Synchronous Fluorescence Spectroscopy for Differentiating Between Brandies and Wine Distillates

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Abstract

Synchronous scanning fluorescence spectroscopy in combination with multivariate data analysis is introduced for the characterization and classification of brandies and wine distillates. Synchronous fluorescence spectra were recorded from 220 to 700 nm with constant difference between excitation and emission wavelength $\Delta\lambda$ = 10–100 nm followed by a classification of samples using principal component analysis (PCA), hierarchical cluster analysis (HCA), and linear discriminant analysis (LDA). Using PCA, correct classification of brandy and wine distillates samples amounting to 99.5% was observed for synchronous fluorescence data set measured at $\Delta\lambda$ = 40 nm. HCA showed, that the brandy and wine distillate samples measured at $\Delta\lambda$ = 40 nm created two clusters. The first cluster included only wine distillate samples and the second one only brandy samples. LDA performed on selected wavelengths provided 99.2% of correct classification.

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

Introduction

Fluorescence spectroscopy is simple, non-destructive, non-invasive and relatively inexpensive analytical method, which can be used to the analysis of fluorescent compounds at very low concentration levels while providing information about structure, formulation, and stability (Luykx and Van Ruth 2008). In conventional fluorescence spectroscopy, two types of spectra are generally measured. When a sample is excited at a fixed wavelength λ_{ex}, an emission spectrum is produced by recording the emission intensity as a function of the emission wavelength λ_{em}. An excitation spectrum may be obtained when λ_{ex} is scanned while the

observation is conducted at the fixed λ_{em}. The broad nature and spectral overlap of conventional fluorescence spectra can be overcome, and enhanced selectivity can be obtained using synchronous fluorescence spectroscopy (SFS). In SFS, the λ_{ex} and λ_{em} are scanned simultaneously. Depending on the scan rate three basic types of SFS technique are possible (Patra and Mishra 2002). Constant-wavelength SFS is very simple technique as the scan rate is constant for both monochromators and, therefore, a constant wavelength interval, $\Delta\lambda$, is kept between λ_{em} and λ_{ex}. Variable-angle SFS is known as the second technique. The excitation and emission wavelengths may be varied simultaneously but at different rates. The third technique, constant-energy SFS, has not been used much. SFS is often considered as a convenient technique for the analysis of multi-component samples without resorting to tedious separation procedures.

Total luminescence and synchronous scanning fluorescence spectroscopic techniques have been widely used for the analysis of biological (Ni et al. 2007), environmental (Hua et al. 2005; Liu et al. 2006; Jaffrennou et al. 2007), and petrochemical samples (Ryder 2004). Recently, a review had been made of the application of fluorescence spectroscopy to foodstuffs (Sádecká and Tóthová 2007). This technique is capable to define various properties of food without use of any chemicals and time-consuming sample preparation. Both solids and liquid samples can be used for direct analysis of some food products. Food products contain a lot of important intrinsic fluorophores. Edible oils (Guimet et al. 2006), dairy products (Liu and Metzger 2007; Diez et al. 2008), honey (Ruoff et al. 2006), eggs (Karoui et al. 2006), meat (Luc et al. 2008), 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 a low or very low concentration (Karoui and De Baerdemaeker 2008). The potential of fluorescence spectroscopy combined with PCA (principal component analysis) and factorial discriminant analysis (FDA) has been used for discriminating wines according to their variety, typicality and vintage. PCA performed on the whole collection of excitation spectra allowed a good discrimination between French and German wines. Using FDA, correct classification of typical and non-typical Beaujolais amounting to 95% was observed for the emission fluorescence data set. These results showed that fluorescence spectroscopy mainly allow the identification of wines according to variety and typicality (Dufour et al. 2006). SFS and multivariate data analysis have been used for classification of differently

stored beer samples (Sikorska et al. 2006) and of different beers from different breweries (Sikorska et al. 2004). SFS is also useful for detection of olive-pomace, corn, sunflower, soybean, rapeseed, and walnut oil in virgin olive oil (Poulli et al. 2005).

The aim of the present study was to assess the potential of synchronous scanning fluorescence spectroscopy using multivariate data analysis methods to differentiate brandy and wine distillate samples. The results indicate that the synchronous fluorescence spectroscopy offers a promising approach for the authentication of brandies.

