Microchemical Journal 143 (2018) 140–144

Contents lists available at ScienceDirect

Microchemical Journal

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

5-Hydroxymethyl furfural determination in Italian honeys by a fast near infrared spectroscopy

Azzurra Apriceno, Remo Bucci, Anna Maria Girelli*, Federico Marini, Ludovica Quattrocchi

Department of Chemistry, Sapienza University of Rome, P.le A. Moro5, 00185 Rome, Italy

ARTICLE INFO

Keywords:
5-Hydroxymethylfurfural
Italian honeys
NIR
HPLC

ABSTRACT

The determination of 5-hydroxymethylfurfural (HMF) content in honey samples of different botanical origin by a NIR-chemometrics-based approach is reported. Spectral regions, statistical models, scatter and derivative correction together with smoothing pre-treatment were examined and selected on the basis of correlation coefficient and root mean square errors for both the calibration (R_c^2 and RMSEC) and cross-validation (R_v^2 and RMSECV). Prediction ability was tested on an external set of honey samples and the best results were achieved with a Partial Least Square regression approach coupled with a multiplicative signal correction of the scattering in the spectral region 4252–4848 cm^1. The correlation coefficient in prediction (R^2 = 0.98) as the residual predictive deviation (RPD = 3.3) suggest that the proposed model proves to be a powerful tool to fulfil quality goals in such a complex matrix as honey.

1. Introduction

Honey comes from bees' transformation or it can be obtained by the secretion of flowers and insects as nectar and honeydew, respectively. It is essentially composed of a carbohydrates mixture (75–80%), mainly glucose and fructose, water (15–18%), organic acids (0.1–1%), minerals, nitrogenous compounds and vitamins [1].

Honey marketing is ensured when it is free from dangerous foreign substances and when its composition still remains the same. Aging is, together with moisture and cleaning, one of the most important features closely related to honey quality. Though honey can be kept for very long time, high temperatures and direct exposition to light can anyway accelerate aging. Indeed, degradation of sugars already starts at 30 °C producing some volatile and noxious furanic compounds such as 5-hydroxymethylfurfural (HMF).

HMF is a cyclic aldehyde usually absent in fresh and untreated foods [2]. Maillard reaction (non-enzymatic browning) [3] and acid-catalysed dehydration of hexones [4] are the main ways of HMF spontaneous formation. Free acidity, botanical origin, water quantity and stress during storage are, in addition to aging, the main causes that may result in an increase of HMF in foods. For this reason, the concentration of this analyte is considered a parameter related to the freshness and quality of honey, since high amounts of HMF give information about quality deterioration [5], adulteration [6], overheating [7] and stress during storage.

In past decades, the National Institute of Environmental Health Science appointed HMF for further analysis in order to have more information about its toxicity because other members of the same class showed evidence for carcinogenic potential [8]. Recent studies establish that HMF could be considered possibly and potentially carcinogenic to humans or it could be metabolized to potentially carcinogenic compounds [9].

The Codex Alimentarius [10] has established a threshold value of 80 mg/kg for the HMF content in honey after processing and blending while the Italian regulation, represented by Legislative Decree No. 179, 21 May 2004, implementing the European Union Directive (110/2001/CE) [11], recommends a limit of 40 mg/kg.

According to the official journal of the Italian Republic, the official method for the determination of HMF is high pressure liquid chromatography (HPLC) coupled with an UV–Vis detector [12]. However, traditional approaches for the evaluation of chemical composition and physical properties of honey are based on time-consuming analytical methods that require considerable sample preparation and analytical skills [13].

Near Infrared Spectroscopy (NIR) is the most attractive techniques to overcome these limitations because it offers some advantageous features: it is reliable, rapid, non-destructive, low cost and suitable for online use. This spectroscopic analytical method was widely used in honey analysis to recognize adulteration, botanical and geographical origin [14,15] or brand identification as well as to determine

* Corresponding author.
E-mail address: annamaria.girelli@uniroma1.it (A.M. Girelli).

https://doi.org/10.1016/j.microc.2018.07.038
Received 4 May 2018; Received in revised form 17 July 2018; Accepted 28 July 2018
Available online 30 July 2018

composition including fructose, glucose, sucrose, maltose, moisture, ash content. Nevertheless, minor components such as free acid, lactone, some oligosaccharides, 5-hydroxymethylfurfural have shown to be not well predicted [16].

In this work, an approach based on coupling NIR spectroscopy with chemometric calibration models is proposed with the aim to improve the determination of 5-hydroxymethylfurfural content for the quality control in 32 samples of Italian honeys of different botanical origin. This is the first effort up to now that focuses research on the exclusive analysis of HMF by near-infrared spectroscopy. In order to build the calibration model, the concentration range was extended to higher values; to this end, some honeys were heated and the final number of samples was increased to 41. Reference values were obtained analysing the same honeys by HPLC-DAD, and this allowed also a comparison between the proposed approach and the official method.

