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Characterization and Classification of Distilled Drinks Using Total Luminescence and Synchronous Fluorescence Spectroscopy

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Abstract

This study shows that brandy, whisky, slivovice, and juniper drink can be discriminated using differences in their synchronous fluorescence spectra. Although differentiation between samples was possible by visual inspection of the spectra, it was accomplished by multivariate data analysis method more easy. Comparison of the results obtained from multivariate data analysis indicated that better classification was obtained from synchronous fluorescence spectra than from the excitation/emission fluorescence spectra. Fluorescence spectroscopy offers a promising approach for the characterisation of distillates as neither sample preparation nor special qualification of the personnel are required, and data acquisition and analysis are relatively simple.

Keywords: brandy, fluorescence, juniper drink, slivovice, whisky

Introduction

Distilled drinks range from pure aqueous alcohol, e.g. vodka, to aromatic products, such as whisky, cognac, brandy, juniper-flavored spirit drink, plum brandy. The flavorful distilled drinks are highly complex mixtures containing hundreds of individual compounds in an ethanol-water matrix. These can be grouped into three categories, the largest of which consists of the volatile congeners - compounds that are produced along with ethanol during fermentation and include higher alcohols, esters, aldehydes, and lactones. The other two categories; consist of volatile phenolics, which mostly arise from lignin breakdown products from smoke if it is used to give a characteristic flavor, and non-volatile cask extractives (Simpkins and Harrison 1995).

According to the Regulation (EC) No 110/2008 of the European Parliament brandy is a spirit drink produced from wine spirit, whether or not blended with a wine distillate, matured for at least one year in oak receptacles or for at least six months in oak casks with a capacity of less than 1,000 litres, and containing the volatile substances derived exclusively from the distillation or re-distillation of the raw materials used.

Whisky or whiskey is a spirit drink produced exclusively by distillation of a mash made from malted cereals with or without whole grains of other cereals and maturation of the final distillate for at least three years in wooden casks not exceeding 700 litres capacity. Only water and plain caramel (for colouring) may be added to final distillate.

Juniper-flavoured spirit drinks are spirit drinks produced by flavouring ethyl alcohol of agricultural origin and/or grain spirit and/or grain distillate with juniper (*Juniperus communis L.* and/or *Juniperus oxicedrus L.*) berries.

Slivovice is produced in the Czech Republic and obtained by the addition to the plum distillate, before the final distillation, of a maximum proportion of 30 % by volume of ethyl alcohol of agricultural origin. This product must be described as 'spirit drink' and may also use the name slivovice in the same visual field on the front label.

Several analytical methods have been published for characterization of distilled drinks. Gas chromatography-mass spectrometry (GC-MS) is a powerful tool in the analysis of volatile components. Various extraction methods have been widely used prior to gas chromatographic analysis, such as liquid-liquid extraction, solid-phase extraction (SPE), solid phase micro extraction (SPME), and ultrasound extraction (Park et al. 1999, Caldeira et al. 2004). HPLC has been widely used for the analysis of phenolic compounds and furanic derivatives of brandies. The method allows classification of the brandies according to the botanical species of the wood barrel (Salagoity-Auguste et al. 1987, Bocchi et al. 1996, Barroso et al. 1996, Canas et al. 2003).

Stable carbon isotopic composition ($\delta^{13}C$) of C3 plant (grape) is very distinct from C4 plant (corn and sugar cane) that are largely used for ethanol production. Thus, the $\delta^{13}C$ can be used to determine the botanical origin of ethanol (Pissinatto et al. 1999).

Analytical method used to characterize the quality of distilled drinks should to perform an analysis without sample pre-treatment. In addition, it should to accomplish a fast data acquisition and carry out the data treatment accurately with relatively low costs. The application of spectroscopic methods is a good way to reach these premises.

Near-infrared (NIR) spectroscopy is characterized by low sensitivity, thus, its application range is limited to the principal constituents. Pure alcoholic beverages (whiskey,

brandy, rum and vodka) and those adulterated with 5 % and 10 % (v/v) of water, ethanol or methanol were successfully classified using NIR spectroscopy and chemometric methods (Barboza and Poppi 2003).