Experimental

Samples

The samples corresponding to eight brandies (B) from 3 different producers (B_1, n=4; B_2, n=2; B_3, n=2) and sixteen wine distillates (D) from 5 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 the local supermarkets. Brandy B_1, a traditional Slovak product from the Small Carpathian viticultural region, is made of the grape using a classic method of aging wine spirit in oak barrels. Brandy B_2 is made of the pure high quality foreign wine spirit matured by natural way in oak barrels. B_3 is made of the wine spirit from the East Slovak viticulture region 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). Samples were stored in the dark at room temperature until the day of analysis, diluted with water (1:100) and measured.

Wood extract

Wood slices (5 g) of approximately 2.5–4 cm thick were taken from the white oak (*Quercus alba*) log. After freeze drying the slices, the powder was obtained by grinding in a mortar with a pestle. The powder was then extracted with 20 ml of methanol:water (2:3) for two months in dark. Extract was filtered through a 0.2 mm membrane filter, diluted with water (1:100) and measured.

Fluorescence spectroscopy

Fluorescence spectra were recorded using a Perkin-Elmer LS 50 Luminescence spectrometer equipped with a Xenon lamp and a quartz cell (10 mm × 10 mm × 45 mm). Excitation and emission slits were both set at 5 nm. Synchronous fluorescence spectra were recorded by simultaneously scanning the excitation and emission monochromator in the excitation wavelength range 220–700 nm, with constant wavelength differences Δλ between them. Spectra were recorded for Δλ interval from 10 to 100 nm, in steps of 5 nm. Fluorescence measurements were done in triplicate for each sample. The spectrometer was interfaced to a computer supplied with FL Data Manager Software (Perkin-Elmer) for spectral acquisition and data processing. Fluorescence intensities were plotted as a function of the excitation wavelength. Contour maps of synchronous scan fluorescence spectra were plotted using Windows-based software OriginPro 7.5 (OriginLab, USA, 2002).

Multivariate analysis of data

PCA and HCA were applied to the fluorescence spectra to investigate differences between the samples. The aims of performing a PCA on multivariate data are two-fold. Firstly, PCA involves rotating and transforming the original, n, axes each representing an original variable into new axes. The transformation is performed in a way so that the new axes are orthogonal, i.e. the new variables are uncorrelated. It is usually the case that the number of new variables, p, needed to describe most of the sample data variance is less than n. Thus PCA affords a method to reduce the dimensionality of the parameter space. Secondly, PCA can reveal those variables, or combination of variables that determine some inherent structure in the data and these may be interpreted in physico-chemical terms (Adams 1995).

When employing hierarchical clustering techniques, the original data are separated into a few general classes, each of which is further divided into still smaller groups until finally the individual objects themselves remain. Such methods can be divisive and agglomerative. Divisive clustering starts with a single cluster, containing all samples, which is successively divided into smaller clusters. Agglomerative clustering starts with individual samples, which are fused to produce larger clusters. There are diverse rules to measure distances and linkages among individual clusters. We used agglomerative cluster analysis, where similarity extent was measured by Manhattan (city-block) distances and cluster aggregation was based on Ward's method (Otto 1999).

Finally, a supervised pattern recognition method, LDA was used to classify samples according to their origin. LDA method is an excellent tool to obtain vectors showing the maximal resolution among categories, maximal separation and compactness of the categories. Statistica software version 6.0 (StatSoft, USA, 2001) was used for statistical analysis.

Results and Discussion

Total synchronous fluorescence spectra

The contour plots of total synchronous fluorescence (TSF) spectra were obtained by plotting the fluorescence intensity (z-axis) as a function of excitation wavelength (x-axis) and wavelength interval $\Delta\lambda$ (y-axis). The TSF spectra of a brandy sample are given in Fig. 1a. It shows that the TSF contour map spreads in the excitation wavelength 220–430 nm and in the wavelength interval 10–100 nm. The plot shows three fluorescence maxima. The maximum fluorescence intensity was observed at excitation wavelength 228 nm ($\Delta\lambda = 90$ nm), 278 nm ($\Delta\lambda = 50$–100 nm) and 347 nm ($\Delta\lambda = 100$ nm) for brandy B_1, 224 nm ($\Delta\lambda = 80$ nm), 279 nm ($\Delta\lambda = 50$–100) and 338 nm ($\Delta\lambda = 100$ nm) for B_2 and 204 nm ($\Delta\lambda = 100$ nm), 278 nm ($\Delta\lambda = 50$–100) and 335 nm ($\Delta\lambda = 100$ nm) for B_3.

