Rapid Method for Sugar Analysis of Doughs and Baked Products

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ABSTRACT

A high-performance liquid chromatography (HPLC) method has been developed to extract and quantitate individual sugars present in dough systems throughout processing. Sugars were extracted from freeze-dried samples by boiling in 60% ethanol (v/v). No defatting of samples was required. All sugars (fructose, glucose, sucrose, maltose, and lactose) were separated in 12 min using an aminopropyl 4-μm column and a 75:25 solution of acetonitrile and water mobile phase. Sodium chloride (0.125%, w/v) was incorporated into the mobile phase to eliminate refractive index interference from salt contained in the samples. Three different yeasted dough systems and one chemically leavened dough system were used to test the methodology. The chemically leavened dough system remained constant in its sugar composition throughout processing. In all of the fermented systems, sucrose was hydrolyzed to fructose and glucose during mixing. Glucose was utilized preferentially by the yeast. Maltose was utilized upon depletion of fructose and glucose, as in the sponge fermentation. Lactose, when present, remained constant throughout the processing stages.

The mandate of nutrition labeling by the Food and Drug Administration (FDA) in 1993 has made sugar analysis (mono- and disaccharides) a necessity for all of the food industry. Previous reports on sugar analysis of various food products were based on the total sugar or sucrose, with little emphasis on the other individual sugars. Colorimetric and iodometric methods (Somogyi 1952, Ting 1956) were unable to quantitate individual sugars. Gas-liquid chromatography (GLC) has been successful in determining individual sugars, but it requires a derivatization procedure (Li et al 1985). Recently, high-performance liquid chromatography (HPLC) has become the preferred method for quantitating simple sugars in a variety of food products (Zygmunt 1982, Picha 1985, Kwak and Jeon 1988, Potus et al 1994).

Sample preparation varies depending on the food product in question. High-fat samples, such as chocolates, require defatting before analysis. High-fat samples have been pretreated by various methods, including boiling water (AACC 1983), a Carrez reagent (Bugner and Feinberg 1992), or a petroleum ether wash (AOAC 1990). Extraction methods for other food products include boiling in 80% ethanol (Picha 1985), 50% alcohol in an 80–85°C water bath (Zygmunt 1982), or a water extraction (AACC 1983). Li et al (1985) extracted samples using hexane followed by water or 80% ethanol. The objectives of our study were to develop an uncomplicated and rapid HPLC method for quantitating individual sugars, particularly in various fermented products, and to monitor those sugars throughout the baking process.

MATERIALS AND METHODS

Product Formulation, Preparation, and Sampling

Four products were made: baking powder biscuits, sponge-and-dough white pan bread, straight-dough white pan bread, and no-time white pan bread. Standard research formulas from the American Institute of Baking Experimental Baking Group were used (Rogers and Langemeier 1995). Product sampling protocols were followed to pinpoint gradual, as well as potentially sudden, changes in sugar content, with doughs being frozen in liquid nitrogen. Samples were held at −20°C before being freeze-dried. Sample weights were carefully monitored at each stage. All results were corrected to as-consumed basis.

Sugar Extraction

Dried samples were ground through a 40-mesh screen (Wiley Jr. Mills, Arthur Thomas Co. Scientific Apparatus, Philadelphia, PA) and analyzed for moisture content (AACC 1983). Aliquots (5–10 g) were boiled for 20 min in 100 ml of 60% ethanol, cooled, and filtered through Whatman 42 paper. Additional sample cleanup was performed on the eluate by sequentially filtering through a Sep-Pak Plus C18 cartridge (Waters, Milford, MA) and a 0.22-μm membrane filter before injection onto the HPLC (modified Picha 1985). At least duplicate samples were extracted for each product and processing stage, and duplicate analysis was performed on the individual extractions.