In contrast to NIR, Fourier transform infrared (FTIR) spectroscopy in the mid-infrared range can give well-resolved, more specific and sensitive response peaks making the analysis of minor components like higher alcohols in spirit drinks possible with satisfactory accuracy (Lachenmeier 2007). FTIR has been employed for the characterization of brandy (Palma and Barroso 2002) or tequila (Lachenmeier 2005).

While there has been a notable growth for NIR and FTIR spectroscopy, there has been little research carried out using either UV-vis absorption or fluorescence spectroscopy in spirit drink authentication applications. Recent work using UV-vis absorption spectroscopy and multivariate analysis proved that different brands of white tequila can be identified from such a spectra, and that 100 % agave and mixed tequilas can be discriminated as well (Barbosa-García et al. 2007).

The application of fluorescence spectroscopy for direct analysis of food has increased during the last decade, probably due to the wide-spread use of multivariate data analysis methods (Christensen et al. 2006). Total luminescence and synchronous scanning fluorescence spectroscopic techniques were tested to characterize and differentiate edible oils, including soybean, sunflower, rapeseed, peanut, olive, grape seed, linseed and corn oils (Sikorska et al. 2005). Fluorescence spectra are also useful for detection of hazelnut (Sayago et al. 2004), olive-pomace, corn, sunflower, soybean, rapeseed, and walnut oil (Poulli et al. 2007) in virgin olive oil. Synchronous scanning fluorescence spectra 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).

Fluorescence spectroscopy provides information on oxidative changes in peanuts, pork scratchings, oatmeal and muesli. The differences between the fresh and oxidized samples are most evident for pork scratchings and oatmeal (Jensen et al. 2004). Tryptophan fluorescence data and infrared spectral data are fingerprints that allow accurate identification of Emmental cheese according to their manufacturing periods (summer and winter) (Karoui et al. 2004).

The aim of this paper is to show that fluorescence spectroscopy and multivariate statistical method (principal component analysis, PCA) can be used for distinguishing between commercial samples of distillates. It appears that front fluorescence spectroscopy may give a quick and non-destructive answer to the product authenticity as spectra can be recorded directly on samples.

Experimental

Samples

The studies were performed on 8 brandy (B), 6 whisky (W), ·8 juniper-flavored spirit drink (J) and 6 slivovice (S) samples purchased from the local supermarkets. 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. Excitation and emission slits were both set at 5 nm. Fluorescence excitation spectra were recorded between 200 and 500 nm (increment 0.5 nm), repeatedly, at emission wavelengths from 300 to 600 nm, spaced by 10 nm interval in the emission domain. Fluorescence emission spectra were recorded from 250 to 700 nm (increment 0.5 nm), repeatedly, at excitation wavelengths from 200 to 500 nm, spaced by 10 nm interval in the excitation domain. Synchronous fluorescence spectra were collected by simultaneously scanning the excitation and emission monochromator in the excitation wavelength range 200–700 nm, with constant wavelength differences $\Delta\lambda$ between them. Spectra were recorded for $\Delta\lambda$ interval from 10 to 100 nm, in steps of 5 nm. Fluorescence intensities were plotted as a function of the excitation wavelength. Samples were placed in 10 mm × 10 mm × 45 mm quartz cell. Fluorescence measurements were done in triplicate for each sample. The spectrometer was connected 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).

Multivariate analysis of data

To investigate the differences between fluorescence spectra of the samples PCA was applied. 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 recognizing the data structure. PCA decomposes a data matrix with n rows (samples) and p columns (variables) into the product of a scores matrix, with n rows (samples) and $d<p$ columns (principal components, PCs), and a 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 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 visualization, classification, and regression of multivariate data.

Results and Discussion

Total luminescence spectra

Fig. 1 shows total luminescence spectra of the brandy, whisky, slivovice, and juniper drink as contour maps, constructed in such a way that x-axis represents the emission and y-axis the excitation wavelength, while the contours are plotted by linking the points of equal fluorescence intensity.

Brandy total luminescence contour map spreads in the excitation wavelength range 380–500 and emission wavelength range 430–610 nm. The contours for brandy are concentrated in the emission wavelength region 510–570 nm and excitation wavelength region 430–480 nm. In general, spectral features and fluorescence intensity values of all brandies are typical of brandies of similar origin and nature. The fluorescence spectra of brandies are characterized by the main fluorophores centered at an excitation/emission wavelength pair of 460/540 nm.

