TECHNICAL COMMUNICATIONS

Measurement of Sugars and Starches in Foods by a Modification of the AOAC Total Dietary Fiber Method

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A separation scheme for the determination of sugars and starch in processed food was developed. It is based on AOAC Method 985.29 for total dietary fiber with these modifications: carbohydrate starches are separated into soluble and insoluble fractions before they are hydrolyzed; acetonitrile is used instead of ethanol to separate sugars from enzyme-resistant carbohydrates, proteins, and other macromolecules; and a solid-phase extraction filter is included to remove substances that interfere with high-performance liquid chromatography (HPLC). Recovery studies indicate a >97% sugar recovery. Twenty foods were analyzed. After enzymatic hydrolysis, fructose, glucose, sucrose, maltose, and lactose were extracted and determined by HPLC using a refractive index detector. Starch content was calculated from the increase in the amount of glucose. The results were compared with values listed on the "Nutrition Facts" panel for that food. The analyzed amounts of sugars and starches were 73–96% of declared values.

Regulations issued by the U.S. Food and Drug Administration on January 6, 1993 (1), made extensive changes to the way foods are labeled. The new food labels contain a "Nutrition Facts" panel that includes serving size, number of servings per container, and information about specific food components. Total carbohydrate, sugars, and dietary fiber are among the mandatory components listed on the new food label. Voluntary components include soluble fiber, insoluble fiber, sugar alcohol, and other carbohydrate.

Although the term "complex carbohydrates" was initially proposed as a mandatory component on the food label (2), the final regulations adopted the term "other carbohydrate," primarily because of the lack of analytical methodology to support the definition of "complex carbohydrates." The term "other carbohydrate" is defined as the amount of carbohydrate remaining after subtraction of dietary fiber, sugars, and sugar alcohol (1). A method to determine "other carbohydrate" has not yet been developed and validated.

The objective of this study was to evaluate a method for analysis of sugars and carbohydrates in processed foods and to compare the results with values listed on the food labels. Digestible carbohydrates such as starch are important components of foods such as cereals. Sugars such as sucrose are also important because they are often added to foods during processing. In this study, we determined carbohydrates that are digestible in the human upper gastrointestinal tract by using enzymes that mimic the human system under laboratory conditions specified in AOAC Method **985.29** for total dietary fiber (3). We also determined amounts of sugars (e.g., fructose, glucose, sucrose, maltose, and lactose) present in foods before enzymatic hydrolysis.

High-performance liquid chromatography (HPLC) with refractive index (RI) detection is a powerful technique for quantitation of various types of sugars and was chosen for this study. Shaw (4) has made an extensive compilation of techniques used for sugar analysis, and Southgate (5) has provided another extensive review. To accurately quantitate carbohydrates, it was necessary to modify starch and dietary fiber methods so that sugars can be separated from components such as proteins, fibers, and other macromolecules that create backflow and that interfere with the resolution of sugar peaks.

Experimental

Materials

(a) *Sugars.*—Fructose, glucose, sucrose, maltose, and lactose (>99.5% purity; Sigma Chemical Co., St. Louis, MO) used in this study were stored in a vacuum desiccator with silica gel as desiccant.

(b) *Enzymes.*—Total Dietary Fiber Kit (Sigma, TDF-100A) was used. This kit includes 10 mL heat-stable α-amylase, 500 mg protease, and 30 mL amyloglucosidase. Each kit is sufficient for 100 determinations (*see* Sigma Technical Bulletin No. TDFAB-2).

(c) *Products examined.*—Plain cereals, sugar-coated cereals, canned fruits, canned vegetables, crackers, and cookies were purchased locally.

Reagents

(a) *Petroleum ether.*—Reagent grade.

(b) *Acetonitrile.*—HPLC grade.

