Total Luminescence Spectroscopy for Differentiating Between Brandies and Wine Distillates

Jana TÓTHOVÁ, Jana SÁDECKÁ and Pavel MÁJEK

Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology, Bratislava, Slovak Republic

Abstract

Tóthová J., Sádecká J., Májek P. (2007): **Total luminescence spectroscopy for differentiating between brandies and wine distillates**. Czech J. Food Sci., **27**: 425–432.

In this study, the differentiation was investigated between brandy and wine distillate samples by fluorescence spectroscopy in combination with multivariate analysis. The samples corresponding to eight brandies from three producers and sixteen wine distillates from five producers were acquired in the local supermarkets. Total luminescence spectra of diluted and undiluted samples were recorded. In order to extract reliable information from the data sets, two multivariate analysis methods, Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA), were applied separately on the excitation and emission spectra. The best differentiation was achieved using the emission spectra (400–470 nm) recorded at the excitation wavelength of 340 nm, or the excitation spectra (240–380 nm) recorded at the emission wavelength of 450 nm. The similarity map defined by the PC1 and PC2 of the PCA performed on the excitation spectra accounted for 94.9% of the total variance (PC1 90.3%, PC2 4.6%) and allowed a good discrimination between the beverages. Although the PCA similarity map defined by the PC1 (84.2%) and PC2 (13.0%) performed on the emission spectra did not lead to a clear discrimination between the beverages, a general trend pointing out the brandies and wine distillates was observed on the map. HCA performed on the excitation spectra provided a better differentiation between the two classes, without any classification error, while HCA performed on the emission spectra allowed 95.8% correct classification.

Keywords: brandy; wine distillate; fluorescence spectroscopy; multivariate data analysis

According to Regulation (EC) No. 110/2008, brandy is a spirit drink produced from wine spirit, whether blended or not with a wine distillate distilled to less than 94.8% vol., provided that that distillate does not exceed a maximum of 50% by volume of the final product. This spirit is aged for at least one year in oak receptacles or for at least six months in oak casks. Wine spirit is a spirit drink produced by the distillation to less than 86% vol. of wine or wine fortified for distillation or by the redistillation of a wine distillate to less than 86% vol. Both brandy and wine spirit shall not contain added ethanol of agricultural origin. In the Slovak Republic, there are two types of these spirits. "Wine distillates" are less expensive, and can be legally produced using wine distillates diluted with ethanol from other sources, whereas "brandy" is more expensive and should contain ethanol from grape only. Fresh wine

Supported by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic and the Slovak Academy of Sciences – VEGA, Grant No. 1/0318/08 and the Grant Agency of the Slovak Republic, Grant No. APVT-20-002904.

distillate is pure and colourless, and as such may also stay if stored in glass bottles. Brandy has to be aged for a certain period of time in oak casks. This aging involves several processes: wood structural biopolymers hydrolyse, lignins decompose with the formation of phenolic compounds, cellulose and hemicellulose depolymerise with the formation of furanic compounds, hydrolysable tannins and coumarins are extracted from the wood, and reactions may occur between the components of wood and spirit. These processes can result in significant alterations in the composition of the ageing spirit and are very important for the quality of the final products (taste, flavour, and colour) (Mosedale & Puech 1998). Wine distillates are produced using wine distillates diluted with ethanol from other sources. They are frequently blended with sugar, brandy aroma, and caramel. Some wine distillates contain honey or colorants.