Whisky total luminescence contour map spreads in the excitation wavelength range 325–500 and emission wavelength range 410–610 nm. The contours for brandy are concentrated in the emission wavelength region 470–520 nm and excitation wavelength region 380–420 nm. The fluorescence spectra are characterized by the main peak centred at an excitation/emission wavelength pair of 410/498 nm.

Slivovice total luminescence contour map lies in the excitation wavelength range 210–500 and emission wavelength range 370–600 nm. The contours are concentrated in the emission wavelength region 440–500 nm and excitation wavelength region 320–370 nm. The fluorescence spectra exhibited the main peak centred at an excitation/emission wavelength pair of 345/474 nm.

Juniper spirit total luminescence contour map spreads in the excitation wavelength range 200–420 and emission wavelength range 280–700 nm. The spectra reveal the presence of three bands at 220/340, 270/324, and 292/406 nm.

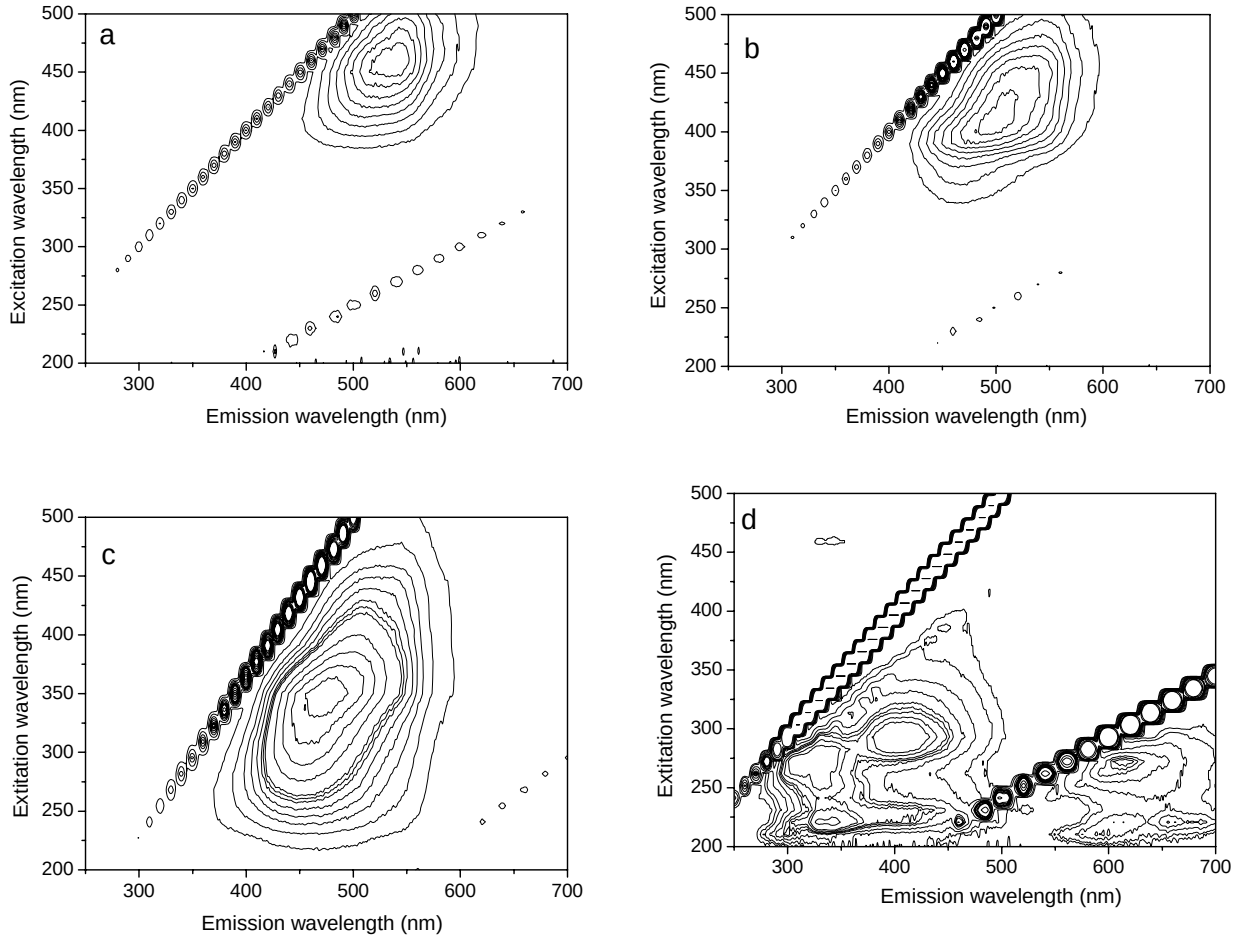

Fig. 1 Contour plots of total luminescence spectra of brandy (a), whisky (b), slivovice (c), and juniper spirit (d) samples. Contours join the points of equal fluorescence intensity.

