

Quantification of Sugars in Soft Drinks and Fruit Juices by Density, Refractometry, Infrared Spectroscopy and Statistical Methods

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ABSTRACT

The amount of sugar in soft drinks and fruit juices has been quantified by density, refractometric and infrared spectroscopic methods. Density and refractometric methods can be used to obtain only the total amount of sugar. However, infrared spectroscopy distinguishes itself as a fast and reliable method for quantitative analysis. Fourier-transformed spectroscopy in combination with a mathematical treatment of the spectra of samples allows the amount of different sugars such as glucose, fructose and sucrose to be determined.

KEYWORDS

Sugar, density, refractometry, mid-FTIR, partial least squares regression.

1. Introduction

Sugars are major sources of energy for all living entities. Plants produce sugars by photosynthesis and convert them into various disaccharides such as sucrose, or convert them into starch for easy storage. Herbivores make use of this energy source and are also attracted to the sweet taste and smell of sugars. Just because of the taste of sugar, humans have gone a step further, adding sugar to food that normally and naturally does not contain it, or has it only in small quantities. People now consume a great deal more refined sugar than their body weight allows. The human body cannot tolerate a large amount of refined carbohydrates, thereby damaging the vital organs. The average healthy digestive system can digest and eliminate two to four teaspoons of sugar daily, usually without noticeable side-effects. An excess of sugar in the diet results in weight gain, thereby increasing the risk of heart disease, diabetes and high blood pressure, apart from dental caries. It is therefore important to know what amount of sugar is present in food and beverages^{1–6} that are commonly consumed.

Fourier-transformed spectroscopic and multivariate data analysis^{7–8} have been used to establish the authenticity of foods such as vegetable oils, olive oil, coffee, honey, raspberry purees, orange juice and meat. The total amount of sugar has been determined using density⁹, refractometric¹⁰ and infrared spectroscopy^{11–13} methods. These methods were used after standardization. The density and refractometric methods were used to obtain the total amount of sugar in samples. However, infrared spectroscopy, coupled with a mathematical treatment, was used in this study to quantify different types of sugars, namely glucose, fructose and sucrose, in soft drinks (SD1–SD10) and fruit juices (FJ1–FJ10).

2. Experimental

Analytical grade D-glucose, D-fructose and sucrose from Aldrich were used for this work. Commercial carbonated soft

drinks and fruit juices, which are readily available and inexpensive, were used as sample materials. Two samples of each of these beverages were analysed. However, a given sample was used for the different methods, as there are variations in sugar content from different samples. The carbonated soft drinks were degassed, and the fruit juices were centrifuged so as to remove the pulp. The clear solution was decoloured by warming with activated charcoal. All solutions and samples used were at room temperature. They posed no chemical exposure hazards and no hazardous wastes were produced. All excess reagents, samples, and waste from this work could be disposed of in the sewage system.

2.1. Standard Solutions

A set of 20 standard solutions with the amount of sucrose varying from 10 to 20 g per 100 mL distilled water were prepared and it was ensured that the sugar solution was homogeneous before being used.

In addition, three sets of standard solutions containing tertiary mixtures of D-glucose, D-fructose and sucrose at concentration levels of 8–17 g per 100 mL distilled water were prepared. The concentration range was chosen to evaluate the adequacy of the method with regard to soft drinks and fruit juices.

2.2. Density and Refractometry Methods

Three density and refractive index determinations were made for each of the standard solutions containing only sucrose. The density values were obtained using a 25 mL pycnometer. The refractive index was obtained using an Abee Refractometer. Graphs were prepared by plotting average density and average refractive index against the concentration of sucrose in the standard solution. The density and refractive index of the soft drinks and juices were obtained as for the standard solutions. These densities, refractive indices and graphs for the standard solutions were used to evaluate the amount of sucrose in the soft drinks and fruit juices.

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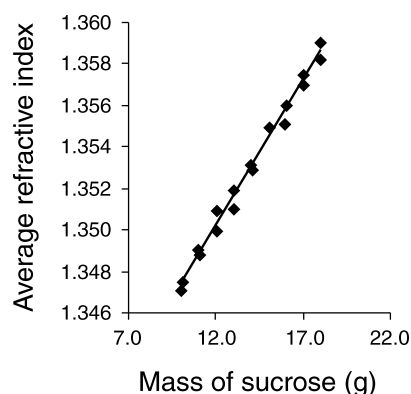


Figure 1 Average refractive index against mass of sucrose in standard sucrose solutions.

