.2 mm) were extracted with refluxing and stirred aqueous 80% methanol (50 mL) for 15–20 min. The insoluble material was then collected on a glass filter (6 cm i.d.) and washed several times with aqueous 80% methanol, and the combined filtrate and washings were concentrated to 100 mL (stock solution). A 10-mL aliquot of the stock solution was concentrated to dryness at 50–60°, and 2-propanol (2 × 0.5 mL) was distilled from the residue.

The dry residue was treated with a solution (500 μL) of pyridine containing hydroxylamine hydrochloride (1.25 g per 50 mL) for 30 min at 70–72°. The oxime derivatives were then treated with a mixture of hexamethyldisilazane (900 μL) and trifluoroacetic acid (100 μL) for 60 min at 70–72°. A portion (5 μL) of the clear supernatant solution was then injected into the gas chromatograph.

## RESULTS AND DISCUSSION

The optimal conditions for the extraction of the saccharides from soya bean ($S_t$) are given in Table II, and the protein contents of the extracts ($S_t$, $S_{2c,d}$) are shown in Table III.

TABLE III

NITROGEN CONTENT OF THE EXTRACTS

| *Soya bean* | *Nitrogen content measured (%)*[a] | | | | |
|---|---|---|---|---|---|
| | *Own data*[b] | | *Literature data* | | |
| | *$H_2O$–MeOH (1:4) (1)*[b] | *$H_2O$–EtOH (1:4) (e)*[b] | *$H_2O$–EtOH (2:3)* | *$H_2O$–EtOH (1:4)* | *$H_2O$* |
| Untreated | | 5.5[7] | 19.8[7] | 25.7[12] | 60.6[7] |
| $S_1$ | 3.7 | 4.4 | | | |
| $S_2$ | 3.8 | 4.5 | | | |
| Acid treated | | | | | |
| $S_{2c}$ | 9.2 | 12.4 | | | |
| Heat-treated | | | | | |
| $S_{2d}$ | 5.2 | 5.3 | | | |

[a]Determined by the Kjeldahl[29] method. [b]See Table II for details of extraction procedure.

TABLE IV

DISTRIBUTION OF SOLUBLE SACCHARIDES IN SOYA BEAN AFTER DIFFERENT DENATURATION TREATMENTS

| *Saccharides*[a] | *Components (% of dry weight)* | | | | | | |
|---|---|---|---|---|---|---|---|
| | *1* | *1a* | *1b* | *1c* | *2* | *2c* | *2d* |
| 1 Arabinose | 0.01 | — | — | 0.70 | — | 0.71 | — |
| 2 Rhamnose | 0 50 | 0.44 | 0.43 | 0.39 | 0.40 | 0.31 | 0.40 |
| 3 Fructose | 1.15 | 1 42 | 1.72 | 1.88 | 0.71 | 3.20 | 1.81 |
| 4 Galactose + Glucose + Mannose | 0.40 | 0.47 | 0.83 | 1 92 | 0.14 | 2.38 | 0.87 |
| 5 Sucrose | 8.14 | 7.12 | 6.12 | 0.34 | 4.32 | 0.19 | 1.56 |
| 6 Cellobiose | 0.45 | 0.33 | 0.31 | 1.11 | 0.18 | 0.54 | 0.16 |
| 7 Galactobiose | 0.41 | 0.34 | 0.34 | 0.87 | 0.20 | 0 50 | 0.15 |
| 8 Melibiose | 0.18 | 0.17 | 0.25 | 0.77 | 0.09 | 0.57 | 0.14 |
| 9 Raffinose | 1.19 | 1.03 | 0.76 | [b] | 1.20 | [b] | 0.40 |
| 10 Cellotriose | 0.07 | 0.08 | 0.06 | [b] | 0.05 | 0.04 | 0.05 |
| 11 Galactotriose | 0.34 | 0.26 | 0.35 | 0.57 | 0.12 | 0.28 | 0.14 |
| 12 Manninotriose | 0.27 | 0.33 | 1.04 | 2.29 | 0.03 | 2.86 | 1.23 |
| 13 Stachyose | 4 55 | 4.39 | 3.48 | 0.41 | 4.82 | [b] | 1.37 |
| 14 Verbascotetraose | 0.03 | [b] | 0.03 | 0.54 | 0.05 | 0.43 | 0.06 |
| 15 Verbascose | 0.31 | 0.22 | 0.16 | [b] | 0.31 | [b] | 0.06 |
| Unidentified | 0.27 | 0.18 | 0.16 | 1.03 | 0.25 | 0.90 | 0.20 |
| Total | 18.27 | 16 78 | 16.04 | 12.82 | 12.87 | 12.91 | 8.60 |
| | 100[c] | 92 | 88 | 70 | 100 | 101 | 67 |
| *A*[d] | 1.69 | 1.63 | 2.27 | 5.93 | 0.89 | 5.62 | 2.06 |
| *B*[d] | 3.24 | 3.02 | 3.78 | 12.6 | 3.06 | 10.0 | 5.26 |
| *A–B* | 1 55 | 1.39 | 1.51 | 6.67 | 2 17 | 4.38 | 3.20 |