The TSF spectra of a wine distillate sample are given in Fig. 1b. The contour map spreads in the excitation wavelength 220–410 nm and $\Delta\lambda$ 30–100 nm. The spectra of wine distillates are characterized by two fluorescence maxima, one at ~ 210 nm and the other at ~ 335 nm, and a shoulder at about 280 nm. The maximum fluorescence intensity was observed at excitation wavelength 210 nm ($\Delta\lambda = 80$ nm) and 340 nm ($\Delta\lambda = 100$ nm) for distillate D_1 and 208 nm ($\Delta\lambda = 90$ nm) and 330 nm ($\Delta\lambda = 100$ nm) for D_2.

Generally, the fluorescence maxima shift to shorter wavelengths with increasing $\Delta\lambda$ for both brandy and wine distillates. Brandies give the longer wavelength high intensive bands while wine distillates give the shorter wavelength less intensive fluorescence bands. Fig. 1 shows the shift and amplification of synchronous fluorescence spectra of brandy and wine distillate sample using different wavelength intervals.
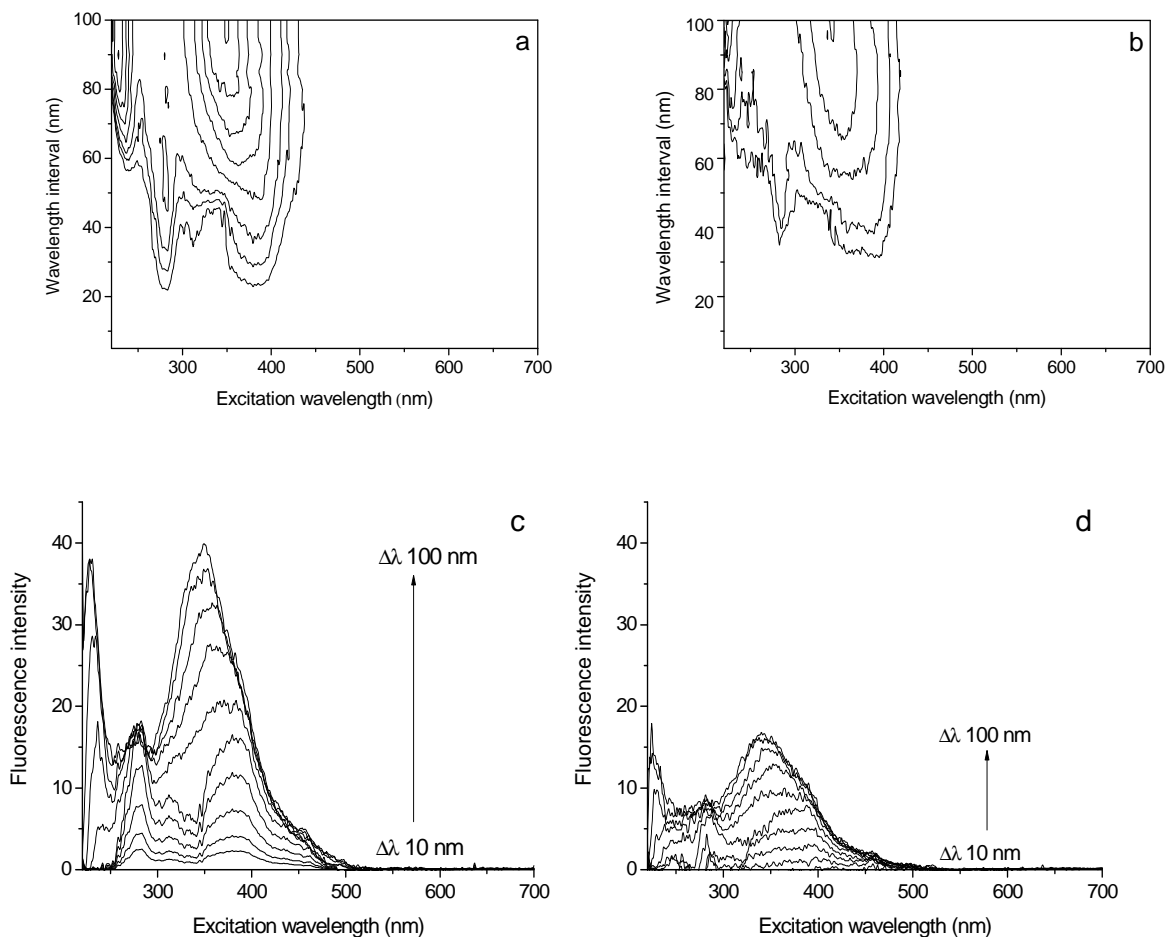
Fig. 1. Contour plots of total synchronous fluorescence spectra of brandy B_1 (a) and wine distillate D_1 (b) samples. Contours join the points of equal fluorescence intensity. Synchronous fluorescence spectra of brandy B_1 (c) and wine distillate D_1 (d) recorded at wavelength interval from 10 to 100 nm in steps of 10 nm.