2.3. Infrared Spectroscopy

A standard solution, pure sucrose or tertiary mixture solution, was placed in the ATR cell of the spectrometer (Avatar 320 FT-IR). The mid-Fourier-transformed infrared absorbance spectrum was then recorded from 2000 cm^{-1} to 800 cm^{-1} in 4 cm increments with air as background. A mathematical treatment was done with the spectra of standard tertiary mixtures and the samples to obtain the amount of different sugars such as glucose, fructose and sucrose in the samples.

2.4. Mathematical Treatment

There are different mathematical methods that can be used for the processing of complex infrared spectral data. Principal component regression (PCR) has often been used for quantitative determination using mid-infrared spectra^{12,14}. However, partial least squares regression appears to be more efficient in predicting ion and sugar contents in biological solutions^{15,16}.

All statistical calculations were carried out with commercial software, The Unscrambler (Camo ASA, Oslo, Norway). The principles of partial least squares regression (PLS) have been described in detail in several papers^{17,18}. This method calculates, from spectral data and concentration or chemical data, new variables termed 'factorial coordinates' which are meaningful with regard to the chemical variables that have to be predicted. The first coordinate is calculated so as to predict as much as possible the chemical variable and the spectral data. The non-predicted part of the variables and of the spectral data, that is the residuals, is calculated. The second coordinate is then determined so as to best predict the residuals of the variables. The new residuals are then calculated and the procedure is repeated

Table 1 Average amount of sugar obtained by density and refractometry methods in soft drinks.

Soft drink	Amount of sugar obtained by density method (g 100 mL ⁻¹)	Amount of sugar obtained by refractometry method (g 100 mL ⁻¹)
SD 1	14.82 (0.92)	14.50 (1.14)
SD 2	13.68 (0.70)	13.43 (0.41)
SD 3	12.73 (0.02)	12.47 (0.16)
SD 4	16.75 (0.07)	16.65 (0.08)
SD 5	16.90 (0.89)	16.71 (0.16)
SD 6	14.07 (1.38)	14.86 (0)
SD 7	12.81 (0.05)	12.75 (0.)
SD 8	14.38 (0.09)	13.82 (0.15)
SD 9	12.15 (0.08)	12.32 (0.15)
SD 10	13.24 (0.01)	13.00 (0.13)

^aValues in brackets are the standard deviation.

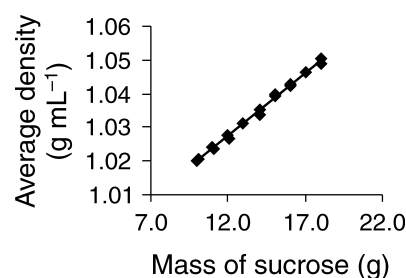


Figure 2 Average density against mass of sucrose in standard sucrose solutions.

until satisfactory precision for the estimation of the chemical variable is obtained.

There are in fact two versions of the PLS algorithm: PLS-1 and PLS-2. In this work, PLS-2 was preferred because it calibrates for all the components simultaneously. Partial least squares regression was used to establish a prediction equation.

PCA and PLS-2 were applied to the MIR spectra in the region 900–125 cm^{-1} (with 181 data points used as principal variables). Spectra were centred prior to further analysis according to:

$$X_{ij} = A_{ij} - A_j,$$

where X_{ij} = centred data, A_{ij} = spectral data ($\log R_0/R$) of spectrum i and wave number j , R_0 corresponds to the background obtained without the sample, R is the reflected intensity from the sample, and A_j = mean value of spectral data at wavelength j for every spectrum.

3. Results and Discussion

3.1. Density and Refractometry

The plots of refractive index and average density (Figs 1 and 2) for the standard solution of pure sucrose against concentration yielded straight lines with R^2 of 0.996 and 0.990, respectively. The regressed lines were used to calculate the amount of sugar in the samples. The results are reported in Tables 1 and 2 for the soft drinks and fruit juices, respectively.