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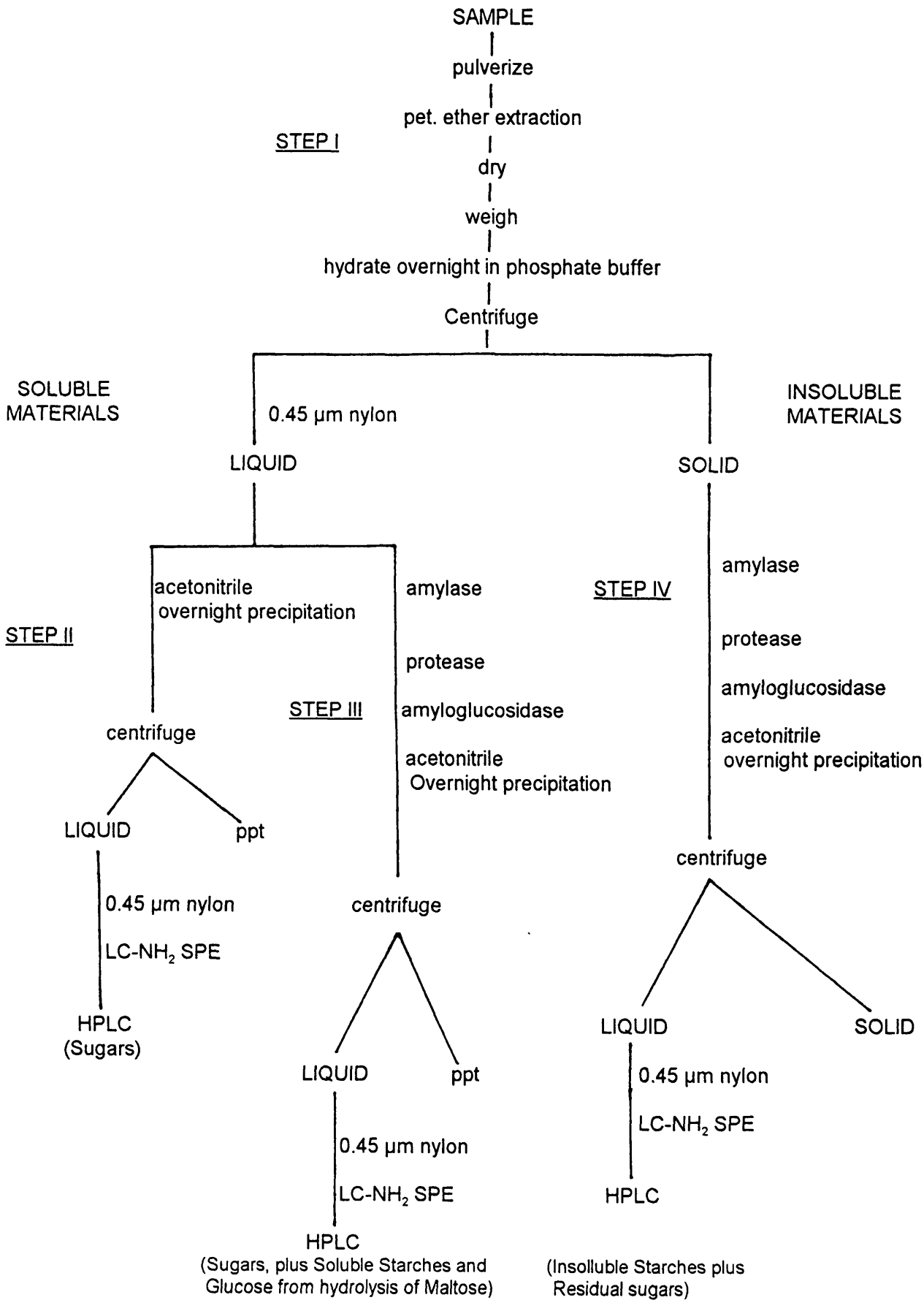

Figure 1. Analytical procedure for determination of sugars and starches.

