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Analytical Methods

# Application of diffusion ordered-$^1$H-nuclear magnetic resonance spectroscopy to quantify sucrose in beverages

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ABSTRACT

This work focuses on a quantitative analysis of sucrose using diffusion ordered-quantitative $^1$H-nuclear magnetic resonance spectroscopy (DOSY-qNMR), where an analyte can be isolated from interference based on its characteristic diffusion coefficient ($D$) in gradient magnetic fields. The $D$ value of sucrose in deuterium oxide at 30 °C was $4.9 \times 10^{-10}$ m$^2$/s at field gradient pulse from $5.0 \times 10^{-2}$ to $3.0 \times 10^{-1}$ T/m, separated from other carbohydrates (glucose and fructose). Good linearity ($r^2 = 0.9999$) was obtained between sucrose (0.5–20.0 g/L) and the resonance area of target glucopyranosyl-α-C1 proton normalised to that of cellobiose C1 proton (100.0 g/L, as an internal standard) in 1D sliced DOSY spectrum. The DOSY-qNMR method was successfully applied to quantify sucrose in orange juice (36.1 ± 0.5 g/L), pineapple juice (53.5 ± 1.1 g/L) and a sports drink (24.7 ± 0.6 g/L), in good agreement with the results obtained by an F-kit method.



## 1. Introduction

Recently, quantitative high-resolution nuclear magnetic resonance (qNMR) spectroscopy has been used to determine the quantitative characteristics of natural or synthetic products (Koskela, 2009; Pauli, Gödecke, Jaki, & Lankin, 2012), which relies on the quantitative feature of $^1$H-NMR resonance area corresponding to the number of protons of the same type in a molecule, after normalisation to an internal standard (IS). The use of an appropriate IS makes qNMR analysis extremely attractive for determination of purity (Campo, Berregi, Caracena, & Santos, 2006; Saito et al., 2009). In food analysis, researchers have tried extensively to apply qNMR to the determination of compounds in natural products (Pauli et al., 2012), foods (Caligiani, Acquotti, Palla, & Bocchi, 2007; Hatzakis, Dagounakis, Agiomyrgianaki, & Dais, 2010) and beverages (Berregi, Santos, Campo, Miranda, & Aizpurua, 2003; Nord, Vaag, & Duus, 2004), because no chemical extraction, fractionation or purification are required. Sugimoto et al. successfully measured quercetin in tartary buckwheat noodle by qNMR, and proved hexamethyldisilane was an effective IS normalising the proton at H-2′ in quercetin, although the use of the IS was limited due to its poor solubility in aqueous solution (Sugimoto et al., 2010).

Even though the typical qNMR approach is rapid, requires no tedious pre-treatment and its specific, it presents problems in complex mixtures, such as foods, because the large number of protons can cause overlapping of resonances (Caligiani et al., 2007; Gil et al., 2004; Siciliano et al., 2013). Diffusion ordered-$^1$H-NMR spectroscopy (DOSY-NMR) might be able to solve this problem because it can distinguish a given proton in an analyte based on its characteristic diffusion coefficient ($D$) in an appropriate gradient of magnetic fields, allowing individual analytes in foods to be detected (Johnson, 1999). Indeed, DOSY-NMR method has been applied to food analysis in characterising prominent food components, such as sugars, organic acids and amino acids in tomato (Sobolev, Segre, & Lamanna, 2003), apple, grape juices and beer (Gil et al., 2004), port wine (Nilsson et al., 2004) and honey (Gresley et al., 2012). It has been proven that the pulse-gradient spin-echo NMR method, similar to DOSY-NMR, is useful for quantitative mixture analysis (Antalek, 2006, 2007; Barrere, Thureau, Thevand, & Viel, 2012), while there has been no report on DOSY-qNMR method for quantitative food analysis.