3.2. Infrared Spectroscopy

The spectra for the standard pure sucrose solutions are illustrated in Fig. 3. It may be found that the intensities of absorbance vary significantly with concentration. This allowed the assumption that the amount of sucrose could be quantified.

However, the sucrose content in soft drinks and fruit juices cannot be quantified just from the height of the peak since the

Table 2 Average amount of sugar obtained by density and refractometry methods in fruit juices.

Fruit juice	Amount of sugar obtained by density method (g 100 mL ⁻¹)	Amount of sugar obtained by refractometry method (g 100 mL ⁻¹)
FJ 1	15.27 (0.19)	14.44 (0.32)
FJ 2	14.49 (0.26)	13.87 (0.41)
FJ 3	12.47 (0.16)	11.40 (0.20)
FJ 4	14.21 (0.25)	13.56 (0.24)
FJ 5	12.48 (0.51)	12.70 (0.41)
FJ 6	16.63 (0.65)	16.51 (0.72)
FJ 7	12.55 (0.25)	11.54 (0.14)
FJ 8	13.14 (0.04)	11.61 (0.05)
FJ 9	13.18 (0.08)	12.27 (0.08)
FJ 10	15.14 (0.42)	14.06 (0.45)

^aValue within bracket are the standard deviation.

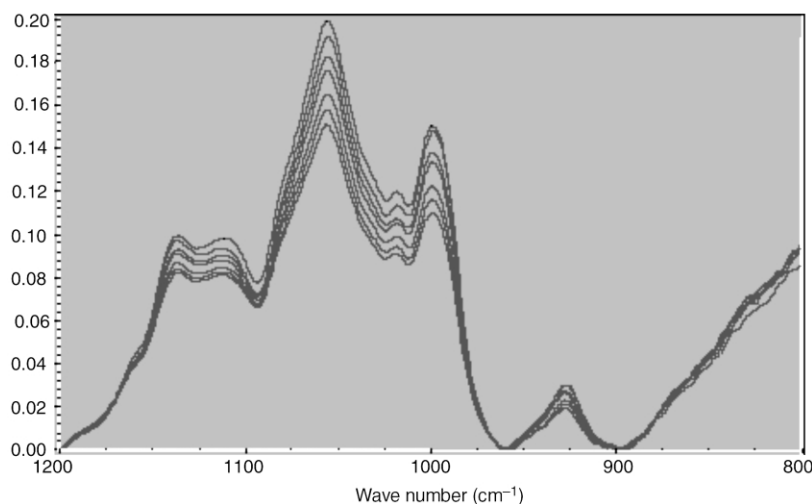


Figure 3 FTIR (800–1200 cm^{-1}) of the standard sucrose solutions.

resulting spectra sometimes include three sugars, namely sucrose, glucose and fructose. The quantification becomes more difficult as the influence of fructose and glucose is not negligible. Hence it is necessary to perform a mathematical treatment of the spectra to obtain the amount of different sugars. This is done by applying PLS. From the prediction equations established by PLS, the glucose, fructose and sucrose contents could then be predicted.

First, PLS-2 was performed on the full set of 23 spectra of standard solutions of sucrose, fructose and glucose at concentrations ranging from 0% to 11%. The prediction equations, that linked sucrose, fructose and glucose to the spectral data, were established on the corresponding first seven axes. The concentrations of the 25 samples from the calibration set were predicted from the prediction equation thus established. The regression model developed here gives sucrose, fructose and glucose contents values that are close to the references values. The plots of the predicted concentrations against measured concentration for the 25 standard solutions are illustrated in Fig. 4.

For the three components, the plots yielded a straight line with R^2 between 0.994 and 0.998.

Determination of sugar contents in different soft drinks and juices could also be considered by using this prediction equation. The average mass of different sugars and the average total amount sugars in the soft drinks and fruit juices as determined by the PLS regression model (see 2.4) are reported in Tables 3 and 4, respectively.

