Analysis of Nonstarch Polysaccharides in Cocoa Powder by a New Micromethod

Commercial cocoa powder was analyzed for nonstarch polysaccharides by means of a new micromethod. The defatted sample was treated with α-amylase from porcine pancreas in order to degrade native starch. The undegraded residue was hydrolyzed in the presence of a cation-exchange resin. Sugars deriving from nonstarch polysaccharides were isolated and subjected to anion-exchange chromatography. Only the neutral fraction was collected. After derivatization to aldonitrile acetates the component sugars were identified and determined semiquantitatively by gas chromatography. The main components of the nonstarch polysaccharides in cocoa powder were constituted of components from glucose, galactose, and arabinose.

A wide range of industrially processed food contains cocoa powder as a natural basestuff. For example, there are cocoa instant powders, chocolate milk drinks, and chocolate puddings on the market. In the course of manufacture some part of these products is treated with polysaccharide gums serving as thickening, gelling, or stabilizing agents (Klose and Glicksman, 1972). Need of ingredients' verification in consumer goods has recently called for simple and versatile methods to analyze for nonstarch polysaccharides and gums in cocoa products.

This paper describes a new micromethod for detection and determination of nonstarch polysaccharides in cocoa instant powders. For performance of this procedure only small portions of sample (ca. 500 mg) are required; a time-consuming extraction step of the crude polysaccharides is not involved. After removal of fat the sample material is subjected to α-amylase treatment in order to degrade the starch fraction. For complete degradation of cocoa starch a highly purified preparation of α-amylase from porcine pancreas, which is free from other carbohydrases, has been shown to be suitable. The undegraded polysaccharide fraction is isolated after the addition of methanol and then hydrolyzed by means of a cation-exchange resin (Porter, 1975; Varma and Varma, 1976). Thus, any protein fractions occurring are also hydrolyzed and then removed by binding to the ion-exchange resin. The sugar components released from the nonstarch polysaccharides are separated into neutral and acidic components by anion-exchange chromatography (Fransson et al., 1968). Only the neutral sugars are isolated and assigned to analysis. The latter is carried out semiquantitatively by gas–liquid chromatography, where the neutral sugars are determined as aldonitrile acetates. Gas–liquid chromatography (GLC) using stainless steel columns has proved effective for the rapid separation of sugar derivatives (Varma and Varma, 1976; Mergenthaler and Scherz, 1980).

EXPERIMENTAL SECTION

Materials. Commercial cocoa powder ("Silber-Kakao, Stollwerck") was used. Locust bean gum and carrageenan were purchased from Roeper Co., Hamburg, West Germany. Sucrose, *myo*-inositol, and α-amylase from porcine pancreas (Type 1 A, PMSF treated) were purchased from Sigma Chemical Co., St. Louis, MO.

Preparation of a Standard Mixture with Polysaccharide Gums. Two grams of commercial cocoa powder was mixed with 6 g of sucrose and 2 g of milk powder. Fifty milligrams of carrageenan and 50 mg of locust bean gum were added and the mixture was finely ground.

Removal of Fat. Five hundred milligrams of cocoa powder was suspended in 100 mL of a mixture of acetone–chloroform (1:1 v/v) and stirred for 30 min at room temperature. The solid material was centrifuged and the described procedure was repeated twice. Finally, the solid material was dried in vacuo over P_2O_5.

Enzymatic Hydrolysis of Starch. The defatted sample was suspended in 50 mL of 0.005 M phosphate buffer solution, pH 6.9, with the aid of ultrasonic treatment. A total of 500 units of α-amylase from porcine pancreas was added and the mixture was agitated for 1 h at room temperature. The enzyme was inactivated by heating the solution on a boiling water bath for 3 min. The nonstarch fraction was precipitated by addition of methanol (250 mL), centrifuged, and washed with a small portion of 85% (v/v) methanol.

