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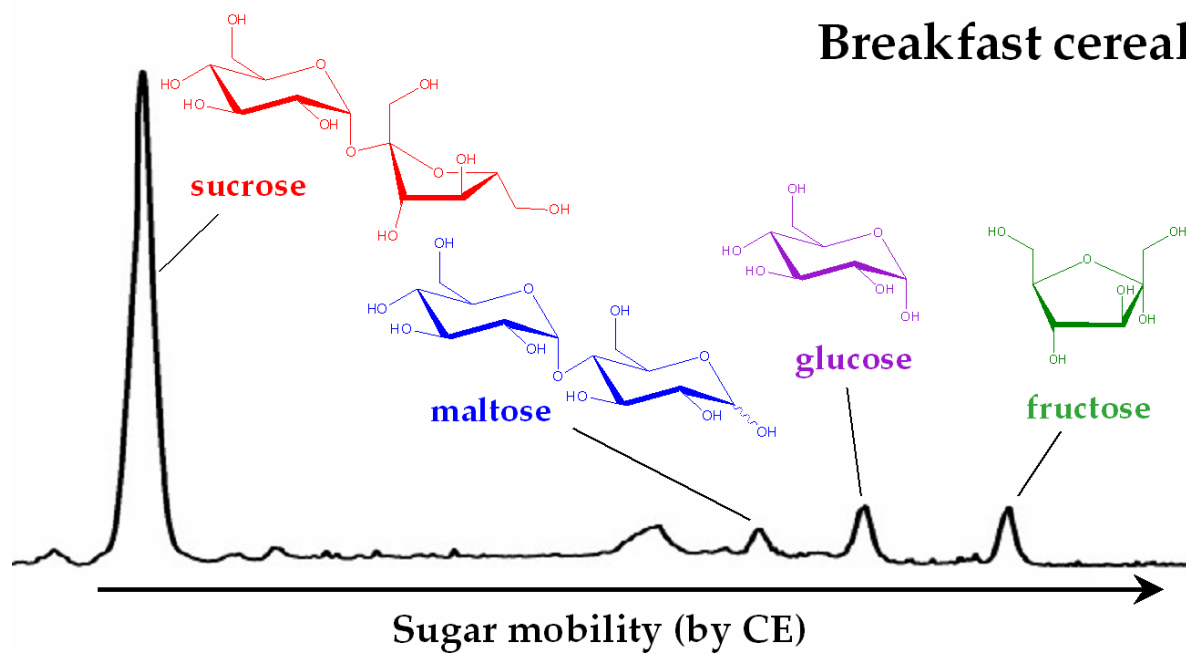
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Graphical abstract



1 Quantification of sugars in breakfast cereals using capillary electrophoresis

2
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18 Abstract

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

31
32 Keywords: Breakfast cereals; Sugars; Sucrose; Glucose; Capillary electrophoresis (CE); Fehling
33 method; 3,5-dinitrosalicylic acid (DNS) assay; High performance liquid chromatography (HPLC)

1 1. Introduction

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

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

20 Due their specificity and ease of operation, enzymatic assays are the preferred reducing sugar
21 method over Fehling method and DNS assay. Glucose and sucrose content has been determined in
22 79 dry, North American BCs using the glucose-oxidase peroxidase (GOD-POD) method.^{6, 7} The sugar
23 content of these samples was previously assayed by the colorimetric condensation reaction with
24 anthrone, which gave unsatisfactory reproducibility.

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

38 In the area of nutrition research, the intrinsic accuracy of the quantities of the different
39 carbohydrates present in a diet is often required for correlation with their metabolic behavior.⁴
40 Separation is used for this purpose. Separation methods have allowed for greater accuracy of sugar
41 analysis in foods. Individual sugars measurements can be summed to calculate the 'total sugar
42 content' for the NIP. Gas chromatography (GC) is a popular method for carbohydrate analysis and is
43 very sensitive. It is the only chromatography method published in the peer-reviewed literature so far

1 for sugar quantification of BCs.¹²⁻¹⁵ The sample preparation requires multiple steps: grinding to pass
2 through a 30-mesh (0.59 mm) screen, drying under vacuum, defatting with *n*-hexane, extraction with
3 water for some of the sample and with aqueous methanol for the rest, centrifugation. In order to
4 make the carbohydrate volatile a multistep derivatization was then needed: concentration under
5 nitrogen flow and then drying under vacuum, reaction with pyridine, hexamethylsilazane in presence
6 of trifluoroacetic acid, followed by another centrifugation. The sample preparation for GC is thus
7 time consuming, laborious and has a significant probability of sample loss.³ High performance liquid
8 chromatography (HPLC) is the other established analytical method for measuring individual sugars in
9 many foods. A number of columns have been tested for normal phase HPLC of carbohydrates, for
10 example ion-exchange columns for BCs,^{16, 17} but they all have their own disadvantages including co-
11 elution, tedious sample preparation and intolerance to salt or acid leading to short column life.^{18, 19}
12 For starchy-food sample matrices, such interfering substances not only disrupt the analysis, they can
13 damage the column leading to a high running cost. High performance anion exchange
14 chromatography (HPAEC) is solving a number of these issues and is rapidly developing for food
15 analysis³ because of its high sensitivity without any required derivatization and its speed in
16 carbohydrates separation.^{20, 21} Released sugars from non-starch polysaccharides in a range of raw
17 and processed foods (including BCs) were determined by HPAEC.¹³

18 Capillary electrophoresis (CE) is becoming increasingly popular for carbohydrate analysis.²² The most
19 useful and simple separation mechanism of CE is free solution (devoid of a gel or polymer network
20 medium). Free solution CE (FSCE), also known as capillary zone electrophoresis (CZE), involves the
21 flushing of an electrophoresis buffer through a narrow-bore capillary prior to sample injection,
22 application of voltage and separation.²³ Like HPLC, FSCE is a fast and repeatable analytical tool for the
23 qualitative and quantitative analysis of carbohydrates in food and beverage samples,²⁴⁻²⁷ including in
24 foodomics²⁸. FSCE sensitivity can be greatly increased using on-line pre-concentration methods.²⁹
25 One FSCE method has been applied to a BC sample (flaked cereals) by coating the capillary with
26 cetrimonium bromide (CTAB).²⁴ This dynamic coating is non-selective for sugars whereby interaction
27 with lipids and other components can become a problem for quantification in complex matrices.
28 Until recently, indirect UV detection of underivatized carbohydrates was considered superior to
29 direct UV detection; detection at low wavelengths after borate complexation generated poor
30 sensitivity. However, direct UV detection at 270 nm in high alkaline conditions has been discovered
31 for the FSCE of sucrose, glucose and fructose in beverage samples³⁰ and the detection has been
32 shown to be specific to carbohydrates^{31, 32}. The method is considered robust by definition: "a method
33 that can be applied to analytes in a wide variety of matrices"³³ and despite the complex matrix, no
34 filtration is necessary during sample preparation³⁴. FSCE with direct UV detection has been applied to
35 acid hydrolyzed plant fiber, fermentation, beverage, pharmaceutical and forensic samples.^{18, 19, 35-37}
36 The CE method was shown to be cost effective, robust and repeatable.

37 The aim of this research was to analyze 13 Australian breakfast cereals using FSCE with direct UV
38 detection¹⁸ and to compare quantitative results with that from traditional chemical analytical
39 methods: Fehling method and DNS assay. Available data from the Food Standards Australia New
40 Zealand (FSANZ) NUTTAB database³⁸ of individual sugar quantities for relevant Australian breakfast
41 cereals was also included for a comparison to our findings.

42

43

1 2. Results and discussion

2 2.1 Detection of individual sugars in breakfast cereals using capillary electrophoresis (CE)

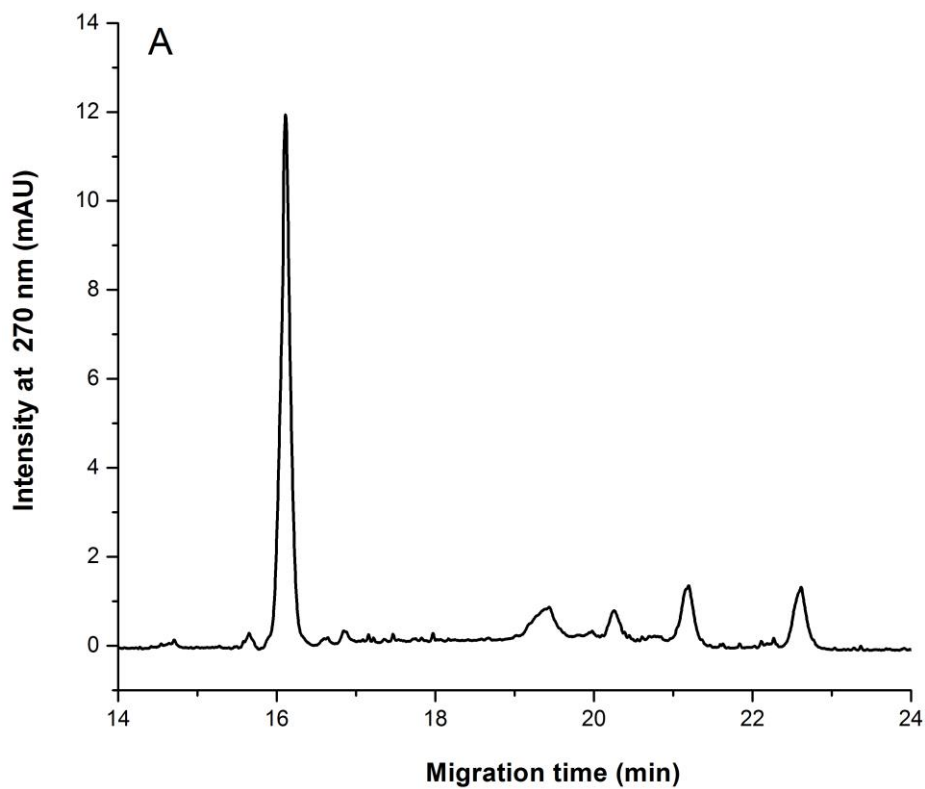
3 The ground breakfast cereals (BC) were simply suspended in water and injected in CE. The sugars in
4 breakfast cereals were separated (Figure 1) by CE. Identification of sugars was validated by
5 comparison of the electrophoretic mobility of observed peaks with that of a standard sugar solution
6 and previous literature (Table 1). A double correction was used to precisely determine the
7 electrophoretic mobility of each sugar peak. The first correction was using a neutral species (DMSO)
8 as an EOF marker (see Equation S-2). The second correction involved a homothetic transformation
9 with an electrophoretic mobility marker (Equation S-3).³⁰ Table 1 demonstrates how the mobility
10 value of a sugar, much like elution time in HPLC, is useful in peak identification. Sucrose was detected
11 in all BCs, while lactose, maltose, glucose and fructose were detected in some. Other components
12 are not detected even though the matrix is complex: proteins, lipids are also present but the direct
13 UV detection has been shown to be specific to carbohydrates since it is due to a photo-oxidation
14 reaction taking place at the detection window³². To identify the carbohydrates, electrophoretic
15 mobility is used, and not migration time, since the former has a higher repeatability than the latter
16 (Table 1). Preliminary sugar identification was confirmed by spiking BC samples (December 2012, see
17 Figure S-2 and S-3). The samples, 'Corn Flakes', 'Froot Loops' and 'Weet-Bix Multigrain' were
18 selected as they contained the greatest number of peaks among the seven BCs used in the first set of
19 experiments (October 2012).