4. Conclusions

In this paper, the total amount of sugar in soft drinks and fruit juices has been obtained by density and refractometric methods. In addition, the amount of different sugars were determined using infrared spectroscopy coupled with PLS analysis. From the results, it is interesting to note that the amount of sucrose is significant in sugar-added fruit juices (FJ1–FJ6) and there is no sucrose in non-sugar-added juices (FJ7–FJ10). Furthermore, the amount of fructose is higher in soft-drinks compared to sugar-added juices.

The infrared spectroscopy method^{19,20} distinguishes itself as a fast and an accurate way of obtaining the amount of different sugars. The results from the three different methods are in agreement and the greater amount of sugar predicted by the density and refractometric methods compared to infrared spectroscopy may be attributed to only sucrose solutions.

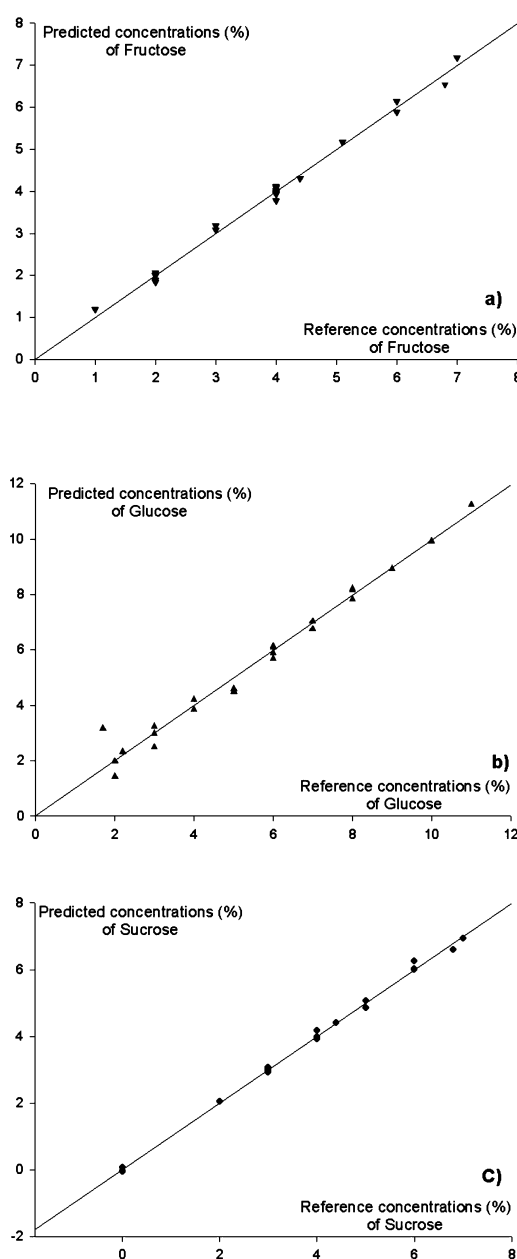


Figure 4 Prediction of sugar concentrations: reference concentrations vs predicted concentrations; a, fructose; b, glucose; c, sucrose.

Table 3 Average amount of different sugars and average total amount sugar in the soft drinks obtained by infrared spectroscopy.

Soft drink	Average amount of sucrose (g 100 mL ⁻¹)	Average amount of glucose (g 100 mL ⁻¹)	Average amount of fructose (g 100 mL ⁻¹)	Average amount of total sugar (g 100 mL ⁻¹)
SD 1	0 (0)	2.82 (0.11)	8.42 (0.13)	11.24 (0)
SD 2	7.39 (0.02)	2.20 (0.03)	2.49 (0.03)	12.08 (0.02)
SD 3	0 (0)	3.92 (0)	8.92 (0.02)	12.84 (0.01)
SD 4	1.61 (0.03)	3.89 (0.02)	7.76 (0.91)	12.65 (1.61)
SD 5	2.58 (0.14)	3.48 (0.06)	7.15 (0.62)	13.21 (1.96)
SD 6	0 (0)	4.19 (0.02)	9.42 (0.42)	13.61 (0.60)
SD 7	1.16 (0)	3.26 (0.02)	6.40 (0.12)	10.82 (0.03)
SD 8	0 (0)	4.28 (0.15)	11.07 (0.64)	15.35 (0.03)
SD 9	6.41 (0.12)	2.70 (0.07)	4.04 (0.88)	13.15 (0)
SD 10	6.55 (0.02)	7.25 (0.02)	4.01 (1.13)	17.84 (0)

Table 4 Average amount of different sugars and average total amount sugar in the fruit juices obtained by infrared spectroscopy.

