A method was developed for determining fructan inulin in various foods (yogurts, honey cakes, chocolates). Warm water was applied for extraction of samples, and mono- and disaccharides were determined by a thin-layer chromatographic densitometric method. A portion of the test solution was hydrolyzed 30 min with 1% oxalic acid in a boiling water bath. Fructose was determined in the hydrolysate. The amount of inulin in a sample was calculated as the difference between the amount of fructose in the sample before and after hydrolysis. The fructose from sucrose formed during the hydrolysis was also considered. The mean recovery from yogurt fortified with 4% inulin was 95.5 ± 4.5% (mean ± standard deviation); from honey cakes extract fortified with 10% inulin, 97.3 ± 5.5%; and from chocolate extract fortified with 30% inulin, 98.6 ± 6.6% (6 replicates in all cases). Determination of glucose is not necessary for analyzing fructans with the composition expressed shortened to $\text{GF}_n$ (G, glucose; F, fructosyl) with the average degree of polymerization $8 \leq n \leq 15$.

Inulin represents a mixture of polysaccharide molecules with the general formula $\text{GF}_{n-1}$, where G is glucose, F is fructosyl ($\text{C}_\text{6H}_{11}\text{O}_\text{5}$), and $n$ is degree of polymerization (polycondensation). Chicory and Jerusalem artichoke are 2 plants rich in inulin in their underground parts.

The human body does not provide enzymes for the depolymerization of inulin. However, the recently recognized health benefits of inulin have made it a desired ingredient in various dietary food products. Inulin together with oligofructoses is classified as a soluble dietary fiber (1).

Depolymerization is neccessary in determining inulin, followed by the determination of its monomeric units. Special care is also needed in recognizing any interference from sugars not originating from inulin. A reliable method for determining mono- and disaccharides, therefore, is of great importance. Many published methods for determining sugars use liquid chromatography (LC) (2–5), capillary electrophoresis (CE; 6, 7) and thin-layer chromatography (TLC; 8–19). Some methods also include determination of oligosaccharides (3–6, 10). However, the results of a recently published interlaboratory study on the determination of sugars in foods by using LC on amino columns, were not very encouraging because of the high repeatability and reproducibility standard deviation (RSD$_r$ and RSD$_R$) values (3). The ion-exchange chromatographic method used in the interlaboratory study of determination of fructans in foods seemed to be better, and the method was adopted first action by AOAC INTERNATIONAL. The method, which includes determination of oligofructoses ($n \leq 10$) and inulin, is quite lengthy (2).

We describe a simple method for determining inulin in yogurts, honey cakes, and chocolates, based only on determination of fructose originating from inulin, using a published TLC method (18). Omitting the determination of glucose greatly facilitates the work. The amount of fructose originating from inulin is exactly equal to the amount of inulin with the average degree of polymerization $n = 11$, and this relation does not change much for $8 \leq n \leq 15$.

**Experimental**

**Apparatus**

All TLC equipment was obtained from Camag (Muttenz, Switzerland).

(a) *Analytical balance.*—Capable of weighing to 0.1 mg.
(b) *Hair dryer.*
(c) *Water bath.*
(d) *TLC application device.*—Linomat IV with 100 µL pipet.
(e) *TLC tank.*—Suitable for development of $20 \times 10$ cm chromatoplate.
(f) *TLC immersion device.*
(g) *TLC plate heater.*
(h) *TLC scanner II.*—Controlled by an external PC via an RS232 interface.
(i) *Standard laboratory glassware (volumetric flasks, pipets, tubes, etc.)*.

**Reagents**

All reagents are reagent grade unless specified.
(a) *Solvents.*—LC grade acetonitrile and methanol.
(b) *Water.*—Deionized via a water purification system
(c) *Oxalic acid.*—5% in water.
Inulin (Panatra, Chemische Fabrik Grünau, Germany).—1 mg/mL in water (soluble at room temperature). Water, mono- and disaccharides were determined in the formulation in separate experiments.

(e) Standards.—D(-)-fructose, D(+)sucrose.—Stock solutions: separate solution of each sugar at 1 mg/mL in 80% methanol. Working standard solution: fructose at 0.2 mg/mL and sucrose at 0.15 mg/mL together in 80% methanol.

(f) Chromatoplate.—Precoated HPTLC Silica Gel 60 (E. Merck, Darmstadt, Germany), 20 × 10 cm, used without pretreatment.

(g) Eluent.—Acetonitrile-diluted phosphate buffer solution (Soerensen, pH 5.5, total phosphate concentration 7 mM) (17 + 3), containing 0.05% 2-aminoethyl diphenylborinate.

(h) Detection reagent.—Dissolve 2 mL aniline and 2 g diphenylamine in 100 mL methanol. Add 15 mL orthophosphoric acid (85%) and mix well.