The classification of brandies has become very interesting for two important reasons. The first one concerns the possibility of differentiating the product from all other similar products on the market, thus protecting its authenticity. The second reason is to find new analytical methods for monitoring the brandy composition during ageing and processing. Several analytical methods have been published for the classification of brandies. Canas *et al*. (2003) proposed and validated a direct HPLC technique for the simultaneous determination of phenolic acids, phenolic aldehydes, and furan derivates in brandies. The analysis of four-year-aged brandies showed the applicability of the method to distinguishing brandies according to the botanical species of the wooden barrel. Ng *et al.* (2000) determined 19 acids and phenolic compounds in alcoholic beverages by GC-MS method. The method required a preconcentration procedure based on solid-phase disk extraction, and in-vial elution and silylation of the analytes. The method was used to quantify age-related analytes. Capillary electrophoresis (CE) was used for the determination of some phenolic compounds in both brandy and wood extracts and the results were compared with those obtained by HPLC. Some disadvantages of CE were the low reproducibility of the migration times, and a lower sensitivity (Bronze *et al*. 1997). These conventional techniques focus on the determination of a certain marker compounds in the test product followed by the comparison of the values obtained with those previously documented for the authentic product. This approach is often time-consuming, expensive, and it requires highly trained staffs, while the range of compounds which must be quantified to ensure authenticity is continuously increasing.

The application of spectroscopic techniques in the study of the origin and differentiation between beverages has developed considerably in recent years. The advantage of these techniques is in their modest demands on the sample preparation, which makes them especially rapid and easy to apply. The analytical information contained in the spectra is multivariate in nature and, therefore, non-selective. In addition, the differences between the samples may cause very slight spectral differences that are difficult to distinguish. Due to this complexity, several chemometric techniques are usually applied to the data treatment like the principal component analysis (PCA), soft independent modelling of class analogy (SIMCA), hierarchical cluster analysis (HCA), canonical analysis (CA), discriminant analysis (DA), principal component regression (PCR), and partial least square analysis (PLS) (Massart *et al.* 1988; Beebe *et al.* 1998; Huerta *et al*. 1998). While there has been a notable growth for the application of near-infrared spectroscopy (Barboza & Poppi 2003; Pontes *et al.* 2006) and Fourier transform infrared spectroscopy in the mid-infrared range (Palma & Barroso 2002; Lachenmeier *et al*. 2005; Lachenmeier 2007), little research has been carried out using either UV-vis absorption or fluorescence spectroscopy in spirit drink authentication. Multivariate analysis applied to the derivatives of the UV-vis absorption spectra of commercially bottled tequilas can be employed to identify different brands of white tequilas, or to discriminate between 100% agave and mixed tequilas (Barbosa-García *et al.* 2007).

Fluorescence spectroscopy as a sensitive, simple, non-invasive and relatively inexpensive analytical method can be used to analyse fluorescent compounds at very low concentration levels (in the parts per billion range) while providing information on the structure, formulation, and stability (Luykx & Van Ruth 2008). The potential of using fluorescence in food research has been increasing during last years, presumably due to the promoted use of multivariate methods. Recently, a review was made of the application of fluorescence spectroscopy to foodstuffs (Sádecká & Tóthová 2007). This technique is able to define various properties of food without the use of any chemicals and time-

consuming sample preparation. Both solid and liquid samples can be used for the direct analysis of some food products. Many food products contain a lot of important intrinsic fluorophores. Edible oils (Guimet *et al.* 2006), dairy products (Diez *et al.* 2008; Karoui & De Baerdemaeker 2008), eggs, fish, (Karoui *et al.* 2006a,b), meat (Moon *et al.* 2006) and beverages (Sikorska *et al.* 2008) contain proteins including tryptophan, tyrosine, and phenylalanine residues, free aromatic amino acids, vitamins A and B, NADH, some nucleotides, chlorophyll, and numerous other compounds that can be found at low or very low concentrations (Karoui & De Baerdemaeker 2008).

The aim of this study is to demonstrate the possibilities of fluorescence spectroscopy in combination with multivariate analysis methods – principal component analysis and hierarchical cluster analysis – to differentiate between brandy and wine distillate samples.

MATERIAL AND METHODS

Samples. The samples corresponding to eight brandies (B) from three different producers (B_1, $n = 4$; B_2, $n = 2$; B_3, $n = 2$) and sixteen wine distillates (D) from five different producers (D_1, $n = 6$; D_2, $n = 6$; D_4, $n = 2$; D_5, $n = 1$; D_6, $n = 1$) were purchased from local supermarkets. Brandy B_1, a traditional Slovak product from the Small Carpathian viticulture region, is made from grapes using a classic method of aging wine spirit in small 300 l oak barrels for a minimum of five years. The wine spirit is then transferred to 20 000 l barrels for next three years. Brandy B_2 is made of pure high quality foreign wine spirit matured by natural way in oak barrels. B_3 is made of wine spirit coming from the East Slovak viticulture region and matured by natural way in oak barrels.

