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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.

### 2.2. Fluorescence spectroscopy

Fluorescence spectra were recorded using a FluoroMax-2 spectrofluorimeter (Spex-Jobin Yvon, Longjumeau, France) mounted with a variable angle front-surface accessory. The incidence angle of the excitation radiation was set at 56° to ensure that reflected light, scattered radiation and depolarisation phenomena were minimised. An aliquot part of 20 g of the honey samples was liquefied at 40 °C for 8 h, allowing the investigated honeys to cool at room temperature. Honey samples were pipetted in 3 mL quartz cuvette and spectra were recorded at 20 °C. Using the excitation wavelengths of 250, 290 and 373 nm, the fluorescence emission spectra were recorded from 280 to 480 nm (increment 1 nm; slits: at excitation, 3.5 and at emission, 2.0), 305–500 nm (increment 1 nm; slits: at excitation, 2.5 and at emission, 2.0) and 380–600 nm (increment 2 nm; slits: at excitation and emission, 1.5), respectively. Fluorescence excitation spectra were recorded between 290 and 440 nm with the emission wavelength set at 450 nm (increment 1 nm; slits: at excitation, 2.0 and at emission, 1.5). For each sample, three spectra were recorded using different aliquots. All spectra were corrected for instrumental distortion in excitation using a rhodamine cell in the reference channel.

### 2.3. Mathematical treatment of data

In order to reduce scattering effects and to compare the investigated honey samples, fluorescence spectra were normalised by reducing the area under each spectrum to a value of 1 according to Bertrand and Scotter (1992). Mainly the shift of the peak maximum and the peak width changes in the spectra were considered following this normalisation. A PCA was applied to the normalised spectra to investigate differences between the samples (Karoui et al., 2005). The PCA transforms the original variables into new axes called principal components (PCs), which are orthogonal, so that the data set presented on these axes are uncorrelated with each other. Therefore, PCA expresses as much as possible the total variation in the data set in only a few PCs and each successively derived PC expresses decreasing amounts of the variance. This statistical multivariate treatment was earlier used to observe similarities among different soft cheeses (Herbert et al., 2000; Karoui & Dufour, 2003), reducing the dimension to two or three PCs, while keeping most of the original information found in the data.

In a second step, factorial discriminant analysis (FDA) was performed on the first 10 PCs resulting from the PCA applied to the fluorescence spectral data sets. The aim of this technique is to predict the membership of an individual to a qualitative group defined as a preliminary (Safar, Bertrand, Devaux, & Genot, 1994). A group was created for each botanical origin of the investigated honeys. The method cannot be applied in a straightforward way to continuous spectra because of the high correlations occurring between the wavelengths. Advantages were found in the preliminary transformation of the data into their PCs. FDA assesses new synthetic variables called "discriminant factors", which are linear combinations of the selected PCs and allows a better separation of the centres of gravity of the considered groups. The individual honey sample can be reallocated within one of the seven groups. For each group, the distance from the various centre of gravity of the groups is calculated. The honey sample is assigned to the group where its distance between the centre of gravity is the shortest. Comparison of the assigned group to the real group is an indicator of the quality of the discrimination. The spectral collection was divided into two groups: two-thirds of the investigated honey samples were used for the calibration set and one-third for the validation set.

Finally, the first 10 PCs of the PCA performed on each of the fluorescence spectral data set were pooled into one matrix and this new table was analysed by FDA (Karoui et al., 2004a). The process consists in putting one beside the other in the same matrix the PCs data sets of each excitation or emission fluorescence spectra to take into account the whole information collected. This concatenation approach helps to improve the discrimination of the investigated honey according to their botanical origin. PCA and FDA were performed using StatBoxPro® Software (version 5.0, Paris, France).

## 3. Results and discussion

### 3.1. Fluorescence spectra of the investigated honey types

The emission spectra (excitation: 250 nm; emission: 280–480 nm) considered in this investigation allowed the study of the fluorescence of aromatic amino acids and nucleic acids (maximum emission at about 340 nm), as well as the fluorescent of other fluorescent products such as furosine, HMF and phenolic compounds present in honey samples. Indeed, as furosine and HMF are bound to the proteins, fluorescence transfer may occur between aromatic amino acids residues in proteins and furosine and HMF exhibiting a maximum excitation wavelength at about 360 nm (Birlouez-Aragon et al., 1998; Kulmyrzeav & Dufour, 2002).