For brandy B_1 (Fig. 1c), three overlapping bands with maxima at 283, 313 and 387 nm are apparent for $\lambda = 10$ nm. For $\Delta\lambda = 50$ nm, the fluorescence intensity of bands increased, changes in their relative intensities were noted, and maxima were 280, 313 and 380 nm, respectively. For $\Delta\lambda = 60$ nm, two bands with maxima at 279 and 371 nm are observed. In addition, a short-wavelength shoulder appeared with a maximum at about 238 nm. At higher $\Delta\lambda$ values, the maximum of the synchronous spectrum is shifted to the blue, with additional fluorescence intensity changes. The shape and intensity of the fluorescence maxima as well as the shape of the spectra varied from one producer to another (data not shown).

For wine distillate D_1 (Fig. 1d), two overlapping bands with maxima at 282 and 375 nm are apparent for $\Delta\lambda = 30$ nm. Increasing the $\Delta\lambda$ values to 50 nm led to an increase of fluorescence intensity; simultaneously, the long-wavelength broad band grows in intensity and its maximum shifts to 370 nm. For $\Delta\lambda = 60$ nm, two bands with maxima at 279 and 358 nm are observed. In addition, a short-wavelength shoulder appeared with a maximum at about 254 nm. At higher $\Delta\lambda$ values, the shoulder and the long-wavelength maxima are shifted to the blue, with additional fluorescence intensity changes. Analogous to brandies, the shape of the spectra varied from one producer to another (data not shown).

Multivariate analysis of synchronous fluorescence spectra

PCA was applied separately on synchronous spectra measured at $\Delta\lambda$ 10–100 nm. The best classification was achieved using fluorescence spectra recorded at $\Delta\lambda = 40$ nm. Fig. 2a shows that the plot of the first two PCs lead to a good discrimination of beverages according to origin. PC1 describes 97.4% of the total variance. The spectral pattern associated with this component shows the importance of the band with a maximum at 280 nm, and of the long-wavelength band at 340–420 nm (Fig. 2b). PC2 describes 2.1% of the total variance and is related to the changes in the 220 nm and also in the 550−700 nm bands.
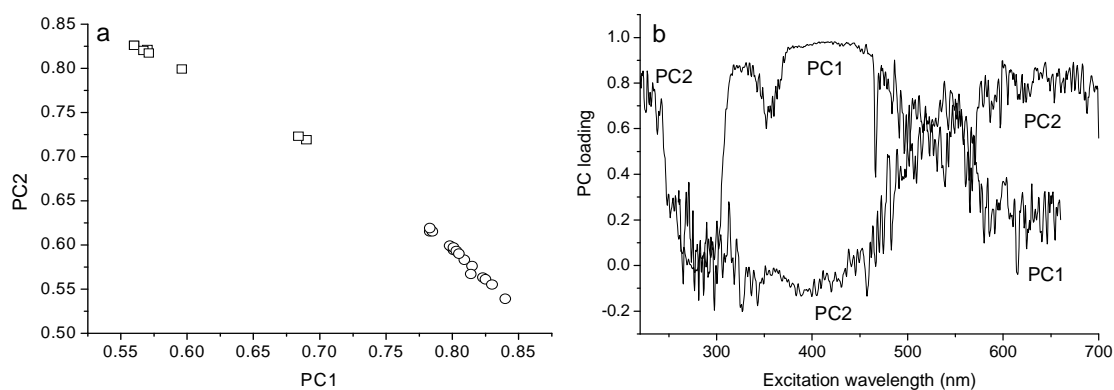

