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Preliminary Investigation on Variety, Brewery and Vintage of Wines using Three-dimensional Fluorescence Spectroscopy

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In this study, the three-dimensional fluorescence spectroscopic technique was applied to discriminate the wines with different variety, brewery and vintage. A total of 42 wines produced in geographic origin of Changli county were analyzed. The results showed that the three-dimensional fluorescence spectra of wine samples with different variety, brewery and vintage had different fluorescence peaks. The fluorescence peaks were found in excitation wavelengths 260 , 290 and 329 nm. However, the number, location and fluorescenece intensity varied with the types of wines. The fluorescence spectra of the wine samples were evaluated using principal component analysis (PCA). PCA performed on the whole collection of three-dimensional fluorescence spectra allowed for a wide range of wine samples. These results showed that three-dimensional fluorescence spectroscopy may provide useful fingerprints that can determine the identity of wines from the Changli region.

Keywords: three-dimensional fluorescence spectroscopy, wine, authentication, principal component analysis (PCA)

Introduction

Nowadays, consumers are concerned with objectivity and authenticity of food information. The determination of food authenticity and the detection of adulteration are major issues in the food industry. Wine quality, especially wines with origin identification, is related to commercial value. Adulteration of foods is possible, which may bring an unfair competition in the wine industry and may harm the rights of consumers. Thus, there is a significant interest in accurate methods for wine characterization that could be used to prevent adulteration and also to classify wine from different geographical origins or countries (Liu *et al*., 2008; Cordella *et al*., 2002; Fischer *et al*., 1999; Reid *et al*., 2006).

Wine is produced by the grape, and the nutrition come directly from the grape fruit, the brewing and the yeast metabolizing (Li, 2000; Li *et al*., 2005). So wine is one of the most complex alcoholic beverages. The main chemical composition of wine involves sugars, acids, volatiles, phenolic compounds and minerals. These substances react with each other to stabilize and determine the peculiar flavour of wine. Further research is focus on these substances and makes them the basis of differences between the wines. Many research methods have been successfully achieved by measuring these chemical compounds using various analytical techniques such as high liquid performance chromatography (HPLC), gas chromatography (GC) and inductively coupled plasma mass spectrometry (ICP-MS) (Alvarez *et al*., 2003; Baxter *et al*., 1996; Castiñeira *et al*., 2004; Cordella *et al*., 2002; De Villiers *et al*., 2005; Fischer *et al*., 1999; Frias *et al*., 2003; Maarse *et al*., 1987). Although these methods provide valuable information on the composition and biochemistry of food products, they are time-consuming, expensive, require highly skilled operators and hardly adapted to on line monitoring. A present trend in analytical chemistry is the development of methodologies able to provide "fitness for purpose" results which take into account aspects related with the importance of time against accuracy achieved (Urbano *et al*., 2006). These aims are often supported on qualitative aspects rather than quantitative results (Trullols *et al*., 2004). A great number of non-invasive and non-destructive instrumental techniques, such as spectroscopic techniques have been developed for the authentication of food products. Techniques such as infrared and fluorescence spectroscopic are fast, relatively low-cost and provide a great deal of information. They are considered as sensitive, nondestructive,

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rapid, environmentally friendly and noninvasive, therefore rendering them suitable for on-line or at-line process control as well as appropriate for process control. For example, near infrared spectroscopy (NIRS) has been widely used in the food field for the determination of physico-chemical parameters of Greak Feta cheeses (Adamopoulos1 *et al*, 2001) and for authentication of foods, e g, identification of different varieties of wheat (Miralbés, 2008); differentiation of frozen and unfrozen beef (Downey and Beauchêne, 1997); discrimination of different edible oils (Armenta *et al*, 2007); and discrimination between different brands of French Emmental cheeses (Karoui *et al*, 2005).