20 Repeatability within the standards was sufficient with relative standard deviation (RSD) values of no
21 more than 1.3 %, providing a reliable set of values on which to base sugar identification. In the
22 analysis of BC samples, higher RSD values were observed in MRT1 'Nutri-Grain' and MVL 'Weet-Bix',
23 yielding measurement errors of 5.6 % and 2.4 % respectively (see also Figure S-1). Apart from these
24 isolated cases, the repeatability of BC sample analysis was good with RSD \leq 1.5 %.

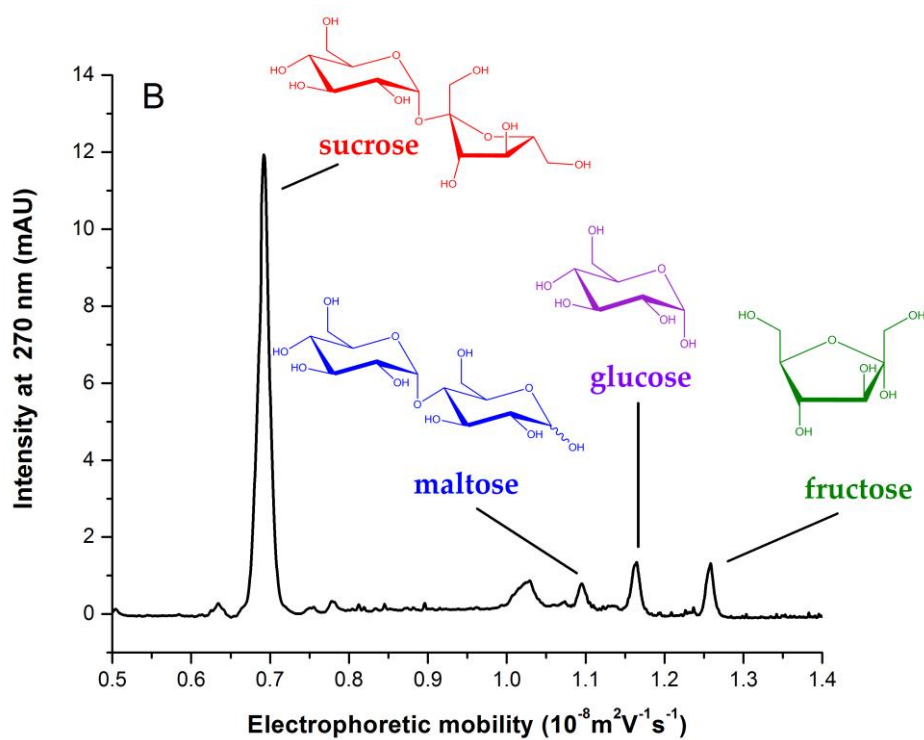
25 Reproducibility of the electrophoretic mobility values has been investigated by comparing the results
26 obtained by two different operators, MVL and MRT, or by the same operator with different sample
27 preparation (differing only in dilution), MRT1 and MRT2, or in the literature. Comparison of analyzed
28 samples shows a reasonable level of reproducibility between values reported in reference³⁰, and
29 those obtained by operator MVL and MRT1, showing a maximum variance between mobilities of
30 approx. 5% (sucrose), with MRT reporting consistently higher mobilities. Identical operator with
31 diluted sample (MRT2) showed reduced repeatability, yielding mobility value variance of 5-10 %
32 compared to MRT1, while comparison with MVL showed variance of 1-5 %, with two values yielding a
33 10 % variance (glucose and fructose in 'Weet-Bix'). Results for diluted 'Coco-Pops' lacked the
34 identification of both glucose and fructose, present in previous experiments, indicating dilution to
35 reduce impacts of overloading is negatively affecting the sensitivity for low concentration sugar
36 identification. Results reported by Oliver et al.¹⁸ present significantly higher electrophoretic
37 mobilities for both glucose and xylose. This illustrates the importance of a standard solution to
38 establish the mobility values of sugars in each session to account for variations between operators,
39 equipment and sessions.

40 The results obtained in this work by both operators MVL and MRT showed a sufficient level of
41 reproducibility between operators to yield identical sugar identifications in all cases. Same operator,
42 with dilution showed a reduced reproducibility, yielding slightly higher variance in mobility, though

- 1 still indicates a sufficient level for the identification of sugars. CE is thus a viable method for the
- 2 identification of sugars in breakfast cereals.



3



4

5 **Figure 1.** Migration time electropherogram (A) and mobility electropherogram (B) of 'Weet-Bix
6 Multigrain'. From the left to the right, peaks correspond to sucrose, (unknown), maltose, glucose and
7 fructose.

1 **Table 1.** Reproducibility of the separation of sugars in five breakfast cereal samples with CE. Operator
 2 MVL (n=2) and Operator MRT (n=3).

Breakfast Cereal	Operator or publication	Average μ_{ep} ($10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$) (RSD in %)					
		Sucrose	Lactose	Maltose	Glucose	Fructose	Xylose
Standard sugar solution	Ref. ³⁰ #	-0.772 (u*)	-	-	-1.176 (u*)	-	-1.365 (u*)
	Ref. ¹⁸ §	-	-	-	-1.518 (0.45)	-	-1.754 (0.40)
	MVL [^]	-0.720 (1.08)	-	-1.116 (0.49)	-1.181 (0.30)	-1.265 (0.30)	-1.395 (1.07)
	MRT ⁺	-0.801 (1.29)	-1.003 (0.77)	-1.172 (0.40)	-1.224 (0.74)	-1.285 (0.40)	-1.419 (u*)
'Coco Pops'	MVL	-0.720 (u*)	-	-	-1.152 (0.77)	1.237 (0.83)	-
	MRT (series 1)	-0.810 (0.109)	-	-	-1.223 (0.006)	-1.289 (0.029)	-1.419 (u*)
	MRT (series 2)	-0.733 (0.470)	-	-	-	-	-1.329 (u)
'Nutri-Grain'	MVL	-0.720 (u*)	-	-	-1.188 (0.77)	-1.283 (0.79)	-
	MRT(series 1)	-0.788 (5.59)	-	-	-1.212 (1.18)	-1.276 (0.792)	-1.419 (u*)
	MRT (series 2)	-0.730 (0.49)	-	-	-1.118 (0.93)	-1.201 (0.19)	-1.333 (u)
'Sustain'	MVL	-0.720 (u)	-	-1.124 (1.06)	-1.195 (1.15)	-1.288 (1.46)	-
	MRT (series 1)	-0.805 (0.070)	-	-1.172 (0.051)	-1.224 (0.038)	-1.285 (0.047)	-1.419 (u*)
'Weet-Bix'	MVL	-0.720 (u*)	-	-	-1.240 (2.20)	1.348 (2.39)	
	MRT (series 1)	-0.798 (0.119)	-	-	-1.221 (0.062)	-1.285 (0.026)	-1.419 (u*)
	MRT (series 2)	-6.834 (1.48)	-	-	-1.090 (0.21)	-1.161 (0.11)	1.272 (u)
'Oats: Apple and Blueberry Bake'	MRT (series 1)	-0.808 (1.061)	-1.025 (1.230)	-	-	-	-1.419 (u*)

3 * u stands for unavailable

4 # mobility correction using methanol as an EOF marker

5 § mobility double correction using DMSO as an EOF marker and lactose as an electrophoretic mobility
 6 marker

7 ^ mobility double correction using DMSO as an EOF marker and sucrose as an electrophoretic mobility
 8 marker

1 + mobility double correction using DMSO as an EOF marker and xylose as an electrophoretic mobility
2 marker

3

4 **Table 2.** Calibration of response at 266 nm (y) as a function of sugar concentration (x) with its
5 correlation coefficient (R^2), for the sugars in standard (capillary of 61.8 cm total length). Xylose (0.5
6 $\text{g}\cdot\text{L}^{-1}$) was used as the internal standard.