[a]The numbers correspond to those in Fig. 1. [b]Not present in measurable amounts. [c]Percentage recovery of the processed samples. [d]Total of the reducing saccharides: *A*, based on g.l.c. data; *B*, obtained according to the method of Kolthoff[30]. Each datum represents the mean of at least three measurements of the same accuracy, as detailed in Table II

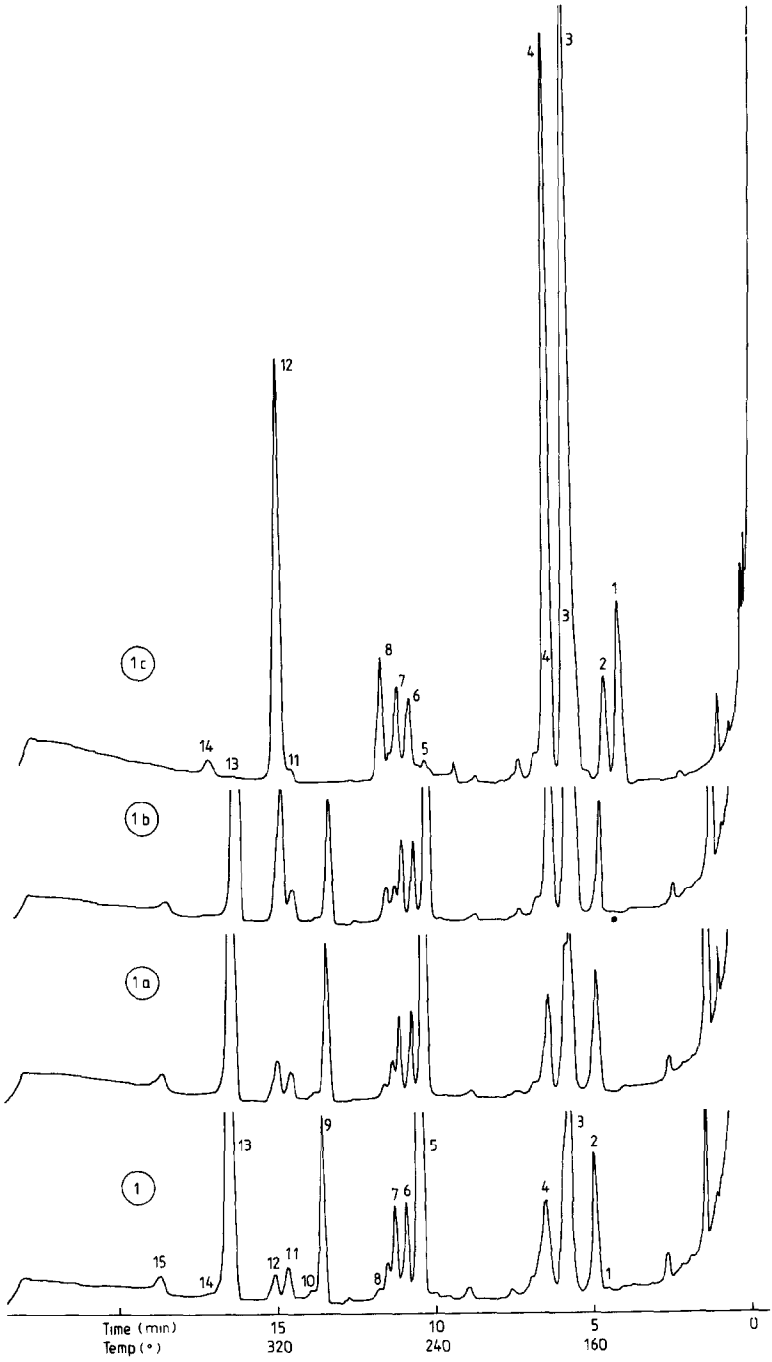


Fig. 1. Gas chromatogram of the trimethylsilylated saccharides in $S_{1a,b,c}$ (See Table IV also).

With the exception of procedures a–c, i, and p, the other eleven extraction procedures were quantitative. Thus, the mean ±s.e. of eleven measurements showed (Table II) that $S_t$ contained 5.08 ±0.21% of sucrose, 1.25 ±0.083% of raffinose, and 4.19 ±0.19% of stachyose.

With the optimal procedure, quantitative extraction was accomplished within 30 min, and the small amount of protein extracted did not interfere with the subsequent g.l.c.

The derivatives most suitable for g.l.c. were the trimethylsilylated oximes, as shown with maltosaccharides[22–27]. We recently reported a new method[28] for the analysis of the components and degradation products of raffinosaccharides with d.p. 1–5. After derivatization, each component is eluted as a single peak. Thus, the quantification of all the main components of soya bean, including soluble saccharides, their metabolites, hydrolysis products of pectins, and cellulose, is possible (Table IV).