Wine distillates are produced using wine spirits diluted with ethanol from other sources. They are frequently blended with sugar, brandy aroma, and caramel. Wine distillates D_1 contain honey and colorants (E 102, E 110, E 120, E 122, E 132, and E 151).

The samples were stored in the dark at room temperature until the day of analysis.

Fluorescence spectroscopy. Fluorescence spectra were recorded using a Perkin-Elmer LS 50 Luminescence spectrometer equipped with a Xenon lamp. The samples were placed in 10 mm × 10 mm × 45 mm quartz cell. The excitation and emission slits were both set at 5 nm.

Fluorescence excitation spectra were recorded between 200 nm and 500 nm (increment 0.5 nm), repeatedly, at the emission wavelengths from 300 nm to 600 nm, spaced by 5 nm interval in the emission domain.

Fluorescence emission spectra were recorded from 250 nm to 700 nm (increment 0.5 nm), repeatedly, at the excitation wavelengths from 200 nm to 500 nm, spaced by 5 nm interval in the excitation domain. Fluorescence measurements were done in triplicates for each sample.

The spectrometer was interfaced to a computer supplied with FL Data Manager Software (Perkin-Elmer) for spectral acquisition and data processing.

Contour maps of total luminescence and synchronous scan fluorescence spectra were plotted using Windows-based software OriginPro 7.5 (OriginLab, USA, 2002).

Mathematical analysis of data. PCA and HCA were applied to the fluorescence spectra to investigate the differences between the samples. PCA is an unsupervised (we have no prior knowledge of the groups to be expected) pattern recognition method that reduces the dimensionality of the original data matrix while retaining the maximum amount of variability as well as recognising the data structure. PCA decomposes the data matrix with n rows (samples) and p columns (variables) into the product of the scores matrix, with n rows (samples) and $d<p$ columns (principal components, PCs), and the loadings matrix, with $d < p$ rows (PCs) and p columns (variables). The scores are the positions of the samples in the space of the principal components and the loadings are the contributions of the original variables to the PCs. All PCs are mutually orthogonal, and each successive PC contains less of the total variability of the initial data set. Usually, only a limited number $d < p$ of PCs are retained, as the variability in the others is due to noise. This reduces the dimensionality of the data considerably, enabling effective visualisation, classification, and regression of multivariate data (Geladi 2003).

HCA is an unsupervised pattern recognition method detecting natural grouping in the data. The objects are grouped in clusters in terms of their similarity. The initial assumption is that the nearness of the objects in the space defined by the variables reflects the similarity of their properties.

There are diverse possibilities and rules used to measure the distances and linkages between the individual clusters. We used hierarchical (agglomerative) cluster analysis, where the similarity extent was measured by Manhattan (city-block) distances and cluster aggregation was based on Ward's method (Otto 1999).

Microsoft Excel 2000 and Statistica software version 6.0 (StatSoft, USA, 2001) were used for statistical analysis.

RESULTS AND DISCUSSION

Undiluted brandy exhibits a high UV-Vis absorption, thus the fluorescence measured using the right-angled geometry is severely distorted due to the inner filter effects. An appropriate dilution of the samples should avoid these effects. On the other hand, dilution reduces the fluorescence intensities arising from the components which are either present at low concentrations or have low fluorescence quantum yields. Figure 1 shows the total luminescence spectra of a brandy and a wine distillate samples. The contour maps of the sample luminescence were constructed so that *x*-axis represents the emission and the y-axis the excitation wavelengths, while the contours are plotted by linking the points of equal fluorescence intensity.

