APPLICATION OF INFRARED ANALYSER AND HPAEC-IPAD FOR THE QUALITY CONTROL OF HONEY SUGAR

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ABSTRACT

Honey is composed mostly by sugars, around 80% of the total content, mainly glucose and fructose. Furthermore, about 25 different oligosaccharides have been detected in the composition of honey. Fructose is present in a higher concentration than glucose and provides the extreme sweetness in honey.

Different chromatographic methods such as HPLC/RI, GC–MS and more recently HPAEC-IPAD (high-performance anion-exchange chromatography with integrated pulsed amperometric detection), have been used to evaluate the sugar content of honey.

In order to characterize the sugar content in honey and correlate it with fast analytical methods, 58 honey samples were evaluated.

The honey's sugar content was analysed in a Dionex ICS3000 ion chromatograph. Separation was performed in a column "CarboPac^{TM} PA20 3x150mm. An electrochemical detector in Integrated Pulsed Amperometric Detection (IPAD) mode was used. The elution was performed with NaOH solution (50 mM). Standard solutions of glucose, fructose and sucrose, were used to identify and quantify the individual sugar components in the honey samples. The same honey samples were also analyzed by infrared analyser (Milkoscan, Foss Instruments, DK).

The chromatographic method showed a good separation between the compounds previously reported by Antunes et al (2012).

The fructose concentrations in analysed honey were higher than the glucose concentrations, with an average value of 43 % (maximum: 39%; minimum: 48%) and 35 % (maximum: 27%; minimum: 38%) respectively for fructose and glucose.

The Fructose/glucose ratio is in accordance for both methods with an average value of 1.25 with amplitudes between 1.05 and 1.62.

The correlation observed for HPAEC-IPAD and infrared analyser were 92% for the fructose/glucose ratio.

The results show that the infrared analyser could be a good technique for screening sugar content in honey. Moreover, more samples were needed in order to have better correlation and consequently be able to use the results as a quick methodology in sugar quality control analysis.

Key words: Honey sugars, Fructose, Glucose, HPAEC-IPAD

INTRODUCTION

According Bogdanov (2009) honey is composed mainly by carbohydrates, comprising about 95 % of honey dry weight, lesser amounts of water and a great number of minor components. Fructose is present in a higher concentration than glucose and provides the extreme sweetness in honey (Cavia et al., 2002). Furthermore, about 25 different oligosaccharides have been detected in the composition of honey (Doner 1977).

The FTIR technique is also used in quality control of the different food products, with promising results for some analytical determinations (Schindler et al, 1998; Nieuwoudt et al, 2004; Velázquez et al, 2009; Lachenmeier et al, 2005; Lachenmeier, 2007; Garrigues 2000; Vonach et al 1998).

The ATR apparatus present advantages in the analysis methods due to the simplicity of instrument operation, data reproducibility and speed of analysis. FTIR-ATR has been applied in many kinds of food and beverages products (Ordóñez and Rupérez, 2012; Shiroma and Saona, 2009).

The aim of this study was to develop multivariate calibration models with data collected with FT-IR spectroscopy to evaluate the free sugars content in honey and have a fast methodology to laboratory quality control of honey sugar content.

MATERIAL AND METHODS

The 58 honeys samples in this study were obtained from commercial markets and also collected directly from beekeepers. The samples were stored in controlled temperature (15 ^{o}C), in glass vessels until analysis.

Standard solutions of glucose, fructose, sucrose, maltose, turanose, trehalose and melezitose were used to identify and quantify the individual sugar components in the honey samples.

The sugar analyse by High performance anion-exchange chromatography pulsed amperometric detection (HPAEC-PAD) was adapted from (Bogdanov et al., 1997).

The different sugars of honey samples were analysed using Dionex ICS3000 equipment. Separation of them was carried out injecting 10 μL of sample on a CarboPac™ PA20 3x150mm column with two precolumns (AminoTrap™ 3 x 30 mm and CarboPac™ PA20 3x30mm), using a flow rate of 0.5 mL min^{-1} of a 50 mM NaOH solution for 25 minutes, followed by column regeneration with 200mM NaOH solution for 10 minutes, and 20 minutes stabilization with 50 mM NaOH solution. An electrochemical detector in Integrated Pulsed Amperometric Detection (IPAD) mode was used. All analyses were carried out in duplicate.

For the protocol with Infrared analyser (MilkoscanTM, Foss Instruments, DK), five grams of honey, previously homogenized, was rigorously weighted (± 0.001g) carefully dissolved and transferred into a 50 millilitres volumetric flask.

The solution is measured in the Milkoscan™ FT120 using the "Juice and Honey calibration" to determine fructose, glucose, and sucrose concentrations.