In contrast to earlier data, it now appears that all the members of the raffinosaccharide series are present in soya bean (Table IV, $S_1$ and $S_2$). On mild treatment with acid (Table IV, $S_{1a}$ and $S_{1b}$), the relative decreases in the amounts of sucrose, raffinose, and stachyose containing terminal sucrose are similar. With more drastic treatment ($S_{1c}$ and $S_{2c}$), manninotriose is a main component in addition to monosaccharides, and a significant amount of melibiose is formed. The accumulation of manninotriose and melibiose reflects the greater resistance of the chains terminating in D-glucose than those terminating in D-galactose. The amounts of galactotriose and galactobiose were also augmented significantly after the more-drastic treatments (Table IV, $S_{1c}$ and $S_{2c}$). They were probably derived by decomposition of the raffinosaccharides and from hydrolysis of D-galactose-containing polysaccharides.

The reducing power of the samples was higher, when measured by the Kolthoff[30] procedure (Table IV, column *B*), than the calculated sum of reducing saccharides identified by g.l.c. (Table IV, column *A*). Thus, oligosaccharides with d.p. >5 and with reducing end-groups may not be detected under our g.l.c. conditions.

ACKNOWLEDGMENTS

We thank Professors U. P. Kralovánszky and J. Mátyás for their help in supplying the soya-bean samples as well as for valuable discussions.

REFERENCES

1 C. ERIKSSON (Ed.), *Maillard Reactions in Food*, Pergamon, New York, 1981, pp. 1–157.
2 D. FRENCH, *Adv. Carbohydr. Chem.*, 9 (1954) 149–184.
3 A. S. PERLIN, *Adv. Carbohydr. Chem.*, 14 (1959) 50–52.
4 H. NIKAIDO AND W. Z. HASSID, *Adv. Carbohydr. Chem. Biochem.*, 26 (1971) 372–376.
5 P. M. DEY, *Adv. Carbohydr. Chem. Biochem.*, 37 (1980) 284–366.
6 W. I. KIM, C. J. B. SMIT, AND T. O. M. NAKAYAMA, *Lebensm.-Wiss. Technol.*, 6 (1973) 201–204.