Studies have been done using NIRS to classify wines and to predict a wine's chemical composition. The results of Cozzolino *et al* showed that assessment of wine composition by Vis and short wavelengths in the NIRS is possible for either qualitative analysis (e.g. low-, medium- and high-quality grading) or for screening of composition during bottling and storage (Cozzolino *et al*, 2007). Cozzolino *et al*. also showed that some macro- and microelements such as Ca, K, Mg, P, Na, S, Fe, B, Mn present in wine might be measured by VIS-NIRS spectroscopy (Cozzolino et al., 2008). NIRS could also be used as rapid alternative method for the prediction of the concentration of phenolic compounds in red wine fermentations (Cozzolino *et al*, 2004). In addition, 16 enological parameters in wine were evaluated by the NIRS (Urbano- Cuadrado *et al*, 2004).

Fluorescence spectroscopy is a rapid and sensitive method, it is 100~1000 times more sensitive than other spectrophotometric techniques. It can be used as a non-destructive analytical technique to provide information on the presence of fluorescent molecules and their environment in all sorts of biological samples. Three-dimensional fluorescence spectroscopy is a rapid, selective and sensitive technique. The outstanding advantage of these analytic technique is that information regarding the fluorescence characteristics can be entirely acquired by changing excitation wavelength and emission wavelength simultaneously. The resulting emission-excitation data matrix (EEM) data consisting of emission spectra registered at different excitation wavelengths. Therefore, the excitation– emission spectra obtained for each sample can be arranged either in data vector arrays or in data matrices for further analysis. Thus, because of its high sensitivity, good selectivity, and non-destruction of samples, EEM fluorescence spectroscopy could be useful in the food industry.

In wine research, the presence of fluorophores in the form of aromatic amino acids, vitamins, cofactors and phenolic compounds etc, makes the technique highly relevant and interesting. Due to the widely use of chemometrics, the application of autofluorescence in analysis of food has increased during the last decade (Karoui *et al*., 2007). Previous research has been shown that front face fluorescence spectroscopy can discriminate 120 wines produced in France and Germany (Dufour *et al*, 2006). Total luminescence and synchronous scanning fluorescence spectroscopic techniques were applied for characterization and differentiation of the intrinsic fluorescence of eight different beers (Sikorska *et al*, 2004). However, no publication has been reported about discrimination of wines with different variety, brewery and vintage using three-dimensional fluorescence spectroscopy.

The objective of the present investigation was to assess the potential of three-dimensional fluorescence spectroscopy using multivariate statistical methods to discriminate wine samples from geographic origin of Changli and to identify wines according to variety, brewery and vintage.

Materials and Methods

Wine samples Wine samples are collected from geographic origin of Changli County, supplied directly by the Huaxia great wall wine company(HGW) and Changli YUQIANNIAN Winery Company(CY). Serial number of wine samples is recorded in Table 1.

Fluorescence measurements Three- dimensional fluorescence spectroscopy was performed using the Spectrofluorometer (F-7000, Hitachi) equipped with 150 W ozone-free xenon lamp (Hamamatsu Photonics K.K.) operated under normal conditions of 5 nm slit widths (excitation and emission), photomultiplier voltage of 700 V, scan speed of 1200 nm per min and auto response time. This instrument has single monochrometers on both excitation and emission spectrometers. The slit of at excition and emission was fixed at 5 nm. Emission data points in the range of 200~600 nm were acquired every 3 nm. Correction of the spectra for instrumental components was performed using the correction protocol recommended by the manufacturer (Hitachi F-7000 owner's manual), which consists of both an excitation and emission calibration.

The wine samples were diluted to one tenth in ultrapure water (Millipore, 18.2 MΩ). The dilution was pipetted in 0.1 cm quartz cell. This short pathlength enabled to obtain absorbance values within the appropriate range ragarding to accuracy and precision specified by the spectrophotometer characteristics manual. Spectra were recorded in three repetition for each wine and data for each wine were averaged.