The 280–480 nm emission spectra of the investigated honeys had broad and maxima located between 330–390 and 395–460 nm (Fig. 1), except for Chestnut which exhibited a maximum located around 380 nm (Ruoff et al., 2005). The alpine polyfloral honeys exhibited the lowest intensity at 402 and 433 nm, whereas the lowland polyfloral honeys exhibited the highest one (Fig. 1).

All the investigated honeys exhibited two maxima located at 402 and 433 nm. These peaks could be due to the presence of fluorescent Maillard reaction products such as furosine, HMF and other unknown fluorescent compounds present in honeys. These molecules (furosine and HMF) have been reported to be present in honey at differ-

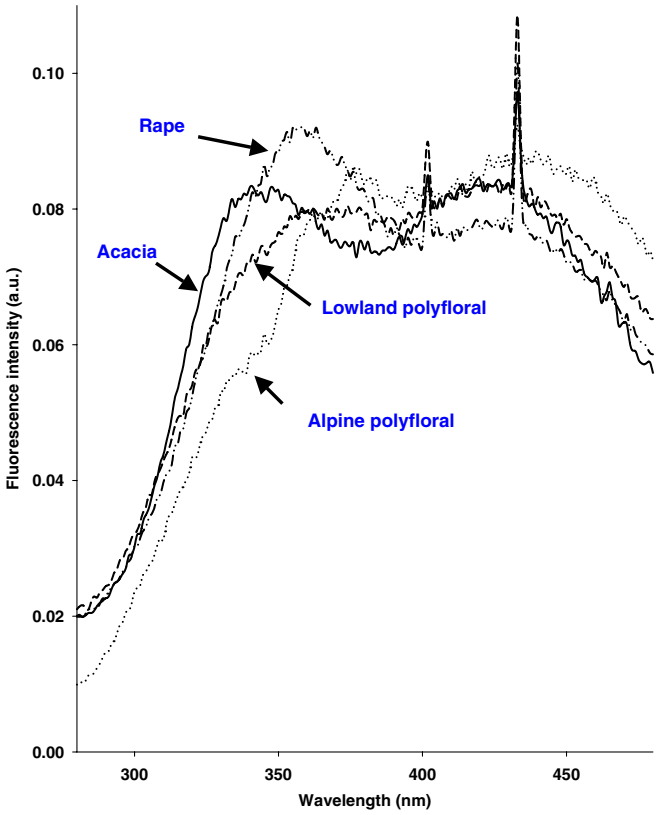


Fig. 1. Normalised fluorescence emission spectra (280–480 nm) recorded following excitation at 250 nm on acacia, alpine polyfloral, lowland polyfloral and rape honeys.

ent levels. Indeed, Sanz, Del Castillo, Corzo, and Olano (2003) reported that the level of furosine varied between 0.25 and 1.2 g × 100 $g^{-1}$ protein for different honey types. In addition, fresh honey samples obtained directly from reliable beekeepers was found to have lower levels of furosine and HMF (0.59 g × 100 $g^{-1}$ of protein and 0.70 mg × 100 $g^{-1}$ product, respectively), whereas commercial honeydew honey samples had a level of furosine and HMF of 1.13 g × 100 $g^{-1}$ of protein and 1.29 mg × 100 $g^{-1}$ product, respectively. The same researchers reported also that a low level of HMF found in fresh samples indicated that the samples are un-heated or submitted to low-heat treatment.

Honey contains a large amount of phenolic compounds. The nature and the quantity of these compounds varied widely according to the floral origins (Amiot, Aubert, Gonnet, & Tacchini, 1989). The phenolic compounds have been reported to be useful markers for the determination of floral origins of some honey samples, particularly in heather, chestnut, eucalyptus, rapeseed and lime-tree honeys (Tomás Barberán, Martos, Ferreres, Radovic, & Anklam, 2001). Since phenolic compounds exhibited emission between 360 and 420 nm after excitation set between 250 and 280 nm (Guilbault, 1973; Rodríguez-Delgado, Malovaná, Pérez, Borges, & García Montelongo, 2001), they can also induce some modification in the shape of the fluorescence spectra after excitation set at 250 nm.