HPLC

The system (Waters) consisted of: model 600 pump, model 600E controller, WISP 700 automatic sample injector, and associated IBM-compatible computer. An Alltec refractive index detector (Knauer, Berlin, Germany) was used. The aminopropyl-bonded phase column (4μm high-performance carbohydrate column, Waters) was operated at 30°C. The mobile phase was an isocratic acetonitrile and water solution (75:25). Sodium chloride was added (0.125%, w/v) to minimize the interference from NaCl. Sugar standards were dried at 60°C in a vacuum oven overnight and dissolved in 60% ethanol (modified Zygmunt 1982). Diverse samples were also spiked with various combinations of standard sugars (1–5 ppm) to monitor recovery. Sugar concentration was calculated based on peak area measurements.

Statistical Analysis

The data was analyzed using analysis of variance (ANOVA) (SigmaStat 1994). Significantly different means were separated using the Student-Newman-Keils test.

RESULTS AND DISCUSSION

Method Development

Sample extraction of fermented products was a major problem in quantitating the individual sugars. In the unbaked doughs, water was effective in extracting the fructose, glucose, sucrose, and lactose. However, maltose values fluctuated widely between repeated samples. Apparently, during the water-extraction process, maltose continued to be formed by β-amylase acting on the dextrins. This was not an issue with any of the bread crumb samples, as the β-amylase activity had been terminated during baking process. High levels of alcohol, as used to extract a variety of other food products (Picha 1985), were effective in minimizing the enzyme activity. However, in studies we performed using 80% alcohol, the total sugars recovery was <90%. Using a higher level of water (60% ethanol) resulted in a 99+% recovery for the products in question, while maintaining a negligible level of amylase activity.

Salt, a major ingredient in any dough system, interfered with the refractive index detection of both fructose and glucose peaks.

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The severity of interference varied according to the level of salt in the sample. To overcome this interference, a constant level of salt (0.125%, w/v) was added to the mobile phase. The salt concentration of all extracted samples was less than that of the mobile phase, resulting in the appearance of a vacancy peak before the fructose peak (Fig. 1). Adequate separation existed between the vacancy and fructose peaks however, so that establishment of the baseline was never a problem.

Refractive index detectors are extremely sensitive to environmental variations. Baseline stability was improved by covering the detector with a styrofoam hood. Premixing the solvents into an isocratic mobile phase removed the pump noise associated with switching from one solvent to the other and resulted in an improvement of detection limits.

Application

The individual sugars were identified by retention times (Table I). Figure 1 illustrates the typical chromatogram of sugars separated from baked products. Baseline separation of all five sugars occurred within 12 min. The system detection limit of each sugar was <1 ppm. The calibrated linear range for each individual sugar was 1–5 ppm.

The sugars in the baking powder biscuit remained constant during processing (Table II). The biscuits were chemically leavened, therefore no change in the sugar composition occurred. Nevertheless, verification of the extraction method with a high fat (26% shortening, based on flour weight) sample was important. No interference from the lipid was observed, eliminating the need to defat the sample as a pretreatment.

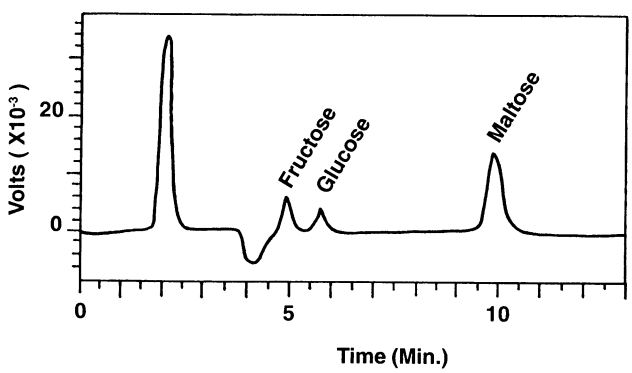

Fig. 1. Sample chromatogram of end of mix, no-time dough containing fructose, glucose, and maltose. The vacancy peak, resulting from the sample salt concentration being less than the mobile phase salt concentration, is before the fructose peak.

TABLE I
High-Performance Liquid Chromatography Retention Times for Sugars

Sugars	Minutes
Fructose	5.0
Glucose	5.8
Sucrose	8.2
Maltose	9.8
Lactose	11.4

TABLE II
Composition of Sugars (%) in Baking Powder Biscuit Throughout Processing, Reported on As-Consumed Basis

	Sucrose	Lactose
End of mix	2.61 a[a]	0.76 a
Crumb	2.55 a	0.67 a

[a]Means followed by the same letters within columns are not significantly different at $P = 0.05$. No fructose, glucose, or maltose were detected in any of the samples.