Table 1. Recovery of sugars added to sample extract determined by HPLC

Spiked extract	Recovery, %[a]				
	Fructose	Glucose	Sucrose	Maltose	Lactose
Buffer[b]	100 ± 0	99 ± 2	101 ± 2	98 ± 2	98 ± 3
Wheat cereal[b]	101 ± 1	101 ± 1	98 ± 1	99 ± 1	100 ± 2
Enzymes[c]	100 ± 1	318 ± 4	97 ± 2	0	99 ± 2

[a] Sugars recovered after acetonitrile extraction filtration through 0.45 μm nylon filter, and LC-NH_2 SPE treatments; $n = 5$.
[b] Analyzed through Step II.
[c] Analyzed through Step IV.

(c) *Phosphate buffer.*—0.08M, pH 6.0. Dissolve 1.400 g anhydrous dibasic sodium (Na_2HPO_4) and 9.68 g monobasic sodium phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$) in 1 L water. Check pH and adjust if necessary.

(d) *NaOH.*—0.275N. Dissolve 11.00 g NaOH in 1 L water.

(e) *HCl.*—0.325M. Dilute 325 mL 1M HCl to 1 L with water.

Instrumentation and Operating Conditions

HPLC was performed with a Shimadzu system consisting of LC-600 pumps, CTP-6A column oven, RID-6A RI detector, and AST-LC computer using Shimadzu EZChrom chromatography data system software, version 3.2. Carbohydrates were separated on a Supelcosil LC-NH_2 25 cm × 4.6 mm column preceded by a Supelguard column containing LC-NH_2 packing. The mobile phase was acetonitrile–water (80 + 20, v/v), filtered through a 0.45 μm nylon filter and degassed before use. The flow rate was 1.0 mL/min.

Quantitation

Solutions of individual sugars (fructose, 4.7 mg/mL; glucose, 4.5 mg/mL; sucrose, 4.5 mg/mL; maltose, 9.6 mg/mL; and lactose, 9.6 mg/mL) in acetonitrile–water (1 + 1) were used as standards. Peak areas were plotted against the corresponding amount of the standard injected (10 μL) into the HPLC system, and linear relationships were obtained from 0 to 160 μg for glucose, fructose, maltose, lactose, and galactose. Food extracts were injected, and a response that fell within the linear range was used in the determination of concentration.

Sample Preparation

All products except canned foods were milled to a fine powder. High-fat crackers and cookies were defatted with petroleum ether as described in AOAC Method **985.29** (3). Sugar values were corrected to reflect weight loss due to defatting. The test portions were dried overnight at 105°C and stored in a desiccator until analysis. Canned foods were homogenized in a Waring high-speed blender and stored in a refrigerator until analysis.

Principle

Duplicate test portions of processed foods were treated with heat-stable α-amylase, protease, and amyloglucosidase to hydrolyze proteins and starch (Figure 1) under laboratory conditions specified in AOAC Method **985.29** (3). Acetonitrile was added to precipitate substances such as soluble proteins and fibers (6). Residues were removed by centrifugation and filtration through a 0.45 μm nylon filter. The filtrate was passed through a Supelco LC-NH_2 solid-phase extraction cartridge (SPE) to remove HPLC-interfering substances. The eluate was analyzed for carbohydrates by HPLC.

Table 2. Carbohydrate analysis of uncoated cereals

Cereal	Step: Material, treatment	Carbohydrate, g/100 g cereal				
		Fructose	Glucose	Sucrose	Maltose	Lactose
Corn flakes	II: Soluble, w/o enzymes	1.3 ± 0.0	1.2 ± 0.0	3.6 ± 0.0	0	0
	III: Soluble, with enzymes	0.1 ± 0.0	12.2 ± 0.2	0.3 ± 0.0	0	0
	IV: Insoluble, with enzymes	0	69.2 ± 1.1	0	0	0
Oat bran	II: Soluble, w/o enzymes	0.1 ± 0.0	0	1.1 ± 0.0	0	0
	III: Soluble, with enzymes	0	0	0	0	0
	IV: Insoluble with enzymes	0	65.1 ± 1.2	0	0	0
Rice (crispy)	II: Soluble, w/o enzymes	0	0.7 ± 0.1	6.0 ± 0.5	0	0
	III: Soluble, with enzymes	0	15.4 ± 0.3	0.2 ± 0.0	0	0
	IV: Insoluble, with enzymes	0	64.0 ± 1.4	0	0	0
Wheat (shredded)	II: Soluble, w/o enzymes	0	0	0.3 ± 0.0	0	0
	III: Soluble, with enzymes	0	3.4 ± 0.0	0	0	0
	IV: Insoluble, with enzymes	0	68.6 ± 1.0	0	0	0

