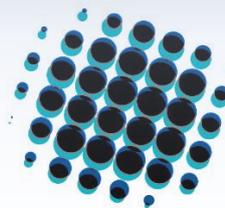


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Application Note: Determination of Sugars in Beverages

Background and Approach

Milkshakes are widely consumed in cafes, restaurants and fast food outlets, where they are typically made from milk and ice cream.

As such, it is expected that they would contain lactose, the sugar found naturally in milk. We developed methods using a combination of techniques to determine whether other sugars were added to the milkshakes and, if so, how the amount varied compared to lactose. Both ion chromatography and nuclear magnetic resonance (NMR) allowed us to identify and quantify the sugars present in vanilla milkshakes obtained from 4 different fast food outlets.



Methodologies

Sample Preparation

In order to purify the sample for further analysis, large molecules, such as proteins, were removed from the samples prior to downstream analysis by treating milkshake with Carrez reagents I and II. Following precipitation and subsequent removal of the unwanted large molecule fraction, the sugar-containing fraction was filtered and then used directly.

Ion Chromatography

Samples were analysed using a Metrohm 930 Compact IC Flex system equipped with amperometric detector and a CarboPac PA20 BioLC (Thermo Scientific) column heated to 30 °C. Samples were eluted isocratically using 12mM sodium hydroxide. Fructose, galactose, glucose, lactose, lactulose and sucrose standards were prepared for reference and calibration. Data were collected and interpreted using MagIC Net 3.2 software (Metrohm). Results were calculated following correction for the dilutions carried out and allowing for the density of each milkshake which was determined using a Mettler Toledo Density2Go handheld density meter (Mettler Toledo).



Metrohm 930 Compact IC Flex

NMR

All samples and standards were prepared for NMR by adding 10 % (v/v) D₂O. NMR spectra were acquired by use of a Bruker Ascend III HD with a room temperature broadband probe operating at 298.15K. 1D spectra were acquired using solvent suppression and 16 transients were collected with 128k points. Carbon HSQC spectra were acquired with 2k points and 256 points in the direct and indirect dimensions respectively.



Bruker Ascend III HD NMR Spectrometer

Findings

Sugar Identification

Comparison of the NMR spectra from each milkshake sample against the spectra collected for each of the standards (fructose, galactose, glucose, lactose, lactulose, sucrose) revealed the presence of lactose, sucrose and glucose in the samples (Figure 1). This was supported by the ion chromatography analysis which showed 3 peaks corresponding to the same sugars (Figure 2). NMR spectra and IC chromatograms were integrated and peak volumes compared against responses calibrated from standard solutions.

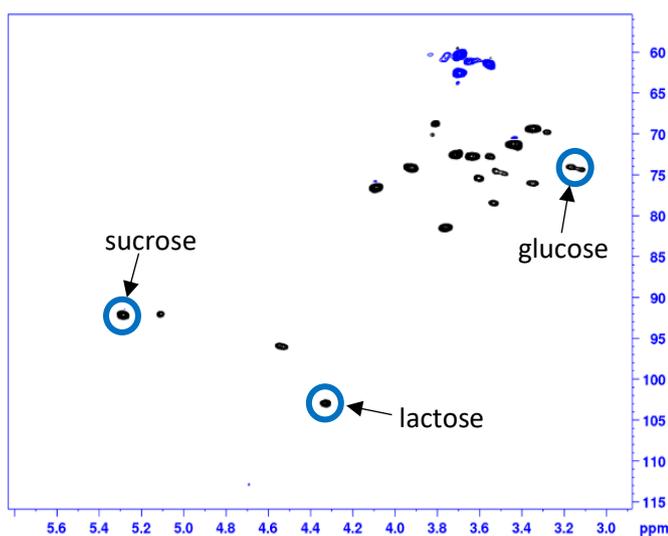


Figure 1. 2D NMR spectrum of milkshake extract with characteristic regions for sucrose, lactose and glucose highlighted confirming their presence

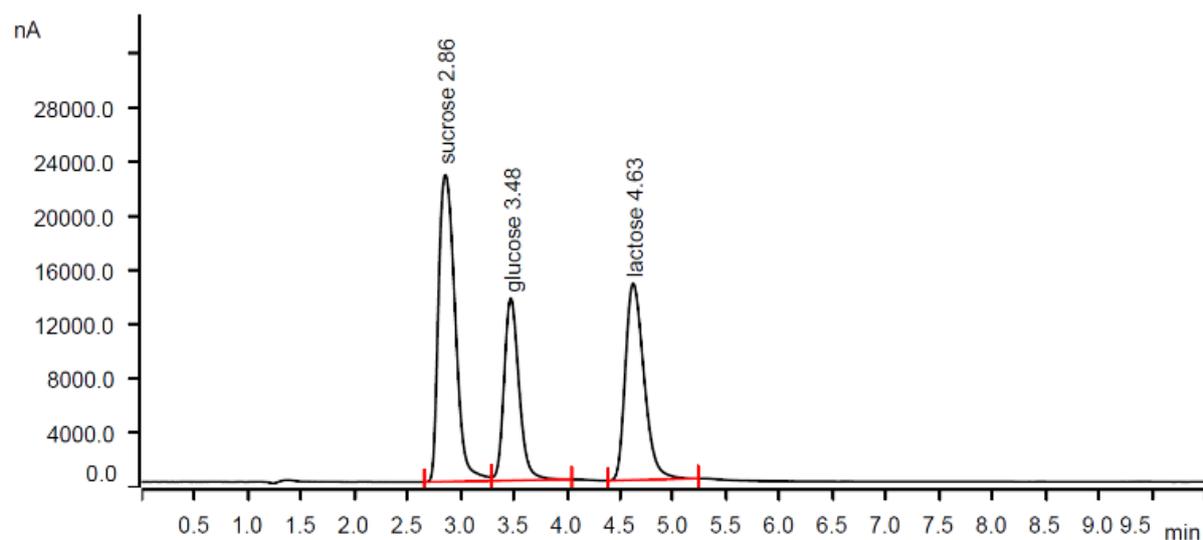


Figure 2. Representative chromatograph of milkshake extract showing peaks for sucrose, glucose and lactose.

Quantification

Milkshakes from all four restaurants contained sucrose, glucose and lactose. In all cases, sucrose was found to be present at the highest concentration ranging from ~60g/L up to ~108g/L. This was followed by lactose which was present at levels of 40 - 50g/L for all milkshakes tested. Finally, glucose was also present in all samples though at lower levels with concentrations ranging from ~20g/L to <10g/L.

Sample	Lactose (g/L)	Sucrose (g/L)	Glucose (g/L)
<i>Restaurant A</i>	41.6	94.5	19.0
<i>Restaurant B</i>	41.0	107.5	0.7
<i>Restaurant C</i>	49.6	98.7	3.1
<i>Restaurant D</i>	48.7	59.5	19.8

Table 1. Concentrations of lactose, sucrose and glucose in four different vanilla milkshakes.

Conclusions

All 4 milkshakes contained high levels of added, processed sugars in the form of sucrose and glucose, taking overall sugar levels to significant percentages of our overall recommended daily amounts.

Both ion chromatography and NMR spectroscopy provide rapid and easy-to-use methods for accurate quantitation of sugars in drinks. These methods can be carried out routinely in the laboratories at JBL Science by scientifically qualified experts.

Analysis of Sugars in Beverages
Application note 202001 from JBL Science at the University of Lincoln

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