Letter to the Editor

A Simple and Rapid HPLC Analysis of Sugars in Soybeans and Factors Affecting Their Standardization

Sir: Several methods for the determination of sugars in soybeans and other oilseeds using high pressure liquid chromatography (HPLC) (1-3) or ion exchange chromatography (4-6) have appeared in recent literature. However, these previously reported methods contain extremely laborious and time-consuming procedures for preparation of soy flour sugar extracts which are sufficiently free from other soluble organic material so as not to cause contamination of the chromatograph or the column. Solvent mixtures such as alcohol and water have been used to extract sugars from soy flour, thereby minimizing the amount of unwanted organic material while preserving the integrity of the sample extract with respect to sugars. However, these alcohol extracts still contain too much soluble organic material to be injected into a liquid chromatograph without at least partially plugging the injector or the filter disk at the front of the column; this results in severely increased back pressures. In addition, the remaining soluble organic material will prematurely reduce column life. Thus, these alcohol extracts required still further treatment for the removal of interfering material.

The following technique has been used successfully for quantitatively extracting sugars from soy flour while leaving behind interfering organic material.

To prepare a sample for analysis, 2.00 g of ground defatted soy flour was weighed into a 50 ml glass-stoppered Erlenmeyer flask, then 25 ml of distilled water was added. The sample was shaken for 1 hr on a Palo laboratory wrist-action shaker. The soy-water slurry was centrifuged in a 50 ml polyethylene centrifuge tube (100 mm x 26 mm) at 2000 rpm for 5 min. The centrifugate was decanted off and 5 ml of the soy extract was pipetted into a 150 mm x 16 mm glass-stoppered test tube. Five ml of acetonitrile (CH_3CN) was added slowly with constant shaking. A flocculent precipitate was formed upon addition of the CH_3CN. The precipitate was filtered under pressure to prevent concentration of the sugars in the solution resulting from evaporation. This was accomplished by forcing a portion of the extract-CH_3CN mixture from a plastic disposable syringe through an attached Waters sample clarification kit (Waters Associates, Inc., Milford, MA). The millipore filter disk (13 mm) was replaced with an equal-sized disk of Whatman No. 1 filter paper. Each sample was filtered into a small plastic-capped vial.

The liquid chromatograph used was Waters Associates model ALC-201 equipped with a differential refractometer. The sugars were separated on a μ BONDAPAK-carbohydrate® column using acetonitrile-water (70-30) at 2.0 ml/min as previously described (3). Stachyose, the largest molecular weight sugar present, was completely eluted in 12 min. Satisfactory quantitation was achieved by repetitive injection of standards and samples.

Average oligosaccharide percentages (dry basis) for 3 different defatted soy flours obtained in triplicate on 3 different days were Amsoy 71: sucrose 8.8, raffinose 1.1, and stachyose 5.2; Beeson: sucrose 7.2, raffinose 0.8, and stachyose 4.9; Corsoy: sucrose 7.3, raffinose 1.0, and stachyose 4.9. The standard deviation for the 3 varieties were: sucrose 0.324, raffinose 0.090 and stachyose 0.124.

The major interfering organic material present in soy-water extracts was identified as soluble protein. In an attempt to remove the soluble protein, several extracts were acidified to reach the isoelectric point of soy protein (pH 4.5). This resulted in the precipitation of ca. 90% of the soluble protein. A total solids analysis (ambient air evaporation) indicated some soy material other than sugars still remained. However, the sample was sufficiently free of extraneous material to preclude any further precipitation after the extract was introduced into the CH_3CN:H_2O mobile phase. Although the organic materials could be successfully removed, this sample preparation technique proved to be only marginal because of hydrolysis of the oligosaccharides in the acidic solution.

Water is obviously an excellent sugar extraction solvent, but soy polysaccharides and proteins are also quite soluble. However, because the HPLC mobile phase (CH_3CN:H_2O) caused precipitation of these interfering materials, an experiment was devised to determine what level of CH_3CN would precipitate most of the interfering soy protein without causing precipitation of any sugars. A simulated soy sugar extract was prepared using sucrose, raffinose and stachyose with small amounts of fructose, glucose, galactose and melibiose also added. The water-sugar solution was mixed 1:1 with CH_3CN and analyzed by HPLC. After adjustment for the dilution and within normal limits of error, the addition of the CH_3CN caused no apparent change in the simulated soy extract, neither by precipitation nor because of altered response of the detector.

To determine the optimal ratio of CH_3CN to use for protein precipitation, a water extract of 2.00 g/25 ml was treated with varying quantities of CH_3CN. at 25% CH_3CN, the mixture yielded only partial precipitation of the soy protein, whereas at 75% the CH_3CN caused an undesirable dilution of the sugars. Based on total solids and sugar analysis, a 1:1 ratio of CH_3CN-soy extract produced protein precipitation in excess of 98% without reducing the sugar concentrations.

Continuous use of a polar-bonded HPLC column naturally leads to a decreasing number of theoretical plates. This constantly diminishing column efficiency causes faster elution times for all sugars until, finally, previously achieved separations cannot be made. This is generally compensated for by increasing the CH_3CN, which does not increase column efficiency, but does increase column resolution. Another reason for increasing CH_3CN would be to separate very closely related sugars such as glucose and galactose on a highly efficient HPLC column.

Data seen in Table I indicate that as the CH_3CN is increased, the response of the differential refractive index detector diminishes rather drastically. The decreased response was evident to some extent for all sugars; however, the relative decrease in response is more severe for galactose and melibiose.

The decreased detector response resulting from increased amounts of CH_3CN in the mobile phase was on a per μg basis and was determined to be independent of the quantity of sugar. Five different levels of each sugar (between 100-1000 μg) yielded the same relative response/μg. These results indicated it was not a solubility phenomenon caused by a nonuniform decrease in the solubility of the various

TABLE I

Effect of Acetonitrile Concentration in the Mobile Phase Upon the Response of the Differential Refractive Index Detector

Sugar (100-1000 μg)	Acetonitrile %)					
	60[a]	65	70	75	80	85
Fructose	443[b]	428	469	425	416	430
Glucose	445	456	458	398	397	363
Galactose	425	418	367	302	259	116
Sucrose	460	479	455	427	424	413
Melibiose	449	478	394	344	267	82
Raffinose[c]	472	453	457	450	—	—
Stachyose[c]	431	430	438	397	—	—

[a]Water solution.

[b]Relative detector response/μg (ave. response/μg of 5 different sugar quantities).

[c]Response data for these sugars unobtainable at high acetonitrile concentrations caused by extreme column retention.

sugars. Response of the detector toward a 1:1 mixture of glucose and galactose, where the 2 peaks were not separated, produced a total response equal to the summed average of the individual responses for each sugar.

The nonlinear change in detector response between sugars indicates the need for greater emphasis on the standardization of each individual sugar, particularly when the CH_3CN in the mobile phase exceeds 70%.

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REFERENCES

1. Rabel, F.M., A.G. Caputo, and E.T. Butts, J. Chromatogra. 126:731 (1976).
2. Cegla, G.F., and K.R. Bell, JAOCS 54:150 (1977).
3. Black, L.T., and E.B. Bagley, Ibid. 55:228 (1978).
4. Brobst, K.M., H.O. Scobell, and E.M. Steele, Proc. Am. Soc. Brew. Chem. 43 (1974).
5. Scobell, H.O., K.M. Brobst, and E.M. Steele, Cereal Chem. 54:905 (1977).
6. Ladisch, M.R., and G.T. Tsao, J. Chromatogr. 166:85 (1978).

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