From Fig. 1, it appeared that each honey type exhibited a specific fluorescence spectrum. It was concluded that emission spectra (280–480 nm) are fingerprints allowing a good identification of the botanical origin of honeys. The obtained results were in agreement with those of Bouseta, Scheirman, and Collin (1996) and Pirini, Conte, Francioso, and Lercker (1992) who reported that amino acids may be used as a tool for the determination of the botanical origins of honeys. Recently, Cometto, Faye, Di Paola Naranjo, Rubio, and Aldao (2003) have confirmed the above findings. Indeed, the above authors discriminated with great success Argentinian honeys according to their geographical and botanical origins since each cluster of honeys appeared to be associated to high or low concentration of different amino acids. On the other hand, Gilbert, Shephard, Wallwork, and Harris (1981) have obtained a good discrimination between honey samples originated from Australia, Argentine and Canada based on the analysis of 17 amino acids.

Fig. 2 showed the emission spectra of tryptophan residues in protein after excitation at 290 nm. Alpine rose and alpine polyfloral as well as rape and acacia (data not shown) honeys had maxima located around 345 nm, while chestnut honeys exhibited a maximum located around 376 nm.

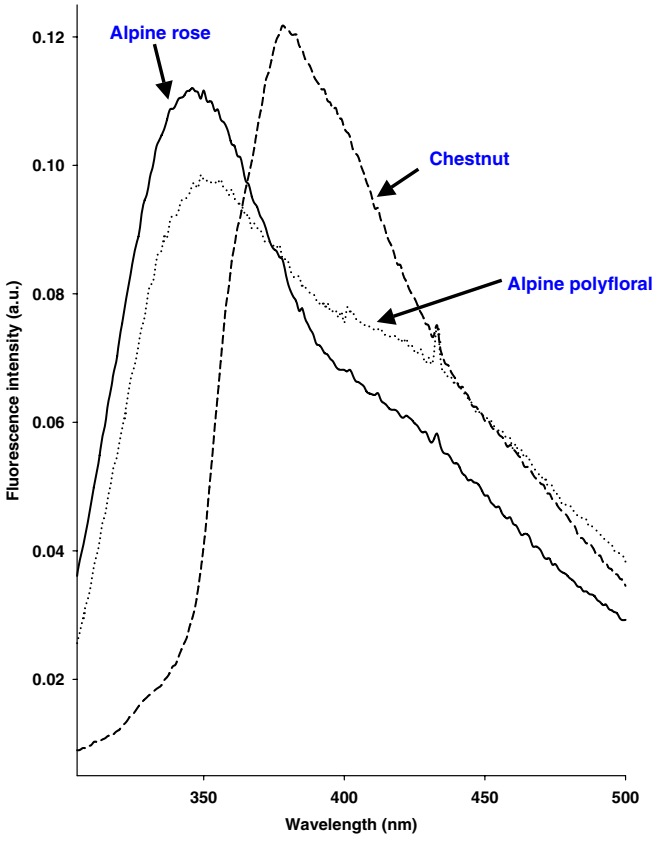


Fig. 2. Normalised fluorescence emission spectra (305–500 nm) recorded following excitation at 290 nm on alpine rose, alpine polyfloral and chestnut honeys.

From the obtained results, it appeared that the environment of tryptophan residues was more hydrophilic for chestnut honeys than for the other samples. An explanation may arise from the difference in moisture content between these honey samples. Indeed, Ouchemoukh, Louaileche, and Schweitzer (in press) reported that water content varied from 14.64 to 19.04 in some Algerian honeys. They attributed these variations in the level of moisture to the climate, season and moisture content of original plant. This difference in the level of water content could induce some changes in the shape of fluorescence spectra. Indeed, it is well known that fluorescence spectra are extremely sensitive to their environment (Dufour & Riaublanc, 1997). This environmental sensitivity enables to characterise conformational changes such as those attributable to the thermal, solvent or surface denaturation of proteins, as well as to the interactions of proteins with other components.

The pH of the investigated honeys can also have an effect on the shape of the emission spectra after excitation at 290 nm. Although honeys are acidic, they have reported to present a pH value in the range 3.5–4.5 (Ouchemoukh et al., in press). This difference in the pH value may also induce some modifications in the tertiary and quaternary structures of protein. Indeed, it has been reported that fluorescence of protein tryptophan recorded by front face fluorescence spectroscopy was a valuable tool to investigate the changes in the protein network structure and protein–water interactions induced by pH modifications during milk coagulation (Herbert, Riaublanc, Bouchet, Gallant, & Dufour, 1999).