In the spectra of undiluted brandy (Figure 1a), a relatively intense band with the excitation at 460 nm and emission at 540 nm is observed. Figure 1b shows the contour map for an undiluted wine distillate sample. This spectrum exhibits a considerably higher intensive band with the excitation at 396 nm and emission at 494 nm. Figure 1c shows the contour map of a diluted brandy sample (1:100 in water). Two emission bands with the excitation between 200–290 nm and emission between 360 nm and 450 nm as well as long-wavelength fluorescence with the excitation at 340 nm and emission at 450 nm are clearly observed. This spectrum exhibits no fluorescence above 450 nm in excitation. Figure 1d shows the contour map of a diluted wine distillate sample (1:100 in water). This spectrum exhibits almost

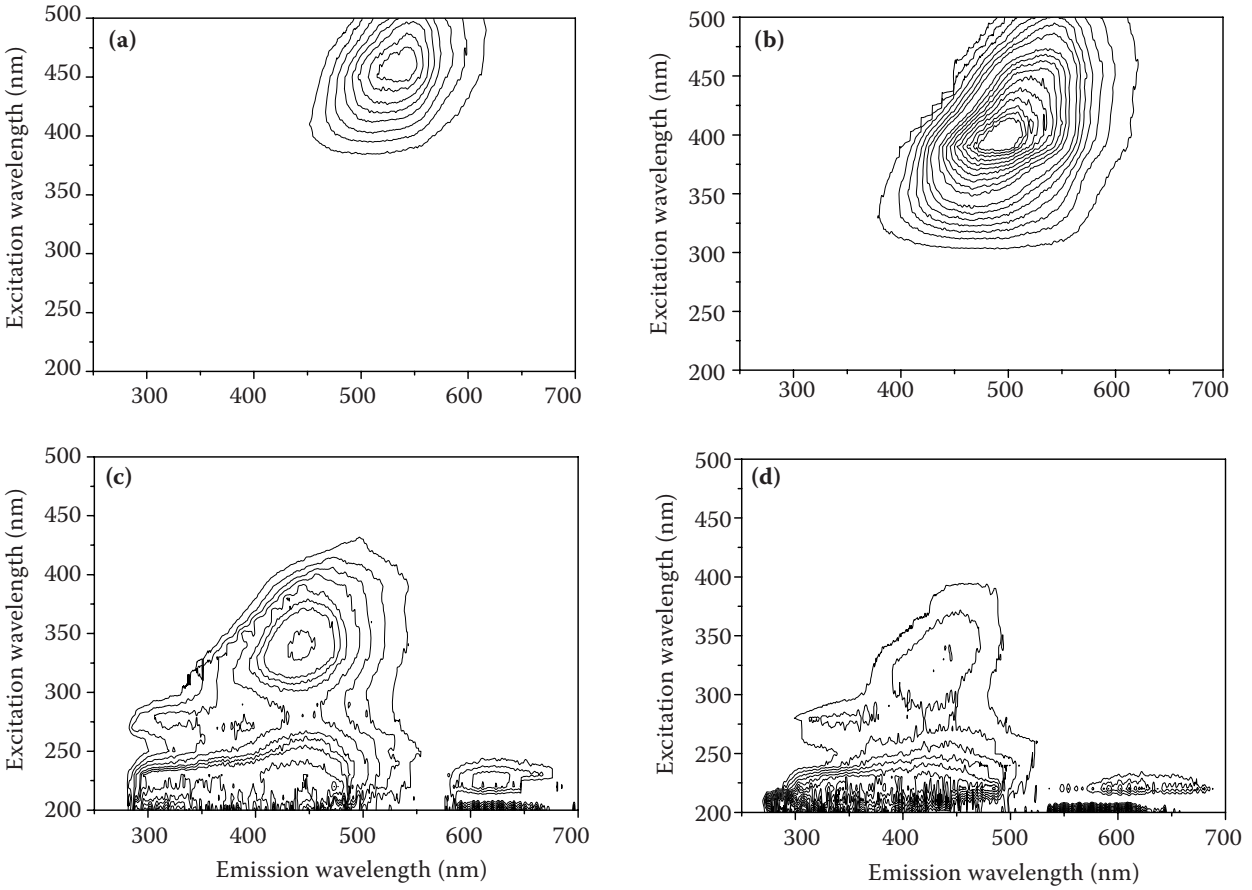

Figure 1. Contour maps of total luminescence spectra of undiluted brandy (a) and wine distillate(b) and diluted (1:100 in water) brandy (c), and wine distillate (d). Contours join the points of equal fluorescence intensity