Fig. 2. Principal component analysis similarity map (score plot) determined by principal components 1 (PC1) and principal component 2 (PC2) (a) and spectral pattern corresponding to PC1 and PC2 (b) for spectra recorded at wavelength interval 40 nm on all 8 brandy samples (□) and all 16 wine distillate samples (o).

Applying HCA to fluorescence spectra recorded at $\Delta\lambda = 40$ nm, the dendrogram shows that the wine distillates are well separated from brandies (Fig. 3). The first main cluster contains wine distillate samples only, while the second one contains brandy samples. Wine

distillates cluster consists of various small groups of very similar products. One small group is constituted of samples D_2 with 95% of similarity among them. Another is constituted of D_1 with 96% of similarity among them and 90% in relation to the previous group. Brandy samples form two small subclusters of the second main cluster. One subcluster is constituted of B_2 with 97% of similarity. Another subcluster is constituted of B_1 and B_3 with 95% of similarity among them and 80% in relation to B_2.
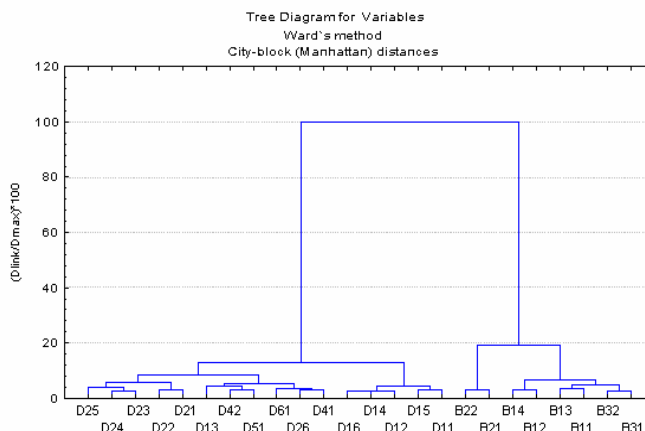

Fig. 3. Hierarchical cluster analysis dendrogram using Manhattan distance for synchronous fluorescence spectra recorded at wavelength interval 40 nm on brandy (B) and wine distillate (D) samples.

Finally, a supervised pattern recognition method, LDA, was applied to fluorescence spectra recorded at $\Delta\lambda = 40$ nm to classify samples according to their origin. LDA starts with number of objects whose group membership is known. The aim of supervised pattern recognition methods is to use these objects to find a rule for assigning a new object of an unknown group to the correct group. The starting point of LDA is to find a linear discriminant function, which is a linear combination of the original variables. For multiple groups canonical discriminant model yields more than one discriminant axis (number of categories minus one). A basic problem in LDA is deciding which variables should be included in the analysis. In stepwise discriminant function analysis, a model of discrimination is built step-by-step (forward or backward). Specifically, at each step all variables are reviewed and evaluated (Fischer's statistics - F to enter and F to remove values) to determine which one will contribute most to the discrimination between groups. This variable will then be included in the model, and the process starts again. After performing backward LDA, a classification function was obtained for individual analyzed beverages containing three variables (excitation

wavelengths): 280 nm, 350 nm, and 387 nm, which provide 99.2% correct predictions for brandies and wine distillates samples. These results show that complete synchronous spectra are not required to discriminate between beverages. Instead of them, fluorescence intensity could be measured at selected wavelengths.