Total soluble solids as brix could also have an influence on the shape of the fluorescence spectra. Indeed, Anupama, Bhat, and Sapna (2003) found negative strong correlation ($r = -0.99$) between moisture content and brix as well as between moisture content and viscosity ($r = -1.0$) of commercial honey samples. As explained herein above moisture content of the honey could have an effect on the shape fluorescence spectra after excitation set at 290 nm. For consequent, it can be supposed that brix can also modify the shape of the 305–500 nm emission spectra. However, much more investigation should be performed on different honey samples presenting different brix in order to confirm this hypothesis.

Again, it appeared that a spectrum recorded on a honey sample following excitation at 290 nm may be considered as a characteristic fingerprint which allows the sample to be identified.

Emission spectra recorded after excitation at 373 nm of the investigated honeys showed a maximum located around 445 nm for all the investigated honeys (Fig. 3). Fluorescent Maillard reaction products such as furosine and HMF compounds can contribute to this band. Indeed, with excitation wavelength set at 360 nm, furosine and HMF have been reported to have maximum emission at 440 and 425 nm, respectively (Birlouez-Aragon et al., 1998; Kulmyrzeav & Dufour, 2002). These molecules have been used as indicator to determine the quality of commercial honey samples (Sanz et al., 2003). Indeed, since HMF was formed during acid hydrolysis of sucrose, the presence of high levels of this compound suggests the possibility that honey samples have been adulterated with invert syrup (Swallon & Low, 1994).

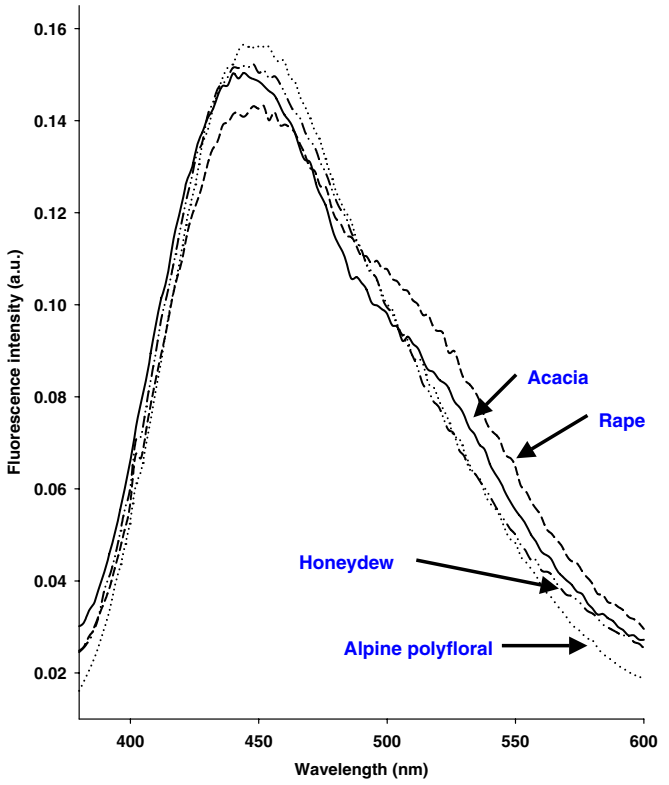


Fig. 3. Normalised fluorescence emission spectra (380–600 nm) recorded following excitation at 373 nm on acacia, alpine polyfloral, rape and honeydew honeys.

The excitation spectra (290–440 nm) recorded after emission at 450 nm are shown in Fig. 4. Most of the investigated honeys showed a maximum located around 370 nm and other narrow peaks located at around 400 nm.

For all the excitation and/or emission wavelengths used in this study, particularly after excitation set at 290 nm, each honey type represent a characteristic spectrum, which can be considered as fingerprints for the determination of the botanical origin of honeys. However, because the numbers of the wavelengths in the fluorescence spectra were much larger than the number of honey samples in the data set, it was necessary to use chemometric tools to extract information from the data set.

### *3.2. Evaluation of the discriminant ability of the investigated spectra recorded on honey types*

In a first step, PCA was applied separately to each excitation or emission wavelength. The plot of the first two PCs did not lead to a good discrimination of honeys according to their botanical origins.

