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Quantification of sugars in breakfast cereals using capillary electrophoresis

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ABSTRACT

About 80% of the Australian population consumes breakfast cereal (BC) at least five days a week. With high prevalence rates of obesity and other diet-related diseases, improved methods for monitoring sugar levels in breakfast cereals would be useful in nutrition research. The heterogeneity of the complex matrix of BCs can make carbohydrate analysis challenging or necessitate tedious sample preparation leading to potential sugar loss or starch degradation into sugars. A recently established, simple and robust free solution capillary electrophoresis (CE) method was used in a new application to 13 BCs (in Australia) and compared with several established methods for quantification of carbohydrates. Carbohydrates identified in BCs by CE included sucrose, maltose, glucose and fructose. The CE method is simple requiring no sample preparation or derivatization and carbohydrates are detected by direct UV detection. CE was shown to be a more robust and accurate method for measuring carbohydrates than Fehling method, DNS (3,5-dinitrosalicylic acid) assay and HPLC (high performance liquid chromatography).

1. Introduction

About 80% of the Australian adult population consume breakfast cereal, either cooked or ready-to-eat, at least five days a week.[1] Breakfast cereals, like most food products, contain a variety of carbohydrates as well as lipids, proteins and minerals. The heterogeneity of this complex matrix can make sugar analysis in breakfast cereals challenging.[2,3]

For sugars, total content is all that is required for the nutrition information panel (NIP), a mandatory labeling requirement of all processed foods in Australia.[3] Methods for the measurement of sugar in foodstuffs were often developed before carbohydrate chemistry was established.[4] Earlier quantitative chemical analytical assays often relied upon the reducing properties of aldehyde or keto group found in monosaccharides and short-chain oligosaccharides. In alkaline solutions, at elevated temperatures, these reducing sugars tautomerize to enediol forms, which are then readily oxidized by oxygen and oxidizing agents (such as metallic salts). An estimate of glucose content was based on the colorimetric measurement of the oxide or the free metal formed. The empirical nature of this reaction allowed analysts to develop methods such as the Fehling method and the 3,5-dinitrosalicylic (DNS) assay, which can produce reproducible and accurate results for samples with simple matrices. Such methods are also inexpensive, technically easy to perform and highly applicable to routine quantification. However, a strict control of experimental conditions (rate of heating, alkalinity and strength of the reagent) in a non-automated setting is necessary to obtain repeatable and reproducible results.[5]

Due to their specificity and ease of operation, enzymatic assays are the preferred reducing sugar method over Fehling method and DNS assay. Glucose and sucrose content has been determined in 79 dry, North American BCs using the glucose-oxidase peroxidase (GOD-POD) method.[6,7] The sugar content of these samples was previously assayed by the colorimetric condensation reaction with anthrone, which gave unsatisfactory reproducibility.

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Most foods, including BCs, contain a mixture of sugars rather than a single type of sugar. Therefore, the methods previously discussed are innately flawed by being either glucose-specific (e.g., GOD-POD) or unable to distinguish between different reducing sugars as is the case for the Fehling method and the DNS assay.[8] In reducing-sugar assays the quantity of product formed and measured is not exactly equivalent to sugar content, and different sugars yield different color intensities; this shows that the chemistry involved in the assay is considerably more complicated than it appears.[9] For certain foods in which the composition of the sugars is known and the requirement of the analysis is routine, e.g. quality control, an estimate of total sugar values expressed as invert sugar or glucose may be sufficient. However, most BCs have sucrose added during manufacture and thus total sugar determination requires a preliminary hydrolysis of non-starch polysaccharides (by acid or enzyme), which may cause sample loss or overestimation of reducing sugars. In addition, mineral ions have been reported to interfere with some reducing-sugar assays,[10,11] a problem for most Australian BCs, which have been fortified.

In the area of nutrition research, the intrinsic accuracy of the quantities of the different carbohydrates present in a diet is often required for correlation with their metabolic behavior.[4] Separation is used for this purpose. Separation methods have allowed for greater accuracy of sugar analysis in foods. Individual sugars measurements can be summed to calculate the 'total sugar content' for the NIP. Gas chromatography (GC) is a popular method for carbohydrate analysis and is very sensitive. It is the only chromatography method published in the peer-reviewed literature so far for sugar quantification of BCs.[12–15] The sample preparation requires multiple steps: grinding to pass through a 30-mesh (0.59 mm) screen, drying under vacuum, defatting with *n*-hexane, extraction with water for some of the sample and with aqueous methanol for the rest, centrifugation. In order to make the carbohydrate volatile a multistep derivatization was then needed: concentration under nitrogen flow and then drying under vacuum, reaction with pyridine, hexamethylsilazane in presence of trifluoroacetic acid, followed by another centrifugation. The sample preparation for GC is thus time consuming, laborious and has a significant probability of sample loss.[3] High performance liquid chromatography (HPLC) is the other established analytical method for measuring individual sugars in many foods. A number of columns have been tested for normal phase HPLC of carbohydrates, for example ion-exchange columns for BCs,[16,17] but they all have their own disadvantages including co-elution, tedious sample preparation and intolerance to salt or acid leading to short column life.[18,19] For starchy-food sample matrices, such interfering substances not only disrupt the analysis, they can damage the column leading to a high running cost. High performance anion exchange chromatography (HPAEC) is solving a number of these issues and is rapidly developing for food analysis[3] because of its high sensitivity without any required derivatization and its speed in carbohydrates separation.[20,21] Released sugars from non-starch polysaccharides in a range of raw and processed foods (including BCs) were determined by HPAEC.[13]