Mathematical analysis of data All statistical procedures were implemented in Matlab 7.0 and SPSS 13.0. EEM spectra demonstrate elliptical contours. The X-axis represents the emission spectra from 200 to 600 nm and the Y-axis is the excitation wavelength from 200 to 600 nm. Fifty contour

Table 1. The collection number of wine samples from geographic origin of Changli

Number	Variety	Brewery	Vintage	Amounts (bottle)
1	*Cabernet Sauvignon*	Huaxia great wall wine company, LTD (HGW)	2004	3
2	*Cabernet Sauvignon*	Huaxia great wall wine company, LTD (HGW)	2005	3
3	*Cabernet Sauvignon*	Changli YUQIANNIAN Winery CO LTD (CY)	2005	3
4	*Merlot*	Huaxia great wall wine company, LTD (HGW)	2004	3
5	*Merlot*	Huaxia great wall wine company, LTD (HGW)	2005	3
6	*Merlot*	Changli YUQIANNIAN Winery CO LTD (CY)	2005	3
7	*Gamay*	Huaxia great wall wine company, LTD (HGW)	2004	3
8	*Gamay*	Huaxia great wall wine company, LTD (HGW)	2005	3
9	*Syrah*	Huaxia great wall wine company, LTD (HGW)	2004	3
10	*Syrah*	Huaxia great wall wine company, LTD (HGW)	2005	3
11	*Cabernet Sauvignon*	Huaxia great wall wine company, LTD (HGW)	1992	3
12	*Cabernet Sauvignon*	Huaxia great wall wine company, LTD (HGW)	1996	3
13	*Cabernet Sauvignon*	Huaxia great wall wine company, LTD (HGW)	2001	3
14	*Cabernet Sauvignon*	Huaxia great wall wine company, LTD (HGW)	2003	3

lines, as the third dimension, are shown for each EEM spectra to represent the fluorescence intensity.

Principal component analysis (PCA) was used to examine the differentiation of wine samples. The PCA has been extensively used for visualisation of hidden trends in a data matrix. It is an unsupervised technique that exuces the dimensionality of the original data matrix retaining the maximum amount of variance. In this experiment, the objects were the wine samples spectra and the variables were the fluorescence intensity. PCA transforms the original variables into new axes, or principal components (PCs), which are orthogonal, therefore, 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. Objects plotted in the new space-score plot constitute a first step in subsequent modelling for samples classification. PCA performed on fluorescence spectra makes it possible to draw similarity maps of the wines.

Results and Discussion

Three-dimensional fluorescence spectra of wines. The fluorescence spectroscopy gives information regarding molecules containing conjugated double bounds. There are many fluorescent molecules in wines, such as phenolic acids, stilbenes, anthocyanins, flavanols and tannins (Dufour *et al*., 2006). The types and amounts of these molecules vary as a function of the variety of grapes. Processing and ageing also affect the wine's phenolic compounds. However, wines contain many other compounds (e.g., proteins, amino acid) that

may fluoresce. So a three-dimensional fluorescence spectroscopy recorded on a wine sample included information on several fluorophores and may be used to discrimination wines according to the variety, brewery and vintage

Fluorescence spectroscopy is a sensitive, rapid fluorescence methods. In fluorescence experimentation, if absorbance is less than 0.1, the intensity of the emitted light is proportional to fluorophore concentration and excitation and emission spectra are accurately recorded by classical right-angle fluorescence device. When the absorbance of the samples exceeds 0.1, emission and excitation spectra are both decreased and excitation spectra are distorted (Karouia *et al*., 2007). Undiluted wine exhibits high UV-Vis absorption, thus fluorescence measured using the right-angled geometry is severely distorted due to primary and secondary inner-filter effects. To avoid these problems, appropriate dilution of samples is required