Soft drink	Average amount of sucrose (g 100 mL ⁻¹)	Average amount of glucose (g 100 mL ⁻¹)	Average amount of fructose (g 100 mL ⁻¹)	Average amount of total sugar (g 100 mL ⁻¹)
FJ 1	5.26(1.13)	5.17 (0.21)	3.96 (0.56)	14.39 (0.66)
FJ 2	3.72 (0.24)	3.77(1.72)	5.29 (1.54)	12.78 (0.58)
FJ 3	3.16 (0.36)	1.00 (0.80)	5.38 (1.05)	9.54 (0.35)
FJ 4	5.79 (0.45)	3.69 (0.63)	3.67 (0.70)	13.15 (0.44)
FJ 5	6.39 (0.21)	4.99 (0.22)	1.72 (0.30)	13.10 (0.23)
FJ 6	4.78 (0.40)	5.98(0.14)	4.65 (0.28)	15.41 (0.78)
FJ 7	0 (0)	3.81 (0.72)	6.76 (0.16)	10.57 (0.68)
FJ 8	0 (0)	3.31 (0.87)	6.64 (0.13)	9.95 (0.98)
FJ 9	0 (0)	3.43 (0.07)	7.08 (0.25)	10.51 (0.29)
FJ 10	0.32 (0.16)	3.81 (0.19)	9.25 (0.72)	13.43 (0.54)

References

- D.Y. Sun, *Yaowu Fenxi Zazhi*, 1997, **17**, 199–203.
- N.H. Low and W. South, *J. AOAC Int*, 1995, **78**, 1210–128.
- K.W. Swallow and N.H. Low, *J. AOAC Int*, 1994, **77**, 695–702.
- N.H. Low, *J. AOAC Int*, 1996, **79**, 724–737.
- N.H. Low and G.G. Wudrich, *J. Agric. Food Chem.*, 1993, **41**, 902–909.
- J.G. Stuckel and N.H. Low, *J. Agric. Food Chem.*, 1995, **43**, 3046–3051.
- L.E. Rodriguez-Saona, F. S. Fry, M.A. McLaughlin and E.M. Calvey, *Carbohydr. Res.*, 2001, **336**, 63–74.
- M. Barker and W. Rayens, *J. Chemometrics*, 2003, **17**, 166–173.
- S.K. Henderson, A.F. Carol and J.D. Domijan, *J. Chem. Educ.*, 1998, **75**, 1122–1123.
- G.P. Meade and J.C.P. Chen, *Sugar Cane Handbook*, John Wiley, New York, 1985.
- F. Cadet, D. Bertrand, P. Robert, J. Maillot, J. Dieudonne and C. Rouch, *Appl. Spectrosc.*, 1991, **45**, 166–172.
- F. Cadet and B. Offmann, *J. Agric. Food Chem.*, 1997, **45**, 166–171.
- L.E.R. Saona, F.S. Fry and E.M. Calvey, *J. Agric. Food Chem.*, 2000, **48**, 5169–5177.
- F. Cadet, *Talanta*, 1999, **48**, 867–875.
- P. Rondeau, S. Sers, D. Jhurry and F. Cadet, *Appl. Spectrosc. Rev.*, 2002, **37**, 119–136.
- P. Rondeau, D. Jhurry, S. Sers and F. Cadet, *Appl. Spectrosc.*, 2003, **57**, 466–472.
- F. Cadet and M.D.L. Guardia, *Encyclopedia of Analytical Chemistry*, (R.A. Meyers, ed.), John Wiley, New York, 2000.
- H.C. Goicoechea and A.C. Olivieri, *Talanta*, 1999, **49**, 793–800.
- M. Tsuta, J. Sugiyama and Y. Sagara, *J. Agric. Food Chem.*, 2002, **50**, 48–52.
- Y. Hasegawa, *Fresh Food Syst.*, 2000, **30**, 74–77.