Thus, the present study is the first work on the application of DOSY-qNMR as a quantitative analytical method in foods. In this study, the sucrose content was determined by DOSY-qNMR in three beverages (orange, pineapple juices and a sports drink). The DOSY-qNMR method was subjected to analytical validation. The results were compared to those obtained by previously proven methods, particularly a commercially available enzymatic F-kit assay and high-performance liquid chromatography (HPLC).

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## 2. Materials and methods

### 2.1. Materials

D-(+)-Glucose, D-(−)-fructose, sucrose, and cellobiose were purchased from Nacalai Tesque Inc. (Kyoto, Japan). 3-Trimethylsilyl-1-propanesulfonic acid-$d_6$ (DSS-$d_6$) was obtained from Wako Pure Chemical Ind. (Osaka, Japan). Deuterium oxide ($D_2O$, 99.8 atom% D) was acquired from Acros Organics (Fair Lawn, NJ, USA). One orange juice, one pineapple juice and one sports drink were commercially available products from the local market in Japan, and were stored at −20 °C prior to use.

### 2.2. 1D $^1$H-NMR

1D $^1$H-NMR spectra were obtained on an ECS-400 spectrometer (JEOL, Tokyo, Japan) composed of fully digitised circuitry including RF generator, NMR lock, and digital matrix shim, equipped with a z-field gradient unit, operating at 400 MHz. Samples were dissolved in $D_2O$ and placed into 5 mm-sample tubes (Nihonseimitsu Scientific Co., Tokyo, Japan). For determination of 90° pulse-width of sucrose, 1D $^1$H-NMR spectra were acquired using a single pulse-sequence without water suppression using the following conditions: acquisition time 2.18 s, acquisition point 16,384, total transients 128, relaxation delay 15 s and gain 26 at 30 °C and non-spinning. The pulse-widths were set from 9.0 to 11.0 μs linearly, with 0.1 μs intervals. The spin–lattice relaxation delay ($T_1$) value was measured on sucrose (10 g/L) and cellobiose (10 g/L) in an arrayed experiment using an inversion-recovery pulse sequence under the same conditions described above, using a relaxation delay of 10 s and pulse-interval times in the range 0.1–1.0 s (Caligiani et al., 2007). All spectra were referenced to the signal of DSS-$d_6$ at 0.0 ppm.

### 2.3. DOSY-qNMR

2D DOSY-NMR measurements with a pulsed-magnetic field gradient spin-echo NMR method (Cabrita, Berger, Brauer, & Karger, 2002), using pulse sequence beginning with a 90° pulse, after a time interval and followed by a 180° pulse (bpp_led_dosy_pfg.ex2 in delta v 4.3.6. library (version 1.1)) were performed with diffusion time of 0.1 s, field gradient pulse-width of $1.0 \times 10^{-3}$ s and field gradient pulse from $5.0 \times 10^{-2}$ to $3.0 \times 10^{-1}$ T/m (logarithmic, points 8, base 2) at 128 scans of 16,384 acquisition points, 90° pulse-width of 10.4 μs, relaxation delay of 4.5 s at 30 °C and non-spinning. Auto-shimming was performed for each measurement with the field-gradient shimming at 4 scans, shimming sets of Z1–Z6, and receiver gain 20. 2D DOSY spectra were acquired on a data inversion program SPLMOD (double precision version 3DP, June 1988), which permits the parallel analysis of large numbers of data sets (Vogel, 1988) with display range from $1.0 \times 10^{-10}$ to $1.0 \times 10^{-8}$ m$^2$/s. Thus, in the 1D sliced NMR spectrum, proton signals detected in analytes with the same $D$ value could be treated equally with the same SPLMOD algorithm (Morris & Johnson, 1993). The $D$ value was calculated according to the Stejskal–Tanner equation Eq. (1) (Lucas, Otto, & Larive, 2002):

$$D = -\frac{1}{(\gamma G\delta)^2(\Delta - \delta/3)} \ln\frac{I(G,\Delta)}{I(0,\Delta)} \quad (1)$$

where $\gamma$ is the gyromagnetic ratio in the observation of nuclei, $G$ is the pulse-field gradient strength, $\delta$ is the pulse-width of the field gradient, $\Delta$ is the diffusion time, and $I$ is the signal intensity.