The contour maps and three-dimensional fluorescence spectroscopy of wines with different variety, brewery and vintage were constructed in Fig. 1. In the three- dimensional fluorescence spectroscopy the x-axis represents the emission wavelengths, y-axis represents the excitation wavelengths, and z-axis represents the fluorescence intensity. Contour maps are plotted by linking points of equal fluorescence intensity. Although wine samples exhibit very similar fluorescence characteristics, differences in band positions, shapes, and relative fluorescence intensities are easily noticeable in Fig. 1. The location, fluorescenece intensities and the numbers of peaks vary with different wine samples. In Table 2, the fluorescence intensity is significantly different with each wine sample. In Fig 1, the wine samples from 2004 and 2005 had the different peak location and peak numbers. The *Cabernet Sauvignon* (sample 1 , 2 and 3) and *Merlot* (sample 4 , 5 and 6) had three peaks of peakI, peakII and peakIII at excitation wavelength 260 , 290 and 329 nm, respectively; *Gamay* (sample 7 and 8) had two peaks, peakI at excitation wavelength 260 and 269 nm, peakIII at excitation wavelength 335 and 332 nm; Syrah in 2004 (sample 9) had three peaks of peakI, peakII and peakIII at excitation wavelength 263, 287 and 332 nm, respectively. *Syrah* in 2005 (sample 10) had two peaks of peakII and peakIII at excitation wavelength 290 and 329 nm. Obviously there are distinct differentiation of different variety and brewery. The wine produced with *Cabernet Sauvignon* from 2001, 2003, 2004 and 2005 (sample 13, 14, 1, 2) had the same peak location and peak numbers, the peaks of peakI, peakII and peakIII at excitation wavelength 260 , 290 and 329 nm, respectively, and the fluorescence intensity were different; in vintage of 1992 and

Table 2. Parameters of fluorescence characteristics

Sample	Peaks Location $\lambda_{EX}/\lambda_{EM}$(nm)			Fluorescence Intensity		
	I	II	III	I_I	I_{II}	I_{III}
1	263/365	290/368	332/425	525.1	665.9	518.8
2	260/368	290/371	326/425	437.4	587.5	606.8
3	260/365	290/368	329/416	690.8	843.8	736.4
4	260/377	287/365	329/398	1293	1255	993.9
5	260/377	284/365	329/425	1427.0	1418.0	699.1
6	260/365	290/368	329/410	576.6	615.2	600.5
7	260/362	——	335/416	727.4	——	570.5
8	269/359	——	332/428	1190.0	——	802.1
9	263/365	287/368	332/398	602.4	597.4	715.9
10	——	290/380	329/401	——	327.2	587.9
11	——	——	332/413	——	——	372.6
12	——	——	323/407	——	——	327.0
13	260/365	290/371	326/416	294.0	409.7	388.2
14	260/362	293/374	332/401	275.6	576.6	685.9