FTIR spectra were acquired with a Bruker FT-IR spectrometer (Alpha) using a diamond single reflection attenuated total reflectance (ATR) device and a zero filling of 2. Duplicate spectra per sample were obtained with 32 scans per spectrum at a spectral resolution of 4 cm^{-1} in the wavenumber range from 400 to 4000 cm^{-1}.

Principal component analyses (PCA) and partial least squares regression (PLS-R) modelling were performed using OPUS Quant 7 (Bruker Optics, Ettlingen, Germany).

The samples were divided into two equal parts, one for calibration (50% of data) and another for validation set (50% of data).

RESULTS AND DISCUSSION

The applied chromatographic method showed a good separation between the compounds previously reported by Antunes et al (2012).

In this study, seven sugars presented in honey samples were quantified: glucose, fructose, trehalose, sucrose, turanose, maltose melezitose (Figure 1 and Table 1).

Table 1 – Mean and standard deviation (Sd) of the observed honey sugar content. (n.d. - not detected).

	mean+Sd	**Min-max**
Trehalose	0.05±0.13	n.d.0.77
Glucose	25.88±3.15	18.13-31.11
Fructose	37.01±2.95	31.07-45.26
Sucrose	0.82±0.48	n.d.-1.96
Melezitose	2.19±1.32	0.70-6.28
Turanose	2.65±0.58	1.23-3.98
Maltose	1.47±0.66	0.38-3.04
Total measured Sugars	69.28±4.08	60.16-81.80
Fructose/glucose ratio	1.49±0.19	1.07±1.79

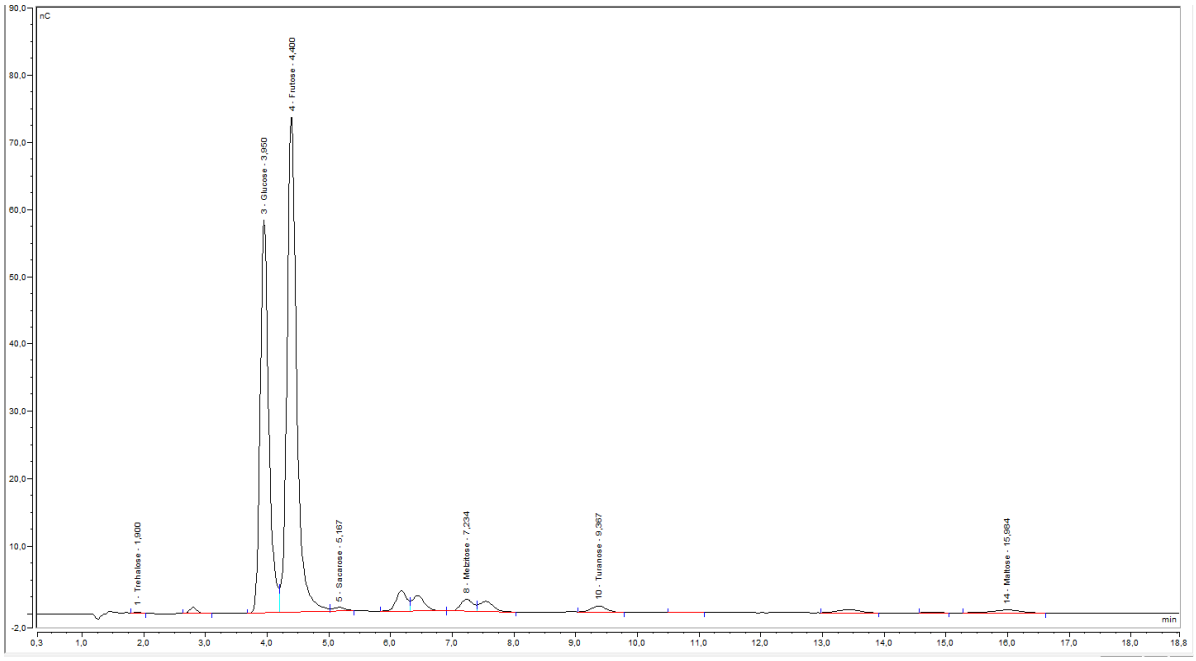

Figure 1 - HPAEC-PAD chromatographic profiles of Sugars in honey samples.

The total measured sugar content present in honey samples varies between 60.2% and 81.8%. These results are in accordance with the reported by other authors (Mendes et al, 1998; Chmielewska, 2007)

Main carbohydrates in analysed honey samples are the monosaccharides, fructose and glucose, which are products of the hydrolysis of the disaccharide sucrose. This results are similar to the previously founds by other authors and indicates that fructose and glucose are the major monosaccharides present in honey (Doner, 1977; Cotte et al 2003; Mendes et al, 1998). Fructose is the most representative monosaccharide in honey

The fructose/glucose ratios ranged from 1.07-1.79, resulting from the variety of floral sources of honey samples. Similar results were observed by Mendes et al (1998) for Portuguese honeys.