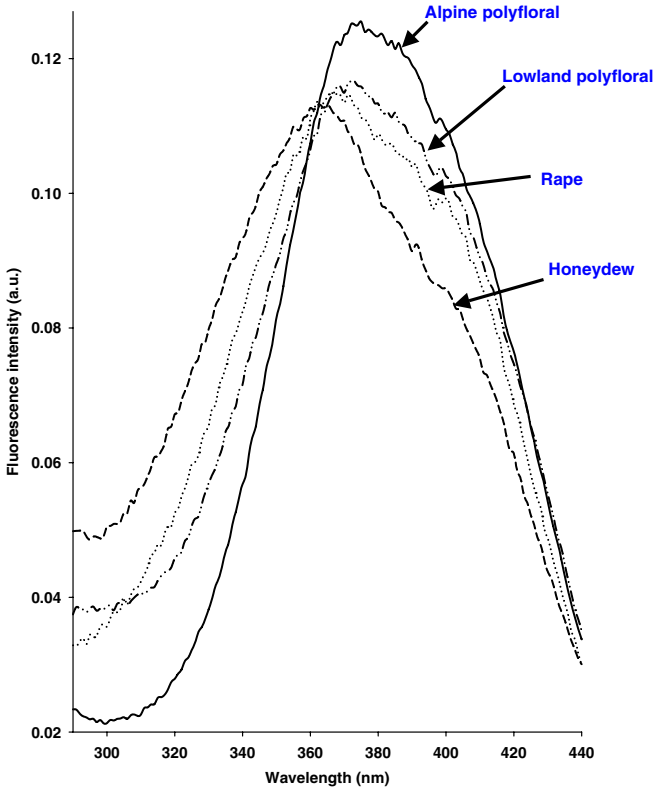


Fig. 4. Normalised fluorescence excitation spectra (290–440 nm) recorded following emission at 450 nm on alpine polyfloral, lowland polyfloral, rape and honeydew honeys.

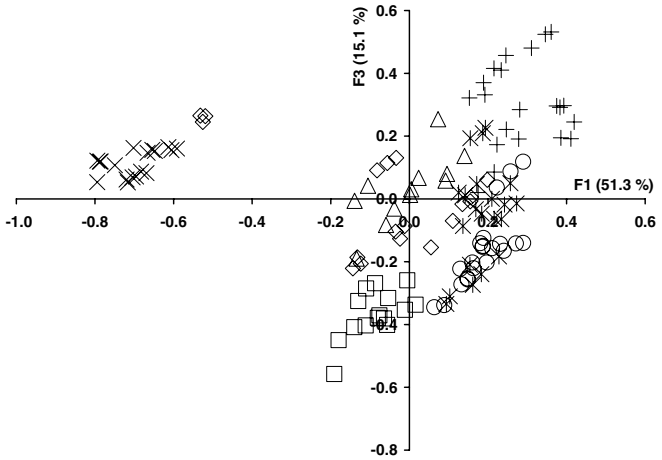


Fig. 5. Discriminant analysis similarity map of the calibration data set determined by discriminant factors 1 (F1) and 3 (F3) for the factorial discriminant analysis (FDA) performed on the fluorescence emission spectra (excitation: 250 nm; emission: 280–480 nm) of acacia (◇), alpine rose (□), alpine polyfloral (△), chestnut (×), lowland polyfloral (∗), rape (○) and honeydew (+) honey types.

In a second step, the ability of fluorescence spectra recorded after excitation set at 250 nm to differentiate honeys as a function of their botanical origins was investigated by applying FDA on the first 10 PCs of the PCA performed on emission spectra recorded between 280 and 480 nm. Before applying FDA, seven groups were defined: acacia, alpine rose, alpine polyfloral, chestnut, lowland polyfloral, rape and honeydew honeys. The map defined by the discriminant factors 1 and 3 took into account 66.4% of the total variance with discriminant factor 1 accounting for 51.3% (Fig. 5). This figure showed a good discrimination between chestnut, alpine polyfloral, alpine rose and honeydew honeys, whereas the other honey types were confused on the map.

Correct classification amounting to 89.7% and 86.7% were observed for the calibration and the validation samples, respectively (Table 1). This table shows that 100% of samples correctly classified were observed for acacia, chestnut, rape, honeydew and alpine rose honey samples. Two samples of alpine polyfloral honeys were classified as belonging to alpine rose group. The worst classification was observed for lowland polyfloral honeys since only 33.3% correct classification was observed. Indeed, among the 9 investigated lowland polyfloral honeys, 3 samples were classified as belonging to rape group and 3 others as alpine polyfloral group. This misclassification has been ascribed to the fact that this type of honey contained small amount of nectar or honeydew produced by various plant species (Ruoff et al., 2005). The same authors reported that rape is one of the most important nectar producing plants in Switzerland. In addition, most lowland polyfloral honeys contains considerable amount of this honey type, which can explains why some lowland polyfloral honeys are misclassified as unifloral rape honeys.