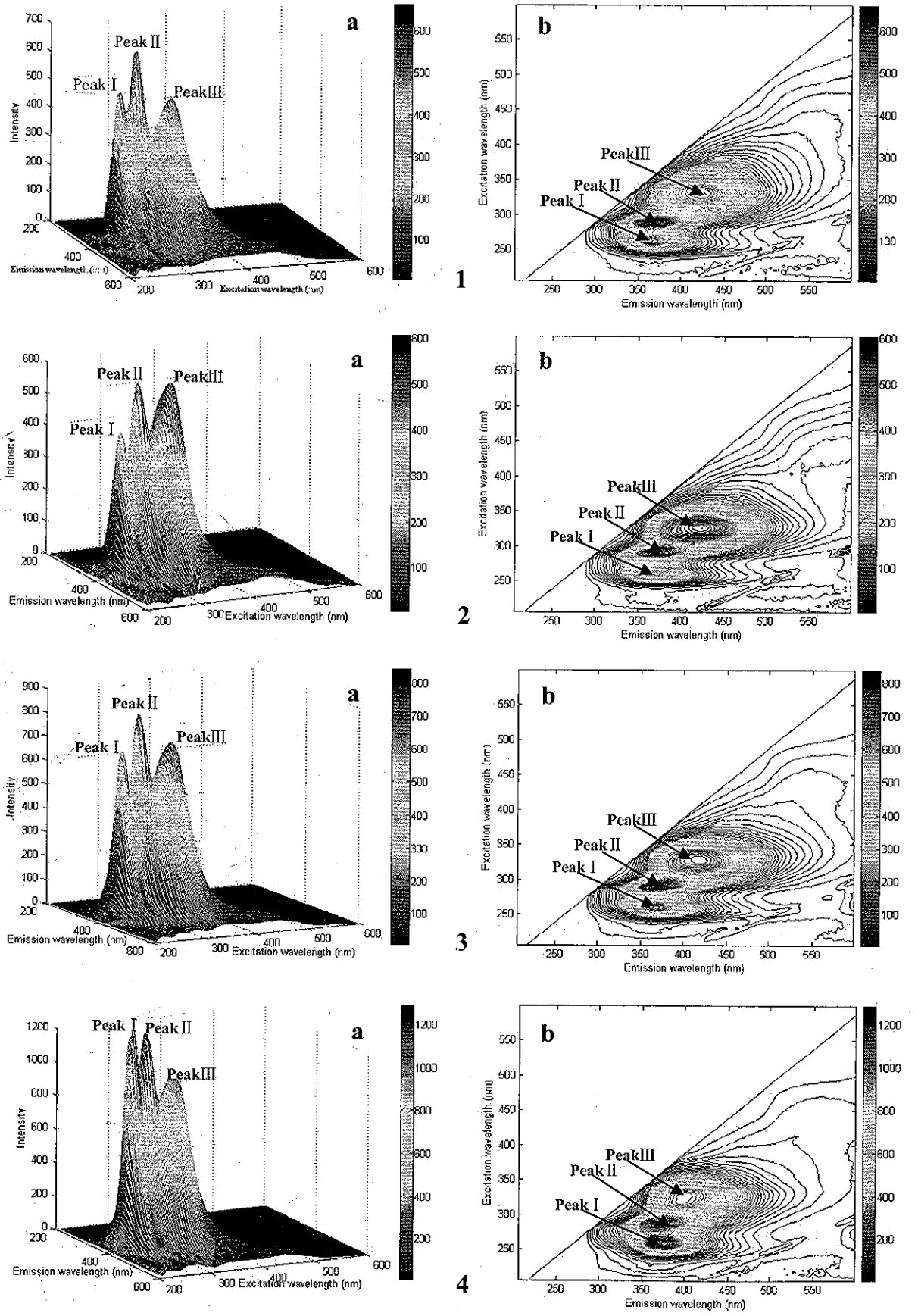

Fig. 1-1. Contour maps and three-dimensional fluorescence spectroscopy of wine samples
(a: three-dimensional fluorescence spectroscopy; b: contour maps; 1~14: the number of different wine samples)