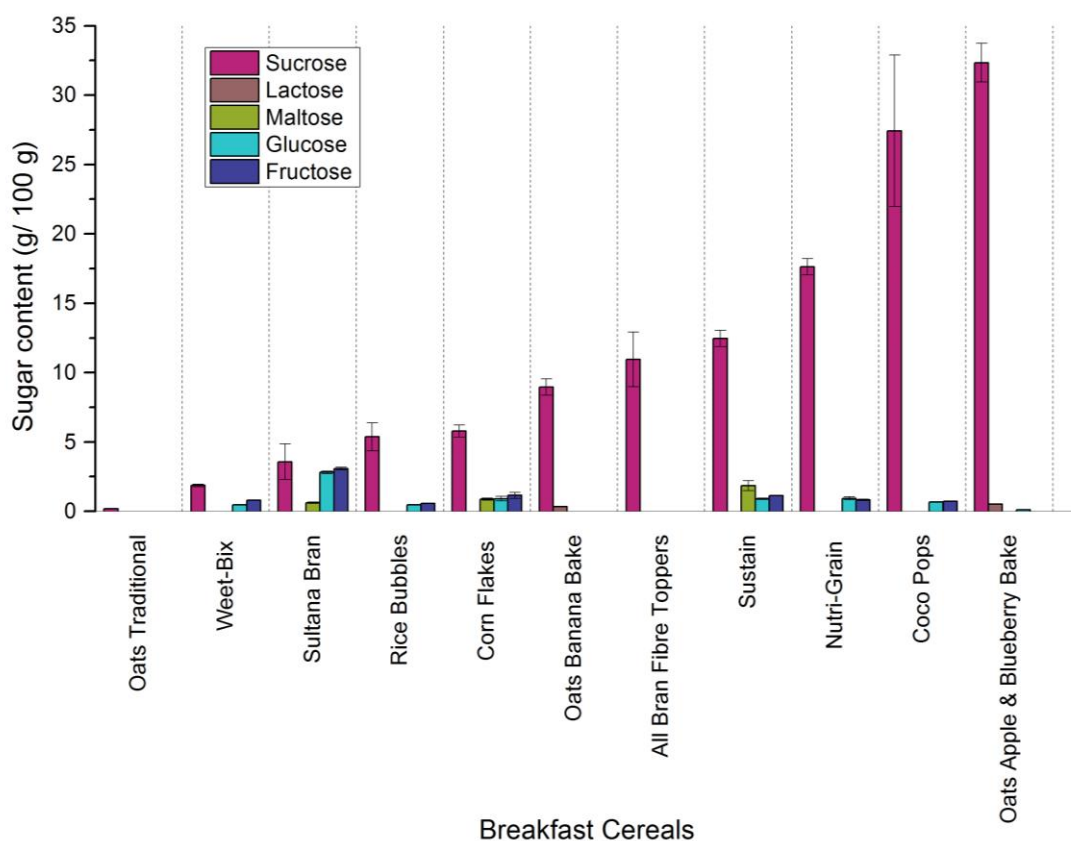
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Sugar	LOD ($\text{mg}\cdot\text{L}^{-1}$)	LOQ ($\text{mg}\cdot\text{L}^{-1}$)	Linear Equation	R^2	Concentration range ($\text{mg}\cdot\text{L}^{-1}$)
Sucrose	5.88	21.6	$y = 0.4517x - 0.0395$	0.999	50 to 1500
Lactose	2.38	19.8	$y = 0.3292x + 0.0208$	0.998	20 to 500
Maltose	20.7	41.7	$y = 0.4104x + 0.0259$	0.995	20 to 500
Glucose	30.0	42.6	$y = 0.2327x + 0.0338$	0.992	20 to 500
Fructose	15.9	44.1	$y = 0.4263x + 0.0324$	0.992	20 to 500

8

9 **2.2 Quantification of individual sugars in breakfast cereals using capillary electrophoresis (CE)**

10 The calibration curve for each sugar was prepared with the sequential analyses of six sugar mixtures
11 injected in triplicate. The linearity and repeatability were determined for 5 sugars, with xylose (0.5
12 $\text{g}\cdot\text{L}^{-1}$) used as the internal standard. Sufficient linearity was achieved for all tested sugars with
13 correlation coefficient (R^2) greater than 0.99 (Table 2) and reasonable standard error on the y
14 estimates (see Figure S-4 to S-6 in supplementary data), as achieved in the literature applying this CE
15 method to different matrices.^{18,39} The calibration for disaccharides, maltose, lactose and sucrose,
16 had slightly better linearity than that for monosaccharides glucose and fructose. The sugar
17 concentrations determined by CE also show good repeatability (see error bars on Figure 2) of the
18 peak area (normalized with respect to the peak area of the internal standard, each peak area being
19 also divided with the corresponding migration time) consistent with the literature (see Table S-3).^{18,30}
20 The use of an electro-osmotic flow marker and the addition of an internal standard are
21 recommended for optimal repeatability of the peak area. The high pH of the NP200 buffer made it
22 prone to carbonation⁴⁰ and it is thus recommended to use buffer within 13 h (or 19 h) of its
23 preparation to be within 10 % (or 15 %) of initial current measurement (Equation S-5 to S-8, Table S-
24 4 and Figure S-7 in supplementary data). Table 2 lists the relative sensitivity of the detection of
25 different sugars along through the limit of detection (LOD) and limit of quantification (LOQ). LOD and
26 LOQ were calculated from the signal-to-noise ratio (SNR) obtained with 32 Karat software. The
27 sensitivity of the direct detection in this study, with LOD values between 2.4 and 30 $\text{mg}\cdot\text{L}^{-1}$, was
28 comparable to studies that had used the same CE method on different types of analytes,^{31,41} (see
29 Table S-1).



1

2 **Figure 2.** Individual sugar quantification of 11 BCs by CE (n=3 or 5 for all BCs).

3

4 The method was applied to the quantification of sugars in several commercial breakfast cereals.
 5 Since fewer than six sugars have been identified in BCs previously, a short capillary, total length 61.8
 6 cm, was employed for all experiments to decrease analysis time to 40 min (including xylose internal
 7 standard). Figure 2 presents the sugar concentration results for ten BCs determined by CE. Each BC
 8 contained sucrose at a higher concentration than any other sugar detected (lactose, maltose, glucose
 9 and fructose). This is likely due to the amount of sugar added during manufacture of the product. BCs
 10 with high sucrose concentrations (measured above 15 g / 100 g), including 'Oats Apple & Blueberry
 11 Bake', 'Nutrigrain', 'Coco Pops', listed sugar as the second highest ingredient after the cereal
 12 component on their packaging.

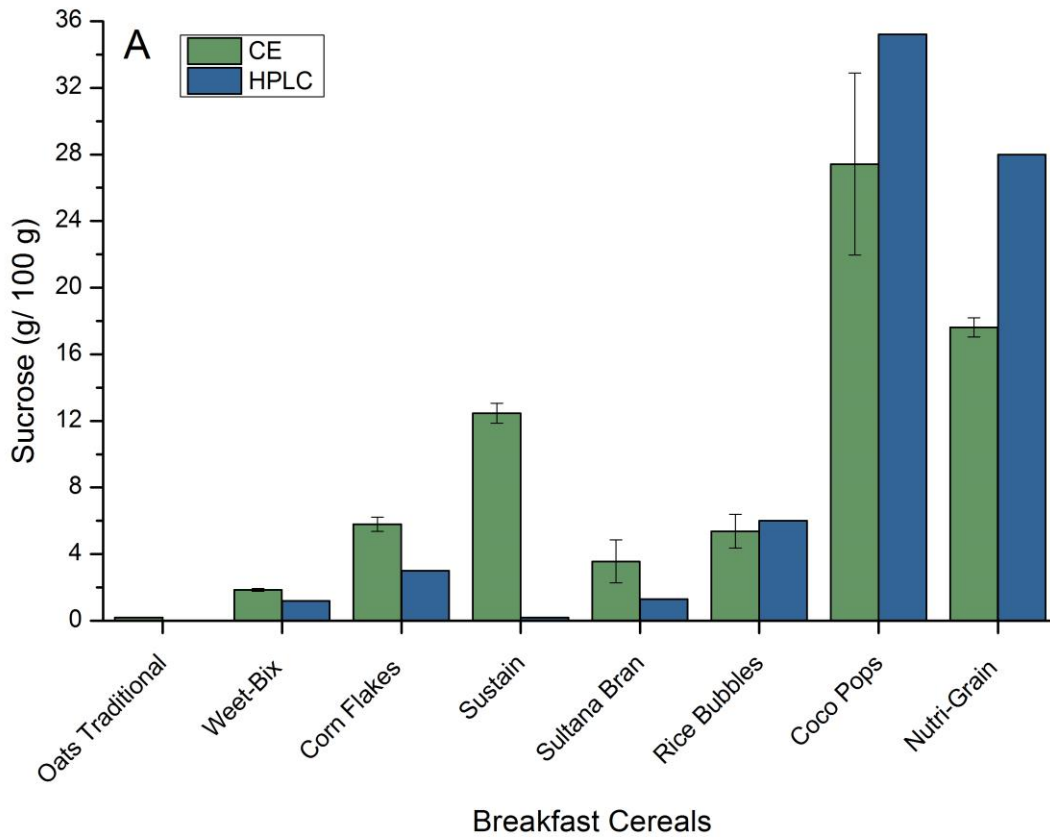
13 Nutritionally insignificant concentrations of maltose, glucose and fructose were detected in 9 of the
 14 11 BCs analyzed by CE. Barley malt extract is listed as an ingredient on BCs 'Sustain', 'Corn Flakes'
 15 and 'Sultana Bran' thus the low concentration values (1.8, 0.9 and 0.6 g / 100 g respectively) for
 16 maltose could be expected. Similarly, trace levels of lactose detected in 'Oats Apple & Blueberry
 17 Bake' (0.5 g / 100 g) and 'Oats Banana Bake' (0.3 g / 100 g) are in agreement with the addition of the
 18 milk powder ingredient in these BCs.

19 Problems were reported with the sugar quantification in GC of North American BCs with sampling
 20 and/or measuring aliquots of individual cereals.¹² The correlation of variation of most samples,
 21 however, was not greater than the standards. They state that RSD was not greater than 2 % for
 22 glucose and sucrose and not greater than 5 % for lactose and maltose. The average RSD from CE
 23 reported in this work is approximately 7 % for lactose, glucose and fructose and about 13 % for

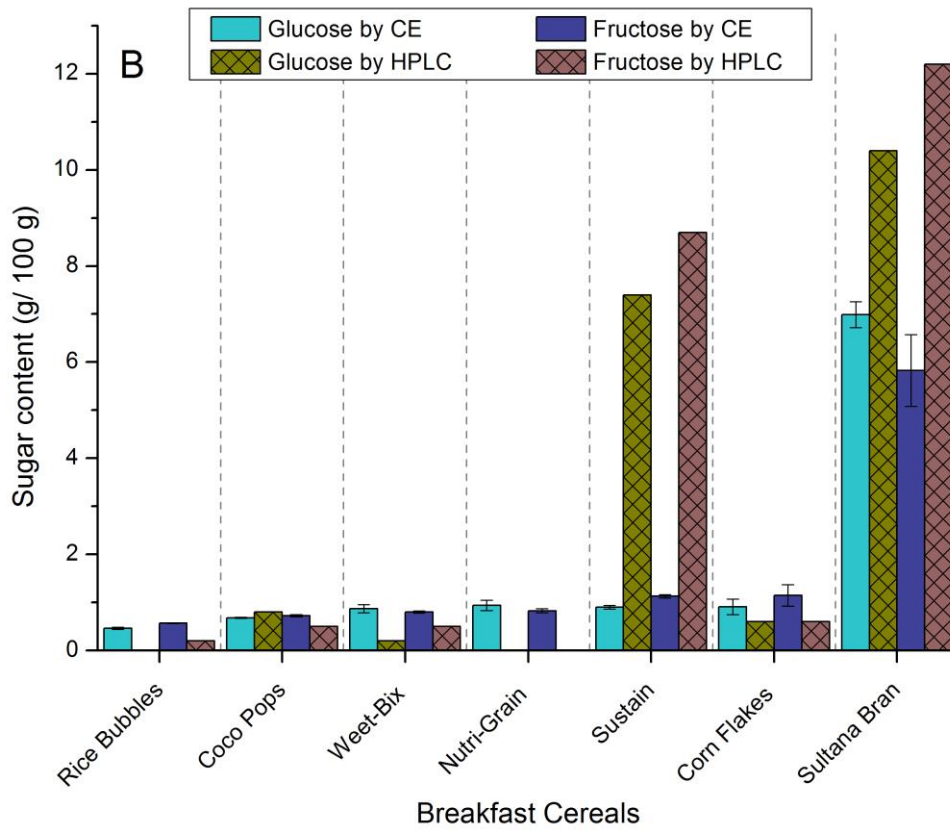
1 sucrose and maltose concentrations. The average overall error of CE at $\pm 9\%$ is comparable to that of
2 the reported GC method at $\pm 7\%$.