As it is clearly shown in Fig. 1 the shape and the fluorescence intensities are different between the four classes of beverages. It appears that total luminescence spectroscopy can be used to characterize these samples using suitable wavelength regions. In general, spectral features and fluorescence intensity values of brandy are similar to those of whisky. However, the excitation/emission wavelength values of the major peaks of the brandies are generally longer than those usually measured for whisky.

Total synchronous fluorescence spectra

Synchronous scanning fluorescence spectroscopy is a useful tool for the analysis of mixtures of fluorescent compounds. Both excitation and emission characteristics are included in the spectrum by simultaneously scanning excitation and emission wavelengths with

constant wavelength differences between them. As a result, the selectivity for individual components is considerably improved and additional information on mixtures of fluorescent compounds is obtained. Although synchronous scanning fluorescence spectroscopy provides less information than the total luminescence spectroscopy, it may offer an alternative due to its inherent simplicity and rapidity. 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. 2a. It shows that the TSF contour map spreads in the excitation wavelength 380–550 nm and in the wavelength interval 10–100 nm. The contours are concentrated in the excitation wavelength region 440–480 nm and wavelength interval 60–100 nm. The plot shows one fluorescence maximum only. The maximum fluorescence intensity was recorded at excitation wavelength 460 nm ($\Delta\lambda$ = 80 nm).

The TSF spectra of a whisky sample are given in Fig. 2b. The contour map spreads in the excitation wavelength 350–530 nm and $\Delta\lambda$ 10–100 nm. The contours are concentrated in the excitation wavelength 390–460 nm and $\Delta\lambda$ 70–100 nm. The spectra of whisky are characterized by two fluorescence maxima, one at ~ 410 nm and other at ~ 470 nm, The maximum fluorescence intensity was observed at excitation wavelength 410 nm ($\Delta\lambda$ = 90 nm). Generally, the fluorescence maxima shift to shorter wavelengths with increasing $\Delta\lambda$ for both brandy and whisky. Whisky gives a shorter wavelength high intensive fluorescence band and brandy gives a longer less intensive band. Comparison of TSF spectra with total luminescence spectra collected from both brandy and whisky showed that these two spirits were better differentiated using TSF spectra.

The TSF spectra of a slivovice sample are given in Fig. 2c. The contour map spreads in the excitation wavelength 310–500 nm and $\Delta\lambda$ 10–100 nm. The contours are concentrated in the excitation wavelength 380–410 nm and $\Delta\lambda$ 70–100 nm. The spectra are characterized by two fluorescence maxima, one at ~ 400 nm and other at ~ 450 nm, The maximum fluorescence intensity was observed at excitation wavelength 390 nm ($\Delta\lambda$ = 100 nm). Generally, the fluorescence maxima shift to shorter wavelengths with increasing $\Delta\lambda$.

The TSF spectra of a juniper spirit sample (Fig. 2d) spreads in the excitation wavelength 200–400 nm and $\Delta\lambda$ 10–100 nm. The shape and intensity of the synchronous fluorescence spectra depend on the difference between excitation and emission wavelengths. The spectra

are characterized by various fluorescence maxima, though the maximum fluorescence intensity was observed at excitation wavelength 280 nm ($\Delta\lambda$ = 45 nm).

