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Identification, classification, and discrimination of agave syrups from natural sweeteners by infrared spectroscopy and HPAEC-PAD

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ABSTRACT

Agave syrups are gaining popularity as new natural sweeteners. Identification, classification and discrimination by infrared spectroscopy coupled to chemometrics (NIR-MIR-SIMCA-PCA) and HPAEC-PAD of agave syrups from natural sweeteners were achieved. MIR-SIMCA-PCA allowed us to classify the natural sweeteners according to their natural source. Natural syrups exhibited differences in the MIR spectra region 1500–900 cm^{-1}. The agave syrups displayed strong absorption in the MIR spectra region 1061–1063 cm^{-1}, in agreement with their high fructose content. Additionally, MIR-SIMCA-PCA allowed us to differentiate among syrups from different *Agave* species (*Agave tequilana* and *Agave salmiana*). Thin-layer chromatography and HPAEC-PAD revealed glucose, fructose, and sucrose as the principal carbohydrates in all of the syrups. Oligosaccharide profiles showed that *A. tequilana* syrups are mainly composed of fructose (>60%) and fructooligosaccharides, while *A. salmiana* syrups contain more sucrose (28–32%). We conclude that MIR-SIMCA-PCA and HPAEC-PAD can be used to unequivocally identify and classified agave syrups.

1. Introduction

Agave syrup is the naturally sweet substance produced when agave pines are cooked. The use of food additives for adulteration or mixing agave syrup with starch, molasses, glucose, dextrin, fructose, or other sugars from sources other than agave plants are not permitted in commercial agave syrup production (Ramos, 2009). Agave syrups are in great demand as sugar substitutes because of their low glycaemic index (Foster-Powell, Holt, & Brand-Miller, 2002), antioxidant capacity (Phillips, Carlsen, & Blomhoff, 2009), and antibacterial properties (Davidson & Ortiz de Montellano, 1983). The popularity of agave syrups has led to the development of new strategies seeking to optimise agave syrup production by elaborating the syrups *via* the enzymatic hydrolysis of agave fructans instead of the traditional methods based on thermal or acid hydrolysis (García-Aguirre et al., 2009).

Vibrational spectroscopic methods [near-infrared (NIR) and mid-infrared (MIR)] in combination with chemometrics (multivariate data analysis) present a nondestructive, rapid, simple, and low-cost approach for screening samples of any type. Infrared (IR) spectroscopy has been applied to determine the presence and quantity of sugars in aqueous mixtures (Kemsley, Zhuo, Hammouri, & Wilson, 1992; Wang, Kliks, Jun, Jackson, & Li, 2010) and to authenticate the botanical and geographical origin of honey samples (Ruoff, Luginbûhl, Bogdanov, et al., 2006; Ruoff, Luginbûhl, Kûnzli, et al., 2006), allowing Irish artisanal honey to be discriminated from such honey adulterated with various sugar syrups (Kelly, Petisco, & Downey, 2006). In addition, IR spectroscopy has the potential to discriminate among and classify adulterants in maple syrups (Paradkar, Sivakesava, & Irudayaraj, 2003).

Principal components analysis (PCA) is a statistical technique that explores unsupervised pattern recognition, enabling the graphical representation of objects or variables in clusters or groups based on similarities (Cheajesadagul, Arnaudguilhem, Shiowatana, Siripinyanond, & Szpunar, 2013; Kelly et al., 2006). The aim of PCA is to express the main information contained within a larger group of variables using a smaller group of variables, defined as principal components (PCs), which describe the main sources of variation in the data. PCs are orthogonal (uncorrelated with each other), hierarchical (the first PC retains the main information of the data, the second PC retains the main information that is not included in the first PC, and so on), and calculated sequentially (Beebe, Pell, & Seasholiz, 1998; Bro & Andersson, 1998).

High-performance anion exchange chromatography with a pulsed amperometric detector (HPAEC-PAD) is recommended for carbohydrate analyses of honey samples because of its low detection limits. Carbohydrate profiles analyses are a valuable tool for characterising and classifying honeys from different botanical

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origins and geographical sources (Nozal, Bernal, Toribio, Alamo, & Diego, 2005). Indeed, HPAEC-PAD can also be used to establish carbohydrate profiles for detecting the adulteration of honey with corn sugar (Megherbi, Herbrereau, Faure, & Salvador, 2009; Morales, Corzo, & Sanz, 2008). Carbohydrate profiles combined with fructose/glucose (F/G) or maltose/isomaltose ratios have been employed to evaluate the adulteration of honey with glucose, sugar cane, or high-fructose syrups (Guler, Bakan, Nisbet, & Yavuz, 2007; Ischayek & Kern, 2006; Nozal et al., 2005).

The aim of this study was to apply NIR and MIR spectroscopic techniques in combination with chemometrics (PCA) to identify, classify, and discriminate agave syrups from other natural sweeteners. We also aimed to simultaneously assess the potential of HPAEC-PAD to establish differences in the oligosaccharide contents and profiles and the monosaccharide ratios of agave syrups and several other natural sweeteners.

2. Materials and methods

2.1. Standards

Glucose, fructose, and sucrose were acquired from Sigma–Aldrich (St. Louis, MO), maltooligosaccharides (MOS: G2–G7) from Supelco (Bellefonte, PA), and fructooligosaccharides (FOS: 1-kestose, 1-nystose and 1-kestopentaose) were obtained from Wako Pure Chemical Industries (Tokyo, Japan).