3 **2.3 Comparison of CE and HPLC for determination of individual sugars**

4 The quantity of individual sugars in Australian BCs as measured by HPLC is available on the Food
5 Standards Australia New Zealand (FSANZ) NUTTAB 2010 database.³⁸ This data was compared with CE
6 results from this work (see Figure 3-5). Among the eight BCs included in this comparison, both
7 methods determined 'Oats Traditional', 'Weet-Bix' and 'Corn Flakes' to have the lowest sucrose
8 concentrations. For these cereals, the HPLC method did not detect any sugar in Oats Traditional
9 whereas CE measured 0.2 % sucrose. CE also detected approximately 36 % more sucrose in 'Weet-
10 Bix' and 48 % more in 'Corn Flakes' compared to HPLC (see Figure 3A). The sucrose content of 'Rice
11 Bubbles' as measured by CE was not significantly different from that from HPLC data. For the two BCs
12 with the highest sucrose concentration ('Nutri-Grain' and 'Coco Pops'), less sucrose was measured by
13 CE compared to HPLC.³⁸ Some of these variations may be due to changes in the recipes or even batch
14 to batch variations. The HPLC data from individual quantification of sugars in BCs are available online
15 to the public. However, the exact methodology is unpublished and unreported. It is very likely that
16 some filtration and / or centrifugation is required to prepare samples for carbohydrate analysis by
17 HPLC. Thus sample loss could have occurred during sample preparation and may have caused an
18 underestimation of sugar content for 'Weet-Bix' and 'Corn Flakes'. CE analysis also yielded a
19 significantly higher sucrose concentration of 12.5 g / 100 g for 'Sustain' compared to HPLC data³⁸ at
20 0.2 g / 100 g (see Figure 3A). In addition, CE data for glucose and fructose concentrations in 'Sustain'
21 were significantly lower (0.9 and 1.1 g / 100 g) compared to HPLC data (7.4 and 8.7 g / 100 g), see
22 Figure 3B. This may be due to differing sample preparation, in a sample which is even more
23 heterogeneous than the other BCs due to the presence of pieces of fruit. During initial sample
24 preparation of BCs for all experimental methods in this study, the fruit pieces and some other large
25 particulate ingredients were resisting grinding and did not pass through laboratory sieves. Thus BCs
26 with added pieces of fruit measured in this study, such as 'Sustain' and 'Sultana Bran', are
27 acknowledged as not being representative of the whole sample. The free form of glucose and
28 fructose is found naturally in plants, including many fruits and vegetables. Typical sugar composition
29 of Australian sultanas, for example, is 38 % fructose and 35 % glucose by dry weight⁴². The majority
30 of sultanas and other pieces of fruit were selectively removed from the BC samples in this study. This
31 likely caused an underestimation of glucose and fructose concentration, as seen in 'Sustain' and
32 'Sultana Bran' (Figure 3B). More extensive sample preparation of BCs that contained fruit led
33 however to up to 55 % error on the measured sugars in the case of GC.¹² The extraction procedures
34 of the BCs including fruits for GC requires aqueous methanol. However, this still led to larger error,
35 especially for maltose quantification.¹² As the total sugar measured by CE and HPLC for 'Sustain' is
36 similar, 16.3 and 18.2 g / 100 g respectively (see Figure 5), the significantly lower sucrose levels by
37 HPLC (see Figure 3A) may be attributed again to sample loss during preparation for HPLC or to a
38 change in the BC composition due to different time of purchase. Lower level of individual sugars
39 quantified by HPLC compared to CE with direct UV detection has been observed for other samples
40 with complex matrices, namely plant fiber¹⁸ and ethanol fermentation¹⁹.



1



2

3 **Figure 3.** Quantification of sucrose (A) and glucose and fructose (B) in BCs by CE (this work)
 4 compared to available HPLC data ³⁸

1 2.4 Estimation of total sugar by CE and Fehling (Lane-Eynon) method

2 Total sugar content is a legal measurement requirement for food labelling in Australia and many
3 other countries. Total sugar content of 11 BCs was determined by the traditional Fehling (Lane-
4 Eynon) method and the high performance CE separation method, and compared with the NIP on the
5 BC packaging label as well as with available HPLC data.³⁸ Comparison of methods is important to
6 determine the accuracy of sugar content in BCs, both to ensure label information is correct and to
7 highlight differences between method in cost and efficiency.

8 The 11 BCs presented in Figure 4 can be grouped into three categories for total sugar content: (high
9 (>12.5 g / 100 g), medium (5 – 12.5 g / 100 g) and low (<5 g / 100 g) sugar content. The high sugar
10 content cereals as measured by CE, in increasing order, were 'Sultana Bran', 'Sustain', 'Nutri-Grain',
11 'Coco Pops' and 'Oats Apple and Blueberry Bake'. 'Sultana Bran' and 'Sustain' had lower levels of
12 total sugar measured by CE and Fehling method in this work compared to the packaging and
13 available HPLC data³⁸ likely due to removal of fruit during sample preparation (as mentioned in
14 section, 2.3). 'Nutrigrain' was consistently ranked amongst the top three BCs for high sugar content
15 among all methods compared. The total sugar content for 'Coco Pops' measured by CE had a
16 relatively high RSD of 20 % and was therefore not significantly different from that measured with
17 other methods in the comparison. 'Oats Apple and Blueberry Bake' was determined to have the
18 highest total sugar content of all BCs by CE, 15% more than labelled on the NIP.

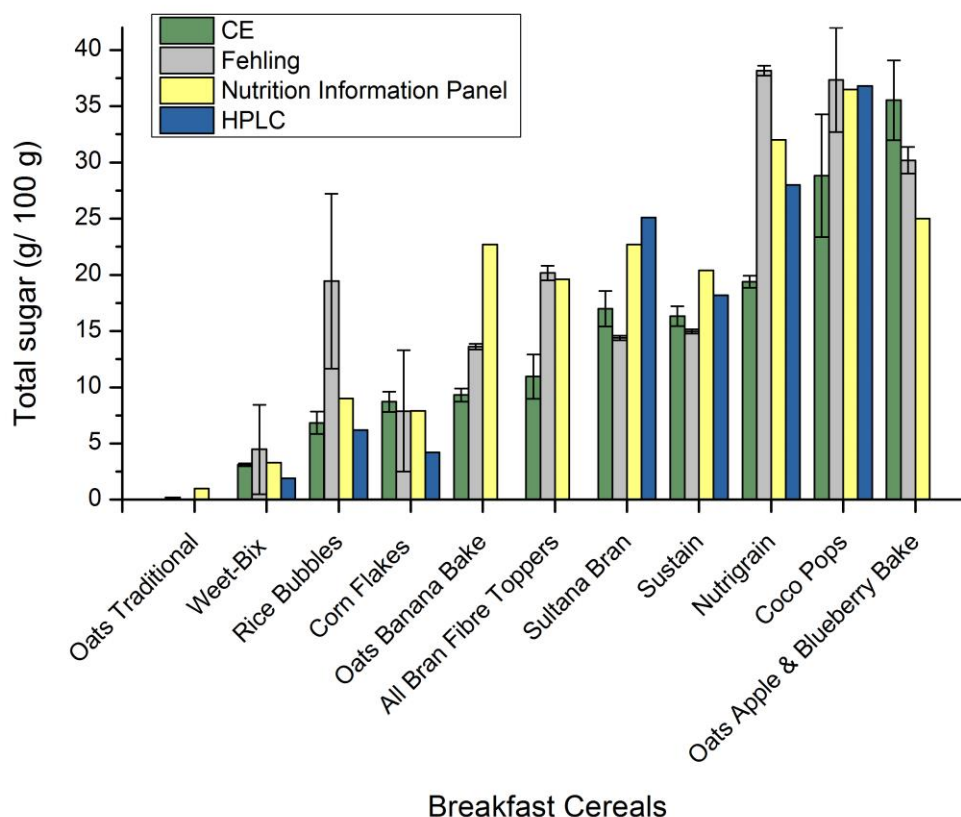
19 The medium sugar content BCs as determined by CE, in increasing order, were 'Rice Bubbles', 'Corn
20 Flakes', 'Oats Banana Bake' and 'All Bran Fibre Toppers'. All methods, except Fehling which gave a
21 large degree of variability between replicates, determined 'Rice Bubbles' to have between 6 and 9 g/
22 100 g total sugar. The CE results for 'Corn Flakes' were in agreement with the NIP which was more
23 than double the amount reported by HPLC; no conclusions could be drawn from Fehling data of this
24 BC due to the large degree of error. Sugar content of 'Oats Banana Bake' and 'All Bran Fibre Toppers'
25 measured by CE was approximately half of that reported on label or measured by Fehling.

26

27 The low sugar content BCs determined by CE were 'Oats Traditional' and 'Weet-Bix'. 'Oats Traditional'
28 had the lowest sugar content measured by CE at 0.2 g/ 100 g. Interestingly, the NIP labelled this BC
29 to have 1 g/ 100 g total sugar, however HPLC data³⁸ reported no sugar at all. Also, sugar contained in
30 'Oats Traditional' was below the limit of detection for the Fehling method. The total sugar content of
31 'Weet-Bix' was consistent between the NIP, the HPLC data and the CE method in this study. At this
32 low level of sugar, the Fehling method had very poor repeatability (RSD = 89 %) as one of the repeats
33 was below LOD.³⁸

34 The overall error for the quantification of sugars in BCs by the CE method was much lower than that
35 of the Fehling method, especially for the BCs with low sugar content. The error for the Fehling
36 method is mainly caused by human technique (such as over-titration), whereas CE is automated and
37 is more affected by operation error. In the case of the low sugar content BCs, the Fehling method
38 was not sensitive enough to produce precise and accurate results. The most criticized aspect of CE in
39 terms of operation error is related to volume variation between injections. Injections in CE are
40 achieved by inserting a capillary into a sample solution vial and using pressure to draw sample
41 solution into the capillary (hydrodynamic injection). Pressure variations lead to differences in
42 injection volume and thus to relatively poor peak area precision. As previously mentioned, an
43 internal standard was used in this work (to correct the peak area) and this eliminated this type of

1 error and greatly improved precision as it had been observed with the CE method applied to plan
 2 fibers¹⁸ or fermentation monitoring¹⁹. There was no available data on the precision of the NIP and
 3 HPLC measurements. However, CE was more comparable to HPLC than NIP or the Fehling method.