The quantitation of sucrose was performed with cellobiose as IS, which was dissolved in 50 μL of $D_2O$ (100.0 g/L) and loaded into a 5 mm-capillary insert glass tube (a specialised NMR sample tube, N-502B, Nihonseimitsu Scientific Co.). The cellobiose-loaded capillary tube, which was used for all the quantitation experiments, was inserted into a coaxial 5 mm-NMR sample tube containing 400 μL of sample solution prior to 2D DOSY-qNMR measurements. The resonance area of the analyte proton normalised to that of the IS proton, in 1D sliced DOSY spectrum taken from $D = 4.9 \times 10^{-10}$ m$^2$/s, was used for the qNMR assay.

### 2.4. Preparation of beverages for DOSY-qNMR

To quantify sucrose, three beverages (orange juice, pineapple juice and a sports drink) were filtered, through a 0.45 μm COSMONICE filter (Nacalai Tesque), to remove any precipitate such as pectins, which might interfere with NMR resolution, and the filtrates were diluted 10-fold with $D_2O$. Each diluted solution was then placed into a 5 mm-NMR sample tube, following insertion of the cellobiose-loaded capillary tube. For recovery, samples were analysed with and without added sucrose. Sucrose (40 or 80 g/L) was added to each beverage, following the pre-treatments described above. The recovery was calculated using the equation: Recovery (%) = (sucrose concentration found in sucrose-added beverage − sucrose concentration found in original beverage)/added sucrose × 100%.

### 2.5. Assay for sucrose in beverages by conventional methods

Two conventional sucrose assays, the F-kit method and an HPLC method, were used to validate the proposed DOSY-qNMR method. For the F-kit enzymatic assay (F-kit sucrose, Roche Diagnostics, Darmstadt, Germany), sucrose content of the filtered beverages was determined spectrophotometrically at 340 nm, according to the manufacturer's protocol.

An HPLC assay was conducted using a PU 2080 liquid chromatograph (Jasco Co., Tokyo, Japan) connected with a refractive-index (RI) detector (Jasco RI-930). LC separation was performed on a COSMOSIL Sugar-D column (4.6 × 150 mm, 5 μm, Nacalai Tesque) at 35 °C, with 75% (v/v) acetonitrile flowing at 1.0 mL/min. Prior to the HPLC assay, aliquots (1.0 mL) of the filtered beverage were dried *in vacuo*. The dried samples were then dissolved in 1.0 mL of 75% (v/v) acetonitrile and aliquots (100 μL) injected on to the HPLC system. Under the HPLC conditions, sucrose was eluted at 4.6 min.

### 2.6. Statistics

Data are expressed as the mean ± standard deviation (SD). Five replicates were analysed for each assay ($n = 5$: five parallel prepared sample from each beverage). Statistical differences between sucrose contents in orange juice, pineapple juice and sports drink obtained by the three assays were analysed by one-way analysis of variance (ANOVA), followed by *post-hoc* Tukey–Kramer's $t$-test. The $p$ value of <0.05 was considered to be statistically significant different. All analyses were performed using Stat View J 5.0 (SAS Institute, Cary, NC, USA).

## 3. Results and discussion

The DOSY-NMR method has been applied to food analysis for characterising prominent food components. Gil et al. reported the DOSY technique could be used for simultaneous characterisation of food compounds, such as organic acids, sugars, amino acids and polyphenols in beverages (Gil et al., 2004). Nilsson et al. analysed the quality of port wine based upon changes in the relative amount of organic acids using the DOSY technique (Nilsson et al., 2004). However, there are few reports on quantitative food