Food Chemistry 136 (2013) 1444–1452

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Rapid analysis of sugars in honey by processing Raman spectrum using chemometric methods and artificial neural networks

Beril Özbalci [a], İsmail Hakkı Boyaci [a,*], Ali Topcu [a], Cem Kadılar [b], Uğur Tamer [c]

[a] Department of Food Engineering, Faculty of Engineering, Hacettepe University, 06800 Beytepe Ankara, Turkey
[b] Department of Statistics, Faculty of Science, Hacettepe University, 06800 Beytepe Ankara, Turkey
[c] Department of Analytical Chemistry, Faculty of Pharmacy, Gazi University, 06330 Ankara, Turkey

ARTICLE INFO

Article history:
Received 9 February 2012
Received in revised form 3 August 2012
Accepted 16 September 2012
Available online 28 September 2012

Keywords:
Honey
Glucose
Fructose
Maltose
Sucrose
Raman spectroscopy
Chemometrics
Artificial neural network

ABSTRACT

The aim of this study was to quantify glucose, fructose, sucrose and maltose contents of honey samples using Raman spectroscopy as a rapid method. By performing a single measurement, quantifications of sugar contents have been said to be unaffordable according to the molecular similarities between sugar molecules in honey matrix. This bottleneck was overcome by coupling Raman spectroscopy with chemometric methods (principal component analysis (PCA) and partial least squares (PLS)) and an artificial neural network (ANN). Model solutions of four sugars were processed with PCA and significant separation was observed. This operation, done with the spectral features by using PLS and ANN methods, led to the discriminant analysis of sugar contents. Models/trained networks were created using a calibration data set and evaluated using a validation data set. The correlation coefficient values between actual and predicted values of glucose, fructose, sucrose and maltose were determined as 0.964, 0.965, 0.968 and 0.949 for PLS and 0.965, 0.965, 0.978 and 0.956 for ANN, respectively. The requirement of rapid analysis of sugar contents of commercial honeys has been met by the data processed within this article.

1. Introduction

Honey is a natural, complex and liquid food product processed by honeybees. This nutritious food is produced by blending the sweetened sap collected from varying natural sources with metabolic gastric juice and sputtering it into beehives made of wax to be ripened. This initial sweet secretory mix is kept in a private environment where climate fluctuation leads to moisture loss. The nectar, gathered in hives, becomes dense, and the initial water content in the range of 30–70%, depending on the plant source, decreases to a final value of 17–18%. Through this maturation period, the enzymes of the gastric juice age the gathered mixture to a final product, which has previously been reported in the literature to contain water, monosaccharides (fructose and glucose), disaccharides (sucrose and maltose), minerals, amino acids, proteins and acids (Doner, 1977; Mesallam & El-Shaarawy, 1987).

The composition of honey varies depending on the region, season, variety of bee, the plant source of nectar, adulteration, etc. At this point, classification and the quality control of such a product gains great priority. The main quality factors for honey are given by Codex Alimentarius as follows: moisture content, mineral content (ash), acidity, hydroxymethylfurfural content and diastase activity (Molina, 1989; White, 1978). Although compositional factors such as sugars, acids and ash were not considered to have primary importance for quality control, some investigators believe them to be crucial (Singhal, Kulkarni, & Rege, 1997). Especially, the profiles of predominant sugars such as glucose, fructose, sucrose and maltose have been associated with a wide variety of second-order quality properties such as viscosity, hygroscopy, granulation and energy value (Ouchemouk, Schweitzer, Bey, Djoudad-Kadji, & Louaileche, 2009). Glucose/water and fructose/glucose ratios were listed as the main factors that characterise the crystallisation of honey samples (Bonvehi, 1989). It has been highlighted that the crystallisation of various honey samples based on the glucose and fructose content varied by crystal size and the rate of crystallisation, which leads to uncertainties for the honey handlers at the processing stage (Bhandari, D'Arcy, & Kelly, 1999). From this aspect, crystallisation should also be considered by the fact that it is an undesirable property in handling, processing and marketing (Assil, 1991). The content of disaccharides (mainly, maltose and sucrose) has been considered as a tool for the characterisation of honey. The maltose content with a complementary of some other oligosaccharides was used to classify Spanish honeys and to differentiate Brazilian honeys from several geographical regions (Da Costa Leite et al., 2000; Mateo & BoschReig, 1997; Ouchemouk et al., 2009; Weston & Brocklebank, 1999). Also, the relative proportions of the main sugars contained

* Corresponding author. Tel.: +90 312 297 61 46; fax: +90 312 299 21 23.
E-mail address: ihb@hacettepe.edu.tr (İ.H. Boyaci).

0308-8146/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2012.09.064

in nectars (fructose, glucose and sucrose) were established to be rather variable. However, they are quite consistent with certain botanical families. The main role of sucrose was pointed out for the characterisation of honey based on origin (Mateo & BoschReig, 1997).

For the determination of the sugar content of honey, many studies have been carried out using high-performance liquid chromatography (HPLC) equipped with a differential refractive index detector (Romero, Manzanares, García, Galdón, & Rodríguez, 2011), gas chromatography equipped with a flame ionisation detector (Kaskoniene, Venskutonis, & Ceksteryte, 2011), nuclear magnetic resonance spectroscopy (NMR), and Fourier transform infrared spectroscopy (FTIR–ATR) (Justino et al., 1998). All the procedures listed above were considered to be time-consuming, not cost effective and labour-intensive due to sample pre-treatment and the need for expensive chemicals.