2.2. Natural syrups

A total of 43 natural sweeteners from different sources were obtained from supermarkets and convenient stores: 25 samples of agave syrup (AS1–AS25), 2 samples of corn syrup (CS1–CS2), 13 samples of honey (HB1–HB13), and 3 samples of sugar cane syrup (SCS1–SCS3). The agave syrup samples came from either *Agave tequilana* Weber blue variety (AS1–AS21) the raw material used for tequila production and hence the most economically important *Agave* species in Mexico (Aguilar-Romo, 2006) or *Agave salmiana* (AS22–AS25), which is distributed in central Mexico and used mainly for aguamiel, pulque, and mezcal production (Gentry, 1982; Martínez-Aguilar & Peña-Álvarez, 2009).

All the samples were stored at 4 °C until analysis. Immediately prior to analysis, the samples were incubated at 50 °C for 1 h, manually stirred, sonicated for 30 min to ensure homogeneity, and then maintained at room temperature (25 °C).

2.3. Physicochemical properties

The physicochemical properties of all the syrups were determined according to the specifications and test methods described in the Mexican Regulations for agave syrup, NMX-FF-110-SCFI-2008 (Ramos, 2009).

2.3.1. Total soluble solids and moisture content

The total soluble solids (°Brix) and moisture content (%*M*) were measured in an Abbe refractometer 736008 from Carl Zeiss (Jena, Germany). Two hundred microlitres of syrup were placed on the refractometer prism, and readings were taken of the °Brix scale and the refractive index. The %*M* was calculated according to the table of equivalences for refractive indexes and humidity (USDA, 1985). Before the measurements were taken, the accuracy of the refractometer was standardised with distilled water at 20 °C.

2.3.2. pH

The pH of the samples was measured on a pH meter Jenway 3510 potentiometer (Bibby Scientific Ltd., Stone, UK). Briefly, 10 g of syrup were dissolved in 75 mL of distilled water free of carbon dioxide. Two millilitres of the solution were then taken, and the pH was determined. Before the analysis, the equipment was calibrated with buffer solutions of pH 4.00 and pH 7.00 (Ramos, 2009).

2.3.3. Colour (DO 560 nm)

The colour designation of the natural syrups was determined according to the United States Standards for Grades of Extracted Honey Approved Colour Standards (USDA, 1985). In short, the light absorbance was measured at 560 nm using a glycerol solution as reference. The syrup colours were classified using the seven categories developed by USDA: water white, extra white, white, extra light amber, light amber, amber, and dark amber.

2.3.4. Statistical analysis

All analyses were carried out in triplicate and the data were expressed as means and standard deviations (SD). ANOVA analyses were performed using the Statgraphics Plus software version 5.1 (2001; StatPoint, Inc., Herndon, VA).

2.4. Infrared spectroscopy

2.4.1. Near-infrared spectroscopy

The samples were liquefied in a water bath at 50 °C for 1 h and then allowed to cool to room temperature before analysis. A 100 mg mL^{-1} solution was prepared for each sample. One millilitre of the prepared solutions was applied to the sampling plate and left to thermally equilibrate for 1 min. NIR spectra were recorded using a Paragon IdentiCheck FT-NIR spectrometer (Perkin Elmer, Beaconsfield, UK). Thirty-two scans with a resolution of 4 cm^{-1} were recorded in transmittance (%*T*) mode for each spectrum in the wavenumber range between 10,000 and 4000 cm^{-1}. Three replicate measures of each sample were taken. Spectral data collections were performed with Spectrum IdentiCheck software (Perkin Elmer).

To exclude measurement noise in the chemometric analysis, NIR spectra models were created for the spectral regions from 8000 to 4000 cm^{-1} and from 5200 to 4200 cm^{-1}, which are the dominant composition wavelength ranges of the relevant sugars (Hollung et al., 2005; Ruoff, Luginbûhl, Bogdanov, et al., 2006). The NIR analysis began with the transformation of all spectra to the absorbance mode with nine-point segment smoothing.

2.4.2. Mid-infrared spectroscopy

Fourier-transformed MIR spectra were recorded using a Perkin Elmer 1600 FT-IR Spectrometer (Perkin Elmer) equipped with a compartment horizontal attenuated total reflectance (HATR) trough top plate by use of a 45° zinc selenide (ZnSe) crystal with an 11 internal-reflections accessory (Perkin Elmer, Beaconsfield, UK). The samples were liquefied in a water bath at 50 °C for 1 h and then allowed to cool to room temperature before analysis. A 100 mg mL^{-1} solution was prepared for each sample. One millilitre was taken from each solution, applied to the flat sampling plate, and left to thermally equilibrate for 1 min. Thirty-two scans were recorded in the range between 4000 and 650 cm^{-1} at a nominal resolution of 4 cm^{-1} in transmittance mode (%*T*). Single-beam spectra of the samples were collected against an air background. Three replicate measures of each sample were taken. Spectral data collections were performed with Spectrum software (Perkin Elmer).

For the chemometric analysis, MIR spectra models were developed for carbohydrates (1185–950 cm^{-1}), proteins (1720–1480 cm^{-1}), and fatty acids (3000–2840 cm^{-1}) (Adt, Toubas, Pinon, Manfait, & Sockalingum, 2006; Kelly et al., 2006; Kemsley et al., 1992; Ruoff, Luginbûhl, Kûnzli, et al., 2006; Tewari &