The calibration used in Foss® equipment was the calibrations for the juice and honey available in the equipment. Honey products may be analysed in this equipment using a 1:10 dilution, to lower sample viscosity.

The Foss calibration predicts the following component with interest of honey: fructose; glucose; sucrose and total sugar content (as the sum of the 3 previously sugar).

The results show that the calibration produced indicated for Juice and Honey samples are not indicate for honey samples. The slope adjustment will produce slightly better values but the residuals range from -9.4 to 8.0% for fructose and from -7.2 to 6.6% for glucose, which is not acceptable for this determination.

Figure 2 show the ATR-FTIR spectrum of the all honeys in this study. The spectrum is dominated by two water bands at 3284 cm^{-1} (OH stretch) and 1641 cm^{-1} (OH deformation) and from about 1500 to 750 cm^{-1} the contribution from mono- and disaccharides of the honey as reported by Anjos et al (2009).

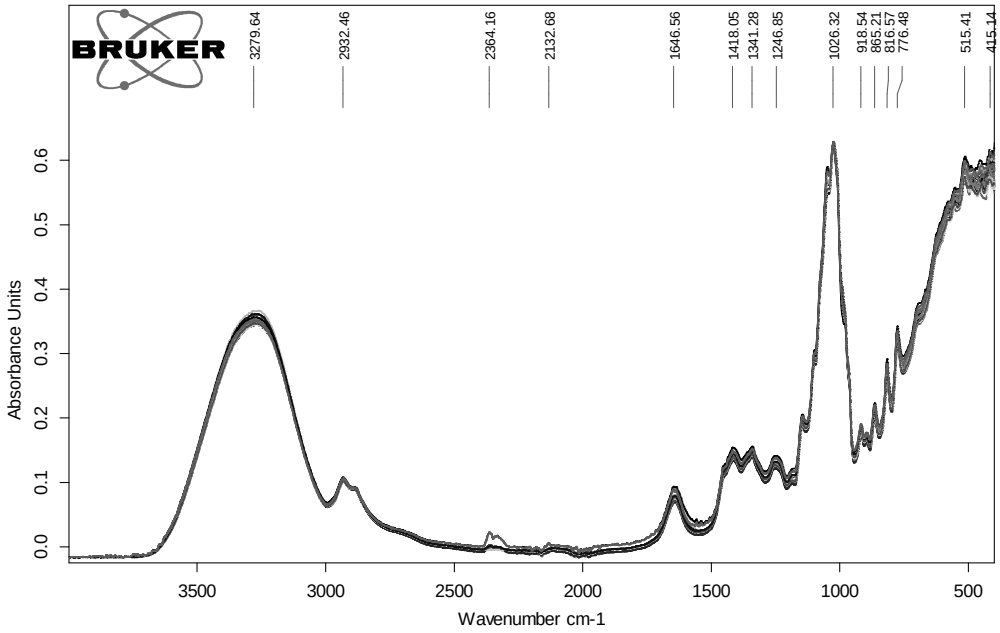

Figure 2 - FTIR- ATR spectrum of the average spectra from honeys acquired from 4000 to 450 cm^{-1}.

The appropriate calibration models for sugar determination in honey were developed based on the highest r^2, higher ratio of the standard deviation of the reference values of the validation samples standard (RPD) and lower number of factors used in the calculation. Table 2 represent the cross validation and test set validation for optimal calibration established.

Table 2 - Results from cross validation and test set validation, respectively, obtained during prediction model of the sugar honey samples.

		Cross validation		**Test set validation**	
Carbohydrate	Preprocess	r^2	RPD	r^2	RPD
glucose	1sr der + MSC	86.01	2.67	87.95	2.88
fructose	1sr der + SLS	84.60	2.55	84.15	2.52
melezitose	1sr der + SLS	92.31	3.61	89.03	3.03
turanose	1sr der + MSC	92.04	3.54	82.46	2.41
maltose	1sr der + MSC	75.74	2.03	60.46	1.59

Calibration model and Validation established with calibration models showed that the chemometric method could accurately predict the carbohydrate content in honey for glucose, fructose, melezitose and turanose content.

CONCLUSION

In the analysed honey samples the sugar content are in accordance with the standard values (Codex Alimentarius, NP-1307).

The models obtained by PLS-R modelling could be a good and faster methodology to predict the *Sugar Content* in honey samples for glucose, fructose, melezitose and turanose as main carbohydrates. For maltose more experiments would be necessary in order to find better calibration models.

The ATR-FT-IR showed to be a good methodology to quantify the sugar content in honey and easily adapted to routine analysis in this product.

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