Table 3. Carbohydrate analysis of sugar-coated cereals

Cereal	Step: Material, treatment	Carbohydrate, g/100 g cereal				
		Fructose	Glucose	Sucrose	Maltose	Lactose
Rice (cocoa)	II: Soluble, w/o enzymes	0.7 ± 0.0	0.9 ± 0.0	35.2 ± 1.0	0	0
	III: Soluble, with enzymes	2.5 ± 0.1	15.4 ± 0.3	0.2 ± 0.0	0	0
	IV: Insoluble, with enzymes	0.1 ± 0.0	28.1 ± 0.6	2.6 ± 0.1	0	0
Wheat flakes	II: Soluble, w/o enzymes	0	0	14.8 ± 0.7	0	0
	III: Soluble, with enzymes	1.3 ± 0.1	4.2 ± 0.1	0	0	0
	IV: Insoluble with enzymes	0	46.9 ± 1.0	1.6 ± 0.1	0	0
Corn flakes	II: Soluble, w/o enzymes	1.0 ± 0.0	1.4 ± 0.1	31.9 ± 1.2	0	0
	III: Soluble, with enzymes	2.0 ± 0.1	11.2 ± 0.4	0.9 ± 0.0	0	0
	IV: Insoluble, with enzymes	0.2 ± 0.0	29.4 ± 1.1	2.1 ± 0.1	0	0
Rice (fruity)	II: Soluble, w/o enzymes	0.1 ± 0.0	0.2 ± 0.0	35.8 ± 0.5	0	0
	III: Soluble, with enzymes	2.1 ± 0.0	13.2 ± 0.5	0	0	0
	IV: Insoluble, with enzymes	0	33.7 ± 1.3	2.8 ± 0.0	0	0

Analytical Procedure

Blanks and test portions were carried through the procedure outlined in Figure 1.

(a) *Step I.*—Duplicate test portions of ca 0.5 g were weighed to 0.1 mg into 16 × 125 mm tubes with screw caps. Ten milliliters of pH 6.0 phosphate buffer was added to each tube. The tubes were stored at 4°C overnight to ensure hydration of the matrix. The tubes were centrifuged to separate particles, and then 5 mL of the aqueous portion from each tube was filtered through a 0.45 μm nylon filter into another 16 × 125 mm tube for analysis through Steps II and III. The remaining 5 mL slurry samples were analyzed through Step IV.

(b) *Step II.*—Two milliliters of the filtered portion was pipetted into a test tube and 2 mL acetonitrile was added. After precipitation overnight, the residue was separated by centrifugation. The aqueous portion was cleaned through an autovial syringless 0.45 μm nylon filter and LC-NH_2 SPE. The resulting filtrate was then analyzed by HPLC for sugars.

(c) *Step III.*—Another 2 mL of the 5 mL filtered aqueous portion from Step I was subjected to enzyme hydrolysis to degrade soluble starch. α-Amylase solution (50 μL) was added, and the tube was placed in a 95°C water bath. After 30 min, it was removed and cooled to ca 60°C and adjusted to pH 7.5 with 0.4 mL 0.275N NaOH. Protease solution (50 μL) was added. The tube was incubated at 60°C for 30 min, and then 0.4 mL 0.325M HCl was added to reduce the pH to 4.5. Amyloglucosidase solution (150 μL) was added, and the tube was incubated at 60°C for 30 min. After the tube had cooled, 3 mL acetonitrile was added. After overnight precipitation, the residue was separated by centrifugation. The liquid portion was filtered through a 0.45 μm nylon filter and then cleaned by SPE. The filtrate was analyzed by HPLC.

