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# The use of front face fluorescence spectroscopy to classify the botanical origin of honey samples produced in Switzerland

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**Abstract**

This study reports the use of front face fluorescence spectroscopy as a tool for the classification of honey samples from Switzerland according to their botanical origins. Honey ($n = 62$) fluorescence spectra from seven floral origins, namely, acacia, alpine rose, chestnut, rape, honeydew, alpine polyfloral and lowland polyfloral were scanned after excitation set at 250 nm (emission: 280–480 nm), 290 nm (emission: 305–500 nm) and 373 nm (emission: 380–600 nm) and emission set at 450 nm (excitation: 290–440 nm). The first 10 principal components (PCs) of the principal component analysis (PCA) extracted from each data set were gathered together into one matrix and analysed by factorial discriminant analysis (FDA). Correct classification of 100% and 90% was observed for the calibration and the validation samples, respectively. The seven honey types were well discriminated indicating that the molecular environment and, for consequent, the physico-chemical properties of the investigated honeys were different. The obtained results showed that front face fluorescence spectroscopy might be a suitable and alternative technique to classify honey samples according to their botanical origins.


*Keywords:* Honey; Botanical origin; Front face fluorescence spectroscopy; Chemometric

## 1. Introduction

Chemically honey is a mixture of sugars (70–80%) and water (10–20%) containing a large number of minor constituents. The monosaccharides, fructose and glucose, are the main sugars found in honey (Nagai, Inoue, Inoue, & Suzuki, 2002). The minor constituents include pollen grains, proteins, amino acids, aliphatic acid salts, lipids and flavouring components. These constituents are due to the maturation of the honey, some are added by the bees and some others are derived from the plants (Anklam, 1998). Amino acids account for 1% and proline is the major contributor with 50–80% of the total amino acids (Hermosin, Chicon, & Dolores Cabezudo, 2003).

The Commission of European Union has adopted a new Council Directive, 2001/110/EC, which repeals the Directive 74/409/ECC. The new Directive establishes the types of honeys that can be found in the European Union market and gives general information related to honey such as humidity, hydroxymethylfurfural (HMF), enzymatic activities and pesticide levels. However, these parameters have not been found to present a real relationship to both the geographical and botanical origins of such product (Fernández-Torres et al., 2005).

Microscopical analysis has been used as the traditional method to determine the botanical origins of honeys. Normally, honeys are classified as monofloral, when the pollen frequency of one plant is over 45%. Other traditional analytical and quantitative techniques including physico-chemical analysis, HPLC, GC with headspace sampling, GC–MS analysis with solid phase microextraction and

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electronic nose have also been used to classify honeys according to their botanical origins and/or their geographical origin (Ampuero, Bogdanov, & Bosset, 2004; Benedetti, Mannino, Sabatini, & Marcazzan, 2004; Bonvehi & Coll, 2003; Corbella & Cozzolino, 2006; Guyot, Scheirman, & Collin, 1999; Moreira, Trugo, Pietroluougo, & De Maria, 2002; Perez, Sanchez-Brunete, Calvo, & Tadeo, 2002; Zhou, Wintersteen, & Cadwallader, 2002). However, these techniques involve a lot of sample preparation, are time consuming and can only be carried out in a specially equipped laboratory environment by well-trained operators. Sensory evaluation by both trained taste panels and consumer panels have also been used to classify honeys as unifloral or polyfloral types (Ampuero et al., 2004). Although, this type of evaluation is important in classifying flavour characteristics according to human perception and consumer behavior, it is very subjective and involves a very costly and time consuming procedure. Taking this into account, the development of new methods for the determination of the botanical origins and/or the geographical origins is of great importance.

In this context, spectroscopic techniques are fast, relatively low-cost and provide a great deal of information with only one test. They are considered as sensitive, non-destructive, rapid, environmentally friendly and non-invasive, making them suitable for on-line or at-line process control and appropriate for process control.