A distilled spirit is a complex mixture consisting of a large variety of substances with different spectroscopic characteristics. Due to this complexity, an intrinsic fluorescence of the beverage is result of the overlapping numerous fluorescence bands. Although complete assignment of the fluorescence bands is beyond the scope of our work, some preliminary assignments could be made, based on comparison of the fluorescence spectra with the fluorescent characteristics of particular beverage constituents

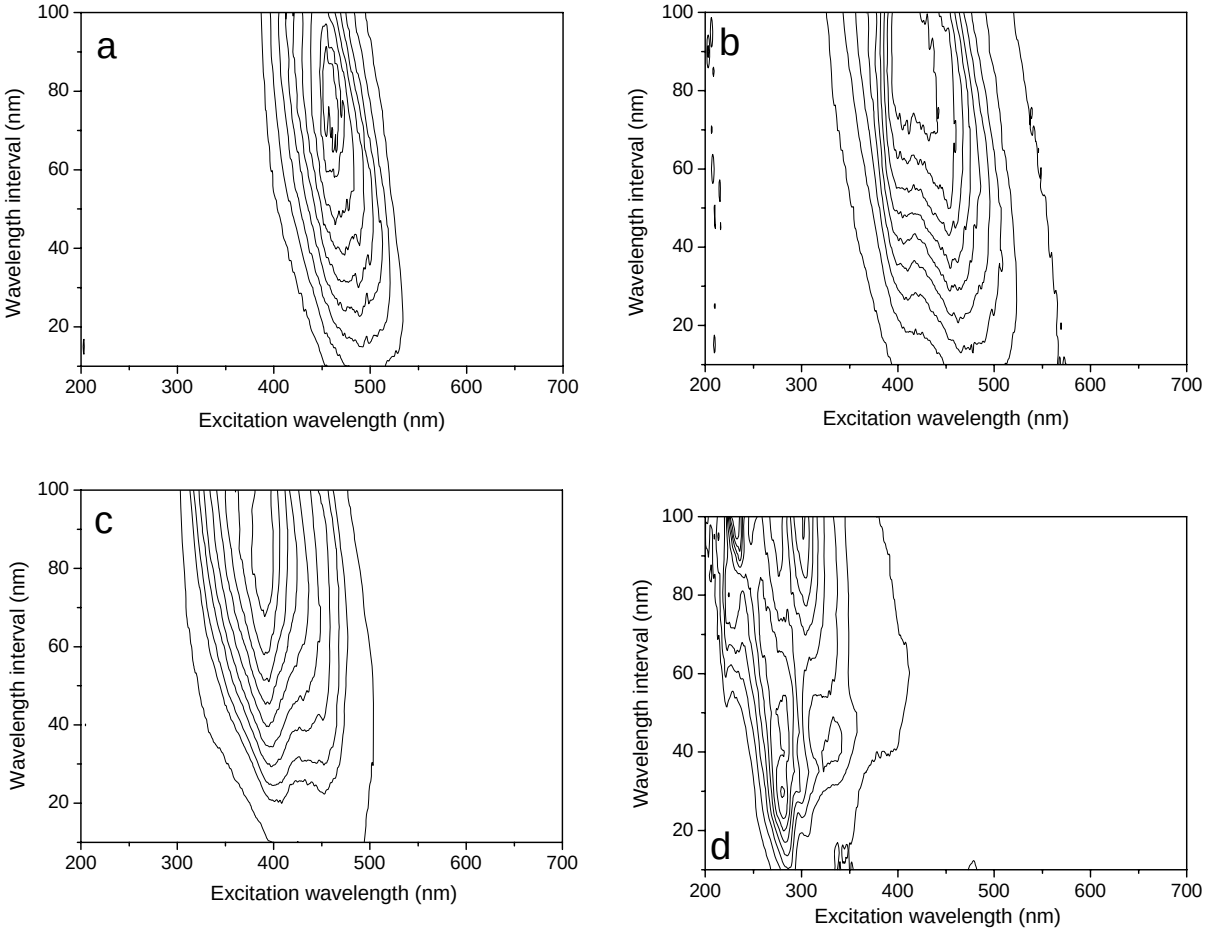

Fig. 2 Contour plots of total synchronous fluorescence spectra of brandy (a), whisky (b), slivovice (c), and juniper spirit (d) samples. Contours join the points of equal fluorescence intensity.

The short-wavelength fluorescence, with excitation at 220 and 270–280 nm and emission maxima located at 320–340 nm, was clearly observed in juniper samples, along with the longer-wavelength fluorescence, with excitation at 290 nm and emission at 406 nm. The

former band was preliminary attributed to the aromatic acids. Since α-tocopherol exhibited emission at 326 nm after excitation at 298 nm it can also modify the shape of the fluorescence spectra after excitation at 280 nm. The latter band could be due to the presence of phenolic acids and coumarins exhibiting emission between 360 and 420 nm after excitation set between 250 and 280 nm. The short-wavelength band could not be observed in the other distillate, while the longer-wavelength band was fairly intense. Reason is that undiluted brandy, whisky, and slivovice exhibit high Vis absorption, thus fluorescence measured using the right-angled geometry is distorted due to the inner-filter effects.