2. Materials and methods

2.1. Samples and chemicals

Thirty-two Italian honey samples were collected, trying to span as much of the botanical variability as possible; indeed, their botanical origin was: chestnut (n = 7); acacia (n = 3); thousand flowers (n = 10); eucalyptus (n = 2); honeydew (n = 2); forest honey (n = 2); lemon (n = 1); orange (n = 1), *Hedysarum coronarium* (n = 1); thyme (n = 1) and thorn (n = 1). Some of them were also thermally processed, by keeping honey immersed in boiling water (~100 °C) for a progressively increasing time. In this way the calibration range was extended, as just reported by Khalil et al. [17], and the final number of investigated samples was increased to 41. The heat treatment of samples was performed. All honey samples were stored at room temperature (25–30 °C). Methanol, ultrapure water and 5-hydroxymethyl-2-furaldehyde were purchased from Sigma-Aldrich (Sigma-Aldrich Italia s.r.l., Milan, Italy). HMF was stored at 4–8 °C.

2.2. Sample preparation

The sample solutions for the HPLC analysis were prepared dissolving 2 g of honey in 10 mL of distilled water. For NIR measurements a drop of each sample was placed between two glass slides and each sample was directly analyzed in triplicate. The results of the statistical treatments were expressed as the mean of the replicate measurements.

2.3. HPLC parameters

Chromatographic analyses were performed on an HPLC system (Shimadzu, LC-6A; Shimadzu Inc., Kyoto, Japan) equipped with a photodiode array detector (Shimadzu, SPD-M6A; Shimadzu Inc., Kyoto, Japan). 20 μL of the honey solution were injected with a micro-syringe (Hamilton Company, Reno, NV) into a system constituted by a C18 pre-column and Alltech C18 column (250 nm × 4.6 mm D.I. 5 μm; Grace, Columbia, MD). The HPLC method included an isocratic mobile phase, 90% water and 10% methanol, with a flow rate of 0.8 mL/min. The detection wavelength was set at 284 nm. The calibration curve of HMF is $y = (6 * 10^6 \pm 1.12 * 10^5)x + (1468 \pm 1.1 * 10^4)$ ($R^2 = 0.996$). The standard error on the quantification of HMF by the chromatographic method was below 5% for all the samples.

2.4. NIR measurements

NIR spectra were acquired on a Nicolet 6700 FT-NIR (Thermo Scientific Inc., Madison, WI) equipped with a tungsten-halogen source, an integrating sphere and an InGaAs detector. The signal was collected in transflectance mode, by positioning the slides that contained honey samples onto the optical window of the sphere and, in order to reflect the radiation back toward the sample, placing a small mirror on top of the slides. Spectra were registered at room temperature in the range 4000–10,000 cm^{-1} region, accumulating 82 scans at a nominal resolution of 4 cm^{-1}, and then converted in pseudo-absorbance (A = log (1/R); R = reflectance) after subtracting the blank spectrum. The acquisition of the spectroscopic data was carried by Omnicare Suite Software (Thermo Fisher Scientific Inc., Waltham, MA) as ASCII files that were processed with TQ-Analyst (Thermo Fisher Scientific Inc.) software.

2.5. Spectra pretreatments and chemometrics

In order to select the optimal model for the quantitative determination of HMF in 31 randomly chosen honey samples, different spectral pretreatments and regression approaches were evaluated. Standard Normal Variate (SNV) and Multiplicative Scatter Correction (MSC), first or second derivative as well as Savitzky-Golay smoothing were considered in order to reduce the effect of noise, avoid peaks overlapping, remove possible scattering effects that could cause scaling differences between samples and, so, enhance sample-to-sample differences. On the other hand, Principal Component Regression (PCR) and Partial Least Square (PLS) were investigated as regression models. In order to avoid over fitting, cross validation was used to select the optimal complexity of the models and for the selection of the best pretreatment. Cross-validation divides the calibration set into a defined number of groups (called “cancelation groups”), so that each group is in turn left out and treated as unknown, i.e. predicted based on a model calculated on all the other samples. In the present study, 2 samples were left out at each cross-validation fold. Model selection was carried out by considering the values of the root mean square error and the coefficient of determination in cross-validation (R^2_{CV} and RMSECV, respectively), since these indices help to evaluate the ability of the model in predicting new samples [18], but also comparing them with their corresponding values in calibration (R^2_c and RMSEC), as a further guarantee of the absence of over-fitting.

Once the best model was identified on the basis of the results in cross-validation, its performances were independently validated on a completely external set of ten honey samples, i.e., not used in any phase of model building and/or selection. The prediction capacity was assessed by calculating the values of the root mean square error and the coefficient of determination in prediction (R^2_p and RMSEP) and the residual predictive deviation (RPD), according to Zhao N. et al. [18].

For repeatability test, one random sample was predicted five times using the optimized calibration models.

3. Results and discussion

3.1.1. HPLC analysis of HMF

The HMF content of 41 samples (32 collected honeys + 9 heated samples) was first determined by the reference method based on HPLC-DAD analysis in which a preliminary treatment was necessary to prepare the honey samples, as reported in the experimental section. Specifically, for what concern heat treatment, it permitted to achieve high HMF values in order to include samples that behave as aged and/or high temperature stored honeys. Indeed, as reported by Shapla et al. [19], natural honey samples can reach high HMF values in function of their storage conditions (time and temperature) and geographical sources.

Results for all samples are summarized in Table 1, where it can be observed that, together with sufficiently representative choice of botanical origins [14] also a wide range of concentrations (2–231 mg/kg) was covered. This is the basis to obtain a reliable approach in which all the sources of variability should be included. However, from the results obtained it is clear, also, that only 5 honey comply with the condition of freshness and quality as they have a value of HMF ≤ 10 mg/kg, while