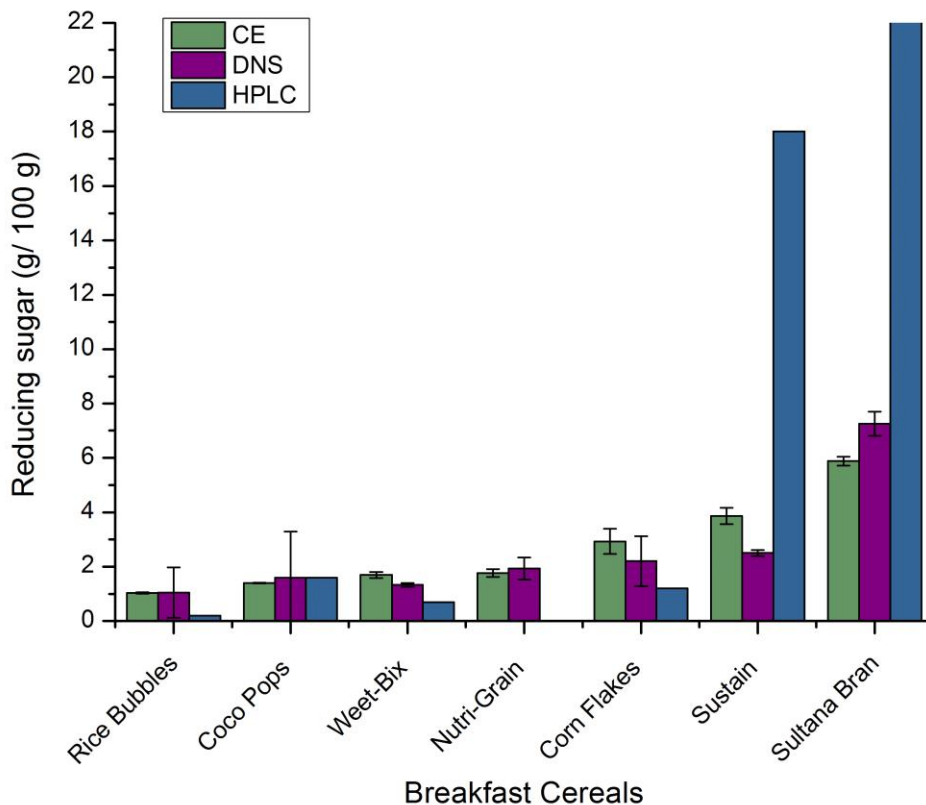


4
 5 **Figure 4.** Total sugar content of 7 BCs by CE, Fehling method, available HPLC data³⁸ and packaging
 6 label information (NIP). No HPLC data was available for ‘All Bran Fibre Toppers’ and ‘Oats Apple and
 7 Blueberry Bake’.

8 **2.5 Estimation of reducing sugar by CE and DNS assay compared with HPLC**

9 A reducing sugar is classified as any sugar that contains or is capable of forming an aldehyde
 10 functional group that can be oxidized to a carboxylic acid functional group. Though the largest
 11 proportion of sugar contained in BCs is sucrose, rather than reducing sugars (lactose, maltose,
 12 glucose and fructose), the quantification of these sugars is important - especially glucose (key
 13 product of digestion). The same ground samples (with fruit removed) were used to measure reducing
 14 sugars with both CE and DNS methods (Figure 5), which may explain the significantly lower content in
 15 reducing sugars in ‘Sultana Bran’ and ‘Sustain’ measured by CE and DNS compared to HPLC data. As
 16 previously discussed, individual CE results confirmed the reducing sugars quantified by HPLC³⁸ were
 17 fructose and glucose (see Figure 3B). The use of the DNS assay for the estimation of reducing sugars
 18 is a widely practiced assay and also recommended by the International Union of Pure and Applied
 19 Chemistry (IUPAC)⁴³. There was no significant difference between the sugar concentrations from CE
 20 and DNS assay. The sugar concentrations were found to agree with that from CE and HPLC in some
 21 cases, but not for the most complex matrices (plant fiber)⁴¹. Overall, the repeatability of the CE
 22 results was better than that of the DNS data in this work. During the DNS assay, relatively harsh
 23 reaction conditions (pH 13.0, 100 °C, 5 - 10 min) is likely to cause starch degradation. In addition,
 24 other side reactions (especially involving minerals) may compete for the availability of the DNS

1 reagent^{10, 11}. The simple suspension in water used for sample preparation in CE ensure no or much
2 more limited degradation for the use of CE on starch food.



3

4 **Figure 5.** Reducing sugar content of 7 BCs by CE, DNS assay and available HPLC data ³⁸.

5 **3. Conclusion**

6 FSCE with direct UV detection was shown to be advantageous for measuring sugar content in
7 breakfast cereals compared to traditional reducing sugar and glucose-specific methods for several
8 reasons: (1) BCs can contain as many as 5 sugars; CE is able to separate and quantify all sugars in a
9 sample compared to traditional methods which give an estimate of total sugar after inversion of
10 sucrose (a great disadvantage when sample composition is unknown), (2) BCs with the lowest
11 measured sugar levels, 'Oats Traditional' and 'Weet-Bix Original', had sugar contents below the LOD
12 of the Fehling method, (3) the precision of data collected by CE is greater than that from traditional
13 methods likely due to automation of the CE. While GC has been used to measure individual sugars in
14 BCs, multi-step sample preparation involving derivatization¹² or enzymatic removal of starch and acid
15 hydrolysis of the non-starch polysaccharides^{13, 14} is time consuming and presents a high risk of sample
16 loss. At present, high performance liquid chromatography (HPLC) is the gold standard for measuring
17 individual sugars in food. This study showed that, for all BCs (excluding those containing fruit), CE
18 was able to detect and quantify more sugars compared to data measured with HPLC³⁸. CE is also
19 more flexible, has a much lower running cost and requires much less sample preparation than HPLC.
20 High performance anion exchange chromatography (HPAEC) has some of the inherent disadvantages
21 associated with normal phase HPLC, but has a greater sensitivity range than CE. In summary, FSCE is,
22 with HPAEC, the method to recommend for the analysis of carbohydrates in breakfast cereals. Future
23 applications of this simple and robust CE method in foodstuffs could be enzymatic hydrolysis
24 monitoring (in vitro digestibility studies) as previously done by NMR ⁴⁴

1 4. Materials and methods

2 4.1 Materials

3 Milli Q quality (Millipore, Bedford, MA, USA) water was used throughout the analysis. Sodium
4 hydroxide pellets (NaOH), disodium monohydrogen phosphate powder (Na₂HPO₄ stored in a
5 desiccator), 100 % pure glacial acetic acid, calcium chloride dihydrate ≥98 %, magnesium chloride ≥99
6 %, sodium acetate ≥99 %, potassium chloride ≥99 %, sodium hydrogen carbonate and sulfuric acid
7 were obtained from Univar (Ingleburn, NSW, Australia); hydrochloric acid and methylene blue (C.I.
8 52015) were sourced from Ajax Chemicals (Auburn, NSW, Australia). Copper (II) sulfate was
9 purchased from Fisons (Homebush, NSW, Australia). Citric acid (anhydrous) was obtained from
10 Chem-Supply Pty Ltd (Gillman, SA, Australia). Zinc acetate ≥99 % was supplied by BDH AnalaR, Merck
11 Pty Limited (Poole, Dorset, England). Sodium potassium tartrate 99 %, sodium bisulfite, phenol, 3,5-
12 dinitrosalicylic acid, Glucose ≥99.5 % and dimethyl sulfoxide (DMSO) ≥99.5% were supplied by
13 Sigma-Aldrich (Castle Hill, NSW, Australia). Xylose ≥99 % was from Alfa Aesar (Ward Hill, MA, USA).
14 Fused-silica capillaries (50 µm internal diameter, 360 µm outside diameter) were obtained from
15 Polymicro (Phoenix, AZ, USA). Infinity™ glucose oxidase liquid stable reagent, pH 7.5 ± 0.1 at 20 °C,
16 was obtained from Thermo Scientific (TR-15221, Worthing, West Sussex, UK).

17 4.2 Initial sample preparation

18 Thirteen breakfast cereals (BC) products were purchased from a local Woolworths supermarket
19 (Marayong, NSW, Australia). Approximately 80-150 g of each BC was milled in a K-mart, m-mini glass
20 jug blender for 20 sec, speed level 1. The ground cereal was passed through a laboratory sieve with
21 pore size 1000 µm and retained in a 500 µm sized sieve, producing BC samples with particle size
22 between 500 to 1000 µm. Samples were stored in a cold room at 4 °C. Basic information of the 13 BC
23 samples, including sugar content, is shown in Table 3.

24 **Table 3.** Breakfast cereal samples according to total sugar content as listed on Nutrition Information
25 Panel (NIP, or packaging label information). Sugar quantity is listed in g per 100 g of BC

<i>Sample Name (Brand Name)</i>	<i>Sugar (g / 100 g)</i>
All Bran Fibre Toppers™ (Kellogg's®)	19.6
Coco Pops® (Kellogg's®)	36.5
Corn Flakes® (Kellogg's®)	7.9
Froot Loops® (Kellogg's®)	38.0
Nutri-Grain® (Kellogg's®)	32.0
Rice Bubbles® (Kellogg's®)	9.0
Sultana Bran (Kellogg's®)	22.7
Sustain® (Kellogg's®)	20.4
Weet-Bix™ (Sanitarium™)	3.3

Weet-Bix™ Multi-grain (Sanitarium™)	9.9
Oats Traditional (Uncle Tobys®)	1.0
Oats apple & blueberry bake (Uncle Tobys®)	25
Oats banana bake (Uncle Tobys®)	22.7

1

2 4.3 Capillary electrophoresis

3 For CE separations, disodium hydrogen phosphate (NP200 - 130 mM NaOH and 36 mM Na₂HPO₄)
 4 buffer was prepared according to ³⁰. This buffer was prepared on the day of use, sonicated for 5 min
 5 and filtered with a Millipore membrane syringe filter (0.2 μm). A stock solution of sugars (standard)
 6 was prepared containing 1.5 g·L⁻¹ sucrose and 0.5 g·L⁻¹ of each maltose, glucose and fructose in
 7 water. Standard curves were obtained using an undiluted standard and standards diluted by factors
 8 of 2, 4, 8, 16, and 32. Sample solutions of 10.0 g·L⁻¹ were prepared by adding 15.0 mg of ground
 9 sample (500-1000 μm particle size) to 1.500 mL of water. To each of the standards and samples, 0.50
 10 g·L⁻¹ xylose was added as an internal standard as well as DMSO (5 μL per 500 μL) to mark the electro-
 11 osmotic flow.