no fluorescence above 420 nm in excitation. In comparison to brandy, wine distillate gives less intensive fluorescence bands centred at the excitation/emission wavelength pairs of 280/350 nm and 330/430 nm, respectively.

It was concluded that the longer-wavelength band was fairly intensive in the bulk samples, while the short-wavelength band could not be observed. To observe this short-wavelength emission, we recorded the total luminescence of diluted samples.

Brandy is a complex mixture consisting of a large variety of substances with different spectroscopic characteristics. Thus, the intrinsic fluorescence of brandy is the result of numerous fluorescence bands overlapping. Although the complete assignment of the fluorescence bands is beyond the scope of this work, some preliminary assignments could be made, based on the comparison of the fluorescence spectra with the fluorescent characteristics of particular beverage constituents. The fluorescence spectral properties of these selected fluorophores are presented in Table 1.

Short-wavelength fluorescence, with the excitation at 280 nm and emission maxima located at 360–370 nm and 450–460 nm, was clearly observed in diluted brandy samples, along with longer-wavelength fluorescence, with the excitation at 340 nm and emission at 450 nm. The former band was preliminarily attributed to the aromatic acids. Since phenolic compounds exhibited the emission between 360 nm and 420 nm after the excitation set between 250 nm and 280 nm, they could also modify the shape of the fluorescence spectra after the excitation at 280 nm. The latter band could be due to the presence of fluorescent cinnamic acids, coumarins – namely scopoletin, Maillard reaction products such as furosine, 5-hydroxymethylfurfuraldehyde, tannins, and other unknown fluorescent compounds present in brandies.

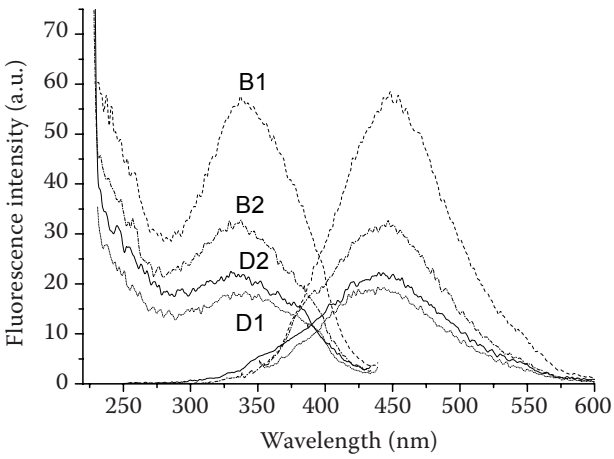

Figure 2. Fluorescence spectra of brandies (B_1, B_2) and wine distillates (D_1, D_2) from two different producers (excitation/emission wavelength pair 340/450 nm)

Although all brandies exhibit very similar fluorescence characteristics, the differences in the band positions, shapes, and relative intensities are easily noticeable. In general, the spectral features and fluorescence intensity values of all brandies are typical of brandies of similar origin and nature. The excitation/emission wavelength values of the major peaks of the brandies are generally longer than those usually measured with wine distillates. Moreover, the wine distillates exhibit considerably lower fluorescence intensities. Further, the spectra of the wine distillates D_1 reveal the presence of an additional band at 200–215 nm in excitation and 536 nm in emission.

As clearly shown in Figure 1, the shape and fluorescence intensities are different between the two classes of beverages. Consequently, total luminescence spectroscopy can be used to characterise brandy and wine distillate samples using suitable wavelength regions.