Sample Preparation

(a) Yogurt (about 4% inulin).—Determination of fructose and sucrose before hydrolysis (if necessary): Dilute 100 mg well-homogenized sample with hot 80% methanol in water in 25 mL volumetric flask. Mix well, cool, and filter to volume with the same solvent. Filter (discard first few milliliters) into a tube and cap. The solution is ready for application on the plate. Ordinary fresh yogurts do not contain fructose or any sugar with the fructose moiety.

Hydrolysis: Weigh to 0.1 mg accuracy about 100 mg sample into 25 mL volumetric flask. Add 4 mL water and 1 mL 5% oxalic acid, cover loosely, and heat in boiling water bath for 30 min. Cool reaction mixture to room temperature, slowly fill to volume with methanol, and mix. Apply filtered portion (discard first few milliliters) on TLC plate.

(b) Honey cakes (about 10% inulin and about 10% fructose).—Grind dried sample to fine powder. Weigh 500 mg sample into 50 mL volumetric flask and fill to volume with hot water (about 70°C). The exact temperature is not important. Note the weight of added water and cap. Shake strongly for 5 min to dissolve inulin. Cool to room temperature and filter. Discard first few milliliters. Pipet 5 mL filtrate into 25 mL volumetric flask, and slowly fill to volume with methanol for the determination of fructose and sucrose before hydrolysis.

Into second 25 mL volumetric flask, pipet 4 mL filtrate, and add 1 mL 5% oxalic acid. Heat loosely covered in boiling water bath for 30 min. Cool to room temperature and fill to volume with methanol. The solution is ready for application on the plate.

(c) Chocolate (about 30% inulin).—Weigh 400 mg ground chocolate into 50 mL volumetric flask, and proceed as for honey cakes.

Recovery Samples Preparation

(a) Yogurt.—Add 4 mL water solution of inulin (1 mg/mL) to 100 mg ordinary yogurt in 25 mL volumetric flask, and proceed with hydrolysis.

(b) Honey cakes.—Add 40 mg inulin to 460 mg honey cakes without inulin in 50 mL volumetric flask, and proceed as with samples.

(c) Chocolate.—Add 120 mg inulin to 240 mg chocolate without inulin in 50 mL volumetric flask, and proceed as with samples.

Thin-Layer Chromatography

Apply standard working solution (1–6 μL) and test solutions (2–5 μL, depending on content of sugars, twice each) alternately on the plate in 8 mm bands, speed 12 s/μL, 6 mm apart, 15 mm from bottom and 20 mm from left edge (12 applications on each plate).

Development: In unsaturated tank, linear, ascendant, 2 times to 7 cm, drying thoroughly with hair dryer between developments. One development takes about 15 min.

Detection: Sink plate into detection reagent for 1 s, dry with hair dryer, and heat plate for 10 min at 120°C. Perform densitometry about 1 h after detection in absorption/transmission mode. Wavelength, 560 nm; band, 30 nm; macro; slit length, 4 mm; slit width, 0.6 mm. Quantitation is based on peak areas.

Calculation

Calculate amount of fructose from the linear regression equation. Determine the difference between the amount of fructose present before and after the hydrolysis, taking account of fructose from sucrose in the sample. The percent of inulin in the sample is equal to the percent of fructose originating from it (Table 1).

Results and Discussion

A standard curve of the contents of interest (fructose at Rf 0.51 and sucrose at Rf 0.27) is always established on the same plate under the same conditions as for samples. There are also lactose at Rf 0.11 in yogurt and chocolate samples, galactose at Rf 0.36 from partly hydrolyzed lactose, and glu-

| Table 1. Dependence of calculation factors on average degree of polymerization, n |
|---------------------------------|-----------------|-----------------|
| n                              | iF1 + G1        | iF1             | iG1  |
| 3                              | 0.93            | 1.40            | 2.8  |
| 5                              | 0.92            | 1.15            | 4.6  |
| 7                              | 0.91            | 1.06            | 6.4  |
| 8                              | 0.91            | 1.04            | 7.3  |
| 9                              | 0.91            | 1.02            | 8.2  |
| 10                             | 0.91            | 1.01            | 9.1  |
| 11                             | 0.91            | 1.00            | 10.0 |
| 15                             | 0.91            | 0.97            | 13.6 |
| 25                             | 0.90            | 0.94            | 22.6 |
| 30                             | 0.90            | 0.93            | 27.1 |
cose at $R_f$ 0.41. The $R_f$ values are apparent because of double development. Double development and addition of 2-aminoethyl diphenylborinate are necessary for better separation of fructose and glucose. Phosphate buffer in the eluent diminishes peak broadening and tailing of fructose (18). There are also other sugars on the plate: pentoses are expected above hexoses and oligosaccharides with more than 3 units near the start of chromatogram. In the determination of sugars in samples before hydrolysis, the spot of inulin on and near the start was seen even when 80% methanol was used for extraction, showing that inulin is partly soluble in this medium. The color of the spot of inulin was brown, like that of fructose. No interferences from the test solutions were observed on the plate. The densitometric quantitation of sugars is performed best when a linear relationship with the peak area (or peak height) exists. This is only in a narrow range of applied amounts and is a drawback compared to the LC technique. The appropriate dilution of sample with methanol must be calculated in advance if possible. More experimental work is needed to find the best conditions for determining the chosen sugar (Figure 2).