The similarity map of the FDA performed on the first 10 PCs of the PCA carried out on emission spectra following excitation at 290 nm showed a good discrimination of acacia, chestnut, rape and honeydew honeys, while the other honey types were overlapped on the map and confused (Fig. 6). The discriminant factor 1 accounting for 61% of the total variance discriminated honeydew honeys located on the positive side from chestnut, acacia and rape honeys which were located on the negative side. The discriminant factor 2 allowed a good discrimination of honeydew and chestnut honeys from rape and acacia honeys.

From the similarity map, it appeared that better trend of discrimination of the investigated honeys was obtained with excitation set at 290 nm than with excitation spectra set at 250 nm. Indeed, after excitation set at 290 nm, correct classification of 86.5% and 90% was observed for the calibration and validation samples, respectively. Table 1 gives the classification of the validation data set. This table shows that acacia, chestnut, rape, honeydew and alpine polyfloral honeys were 100% correctly classified. The worst classification was obtained with lowland polyfloral and alpine rose honeys since only 66.7% correct classification was obtained for each of them. As observed with excitation wavelength set at 250 nm, 3 samples of lowland polyfloral honeys were classified as belonging to alpine polyfloral group after excitation set at 290 nm. Discriminant analysis similarity map of the calibration data set determined by discriminant factors 1 and 2 of the FDA performed on

Table 1
Classification table for honey type samples based on fluorescence at different excitation and emission wavelengths of the validation data sets

| Observed[a] | Predicted[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Acacia | Chestnut | Rape | Honeydew | Alpine polyfloral | Lowland polyfloral | Alpine rose | % Correct classification |
| *Excitation: 250 nm; emission: 280–480 nm* | | | | | | | | |
| Acacia | **9** | – | – | – | – | – | – | 100 |
| Chestnut | – | **9** | – | – | – | – | – | 100 |
| Rape | – | – | **9** | – | – | – | – | 100 |
| Honeydew | – | – | – | **9** | – | – | – | 100 |
| Alpine polyfloral | – | – | – | – | **4** | – | 2 | 66.7 |
| Lowland polyfloral | – | – | 3 | – | 3 | **3** | – | 33.3 |
| Alpine rose | – | – | – | – | – | – | **9** | 100 |
| Total | – | – | – | – | – | – | – | **86.7** |
| *Excitation: 290 nm; emission: 305–500 nm* | | | | | | | | |
| Acacia | **9** | – | – | – | – | – | – | 100 |
| Chestnut | – | **9** | – | – | – | – | – | 100 |
| Rape | – | – | **9** | – | – | – | – | 100 |
| Honeydew | – | – | – | **9** | – | – | – | 100 |
| Alpine polyfloral | – | – | – | – | **6** | – | – | 100 |
| Lowland polyfloral | – | – | – | – | 3 | **6** | – | 66.7 |
| Alpine rose | 3 | – | – | – | – | – | **6** | 66.7 |
| Total | – | – | – | – | – | – | – | **90** |
| *Excitation: 373 nm; emission: 380–600 nm* | | | | | | | | |
| Acacia | **9** | – | – | – | – | – | – | 100 |
| Chestnut | – | **9** | – | – | – | – | – | 100 |
| Rape | – | – | **9** | – | – | – | – | 100 |
| Honeydew | – | – | – | **9** | – | – | – | 100 |
| Alpine polyfloral | – | – | – | 1 | **4** | 1 | – | 66.7 |
| Lowland polyfloral | – | – | 3 | 3 | – | **3** | – | 33.3 |
| Alpine rose | – | – | 3 | – | – | – | **6** | 66.7 |
| Total | – | – | – | – | – | – | – | **81.7** |
| *Emission: 450 nm; excitation: 290–440 nm* | | | | | | | | |
| Acacia | **9** | – | – | – | – | – | – | 100 |
| Chestnut | – | **9** | – | – | – | – | – | 100 |
| Rape | – | – | **9** | – | – | – | – | 100 |
| Honeydew | – | – | 1 | **7** | – | 1 | – | 77.8 |
| Alpine polyfloral | – | – | 2 | 1 | **1** | 2 | – | 16.7 |
| Lowland polyfloral | – | – | 3 | – | 6 | – | – | 0 |
| Alpine rose | – | – | – | – | – | – | **9** | 100 |
| Total | – | – | – | – | – | – | – | **73.3** |
| *Concatenation of the four investigated excitation and emission wavelengths* | | | | | | | | |
| Acacia | **9** | – | – | – | – | – | – | 100 |
| Chestnut | – | **9** | – | – | – | – | – | 100 |
| Rape | – | – | **9** | – | – | – | – | 100 |
| Honeydew | – | – | – | **9** | – | – | – | 100 |
| Alpine polyfloral | – | – | – | – | **3** | 3 | – | 50 |
| Lowland polyfloral | – | – | 3 | – | – | **6** | – | 66.7 |
| Alpine rose | – | – | – | – | – | – | **9** | 100 |
| Total | – | – | – | – | – | – | – | **90** |