The possibility of differentiating brandies from wine distillates was investigated by using two

Table 1. Fluorescence spectral characteristics of potential intrinsic fluorophores in brandy

Compound	λ_{EX} (nm)	λ_{EM} (nm)
Benzoic acids	250–280	350–360
Cinnamic acids	260–280, 320	420
Cuomarins	340	425
Maillard reaction products	340–370	420–440
Tryptophan, tryptophol	280	357–361
Tyrosine, tyrosol	276	302

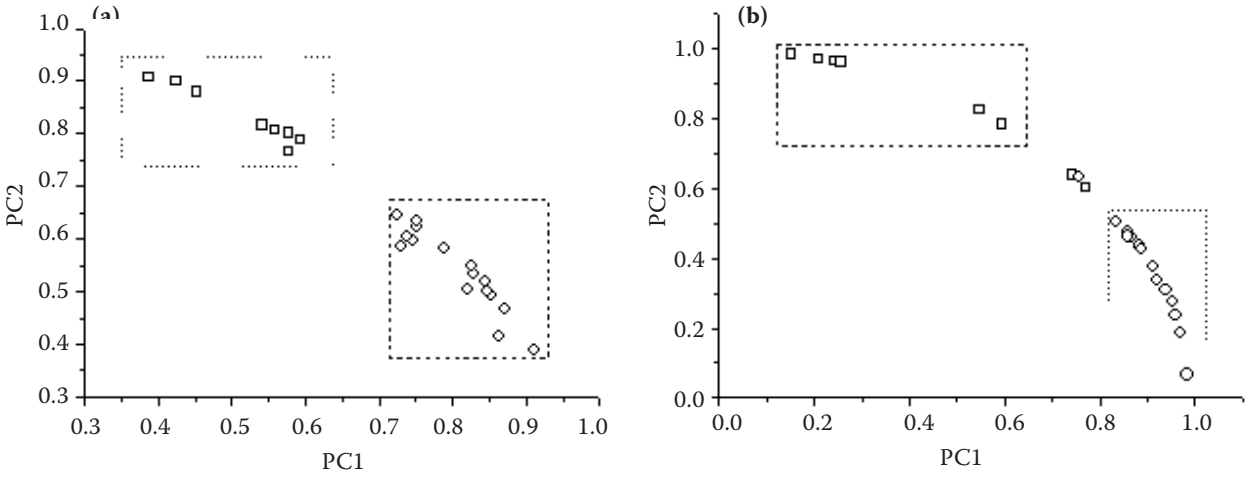

Figure 3. Principal component analysis similarity map (score plot) determined by principal component 1 (PC1) and principal component 2 (PC2) for excitation fluorescence spectra recorded at emission wavelength 450 nm (a) and emission spectra recorded after excitation at 340 nm (b) on brandy (□) and wine distillate (○) samples

multivariate analysis methods: PCA and HCA. The PCA was applied separately on the excitation and emission spectra. The best differentiation was achieved using the emission spectra (400–470 nm) recorded at the excitation wavelength 340 nm or the excitation spectra (240–380 nm) recorded at the emission wavelength 450 nm. Figure 2 illustrates the differences between the fluorescence spectra of brandies and wine distillates obtained from two different producers, as recorded at the excitation/emission wavelengths pair 340/450 nm. In general, brandies had a higher fluorescence intensity regardless of the wavelength but they were also more heterogeneous in this respect.

The similarity map defined by the PC1 and PC2 of the PCA performed on the excitation spectra accounted for 94.9% of the total variance (PC1 90.3%, PC2 4.6%) and allowed a good discrimination between the beverages (Figure 3a). While the discrimination between the brandy and wine distillate samples was achieved with the excitation data collection, a different trend was observed with the emission spectra. Although the PCA similarity map defined by the PC1 and PC2 (84.20427% and 13.0% of the total variance, respectively) did not lead to a clear discrimination between the beverages, a general trend pointing out the brandies and wine distillates was observed on the map – Figure 3b).