As mentioned, whisky is colored with plain caramel. Therefore we assumed that the band at 410 nm originate from this colorant. To support this assumption, spectra of caramel in water were recorded. Emission spectra recorded after excitation at 410 nm showed a maximum located around 498 nm. Comparison shows that the maxima observed for caramel at different Δλ-values are consistent with the respective maxima in whisky, so confirming a correct assignment of the shorter-wavelength band to caramel. The fluorescence intensity at 470 nm could be due to the presence of coumarins, tannins and other unknown fluorescent compounds originating from wooden casks. Similar spectral profiles were obtained for the slivovica. However, slivovice exhibits higher fluorescence at ~ 400 nm, while whisky shows higher fluorescence at ~ 470 nm.

Multivariate analysis of synchronous fluorescence spectra

PCA was applied separately on synchronous spectra measured at Δλ 10–100 nm. The best classification was achieved using fluorescence spectra recorded at Δλ = 40 nm, where synchronous fluorescence spectra showed different shapes. Fig. 3 illustrates differences in the synchronous fluorescence spectra of distillates obtained at Δλ = 40 nm. Fig. 4 shows that the plot of the first two PCs lead to a good discrimination of distillates according to origin. PC1 and PC2 describe 85.0 % and 12.4 %, respectively, of the total variance.

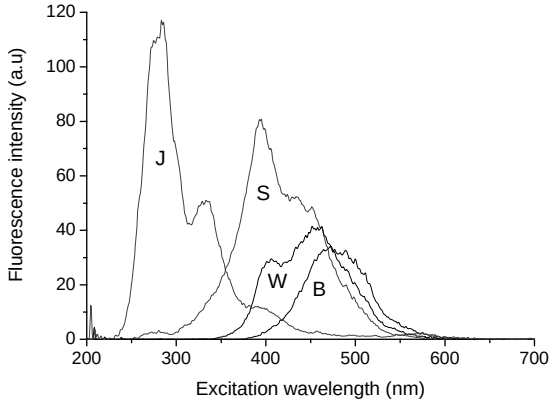

Fig. 3 Synchronous fluorescence spectra of brandy (B), whisky (W), slivovice (S), and juniper spirit (J) recorded at wavelength interval 40 nm.

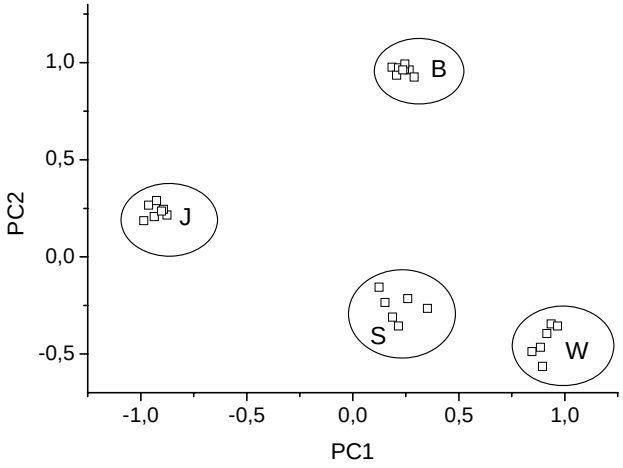

Fig. 4 Principal component analysis similarity map (score plot) determined by principal components 1 (PC1) and principal component 2 (PC2) for synchronous fluorescence spectra recorded at wavelength interval 40 nm on brandy (B), whisky (W), slivovice (S), and juniper spirit (J) samples.

This preliminary study shows that brandy, whisky, slivovice, and juniper drink can be discriminated using differences in their synchronous fluorescence spectra. Although differentiation between samples was possible by visual inspection of the spectra, it was accomplished by multivariate data analysis method more easy. Comparison of the results obtained from multivariate data analysis indicated that better classification was obtained from synchronous fluorescence spectra than from the excitation/emission fluorescence spectra. Fluorescence spectroscopy offers a promising approach for the characterization of distillates as neither sample preparation nor special qualification of the personnel are required, and data acquisition and analysis are relatively simple.

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