[a] The number of observed honey samples.
[b] The number of predicted honey samples.

the fluorescence emission spectra (excitation: 373 nm; emission: 380–600 nm) allowed only a clear discrimination of acacia and chestnut honeys (data not shown). Correct classification was observed for 82.5% and 81.7% for the calibration and validation samples, respectively.

The percentage of samples correctly classified with the excitation spectra set at 450 nm was 89.1% and 73.3% of the calibration and validation samples, respectively. Table 1 shows that some misclassifications occurred between honeydew, alpine polyfloral and lowland polyfloral honeys, whereas acacia, chestnut, rape and alpine rose honey types were completely classified. For honeydew samples, 1 sample was assigned to rape group and another one was classified as belonging to lowland polyfloral group. Considering alpine polyfloral honey type, 5 samples were misclassified: 2 samples as rape group, 2 other samples as lowland polyfloral group and 1 sample as honeydew honey group. The worst classification was observed for lowland polyfloral honeys, where no sample was correctly classified.

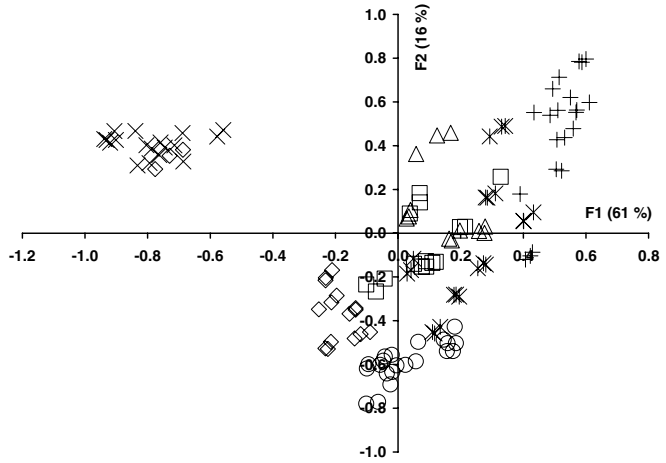


Fig. 6. Discriminant analysis similarity map of the calibration data set determined by discriminant factors 1 (F1) and 2 (F2) for the factorial discriminant analysis (FDA) performed on the fluorescence emission spectra (excitation: 290 nm; emission: 305–500 nm) of acacia (◇), alpine rose (□), alpine polyfloral (△), chestnut (×), lowland polyfloral (∗), rape (○) and honeydew (+) honey types.

From the obtained results, it appeared that excitation spectra set at 290 nm was the most interesting probe for the determination of the botanical origins of the investigated honeys. The current work confirms the high potential of this probe as a useful tool for differentiating between honey samples. It was concluded that emission spectra recorded between 305 and 500 nm are fingerprints allowing a good discrimination of Swiss honey samples according to their botanical origins.

### 3.3. Global analysis of the fluorescence spectral data sets recorded on different honey types: concatenation

Honey is a remarkably complex natural liquid that is reported to contain at least 181 substances (White, 1975); among these latter, many molecules can fluoresce. It is well known that tryptophan, aromatic amino acids and nucleic acids give information about the tertiary structure of proteins (Dufour & Riaublanc, 1997). Other molecules such as Maillard reaction products (furosine, HMF) which are present in honey (Sanz et al., 2003) provide information on the degree of lipid oxidation in the product. For exploring all these data sets, it is common to perform a multidimensional analysis on each data table as done in Section 3.2. Although, the FDA applied, separately, to the first 10 PCs of the PCA performed on each data table can lead to some discrimination between the investigated honey types, it obviously cannot allow studying all the information contained on these tables, which could contain complementary information allowing a good identification of honey samples according to their geographical origins. This can be done by other kind of methods such as concatenation, which has been used with success for the determination for the geographical origins of Emmental cheeses produced during summer and winter seasons and originating from different European countries (Karoui et al., 2004a). Thus, the first 10 PCs of the PCA applied to each of the four data sets were gathered into one matrix (40 variables) and this table was analysed by the FDA.