Fig. 4 illustrates differences in the synchronous fluorescence spectra of brandy and wine distillate from various producers obtained at $\Delta\lambda = 40$ nm. The synchronous fluorescence spectra showed different shapes. In addition to qualitative variance, the samples also differ in fluorescence intensities of particular components. Brandies had higher fluorescence intensity regardless of wavelength but they were also more heterogeneous in this respect. Despite their general similarity, the profiles of synchronous fluorescence spectra of individual brandies vary significantly, leading to the unique spectral patterns (Fig. 4c).
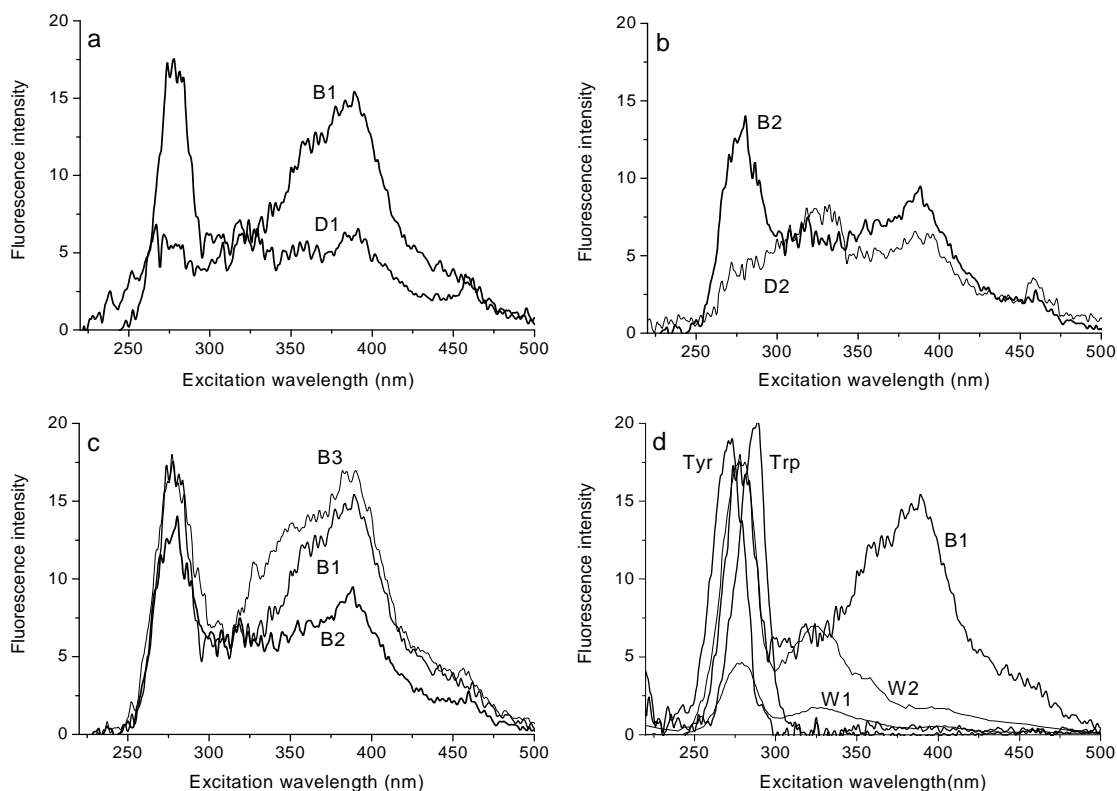

Fig. 4. Synchronous fluorescence spectra of brandy (B) and wine distillate (D) samples from three (1, 2, 3) producers (a, b, c) and of brandy, tryptophan (Trp), tyrosine (Tyr) and oak wood (W) extract (d) obtained at wavelength interval 40 nm. W1 − extracted for a week, W2 − extracted for two months.

In our previous study we assumed that the relatively narrow short-wavelength band at ~280 nm originate from the amino acids (Tóthová et al. 2009). To support this assumption,

spectra of tyrosine and tryptophan in water were recorded at $\Delta\lambda = 40$ nm. Comparison shows (Fig. 4d) that the maxima observed for tyrosine (272 nm) and tryptophan (289 nm) are not consistent with the respective maxima in brandies (278 nm). Both tryptophan and tyrosine are excited at about 280 nm, their emission spectra have an overlap in the spectral region between 300 and 320 nm. In all conventional spectroscopic techniques, the tyrosine component is completely masked by the strong tryptophan fluorescence. Therefore, the identification of the tyrosine component in the presence of tryptophan component is a challenging task.