The fermented products showed changes in sugar composition throughout processing (Tables III–V). In agreement with Koch et al (1954), sucrose was hydrolyzed completely by yeast invertase before the first sampling (end of mix) of all the methods. Glucose was preferentially used by the yeast in all the fermented systems.

The no-time doughs had a significant increase in maltose from the mixing to the crumb stage (Table III). β-Amylase acting on dextrins from damaged starch created maltose during the half-hour fermentation, proof, and the first minutes of baking. Baker's yeast prefers monosaccharides, therefore the maltose, a dissaccharide, accumulated in the doughs. Glucose decreased significantly during proofing.

The straight-dough had a consistent level of lactose throughout the process (Table IV). The fructose was significantly reduced by the end of the proofing stage. Glucose decreased by the end of the 2-hr fermentation and continued to decrease throughout processing. Maltose increased significantly by the end of fermentation.

The trends of sugar utilization differed in the sponge-and-dough bread system, compared to that of the other fermented systems. There was no added sugar with the sponge ingredients; the small amount of sugar present in the sponge sample was from the flour. During the 4-hr sponge fermentation, the yeast consumed all of the sugar present (Table V), including the maltose. Only a small portion of the high-fructose corn syrup (fructose and glucose) added at the dough side was utilized by the yeast during the dough fermentation and proofing. The yeast, having been fully adapted to ferment maltose during the sponge fermentation, continued to use the maltose during the remainder of processing at a rate of fermentation similar to that of glucose and fructose (Koch et al 1954).

TABLE III
Composition of Sugars (%) in No-Time Bread Throughout Processing, Reported on As-Consumed Basis

	Fructose	Glucose	Maltose
End of mix	1.34 a[a]	1.50 a	0.72 a
End of proof	1.32 a	0.92 b	1.48 b
Crumb	1.31 a	0.62 b	2.01 c

[a]Means followed by the same letters within columns are not significantly different at $P = 0.05$. No sucrose or lactose were detected in any of the samples.

TABLE IV
Composition of Sugars (%) in Straight-Dough Bread Throughout Processing, Reported on As-Consumed Basis

	Fructose	Glucose	Maltose	Lactose
End of mix	1.31 a[a]	1.28 a	0.52 a	0.66 a
End of fermentation	1.15 a	0.67 b	1.72 b	0.59 a
End of proof	0.52 b	0.10 b,c	1.88 b	0.49 a
Crumb	0.44 b	0.06 c	2.04 b	0.52 a

[a]Means followed by the same letters within columns are not significantly different at $P = 0.05$. No sucrose was detected in any of the samples.

TABLE V
Composition of Sugars (%) in Sponge-and-Dough Bread Throughout Processing, Reported on As-Consumed Basis

	Fructose	Glucose	Maltose
End of sponge mix	0.09 a[a]	0.09 a	0.86 d
End of sponge Fermentation	0.00 a	0.00 a	0.00 a
End of dough mix	1.11 b	1.53 d	0.25 c
End of dough Fermentation	1.35 c	1.62 d	0.23 b,c
End of proof	1.42 c	1.33 c	0.08 a,b
Crumb	1.06 b	1.06 b	0.11 a,b

[a]Means followed by the same letters within columns are not significantly different at $P = 0.05$. No sucrose or lactose were detected in any of the samples.

CONCLUSIONS

The HPLC method for analyzing fermented products is a composite of methods previously applied to various food products, with modifications. Extracting sugars by boiling in 60% ethanol (v/v) eliminated fluctuations in maltose while maintaining 99+% recovery of all five mono- and disaccharides. Adding 0.125% NaCl (w/v) to the mobile phase eliminated salt interferences from the chromatograms. Utilizing an isocratic solvent system improved detection limits. Analyses could be completed without preliminary defatting of samples.

No sucrose was present in any of the fermented samples. Glucose decreased faster than fructose in yeasted products. Maltose decreased only in the sponge-and-dough process. Lactose values did not vary.

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