The near infrared (NIR) spectroscopy has been used to: (i) classify the floral origin of Uruguayan honey samples (Corbella & Cozzolino, 2005) and European honey samples (Davies, Radovic, Fearn, & Anklam, 2002) and (ii) detect honey adulteration by addition of fructose and glucose (Downey, Fouratier, & Kelly, 2003). The mid infrared (MIR) has also been utilised for the determination of beet medium invert sugar adulteration in three different varieties of honey (Sivakesava & Irudayaraj, 2001) and for the prediction of major components of more than 1600 honey samples (Lichtenberg-Kraag, Hedtke, & Biene, 2002). The above latter authors reported that chemical composition as well as physical properties of honeys can be determined with high degree of accuracy by using Fourier-transform infrared spectroscopy. Recently, Tewari and Irudayaraj (2005) found that MIR spectra recorded directly on honeys in the 4000–600 $cm^{-1}$ spectral region by using attenuated total reflection was successfully utilised to classify honeys into seven different floral sources.

The presence of fluorophors in the form of aromatic amino acids, vitamins, cofactors and phenolic compounds in honey makes front face fluorescence spectroscopy a valuable technique to determine the botanical origins of such product. The application of autofluorescence in analysis of food has increased during the last decade, probably due to the propagated use of chemometrics. In addition, fluorescence spectroscopy offers several inherent advantages for the characterisation of molecular interactions and reactions. First, it is 100–1000 times more sensitive than other spectrophotometric techniques (Strasburg & Ludescher, 1995). Second, fluorescent compounds are extremely sensitive to their environments. It has been shown that front face fluorescence spectroscopy can discriminate milk samples subjected to heat treatment from those subjected to homogenisation (Dufour & Riaublanc, 1997) and to characterise mild heat treatment applied to milk (Kulmyrzeav, Levieux, & Dufour, 2005). Front face fluorescence spectroscopy has also been used for the determination of the geographical origins of milk (Karoui, Martin, & Dufour, 2005c), French Jura hard cheese, PDO Gruyère and L'Etivaz PDO cheeses (Karoui, Bosset, Mazerolles, Kulmyrzaev, & Dufour, 2005a) and of Emmental cheeses originated from different European countries and manufactured during winter and summer seasons (Karoui et al., 2005; Karoui et al., 2004a, 2004b).

At our best knowledge only a preliminary study has investigated the potential of front face fluorescence spectroscopy to determine the botanical origins of honey (Ruoff et al., 2005). In their research, the fluorescence spectra were analysed by using principal component analysis (PCA) and linear discriminant analysis (LDA). However, honey can be considered as complex system that has to be described by several kinds of measurements. Since several intrinsic probes were investigated in the above mentioned study, appropriate chemometric methods were required to cope with honey complexity in a very efficient way. The aim of this paper was to propose a complementary chemometric method (concatenation) to manage the whole information provided by four intrinsic probes scanned on 62 honey samples. The samples investigated in this study included those studied in a previous research (Ruoff et al., 2005) and some additional samples that have been analysed in the same condition ($n = 5$).

## 2. Materials and methods

### 2.1. Sampling and botanical classification by reference methods

A total of 62 honey samples produced in Switzerland between 1998 and 2001 were collected and stored at 4 °C until analysis. To classify these honey samples, the following measurements were determined according to the harmonised methods of the European Honey Commission (Bogdanov, Martin, & Lüllmann, 1997): electrical conductivity, sugar composition, fructose/glucose ratio, pH-value, free acidity and proline content. Pollen analysis was carried out according to DIN 10760 (DIN, 2002; Von der Ohe, Persano Oddo, Piana, Morlot, & Martin, 2004). On the basis of these analytical results and sensory panel composed of four specialists, the investigated honey samples were assigned to one of the seven honey types: acacia (*Robinia pseudoacacia*) ($n = 9$), alpine rose (*Rhododendron ferrugineum*) ($n = 8$), sweet chestnut (*Castanea sativa*) ($n = 9$), rape (*Brassica napus* var *oleifera*) ($n = 10$), honeydew ($n = 10$), alpine polyfloral ($n = 6$) and lowland polyfloral ($n = 10$) honeys.