The synchronous fluorescence technique can be applied to the resolution of tyrosine and tryptophan fluorescence. It was found that at small wavelength intervals, the synchronous fluorescence spectra of a tyrosine-tryptophan mixture solution are characteristic of tyrosine, while at large wavelength intervals, the spectra are similar to that of tryptophan (Chou et al. 1995). Fig. 5 shows the spectra of the brandy B_1 for 20- and 80-nm intervals with corresponding spectra of free tyrosine and tryptophan solution for comparison.
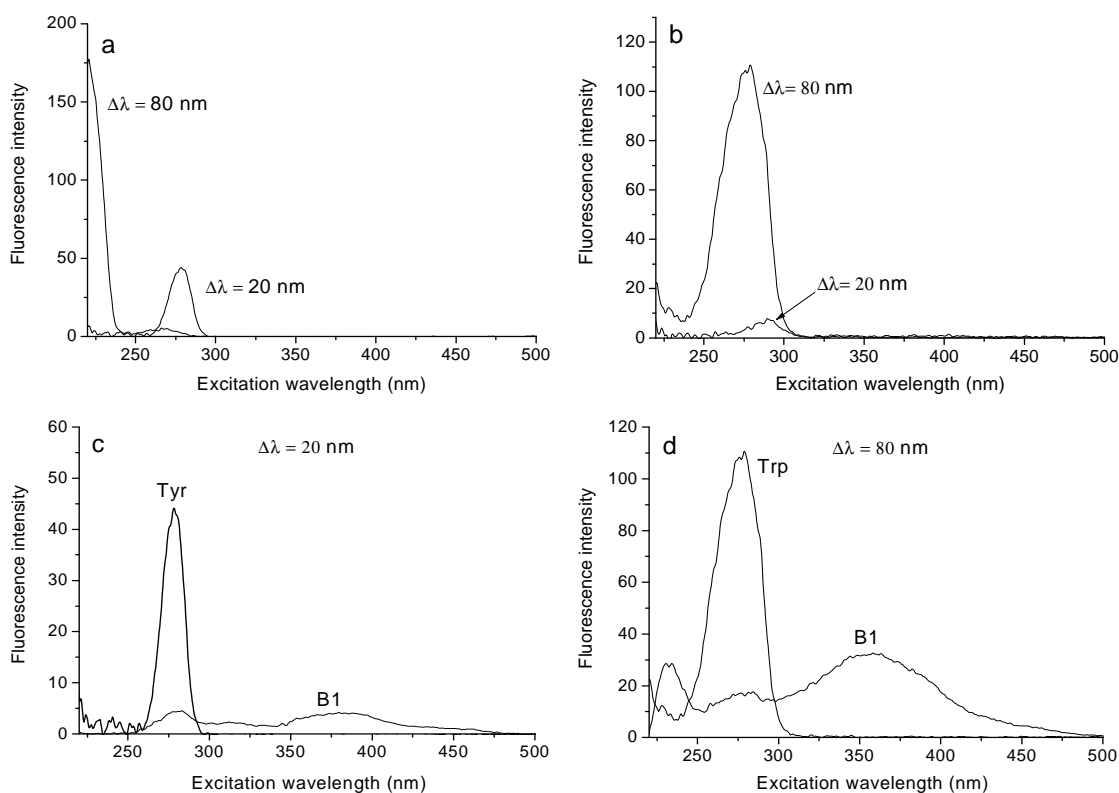

Fig. 5. Synchronous fluorescence spectra of tyrosine (a), tryptophan (b) obtained at wavelength interval 20 nm and 80 nm, brandy and tyrosine (c) at wavelength interval 20 nm, and brandy and tryptophan (d) at wavelength interval 80 nm. B1 – brandy, Tyr – tyrosine, Trp – tryptophan.

The emission of brandy at $\Delta\lambda = 20$ nm can be the contribution of tyrosine. The band appears at 280 nm and its position is consistent with the tyrosine in the aqueous solution. At $\Delta\lambda = 80$ nm, the band observed at 279 nm can be attributed to tryptophan. By comparing the fluorescence spectra of brandy with those of gallic, vanillic and syringic acids, tryptophol, tyrosol and lignin we assumed that these compounds can also contribute to the fluorescence at 280 nm. The broad band at 340–420 nm can be ascribed to the ferrulic, p-coumaric, and caffeic acids, chlorophyll, lignin, scopoletin, umbelliferon and 4-methylumbelliferon. Regardless of bands assignment, Fig. 4d proves that the fluorescent molecules in brandy originate from oak wood.

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