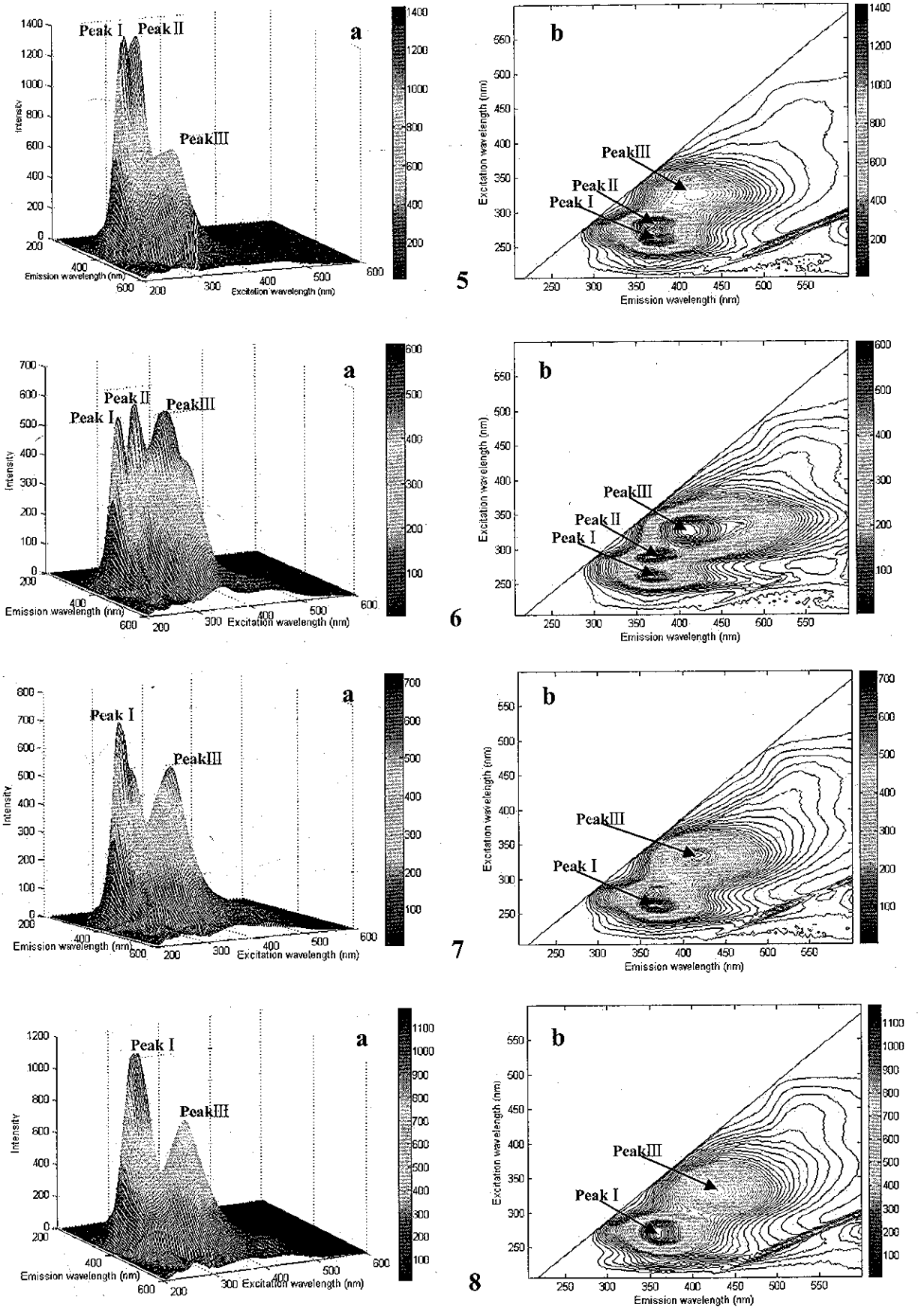

Fig. 1-2. (Continued)