(d) *Step IV.*—The insoluble residue slurry from Step I was subjected to enzyme hydrolysis in the same way as described for Step III, except that 1 mL 0.275N NaOH, 1 mL 0.325M HCl, and 7 mL acetonitrile were used.

(e) *Recovery.*—Three sugar recovery determinations were performed: phosphate buffer solution without enzyme treatment, a sample of wheat cereal in buffer without enzyme treatment, and buffer with enzyme treatment (Table 1). The standard mixture of sugars was added to each mixture. The sugars in the buffer–enzyme solution were quantitatively recovered as described in Step IV. The other 2 were recovered through Step II.

Table 4. Carbohydrate analysis of canned fruits

Fruit	Step: Material, treatment	Carbohydrate, g/100 g fruit				
		Fructose	Glucose	Sucrose	Maltose	Lactose
Fruit cocktail	II: Soluble, w/o enzymes	4.5 ± 0.2	5.0 ± 0.2	1.5 ± 0.1	0	0
	III: Soluble, with enzymes	0.1 ± 0.1	1.9 ± 0.1	0	0	0
	IV: Insoluble, with enzymes	1.7 ± 0.1	1.0 ± 0.0	0.8 ± 0.0	0	0
Pear halves	II: Soluble, w/o enzymes	3.3 ± 0.1	3.5 ± 0.1	3.1 ± 0.0	0	0
	III: Soluble, with enzymes	0.4 ± 0.0	2.5 ± 0.1	0	0	0
	IV: Insoluble with enzymes	0.1 ± 0.0	0.1 ± 0.0	1.5 ± 0.0	0	0
Sliced peaches	II: Soluble, w/o enzymes	3.0 ± 0.1	3.4 ± 0.1	2.2 ± 0.0	0	0
	III: Soluble, with enzymes	0.3 ± 0.0	1.6 ± 0.1	0	0	0
	IV: Insoluble, with enzymes	1.0 ± 0.0	1.6 ± 0.1	1.1 ± 0.0	0	0

Table 5. Carbohydrate analysis of canned vegetables

Vegetable	Step: Material, treatment	Carbohydrate, g/100 g vegetable				
		Fructose	Glucose	Sucrose	Maltose	Lactose
Carrots	II: Soluble, w/o enzymes	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0
	III: Soluble, with enzymes	0	2.3 ± 0.3	0	0	0
	IV: Insoluble, with enzymes	0.1 ± 0.0	0	0	0	0
Corn	II: Soluble, w/o enzymes	0.1 ± 0.0	0	4.9 ± 0.1	0.1 ± 0.0	0
	III: Soluble, with enzymes	0.9 ± 0.0	7.1 ± 0.6	0.2 ± 0.0	0	0
	IV: Insoluble with enzymes	0	0	0	0	0
Green beans	II: Soluble, w/o enzymes	0.4 ± 0.0	0.4 ± 0.0	0.1 ± 0.0	0	0
	III: Soluble, with enzymes	0	1.1 ± 0.1	0	0	0
	IV: Insoluble, with enzymes	0.1 ± 0.0	0	0	0	0

Calculation

Six analytical values per food product were obtained. Sugars were calculated with the following formula:

$$\text{Sugars, } \mu g/g = \frac{\text{peak area} \times \text{std factor} \times \text{volume factor}}{\text{test portion weight, g}}$$

where peak area = chromatographic peak area of sugar, std factor = conversion factor to convert peak area to sugar value in micrograms based on sugar standard curve (slope), and volume factor = 10.0 for Step II, 15.125 for Step III, or 14.25 for Step IV.

The sugar contents determined are given in Tables 2–7.

(**a**) *Soluble materials.*—The amount of individual sugars in Step III is the amount remaining after subtraction of the amount of the corresponding sugar determined in Step II. Because of hydrolysis by enzymes used in Step III, the amount of glucose derived from maltose (determined in Step II) is subtracted from the amount of glucose in Step III. A maltose-to-glucose conversion factor of 0.9 is used in this case.