12 Separations were performed on a Beckman P/ACE MDQ capillary electrophoresis system (AB Sciex
 13 Separations, Mount Waverley, Australia) monitoring at 191 nm, 266 nm and 270 nm with a 10 nm
 14 bandwidth. A capillary with a total length of 61.8 cm (51.8 cm effective length) was used. The
 15 capillary was preconditioned before use by flushing with 1 M NaOH, 0.1 M NaOH, water and NP200
 16 buffer for 20 min each. The cassette temperature was set to 15 °C. Samples were injected by
 17 applying 34 mbar for 4 s followed by injection of NP200 buffer in the same manner. A voltage of 16
 18 kV was ramped up over 2 min. Between consecutive separations, the capillary was flushed with
 19 NP200 buffer for 5 min. After the final injection, the capillary was flushed with 1 M NaOH for 1 min,
 20 followed by water and then air (10 min each). Carbohydrates were monitored at 266 and 270 nm and
 21 the EOF was monitored at 191 nm by 32 Karat software. The data was processed first using either
 22 Origin 8.5.1 ('manual' treatment) or 32 Karat ('automated' treatment). Quantification of the
 23 carbohydrates was not done at 270 nm as in earlier literature but at 266 nm since it gave the optimal
 24 signal-to-noise ratio as also observed in the most recent literature.^{19, 32, 39} Both data treatment
 25 yielding the same results (data not shown) the latter treatment was used for all the results presented
 26 in this work. Concentration of identifiable sugars in BCs was determined from normalized peak area
 27 (relative to the internal standard) and the standard curve. Outliers were removed, where relevant,
 28 after applying a Grubb test (see Equation S-4).

29

30 4.4 DNS assay in micro plate format

31 Reducing sugars in BCs were quantified by the DNS assay in microplate format. The DNS reagent was
 32 prepared exactly according to ⁹. Sample solutions of 10.0 g·L⁻¹ were prepared by adding 15 mg of
 33 ground sample (500-1000 μm particle size) to 1.5 mL of water. Triplicates of 100 μL were made up as
 34 both undiluted samples and diluted samples (with dilution factors of 1, 2, 4, 8, 16, 32). Glucose
 35 standard solutions of 0.2, 0.4, 0.6, 0.8, 1.0 g·L⁻¹ were prepared in triplicates for the purpose of
 36 procuring a standard curve. A blank and a set of standards were included with the samples tested on

1 each microtitre plate. The contents of the plates were then mixed on a plate mixer for 5 s, sealed and
2 incubated at 100 °C in a water bath for 10 min. The plates were then cooled to room temperature to
3 stop the reaction by being placed on ice. Absorbance values were measured at 640 nm on a
4 microplate reader with the standard containing no glucose as the blank.

5 **4.5 Fehling (Lane-Eynon) method**

6 Estimation of total sugar content in BCs was carried out using Fehling's solution reagents as
7 described in ⁴⁵. Fehling solution A contained 69.3 g of copper sulfate in 1 L of water, Fehling solution
8 B contained 346 g of Rochelle salt (potassium sodium tartrate) in 1 L of water. Carrez solution 1
9 contained 21.9 g of zinc acetate and 3 mL of glacial acetic acid in 100 mL of water, Carrez solution 2
10 contained 10.6 g of potassium ferrocyanide in 100 mL of water. Each ground BC sample (5 g) was
11 added to 100 mL water, mixed with 5 mL each of Carrez solutions 1 and 2 and made up to a total
12 volume of 250 mL with water. The mixture was then decanted and filtered through Whatman® 540
13 filter paper. The clarified solution (25 mL of it) was transferred to a conical flask, mixed with 2.5 g
14 citric acid and gently boiled on a hot plate for 5 min to ensure the inversion of any sucrose present.
15 The sample solution was then neutralized to pH 6.5 - 7.5 with 1 M NaOH and made up to a total
16 volume of 250 mL with water. This gave an approximate sample concentration in sample preparation
17 of 2 g·L⁻¹. Fehling's solution was titrated against the sample solution as reported previously for the
18 standard glucose solution. The total sugars content was calculated following Equation S-1 (see
19 supplementary data).

20 Standardization of Fehling's solutions was carried out using a 10 g·L⁻¹ glucose solution. Fehling's
21 solutions (2.5 mL each of A and B) were mixed with 30-50 mL water in a ceramic crucible and heated
22 to 100 °C over a Bunsen burner for 15 s. Three drops of methylene blue indicator were added
23 followed by dropwise addition of the standard glucose solution until only a faint blue color remained.

24

25 **Supplementary data**

26 The supplementary data includes the details of the calculation of total sugars in Fehling method, CE
27 electropherograms with spiked BC samples, repeatability of CE electropherograms and tables with
28 the RSDs of the electrophoretic mobility, the comparison of LODs and LOQs with published values
29 and the statistics applied to detect outliers.

30

31 **Acknowledgements**

32 The authors acknowledge a UWS School of Science and Health Equipment Grant for the CE
33 instrument.

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- 24

SUPPLEMENTARY DATA

For

Quantification of sugars in breakfast cereals using capillary electrophoresis

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Supplementary information relating to the Fehling method and then Capillary Electrophoresis is given below.

1. Calculation of total sugars in Fehling method.

The total sugar content was determined as total invert sugars (IS_{tot} , in g per 100 g of BC) as a function of the mass of invert sugars in the standard (IS_{stan} , in g), the dilution factor D_f , the measured titre T (in mL), and the mass of the BC sample (m_{BC} , in g) according to Equation S-1. The initial volume of the sugar solution is 100 mL.

$$IS_{\text{tot}} = \frac{IS_{\text{stan}} \times D_f \times 100}{T \times m_{\text{BC}}} \quad (\text{Equation S-1})$$

2. Capillary electrophoresis.

2.1 Sample preparation and ageing

Suspensions of breakfast cereals in water were injected as 1 to 2 days old samples throughout this work. It was observed that samples aged for several months contain additional peaks (data not shown) which are assumed to be degradation products.

2.2 Electrophoretic mobility

Electrophoretic mobility (μ) is calculated relative to the migration of a neutral molecule representing the electro-osmotic flow, calculated by equation S-2.

$$\mu = \frac{l_d l_t}{V} \left(\frac{1}{t_m} - \frac{1}{t_{eof}} \right) \quad (\text{Equation S-2})$$

where l_d is the capillary length to the detection window (effective length), l_t is the total capillary length, V is voltage, t_m is migration time, t_{eof} is migration time of the electro-osmotic flow (EOF) marker.

Double correction of electrophoretic mobility:

Electrophoretic mobility is then normalized using a mobility marker as shown in equation S-3.

$$\mu^{normalized} = \frac{\mu^{ref\ mobility\ marker} \times \mu}{\mu^{mobility\ marker}} \quad (\text{Equation S-3})$$

where μ is the electrophoretic mobility (calculated with Equation S-2) which has already been corrected according to the EOF marker, $\mu^{normalized}$ refers to the electrophoretic mobility normalised by a reference mobility marker, $\mu^{mobility\ marker}$ is the electrophoretic mobility of the mobility marker, $\mu^{ref\ mobility\ marker}$ refers to the electrophoretic mobility of the reference mobility marker (taken as an average of all mobility markers for a set of injections).

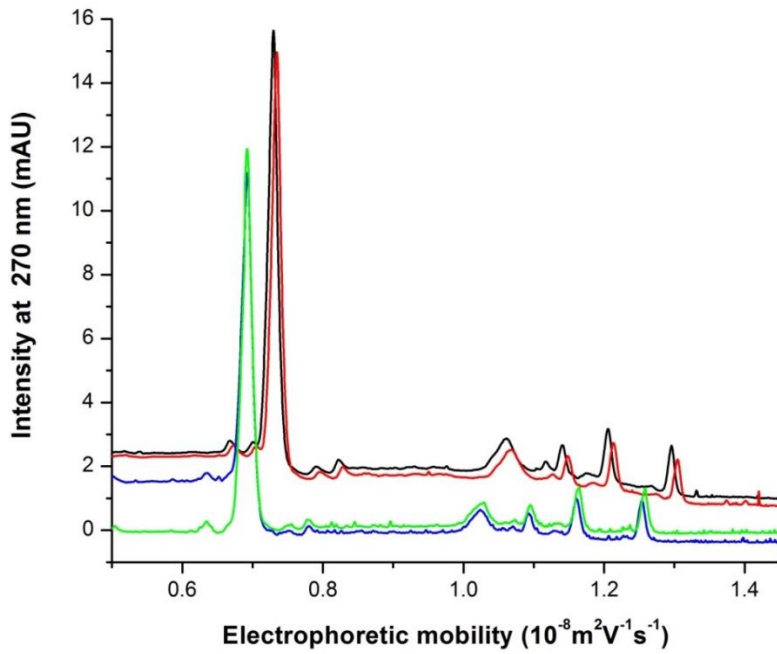


Figure S-1. Mobility electropherogram of 'Weet-Bix Multigrain' (4 repeats)

2.3 Spiking

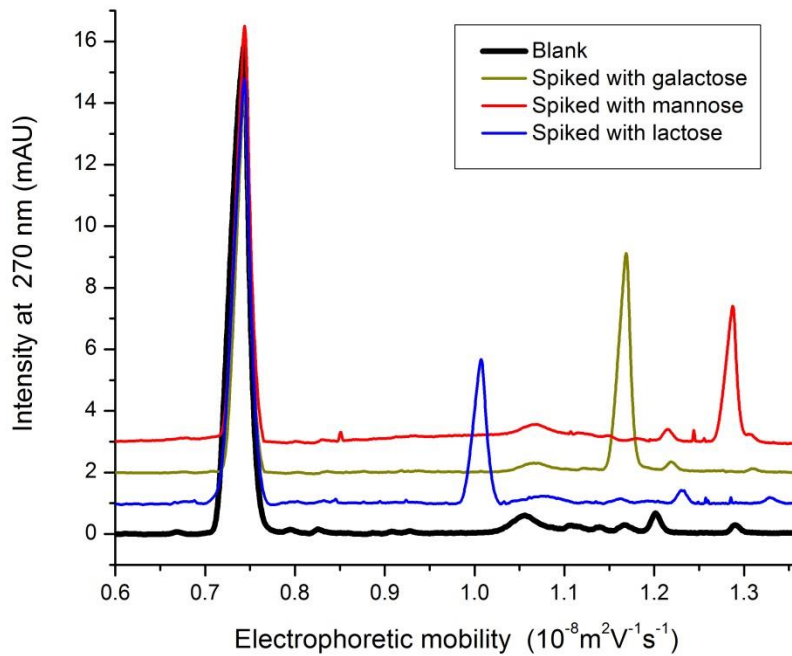


Figure S-2. CE with direct UV detection of 'Cornflakes', unspiked (blank) as well as spiked with lactose, galactose and mannose.