Nowadays, the Raman spectroscopy is increasingly used as an analytical technique for the evaluation of food safety and quality. The principle of Raman signals arises from the inelastic scattering of the incident light from a sample and the frequency shift of the scattered light shifts in a manner of characteristic molecular vibrations (Kneipp, Kneipp, Itzkan, Dasari, & Feld, 1999). The general advantages of Raman spectroscopy over other spectroscopic systems are the non-interference from water present in the sample with the Raman measurement, ease of sampling and measurement, and minimal fluorescence interference of sample matrix varying from sample to sample. Combining Raman spectroscopy with chemometric methods and vibrational spectroscopy has enabled enormous progression for both quantitative and qualitative measurements of food components (Yang, Irudayaraj, & Paradkar, 2005).

Multivariate analysis is used in spectroscopy to extract information from complex spectra containing overlapping regions (Irudayaraj & Paradkar, 2002). The most commonly used multivariate calibration methods are principle component analysis (PCA) and partial least squares (PLS). These techniques are used to reveal the differences between apparently similar spectra by using the correlation between the collected data and the change made on variables of interest. This way, a huge amount of collected data on a spectrum can be reduced to principal components that represent the whole picture of the data (Irudayaraj & Sivakesava, 2001). The success of these methods depends upon the choice of proper spectral range, and the number of variables employed in the model (Irudayaraj & Paradkar, 2002).

In the last decade, artificial neural networks (ANNs), known as a knowledge-based approach, have been applied for the control of food quality (Huang et al., 2010; Kilic, Boyaci, Koksel, & Kusmenoglu, 2007). ANNs have been developed as generalisations of mathematical models of the human cognition system or neural biology (Fausett, 1994). Because of the outstanding utilities of this approach, it is not necessary to use any threshold values for inspection of the sample and there is no strict mathematical equation, which causes difficulties in the analysis (Baş, Dudak, & Boyacı, 2007).

The determination of glucose content in the beverage industry (Delfino et al., 2011) and the discrimination of sugar additives in honey as adulteration (Irudayaraj & Paradkar, 2002) by using Raman instrumentation have been performed previously. Considering these studies on quantification by Raman spectroscopy, the quality control of honey by determining the sugar contents seems to be a promising method. From this point of view, the determinations of glucose, fructose, sucrose and maltose contents of varying honey samples are presented in this work. The spectra obtained from 40 model mixtures and 90 honey samples were used. The collected data, from both prepared sugar mixtures and honey solutions, were processed through PCA, PLS and ANN to develop prediction models in an attempt to achieve multicomponent quantitative analysis in complex mixtures. The results were compared with the results of HPLC as a reference method.

2. Experimental

2.1. Chemicals

High-purity glucose and fructose (>99%) were purchased from Sigma–Aldrich (Taufkirchen, Germany) and used without any further treatment. Biochemical-grade sucrose and maltose powder was purchased from Acros Organics (Geel, Belgium). All solutions were prepared with ultra-pure water (18 MΩ cm) to reach the desired concentrations.

2.2. Samples

In the first part of the study, the 40 model solutions, each consisting of different ratio of glucose, fructose, sucrose and maltose mixtures, were prepared. The total sugar contents of each model solution was 20% and separately in the range of 0–14%. Model solutions were used for PCA evaluation to check possible sample grouping of Raman spectra.

In the second part of the study, 22 different honey samples (14 flower and 10 pine honeys) of varying brands were purchased from a Turkish market and a total number of 90 honey samples were prepared by blending the different brands. To obtain higher bands in Raman spectra the sugar concentrations were set to 10 times higher than that of HPLC measurement. A total of 30 g of honey was dissolved in a final volume of 100 mL for Raman, whereas 3 g was dissolved in a final volume of 100 mL for HPLC measurements. The strong bands obtained with the 30% honey concentration used during Raman have thought to increase the prediction capabilities of this method. Subsequently, these prepared solutions were centrifuged at 18,000 rpm for 10 min at room temperature and supernatants were used for measurements.

2.3. Instrumentation and sample analysis

DeltaNu Examiner Raman microscope (Deltanu Inc., Laramie, WY, USA) with a 785 nm laser source, a motorised microscope stage sample holder, and a CCD detector was used. Instrument parameters were as follows: 200 μL solution in glass Raman cuvette, 75 mW laser power, and 30 s acquisition. Spectra were obtained in the range of 200–2000 cm^{-1} at a resolution of 2 cm^{-1}. Each sample was manipulated with the built-in "automatic baseline correct" functions of the software.

Glucose, fructose, sucrose and maltose contents of the sample were also determined by the HPLC method. SpectraSystem HPLC (ThermoFinnigan Inc., CA, USA) integrated with an auto sampler including temperature control for the column (SpectraSystem AS3000), a degasser system (SpectraSystem SCM1000), a quaternary gradient pump (SpectraSystem P4000), a refractive index detector (Shodex RI-101, Showa Denko, NY, USA), and a personal computer with a software package for system control and data acquisition (ChromQuest 4.2.34) were used for analyses. The analysis of glucose, fructose, and sucrose + maltose were performed isocratically at 0.6 mL/min flow rate and at 80 °C with a 300 × 7.8 mm i.d. cation exchange column (Rezex RCM column, Ca^{2+}, 8 μm, Torrance, CA). Sucrose and maltose separation was not successful and coeluted. So, Rezex RSO column (Ag^{+}, 12 μm, 200 × 10 mm, Torrance, CA) was used for maltose determination, and separation was achieved isocratically at a flow rate of 0.3 mL/min and at 80 °C. For both HPLC columns, deionised water was used as mobile phase and injection volume was 20 μL. Quantification of sugars was based on the external standard method.