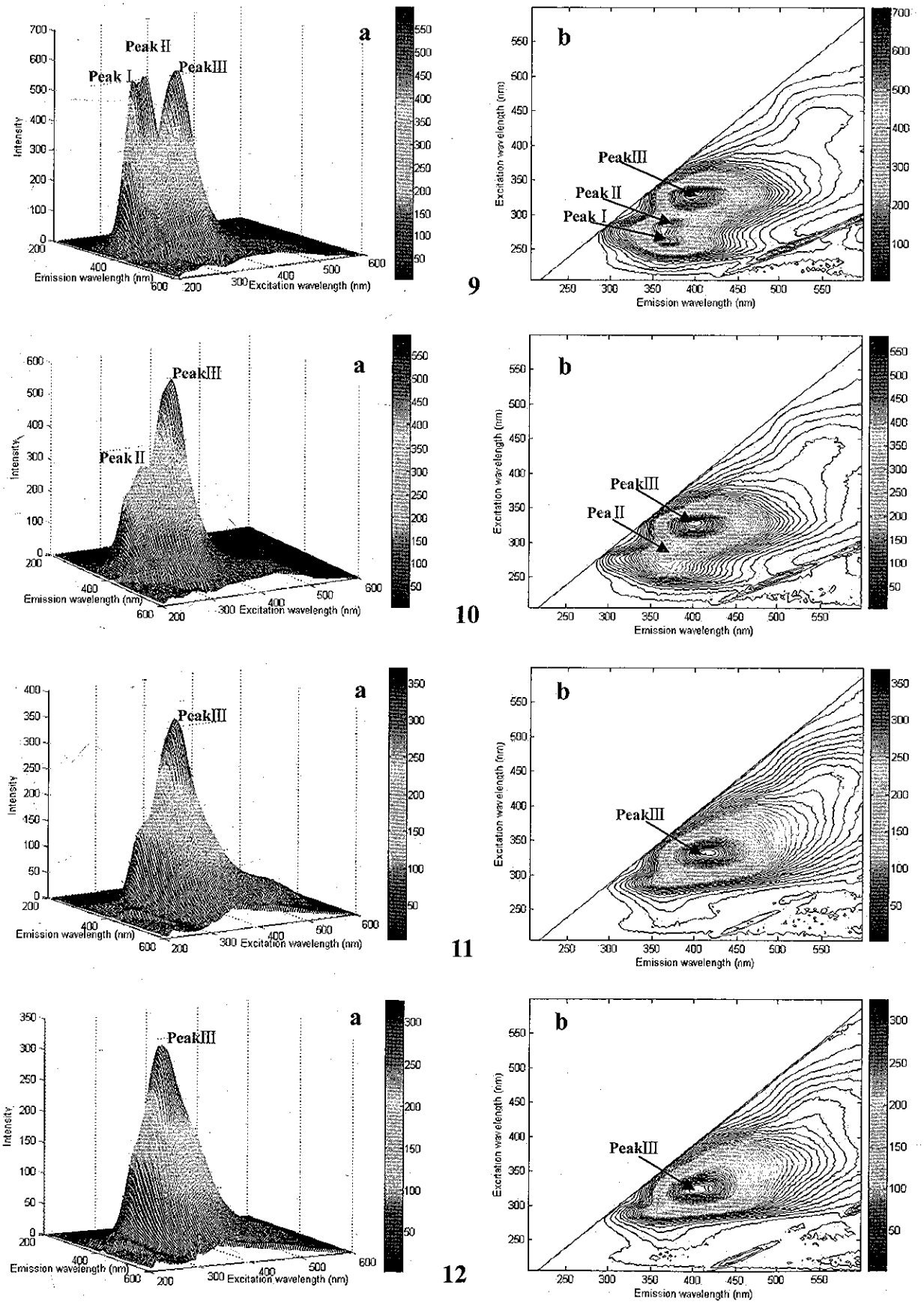

Fig. 1-3. (Continued)