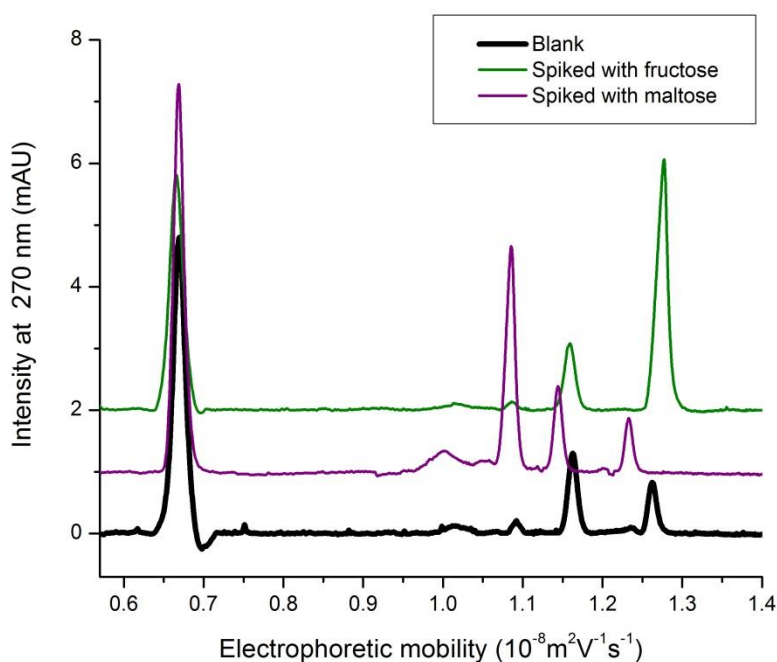


Figure S-3. CE with direct UV detection of 'Froot Loops', unspiked (blank) as well as spiked with maltose and fructose.

2.4 Sensitivity

Table S-1. Limit of detection (LOD) and limit of quantification (LOQ) of the CE with direct UV detection used in this work and in comparison with the literature (see page before last for reference list). LOD is defined as the concentration for which the signal-to-noise ratio (SNR) is equal to 3, LOQ as the concentration for which SNR is equal to 10.¹

	CE (this work) [¶]		CE (this work) [†]		CE ² LOD (mg·L ⁻¹)	CE ³ LOQ (mg·L ⁻¹)		CE ⁴ LOD* (mg·L ⁻¹)	CE ⁵ LOD (mg·L ⁻¹)	
	LOD (mg·L ⁻¹)	LOQ (mg·L ⁻¹)	LOD (mg·L ⁻¹)	LOQ (mg·L ⁻¹)		LOQ (mg·L ⁻¹)	LOD (mg·L ⁻¹)		LOQ (mg·L ⁻¹)	
Sucrose	4.50	19.0	5.88	21.6	-	-	9.93	1.6	34.4	167
Lactose	8.21	20.9	2.38	19.8	3.6	12	10.9	-	-	-
Maltose	13.3	30.4	20.7	41.7	-	-	-	2.8	-	-
Glucose	23.5	33.8	30.0	42.6	3.6	12	5.95	1.8	-	-
Fructose	14.4	35.9	15.9	44.1	-	-	8.47	-	-	-

* Noise level in 130 mM NaOH = 0.01 mAU.

[¶] Calculated manually by calculating SNR from raw data (peak height and average baseline noise height visually read from computer screen).

[†] Calculated manually by automatic SNR produced by 32 Karat software.

2.5 Linearity of the calibration curves

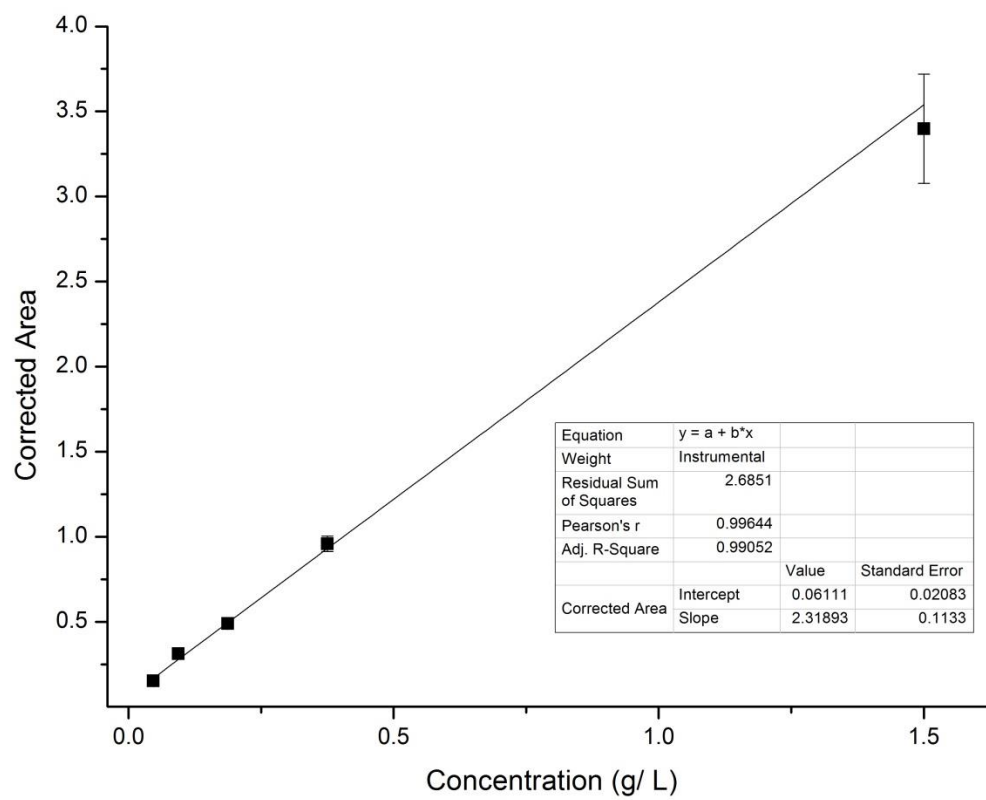


Figure S-4. Calibration curve for sucrose with the error bars from the standard deviation (n=3)

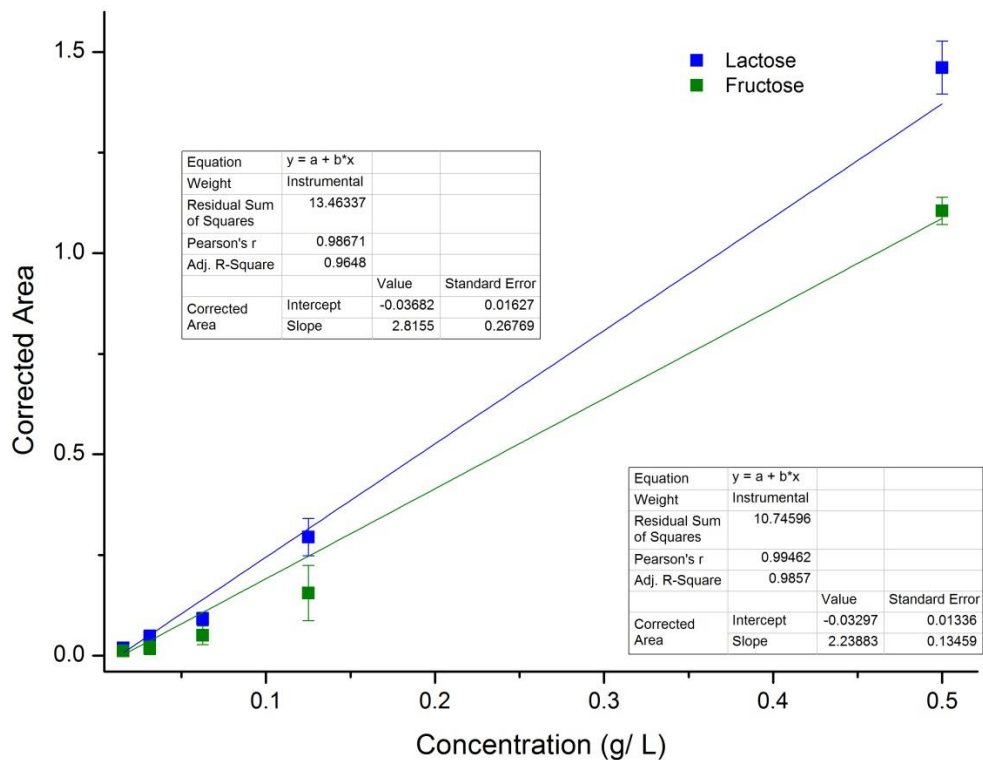


Figure S-5. Calibration curves for lactose and fructose with the error bars from the standard deviation (n=3)