Solubility of inulin in water depends on the degree of polymerization (polymers with lower $n$ are more soluble) and on the water temperature. Inulin is more soluble in hot than in cold water. In hot water, starch from samples (e.g., cakes) passes into the water, which is undesirable. A high mass proportion of water to the inulin sample (about 1000) was applied for extraction of honey cakes and chocolates. However, inulin dissolved in hot water at this concentration does not precipitate when the solution is cooled to 20°C. Oxalic acid was used for the hydrolysis of inulin as recommended because of the instability of fructose when boiled with strong mineral acids (20).

Accuracy of the method was controlled by adding a solution of commercial inulin formulation to a water suspension of yogurt, cakes, or chocolate without inulin at concentrations similar to those in the samples. The mean recovery from yogurt fortified with 4% inulin was 95.5 ± 4.5% (mean

Figure 1. CCD image of HPTLC plate with standard and sample test solutions (INU - inulin, LAC - lactose, SUC - sucrose, GAL - galactose, GLU - glucose, FRU - fructose). Odd lanes: 0.2–1.2 µg fructose and 0.15–0.9 µg sucrose; lane 2, yogurt without inulin after hydrolysis; lane 4, yogurt with 4% inulin after hydrolysis; lane 6, honey cakes with 10% inulin before hydrolysis; lane 8, the same as 6 but after hydrolysis; lane 10, chocolate with 30% inulin before hydrolysis; lane 12, the same as 10 but after hydrolysis.

Figure 2. Densitograms obtained from HPTLC plate: A, lane 10, chocolate with 30% inulin before hydrolysis, 4 µL applied; B, lane 12, chocolate with 30% inulin after hydrolysis, 2 µL applied.
± standard deviation); from honey cakes extract fortified with 10% inulin, 97.3 ± 5.5%; and from chocolate extract fortified with 30% inulin, 98.6 ± 6.6% (6 replicates in all cases).

Results were calculated only from fructose originating from inulin and are based on the determination of fructose and sucrose before and fructose after hydrolysis.

Determination of glucose is unnecessary if we assume the chemical formula of inulin to be \( GF_{\text{n-1}} \) (see above):

\[
n = \frac{F_i}{G_i + 1}
\]

where \( n \) is average degree of polymerization, \( F_i \) is content of fructose originating from inulin in sample, and \( G_i \) is content of glucose originating from inulin in sample.

\[
i = \frac{[180 + 162*(n - 1)]/[180*n]}{(F_i + G_i)}
\]

where \( i \) is content of inulin in sample.

Combining Equations 1 and 2, we obtain the following:

\[
i = 0.9*F_i + G_i \quad \text{or} \quad (3)
\]

\[
i = 0.9*F_i + F_i/n - 1 \quad \text{or} \quad (4)
\]

\[
i = 0.1*G_i + 0.9*n*G_i \quad \text{or} \quad (5)
\]

If \( F_i \) and \( G_i \) are accurately determined, then \( i \) and \( n \) can be calculated. Accurate and precise determination of \( G_i \) can be difficult in some cases when it is obtained as a small difference between 2 much greater values, or if a chromatographic interference is present. Table 1 shows that the content of \( F_i \) is exactly the content of inulin for \( n = 11 \) and an acceptable approximation for \( n \) from 8 to 15. Determination of inulin based only on determination of its glucose content is impossible without knowing \( n \).

If the formulation of inulin applied as a food ingredient is known and at one’s disposal (very useful), \( n \) is more easily determined from the formulation by applying the following relationship:

\[
n = \frac{(i + 0.1*F_i)}{(i - 0.9*F_i)}
\]

where \( i \) is content of inulin in the formulation sample.

The determination of \( n \) from the formulation of inulin is naturally possible also from \( F_i \) and \( G_i \), according to Equation 1. Sweet and quite water-soluble inulins have, fortunately, an average degree of polymerization of about 10 compared to nonsweet and less soluble inulin with \( n = 30 \).

Simultaneous determination of fructose and glucose is necessary only for determination of smaller molecules of GF_{n-1}. These compounds (oligofructoses) serve as a dietary substitute for sucrose. They can be determined in samples without hydrolysis individually by LC or TLC. Only appropriate standards are needed.

References