(**b**) *Insoluble materials.*—In Step IV, the amount of individual sugars is the amount remaining after subtraction of the amount of the corresponding sugar determined in Step III and of the amount of glucose derived from maltose determined in Step II.

The amounts of soluble starches are obtained by multiplying the increased amount of glucose from hydrolysis of soluble material by 0.9. The amount of insoluble starches are obtained by conversion of the increased amount of glucose in the insoluble material. The amount of glucose derived from maltose is not included in this determination.

Results and Discussion

The analytical scheme shown in Figure 1 allowed the measurement of total carbohydrate in processed foods. In previous studies, ethanol was used to extract sugars. The ethanol extracts contained fairly high amounts of soluble nonsugar components. When the extract is injected into the HPLC system, the acetonitrile (80%) mobile phase precipitates the components, causing an increased back pressure. To overcome this problem, the filtered enzyme digestate fractions from fiber analytical procedures of foods were treated with acetonitrile instead of ethanol before injection into the HPLC system. Another advantage of using acetonitrile is that the solvent/sample solution ratio is 1/1, whereas the ethanol/sample ratio used in the fiber method is 4/1. An experiment was conducted and found no difference in carbohydrate analyses when 80% ethanol or acetonitrile is used. This new procedure provided a food extract containing all sugars present in the food as simple sugars and

Table 6. Carbohydrate analysis of crackers

Cracker	Step: Material, treatment	Carbohydrate, g/100 g cracker				
		Fructose	Glucose	Sucrose	Maltose	Lactose
Cheese	II: Soluble, w/o enzymes	0.1 ± 0.0	0.1 ± 0.0	1.0 ± 0.0	0.6 ± 0.0	0
	III: Soluble, with enzymes	0.1 ± 0.0	4.2 ± 0.1	0	0	0
	IV: Insoluble, with enzymes	0	43.0 ± 1.4	0	0	0
Wheat	II: Soluble, w/o enzymes	0	0.3 ± 0.0	1.2 ± 0.1	0.3 ± 0.0	0
	III: Soluble, with enzymes	0.3 ± 0.0	9.4 ± 0.4	0	0	0
	IV: Insoluble with enzymes	0	42.1 ± 1.9	0	0	0
Saline	II: Soluble, w/o enzymes	0	0.7 ± 0.1	0	0.8 ± 0.1	0
	III: Soluble, with enzymes	0.2 ± 0.0	3.1 ± 0.1	0	0	0
	IV: Insoluble, with enzymes	0	60.5 ± 1.9	0	0	0

Table 7. Carbohydrate analysis of cookies

Cookie	Step: Material, treatment	Carbohydrate, g/100 g cookie				
		Fructose	Glucose	Sucrose	Maltose	Lactose
Chocolate	II: Soluble, w/o enzymes	0.5 ± 0.0	0.2 ± 0.0	21.5 ± 0.8	0	0
	III: Soluble, with enzymes	4.6 ± 0.2	4.1 ± 0.2	0	0	0
	IV: Insoluble, with enzymes	0	31.0 ± 1.4	4.0 ± 0.1	0	0
Wafer	II: Soluble, w/o enzymes	0.1 ± 0.0	0	17.4 ± 0.6	0	0
	III: Soluble, with enzymes	4.5 ± 0.2	2.5 ± 0.1	0.2 ± 0.0	0	0
	IV: Insoluble with enzymes	0	36.5 ± 1.2	3.8 ± 0.1	0	0
Vanilla	II: Soluble, w/o enzymes	0.9 ± 0.0	0.8 ± 0.0	12.0 ± 0.5	0	0
	III: Soluble, with enzymes	3.6 ± 0.2	3.7 ± 0.1	0	0	0
	IV: Insoluble, with enzymes	0	46.4 ± 1.0	4.0 ± 0.2	0	0

digestible carbohydrates. Although acetonitrile may be more hazardous than ethanol, we think that with proper use the advantages of using acetonitrile outweigh the somewhat increased risk.