Capillary electrophoresis (CE) is becoming increasingly popular for carbohydrate analysis.[22] The most useful and simple separation mechanism of CE is free solution (devoid of a gel or polymer network medium). Free solution CE (FSCE), also known as capillary zone electrophoresis (CZE), involves the flushing of an electrophoresis buffer through a narrow-bore capillary prior to sample injection, application of voltage and separation.[23] Like HPLC, FSCE is a fast and repeatable analytical tool for the qualitative and quantitative analysis of carbohydrates in food and beverage samples,[24–27] including in foodomics.[28] FSCE sensitivity can be greatly increased using on-line pre-concentration methods.[29] One FSCE method has been applied to a BC sample (flaked cereals) by coating the capillary with cetrimonium bromide (CTAB).[24] This dynamic coating is non-selective for sugars whereby interaction with lipids and other components can become a problem for quantification in complex matrices. Until recently, indirect UV detection of underivatized carbohydrates was considered superior to direct UV detection; detection at low wavelengths after borate complexation generated poor sensitivity. However, direct UV detection at 270 nm in high alkaline conditions has been discovered for the FSCE of sucrose, glucose and fructose in beverage samples[30] and the detection has been shown to be specific to carbohydrates[31,32] The method is considered robust by definition: 'a method that can be applied to analytes in a wide variety of matrices'[33] and despite the complex matrix, no filtration is necessary during sample preparation.[34] FSCE with direct UV detection has been applied to acid hydrolyzed plant fiber, fermentation, beverage, pharmaceutical and forensic samples.[18,19,35–37] The CE method was shown to be cost effective, robust and repeatable.

The aim of this research was to analyze 13 Australian breakfast cereals using FSCE with direct UV detection[18] and to compare quantitative results with that from traditional chemical analytical methods: Fehling method and DNS assay. Available data from the Food Standards Australia New Zealand (FSANZ) NUTTAB database[38] of individual sugar quantities for relevant Australian breakfast cereals was also included for a comparison to our findings.

2. Results and discussion

2.1. Detection of individual sugars in breakfast cereals using capillary electrophoresis (CE)

The ground breakfast cereals (BC) were simply suspended in water and injected in CE. The sugars in breakfast cereals were separated (Fig. 1) by CE. Identification of sugars was validated by comparison of the electrophoretic mobility of observed peaks with that of a standard sugar solution and previous literature (Table 1). A double correction was used to precisely determine the electrophoretic mobility of each sugar peak. The first correction was using a neutral species (DMSO) as an EOF marker (see Equation S-2). The second correction involved a homothetic transformation with an electrophoretic mobility marker (Equation S-3).[30] Table 1 demonstrates how the mobility value of a sugar, much like elution time in HPLC, is useful in peak identification. Sucrose was detected in all BCs, while lactose, maltose, glucose and fructose were detected in some. Other components are not detected even though the matrix is complex: proteins, lipids are also present but the direct UV detection has been shown to be specific to carbohydrates since it is due to a photo-oxidation reaction taking place at the detection window.[32] To identify the carbohydrates, electrophoretic mobility is used, and not migration time, since the former has a higher repeatability than the latter (Table 1). Preliminary sugar identification was confirmed by spiking BC samples (see Figs. S-2 and S-3). The samples, 'Corn Flakes', 'Froot Loops' and 'Weet-Bix Multigrain' were selected as they contained the greatest number of peaks among the seven BCs used in the first set of experiments.

Repeatability within the standards was sufficient with relative standard deviation (RSD) values of no more than 1.3%, providing a reliable set of values on which to base sugar identification. In the analysis of BC samples, higher RSD values were observed in MRT (series1) 'Nutri-Grain' and MVL 'Weet-Bix', yielding measurement errors of 5.6% and 2.4%, respectively (see also Fig. S-1). Apart from these isolated cases, the repeatability of BC sample analysis was good with RSD $\leq$1.5%.

Reproducibility of the electrophoretic mobility values has been investigated by comparing the results obtained by two different