Resin-Catalyzed Hydrolysis of the Nonstarch Fraction. Hydrolysis was carried out in a sealed glass tube with screw cap and septum (volume ca. 30 mL). The nonstarch fraction was suspended in 10 mL of deionized water, containing 2 mg of *myo*-inositol as the internal standard. Ten milliliters of a 40% (w/v) suspension of AG 50 W-X8 (H^+-form) resin in 0.02 N HCl was added to the sample solution. The mixture was shaken thoroughly and then heated to 100 °C in an oven for 48 h. After being cooled, the mixture was centrifuged and the centrifuged material washed several times with small portions of water. The combined aqueous solutions were evaporated to dryness under reduced pressure.

Passage through an Anion-Exchange Column. The residue after evaporation was dissolved in 5 mL of deionized water and added to a column (15 cm × 1.5 cm i.d.) packed with AG 1-X8 ($HCOO^-$ form). The column was eluted with 20 mL of 0.5 N formic acid at a flow rate of 0.5 mL/min. Fractions with a volume of 1 mL were collected; each fraction was tested for carbohydrates by the carbazole reaction. Those fractions that contained the neutral carbohydrates were collected, evaporated in vacuo, dried in a desiccator over P_2O_5, and weighed. (Anion-exchange chromatography was standardized by application of an appropriate standard mixture of neutral and acidic monosaccharides.)

Derivatization to Aldonitrile Acetates. The derivatization was carried out in a glass tube sealed with screw cap and septum. To 10 mg of the dried sugar mixture were added 10 mg of hydroxylamine hydrochloride and 0.5 mL of dry pyridine. The tube was heated in an oven to 90 °C for 30 min. After the mixture was cooled, 1.5 mL of dry acetic anhydride was added and the sealed tube was heated to 90 °C for another 30 min. The cooled solution was evaporated to dryness under reduced pressure at a maximum of 70 °C. The oily residue was dissolved in 200 μL of methylene chloride and 1-2 μL was injected into the gas chromatograph.

Gas Chromatography. GLC analysis was carried out on a Hewlett-Packard Model 5700 A equipped with a flame ionization detector. A stainless steel column (7 ft × $^1/_8$

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Table I. Percental Amounts of Total Nonstarch Polysaccharides in Cocoa Powders

sample	%[a] micro-method	extraction method
(1) cocoa powder	6.5	8.0
(2) standard mixture	2.1	1.8

[a] Percent of total material; mean values; after hydrolysis and anion-exchange chromatography.

Table II. Distribution of Neutral Sugars in Nonstarch Polysaccharides[a]

component sugar	(1) cocoa powder	(2) standard mixture
galactose	1.0 (1.0)	1.0 (1.0)
glucose	3.7 (4.1)	2.0 (2.0)
mannose	0.1 (0.2)	0.5 (0.6)
arabinose	0.8 (0.6)	0.9 (0.6)
xylose	0.2 (0.2)	0.1 (0.2)
rhamnose	0.1 (0.2)	tr (0.1)

[a] All values are related to galactose; values obtained by the extraction method are in parentheses; tr = traces.

in.) packed with 3% poly(neopentyl glycolsuccinate) on Chromosorb WAW (100–120 mesh) was used. Helium was used as the carrier gas at a flow rate of 40 mL/min. The temperature was programmed from 190 to 230 °C at 2 °C/min.

RESULTS AND DISCUSSION

The seeds of crude cocoa beans are known to contain starch, β-glucans, pectins, and other nonstarch polysaccharides, of which the latter have been investigated by different workers [e.g., Whistler et al. (1956)]. The neutral constituents of these nonstarch polysaccharides include rhamnose, galactose, arabinose, and mannose as component sugars.

The present work deals with detection and determination of nonstarch polysaccharides occurring in commercial cocoa powders. For this purpose a new micromethod was developed, which comprises essentially an enzymatic removal of the native starch and a resin-catalyzed hydrolysis of the nonstarch fraction.

The results obtained by the micromethod were compared with corresponding results that had been obtained by a previously applied extraction method (Mergenthaler and Scherz, 1980). Table I summarizes the percent amounts of isolated total nonstarch polysaccharides (after hydrolysis and anion-exchange chromatography).