7 G. F. CEGLA AND K. R. BELL, *J. Am. Oil Chem. Soc.*, 54 (1976) 150–152.
8 E. J. CONKERTON, F. W. PARRISH, D. C. CHAPITAL, AND R. L. ORY, *J. Food. Sci.*, 48 (1983) 1269–1271.
9 J. DELENTE AND K. LADENBURG, *J. Food. Sci.*, 37 (1972) 372–374.
10 S. KU, L. S. WEI, M. P. STEINBERG, A. I. NELSON, AND T. HYMOWITZ, *J. Food Sci.*, 41 (1976) 361–364.
11 T. HYMOWITZ, F. I. COLLINS, J. PANCZNER, AND W. M. WALKER, *Agron. J.*, 64 (1972) 613–616.
12 S. KAWAMURA, *Tech. Bull. Fac. Agr. Kagawa Univ.*, 18 (1967) 117–137.
13 M. TANAKA, D. THANANUNKUL, T.-C. LEE, AND C. O. CHICHESTER, *J. Food Sci.*, 40 (1975) 1087–1088.
14 D. FRENCH AND G. M. WILD, *J. Am. Chem. Soc.*, 75 (1953) 2612–2616.
15 F. CHAMPAGNOL AND M. BOURZEIX, *J. Chromatogr.*, 59 (1971) 472–475.
16 S. KAWAMURA, *Tech. Bull. Fac. Agr. Kagawa Univ.*, 18 (1967) 138–141.
17 S. KAWAMURA, *Tech. Bull. Fac. Agr. Kagawa Univ.*, 18 (1967) 142–146.
18 T. HYMOWITZ, W. M. WALKER, F. I. COLLINS, AND J. PANCZNER, *Commun. Soil Sci. Plant Anal.*, 3 (1972) 367–373.
19 T. HYMOWITZ AND F. I. COLLINS, *Agron. J.*, 66 (1974) 239–240.
20 T. DÉVÉNYI, F. KOCSIS, K. KRALOVÁNSZKY-BÓCSA, S. PONGOR, G. SZABOLCSI, AND M. SUCH, Hung. Pat. 3255/8 (A23 J1/00 A23 K1/00), 21 Dec. 1979; Appl. 30. Dec. 1981; p. 13.
21 J. BENEDEK, T. DÉVÉNYI, J. MAGYAR, M. NEDELYKOV, AND G. SZABOLCSI, Hung. Pat. 2885 (A23 J3/00 A23 K1/00), 6 Oct. 1981; Appl. 3. Feb. 1984; p. 20.
22 C. C. SWEELY, R. M. BENTLEY, M. MAKITA, AND W. W. WELLS, *J. Am. Chem. Soc.*, 85 (1963) 2497–2507.
23 K. ZÜRCHER AND H. HADORN, *Dtsch. Lebensm.-Rundsch.*, 70 (1974) 425–431; 71 (1975) 68–71; 71 (1975) 393–399.
24 J. HAVERKAMP, J. P. KAMERLING, AND J. F. G. VLIEGENTHART, *J. Chromatogr.*, 59 (1971) 281–287.
25 Z. L. NIKOLOV AND P. J. REILLY, *J. Chromatogr.*, 254 (1983) 157–162.
26 K. M. BROBST AND C. F. LOT, JR., *Cereal Chem.*, 43 (1966) 35–43.
27 I. M. PERL AND M. P. SZAKÁCS, *J. Chromatogr.*, 216 (1981) 219–228.
28 I. M. PERL, M. P. SZAKÁCS, Á. KÖVÁGÓ, AND I. PETRÓCZY, *J. Chromatogr.*, 295 (1984) 433–443.
29 J. KJELDAHL, *Z. Anal. Chem.*, 22 (1883) 366–382.
30 I. M. KOLTHOFF, *Z. Unters. Lebensm.*, 45 (1923) 131–141.