Figure 2 shows typical HP liquid chromatograms of fructose, glucose, sucrose, maltose, and lactose. The extraction, cleanup, and HPLC procedures were validated with these sugars. Table 1 shows the ability of the procedure to recover these added sugars. With the exception of glucose recovered from the

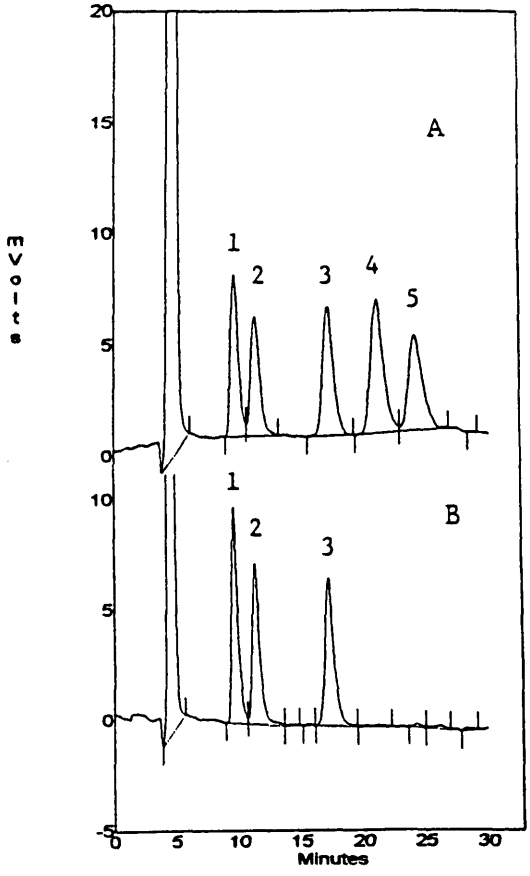

Figure 2. High pressure liquid chromatograms of (A) reference standard of (1) fructose, (2) glucose, (3) sucrose, (4) maltose, and (5) lactose; (B) enzyme-hydrolyzed canned fruit carbohydrates: (1) fructose, (2) glucose, and (3) sucrose (Step IV of analytical procedure).

buffer solution with enzymes, recoveries of sugars ranged from 97 to 101%. The enzymes cleaved maltose quantitatively to glucose. Recovery of this sugar is higher because of the addition of water to the glucose fragments by hydrolysis. The theoretical recovery of glucose from maltose is 222%. With added glucose from a standard mixture of sugars, we found a recovery of 318%, 98.8% of the theoretical value of 322% (Table 1). These recovery data demonstrate that the procedures outlined in Figure 1 yield extracts without loss of sugars.

This method was applied to various processed foods, including cereals, fruits, vegetables, crackers, and cookies (Tables 2 to 7). These tables include treatment steps and food sources (e.g., soluble and insoluble) from which sugars were extracted. The values are the amounts of sugars extracted before or after enzymatic treatment. The use of enzymes in Step III and Step IV resulted in increased amounts of glucose, indicating hydrolysis of starches present in soluble and insoluble food materials. Small increases in the amounts of sucrose and fructose were found after hydrolysis in some of the food products, such as cookies. Additional studies were performed to determine whether more of these sugars could be extracted. Prolonged incubation with α-amylase (up to 2½ h) did not result in any further increase of the amounts. Also, when protease, one of the 3 enzymes used in this study, was omitted for the purpose of testing with cookies (chocolate, wafers, and vanilla), up to 6% (6, 4, and 2%, respectively) of the total amount of sugars could not be extracted after hydrolysis with the other 2 enzymes, α-amylase and amyloglucosidase. This finding indicates that these sugars were probably associated with proteins and cannot be isolated simply by extraction with buffer in Step I. All protease-freed sugars were simply combined with those extracted in Step II. Lactose was not detected in cheese cracker. According to the product information provided by the supplier, the cracker contained mostly wheat and barley flour, a small amount of skim milk cheese, and no cholesterol. Table 8 summarizes the results of carbohydrate analyses of the food extracts. The total amounts of sugars and starches vary from those listed on the food products' nutrition labels by 4 to 28%.