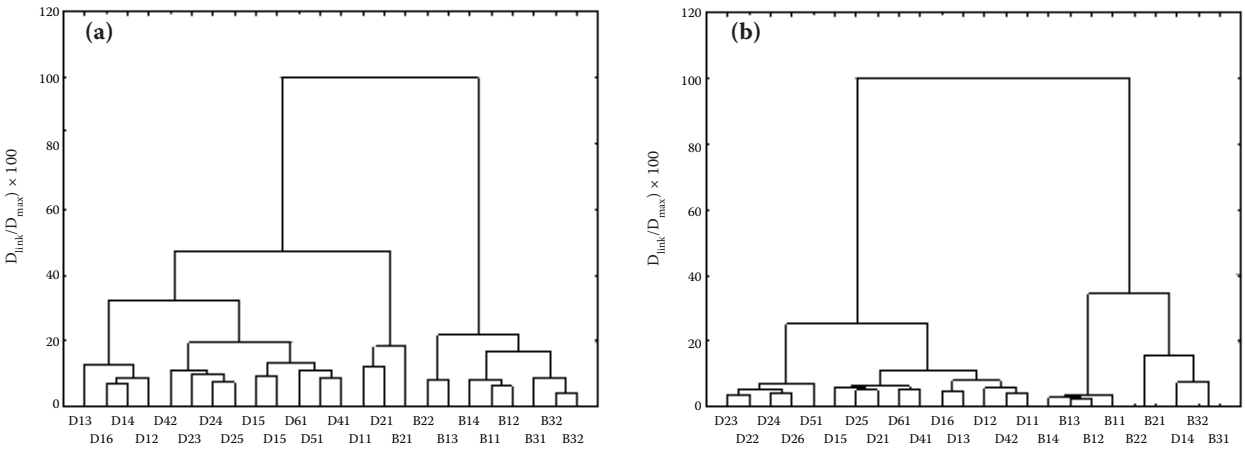

Figure 4. Hierarchical cluster analysis dendrogram using Manhattan distance for excitation fluorescence spectra recorded at emission wavelength 450 nm (a) and emission spectra recorded after excitation at 340 nm (b) on brandies (B) and wine distillates (D)

In the second step, HCA was applied separately on the excitation and emission spectra. The evaluation of the above mentioned excitation spectral region provided two main clusters (Figure 4a). The first main cluster contains wine distillate samples, while the second one contains brandy samples. Wine distillates form three smaller groups. One small group is constituted by samples D_1 with 88% of similarity between them. Another group is constituted by D_2, D_4, D_5, and D_6 with 80% of similarity between them and 68% in relation to the group D_1. The brandy samples form three small subclusters of the second main cluster. One subcluster is constituted of B_2 with 92% of similarity. Another subcluster is constituted by B_1 and B_3 with 84% of similarity between them and 78% in relation to B_1. Figure 4b shows the results from HCA concerning the emission spectra. All wine distillates are linked together in the first main cluster except sample D_{14}. This main cluster is heterogeneous enough but it consists of various small groups of very similar products. All brandies are linked together in the second main cluster. One subcluster is constituted by B_1 (brandy from producer 1) with 96% of similarity. Another subcluster is constituted of brandies B_2 and B_3 along with wine distillate D_{14} with 84% of similarity between them and 65% in relation to B_1.

CONCLUSION

Fluorescence spectroscopy coupled with multivariate data analysis methods has the potential to differentiate between brandy and wine distillate using the differences between their excitation and emission fluorescence spectra. The comparison of the results obtained from multivariate data analysis indicated that a better classification was obtained from the excitation fluorescence spectra. Thus, fluorescence spectroscopy offers a promising approach for the authentication of brandies as neither the sample preparation nor special qualification of the personnel are required, and the data acquisition and analysis are relatively simple.

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Received for publication July 11, 2008
Accepted after corrections October 13, 2009

Corresponding author:

Doc. Ing. Jana Sádecká, Ph.D., Slovenská technická univerzita v Bratislave, Ústav analytickej chémie, Radlinského 9, 812 37 Bratislava, Slovenská Republika
tel.: + 421 259 325 722, fax: + 421 252 926 043, e-mail: jana.sadecka@stuba.sk