The neutral sugars from the hydrolyzed polysaccharides were derivatized to aldonitrile acetates and analyzed by gas chromatography. The results of the semiquantitative analysis are given in Table II. A typical chromatogram is shown in Figure 1. It should be mentioned here that the precision of the applied micromethod was sufficient within the limits of the aldonitrile acetate method (Varma and Varma, 1976). The detection limit for nonstarch polysaccharides in cocoa powder (sample 1) amounted to 1.1 mg of polysaccharide/1000 mg of sample.

As shown in Table II, the main constituents of the nonstarch polysaccharides in cocoa powder (sample 1) were glucose, galactose, and arabinose. In comparison to the untreated cocoa powder, the different distribution of component sugars in the standard mixture (sample 2) indicated the addition of polysaccharide gums. The exceeding portion of mannose gave evidence for the addition of a special gum. β-Glucans could account for the glucose contents in both samples.

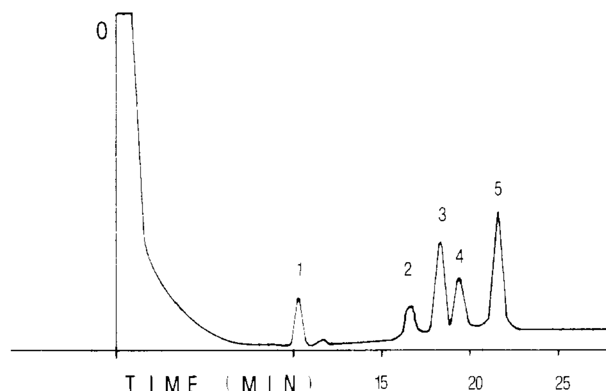

Figure 1. GLC separation of the aldonitrile acetates of the component sugars in nonstarch polysaccharides of a cocoa instant powder (standard mixture 2): 0 = solvent; 1 = arabinose; 2 = mannose; 3 = glucose; 4 = galactose; 5 = *myo*-inositol (internal standard).

Concerning the most component sugars in both samples, the obtained mean values were in sufficient agreement with the corresponding results from the extraction procedure. The only remarkable exception with regard to both samples appeared to be the relative amount of arabinose, which was lower in the previous experiments. Possibly, this finding could be ascribed to labile arabinofuranosyl bondings occurring frequently in nonstarch polysaccharides. Perhaps a great deal of these bondings was cleaved in the course of the rather rigorous extraction procedure. On the other hand, the new micromethod might proceed under more gentle conditions, preventing losses of sugar components.

It became evident that the micromethod offers some advantages when applied to analysis of polysaccharides in food. Setting forth with small quantities of sample material, one can determine semiquantitatively the nonstarch polysaccharides in cocoa powders by gas chromatography. Moreover, the new method enables a great number of samples to be investigated simultaneously. Eventually its scope of applicability may be extended to other industrially finished food products. For example, canned tomato sauces, fruit juices, cream puddings, and yogurts may become amenable to the new micromethod.

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LITERATURE CITED

Fransson, L. A.; Rodén, L.; Spach, M. L. *Anal. Biochem.* **1968**, *21*, 317–330.
Klose, R. E.; Glicksman, M. In "CRC Handbook of Food Additives"; Furia, T. E., Ed.; CRC Press: Cleveland, OH, 1972; pp 325–359.
Mergenthaler, E.; Scherz, H. *Z. Lebensm.-Unters. -Forsch.* **1980**, *170*, 280–286.
Porter, W. H. *Anal. Biochem.* **1975**, *63*, 27–43.
Varma, R.; Varma, R. S. *J. Chromatogr.* **1976**, *128*, 45–52.
Whistler, R. L.; Masak, E.; Plunkett, R. A. *J. Am. Chem. Soc.* **1956**, *78*, 2851–2854.

Hendrik Schäfer[1]

Deutsche Forschungsanstalt für Lebensmittelchemie
D-8046 Garching, Federal Republic of Germany
[1]Present address: Bruggfeldstrasse 5
A-6500 Landeck, Austria

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