The map defined by the discriminant factors 1 and 2 represented 66.7% of the total variance with discriminant factor 1 accounting for 40.2% of the total variance (Fig. 7). This figure shows that all honey types can be visually discriminated.

Correct classification was observed for 100% and 90% for the calibration and validation samples, respectively (Table 1). This table gives the classification of the validation spectra for the seven groups. One hundred percent (100%) classification accuracy was achieved for acacia, chestnut, rape, honeydew and alpine rose honeys. Three alpine lowland honey types were misclassified as belonging to alpine polyfloral honeys. Considering lowland polyfloral honeys, 3 samples were classified as belonging to rape honey group. The worst classification of alpine polyfloral honeys could be due to the lower number of samples used in the validation data set.

From the obtained results, it appeared that the approach using concatenation of the four fluorescence spectral data sets allowed us to manage in a very efficient way the whole information collected on the investigated honeys. Each of the investigated probe provides information which can be used for recognising the botanical origin. It was concluded that the relationships between all the four data tables mainly lead to the assessment of two discriminant factors (F1 and F2) allowing an exhaustive characterisation of the various botanical origin of honey samples under study. Each of these discriminant factors was built using a set of information related to different aspects of the investigated honey samples. These results, which were not obtained by the FDA performed, separately, on each of the fluorescence spectral data sets showed the utility of using the concatenation methodology.

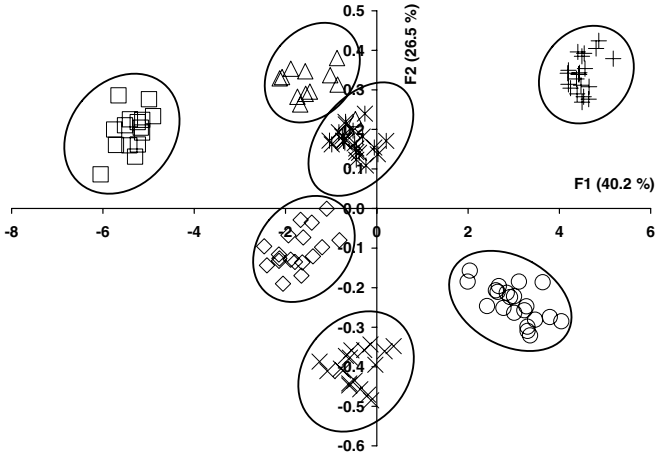


Fig. 7. Discriminant analysis similarity map of the validation data set determined by discriminant factors 1 (F1) and 2 (F2). Factorial discriminant analysis (FDA) was performed on the 40 concatenated PCs corresponding to the PCA performed on the emission spectra after excitation at 250, 290 and 373 nm and excitation spectra after emission at 440 nm of acacia (◇), alpine rose (□), alpine polyfloral (△), chestnut (×), lowland polyfloral (∗), rape (○) and honeydew (+) honey types.

## 4. Conclusion

This study demonstrates the interest of coupling different intrinsic probes recorded on honey samples to allow us to cope with honey complexity in a very efficient way. Fluorescence spectroscopy has demonstrated its feasibility to classify Swiss honeys from seven different floral sources (acacia (*R. pseudoacacia*) ($n = 9$), alpine rose (*R. ferrugineum*) ($n = 8$), sweet chestnut (*C. sativa*) ($n = 9$), rape (*B. napus* var *oleifera*) ($n = 10$), honeydew ($n = 10$), alpine polyfloral ($n = 6$), and lowland polyfloral honeys ($n = 10$)). However, the relatively limited number of samples involved in the present work led us to be caution in terms of extrapoling the results of this work to other floral honeys. A second study should be based on a sample collection system that would contain a high number of samples in order to provide a robust model to discriminate between floral origins using front face fluorescence spectroscopy.

Fluorescence spectroscopy is a very practical technology which is currently in use in many areas of agriculture and food production in the world-wide. The data analysis system is so complex that it would be almost impossible to cheat. It would obviously take some time before it would be used in routine but it mains use would be as a rapid screening tool for the identification of suspect samples which could be investigated by established techniques.

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