Because current food labeling regulations do not require that the amounts of individual soluble and insoluble carbohydrates or starches be declared on the label, only Steps I, II,

Table 8. Carbohydrate content of food products[a]

Product	Soluble starches	Insoluble starches	Total starches	Total sugars	Starches and sugars	Label value[b]	Percent of label value
			Uncoated cereals				
Corn	11.0 ± 0.2	62.3 ± 1.0	73.3 ± 1.2	6.5 ± 0.0	79.8	84.5	94
Oat	0	58.6 ± 1.1	58.6 ± 1.1	1.2 ± 0.0	59.8	67.9	88
Rice	13.9 ± 0.3	57.6 ± 1.2	71.5 ± 1.5	6.9 ± 0.6	78.4	88.0	89
Wheat	3.1 ± 0.0	61.7 ± 0.9	64.8 ± 0.9	0.3 ± 0.0	65.1	77.7	84
			Sugar-coated cereals				
Rice (cocoa)	13.9 ± 0.3	25.3 ± 0.5	39.2 ± 0.8	42.2 ± 1.2	81.4	86.2	94
Wheat	3.8 ± 0.1	42.2 ± 0.9	46.0 ± 1.0	17.7 ± 0.8	63.7	76.9	83
Corn	10.1 ± 0.4	26.5 ± 0.9	36.6 ± 1.3	39.5 ± 1.5	76.1	93.3	82
Rice (fruity)	11.9 ± 0.4	30.3 ± 1.1	42.2 ± 1.6	41.0 ± 0.6	83.2	88.9	94
			Canned fruits				
Fruit cocktail	1.7 ± 0.1	0.9 ± 0.0	2.6 ± 0.1	13.6 ± 0.5	16.2	18.1	90
Pear halves	2.3 ± 0.1	0.1 ± 0.0	2.4 ± 0.1	11.9 ± 0.2	14.3	18.1	79
Sliced peaches	1.4 ± 0.1	1.4 ± 0.1	2.8 ± 0.2	11.0 ± 0.2	13.8	18.1	76
			Canned vegetables				
Carrots	2.1 ± 0.3	0	2.1 ± 0.3	0.9 ± 0.1	3.0	4.1	73
Corn	6.4 ± 0.5	0	6.4 ± 0.5	6.2 ± 0.1	12.6	16.0	79
Green beans	1.0 ± 0.1	0	1.0 ± 0.1	1.0 ± 0.0	2.0	2.5	80
			Crackers				
Cheese	3.8 ± 0.1	38.7 ± 1.3	42.5 ± 1.4	1.9 ± 0.1	44.4	50.0	89
Wheat	8.5 ± 0.4	37.9 ± 1.7	46.4 ± 2.1	2.1 ± 0.2	48.5	56.3	86
Saline	2.8 ± 0.1	54.5 ± 1.7	57.3 ± 1.8	1.7 ± 0.2	59.0	64.3	92
			Cookies				
Chocolate	3.7 ± 0.2	27.9 ± 1.2	31.6 ± 1.4	30.8 ± 1.1	62.4	66.7	94
Wafer	2.3 ± 0.1	32.9 ± 1.1	35.2 ± 1.2	26.0 ± 0.9	61.2	64.5	95
Vanilla	3.3 ± 0.1	41.8 ± 0.9	45.1 ± 1.0	21.3 ± 0.9	66.4	69.0	96

[a] In g/100 g product ± standard deviation; $n = 6$.
[b] Each label value was calculated from label declaration of total carbohydrate per serving divided by serving size times 100. Content of total dietary fiber is not included.

and IV can be used to obtain total amounts of starches and of sugars. The amount of glucose derived from maltose determined in Step II is subtracted from the total amount of glucose in Step IV. The remaining glucose content is converted to total starch content. The total amount of sugars is determined by combining the amounts of the remaining sugars in Step IV including maltose. This study demonstrates that the method is applicable to determination of digestible carbohydrates and sugars in the foods tested. Currently, we do not know how the labeled sugar and starch contents were determined by the industries. Our analytical data were between 73 and 96% of the declared values.

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