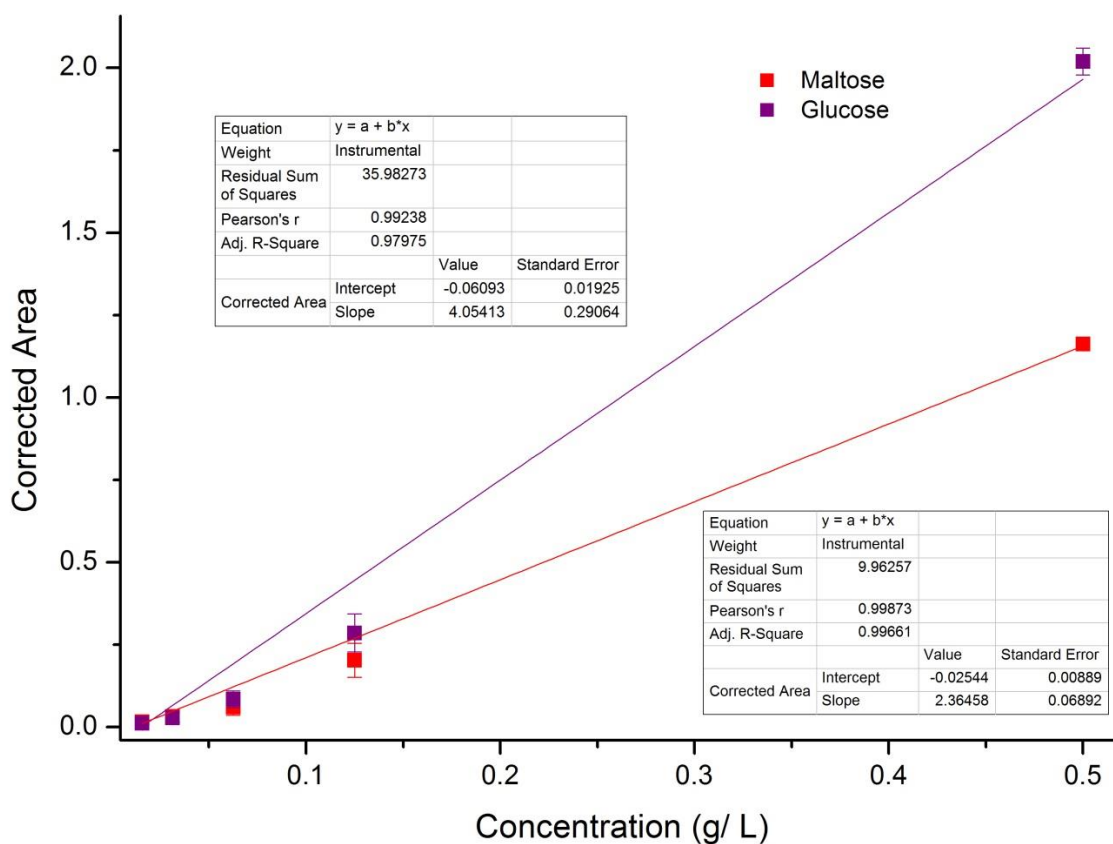


Figure S-6. Calibration curves for maltose and glucose with the error bars from the standard deviation (n=3)

2.6 Determination of outlier – Grubbs Test.

This test was used to determine outliers for CE data. The mean, \bar{x} , and standard deviation, s , were calculated of the sample with the point included. If more than one point was a possible outlier, then the furthest from the mean was taken in priority.

Hypothesis – statement of truth “selected data point comes from the same sample as all data points in set” i.e. the point, in question, is not an outlier.

Calculation of G, the statistic value which represents the “number of standard deviation” the point in question is from the mean, see Equation S-2.

$$G = \left| \frac{(\text{value of outlier}) - \bar{x}}{s} \right| \quad (\text{Equation S-2})$$

If $G >$ critical value, the hypothesis is false and the suspect point is an outlier and is omitted.

Table S-2. Critical values of G

Sample size	3	4	5	6	7	8	9	10
Critical value	1.15	1.481	1.715	1.887	2.02	2.126	2.215	2.298

Sample: Oats Traditional, 0.349188679, 0.195632075, 0.191679245, 0.214537736, 0.1625

Mean = 0.222707547 s = 0.073118069 Result = 0.349188679

Determine if point 0.349188679 is an outlier. N = 5; critical value = 1.715

$$G = \left| \frac{0.349188679 - 0.222707547}{0.073118069} \right| = 1.730 > 1.715 \text{ (critical value)}$$

=> hypothesis is false => point is NOT from the sample population => point is an outlier

New sample population (outlier discarded) 0.195632075, 0.191679245, 0.214537736, 0.1625

Mean = 0.19109 s = 0.02151 Result = 0.19563

Determine if point 0.19563 is an outlier. N = 4; critical value = 1.481

$$G = \left| \frac{0.196 - 0.19109}{0.02151} \right| = 1.730 > 1.715 \text{ (critical value)}$$

=> hypothesis is true => point is from the sample population => point is NOT an outlier

2.4 Precision

Table S-3. Precision of sugar concentration values obtained by CE for breakfast cereals (this work) compared to complex fermentation samples sourced from literature

<i>Data from ⁶</i>				<i>This work</i>			
<i>Sample*</i>	<i>Sugar</i>	<i>C</i> (g L ⁻¹)	<i>RSD</i> (%)	<i>Sample</i>	<i>Sugar</i>	<i>C</i> (g L ⁻¹)	<i>RSD</i> (%)
Fermentation sample 1	Glucose	10.9	14.8	All Bran Fibre Toppers	Sucrose	11.0	18
	Fructose	9.24	3.14				
Fermentation sample 2	Glucose	9.56	11.3				
	Fructose	10	3.7				
Fermentation sample 3	Glucose	7.36	4.77	Sultana Bran	Glucose	6.98	3.9
	Fructose	7.86	2.01				
Fermentation sample 4	Glucose	0.93	2.75	Nutri-Grain	Glucose	0.937	11
	Fructose	7.02	2.38	All Bran Fibre Toppers	Glucose	2.81	2.9
Fermentation sample 5	Glucose	0.57	2.01		Fructose	3.07	3.0

	Fructose	2.13	8.13	Sultana Bran	Maltose	0.616	8.2
Fermentation sample 6	Glucose	BDL ^	BDL	Coco Pops	Glucose	0.682	1.4
	Fructose	0.54	0.56 3				

* Fermentation media was incubated for different lengths of time with a microbe to change the carbohydrate composition.

^ BDL stands for “below detection limit”.

2.5 NP200 Buffer Decomposition – change in current over time

The plateau value of the current, c , for a given CE experiment, was measured and plotted against time that had lapsed since the preparation of the buffer (see Figure S-3). The initial current is noted as c_i while the final current is noted as c_f . The evolution of the current with time was fitted with linear regression and the slope, a , and intercept, b were determined. The following applies:

$$c_i = a \times 0 + b = b \quad \text{Equation S-5}$$

$$c_f = 0.9 \times c_i \quad \text{Equation S-6}$$

This allows calculating the time after which the current decrease by 10 % as:

$$x = - \frac{0.1 \times b}{a} \quad \text{Equation S-7}$$

and the time taken to lose 15% current from initial current measurement as:

$$x = - \frac{0.15 \times b}{a} \quad \text{Equation S-8}$$

Table S-4. Results of Buffer Decomposition

Experiment series	a	b	Time x for 10 % current loss	Time x for 15 % current loss
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			(min)	(h)	(min)	(h)
1	-0.0146	115.78	793	13.2	1190	19.8
2	-0.0163	118.06	724	12.1	1086	18.1
3	-0.0155	116.74	753	12.6	1130	18.8
4	-0.014	115.86	828	13.8	1241	20.7
5	-0.021	109.88	523	8.7	785	13.1
6	-0.0115	117.23	1019	17.0	1529	25.5
AVERAGE			773	12.9	1160	19.3
SD			161	2.7	241	4.0

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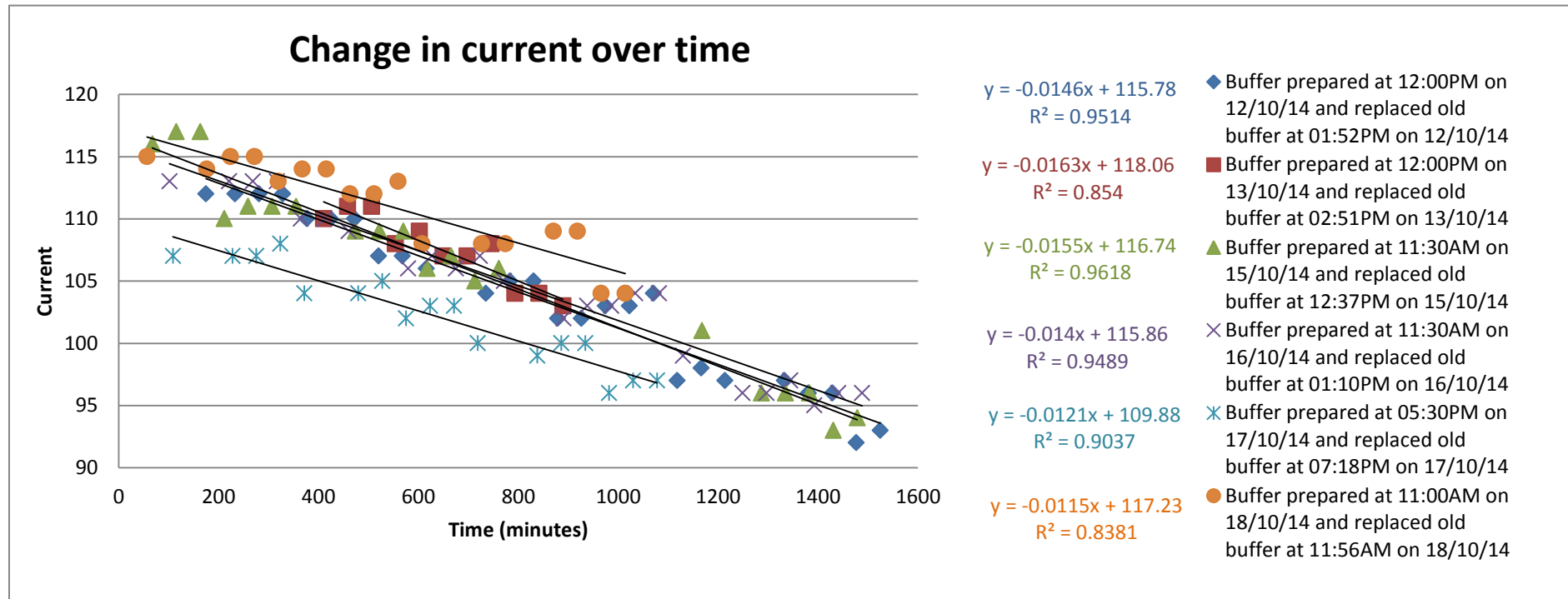


Figure S-7: Current against time since the buffer was prepared.