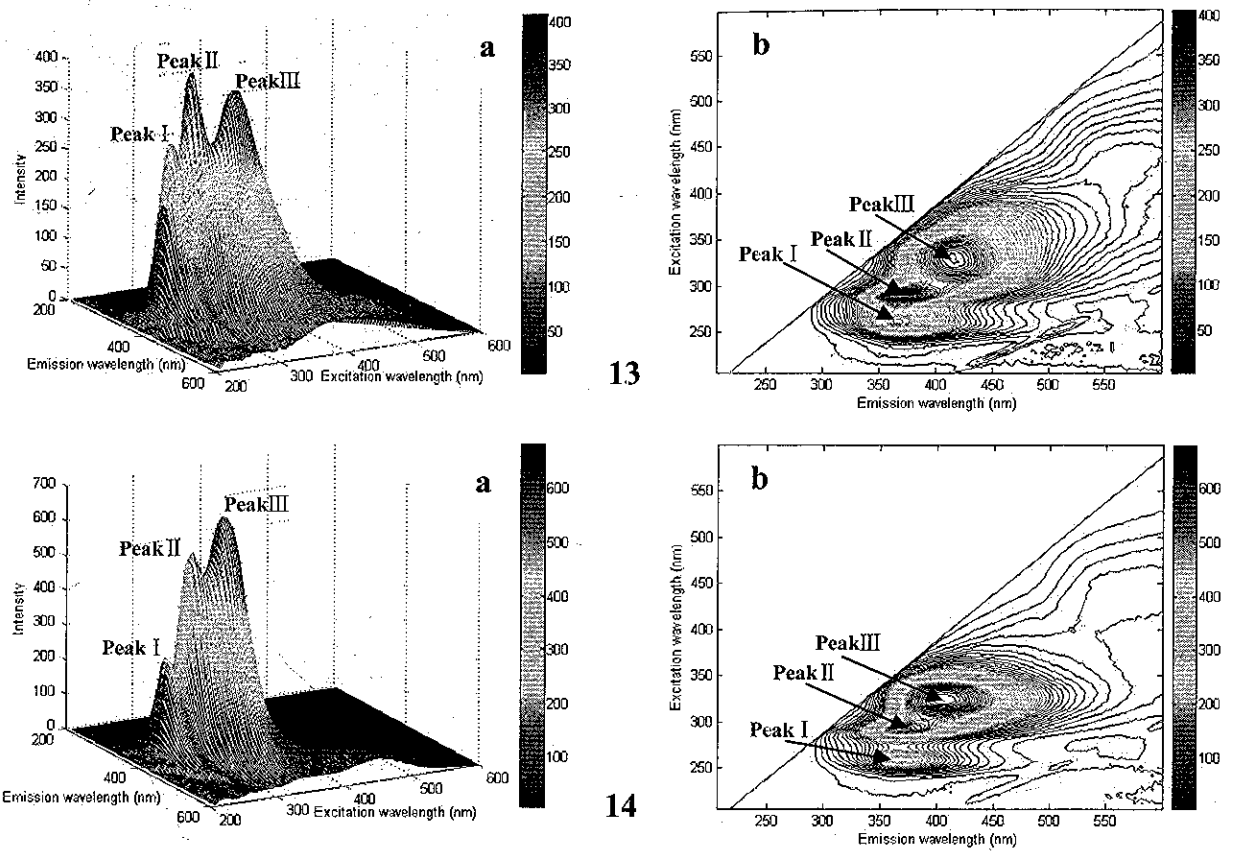

Fig. 1-4. (Continued)

1996(sample 11, 12) there is one peak of peakIII at excitation wavelength 332nm. This shows that there is distinct differentiation between vintages

Principal component analysis of wine fluorescence spectra Multivariate techniques are increasingly being applied to complex instrumental data to achive classification of agricultural products. Spectroscopic techniques are often favoured for the interaction of all absorbing species in the sample matrix is captured and taken into consideration when the analysis is carried out. However, it requires the use of multivariate analysis in order to interpret and extract the information of such complex matrices. Principal component analysis is a main method to do that.

PCA was applied separately on the normalised three-dimensional fluorescence spectra. The maps defined by PC1-PC2 for the PCAs performed on fluorescence data are shown in Fig. 2, 3, 4 and 5 respectively. For the three-dimensional fluorescence data collection, a discrimination of the wine samples with different variety, brewery and vintage was observed. In Fig. 2 the wine samples 1, 4, 7 and 9 were made up with different variety in 2004. The first two principal components took into account 51.02% and 24.5% of the total variance. In Fig. 3 the wine samples 2, 5, 8 and 10 were made up with different variety in 2005. The first two principal components took into account 64.1% and 21.58% of the total variance. In Fig. 4 the wine samples 2, 3, 5 and 6 were made up with different brewery in 2005. The first two principal components took into account 49.64% and 27.17% of the total variance. In Fig. 5 the wine samples 1, 2, 11, 12, 13 and 14 were made up with different vintage of Cabernet Sauvignon. The first two principal components took into account 50.12% and 20.75% of the total variance. The PCA similarity map (Fig.2, 3, 4 , 5) showed a clear discrimination of wines with different variety, brewery and vintage.

The ability of the three-dimensional fluorescence spectra to discriminate wine samples with variety, brewery and vintage was in investigated. Through the Fig.2, 3, 4 and 5, we found that the spectra retain complementary and useful information allowing the discrimination of the wine collection. The fluorescence spectrum recorded directly on a wine sample can be considered as a fingerprint.

Conclusion

Three-dimensional fluorescence measurements applied directly on wines have been used for discriminate the variety, brewery and vintage of a collection of Changli wines

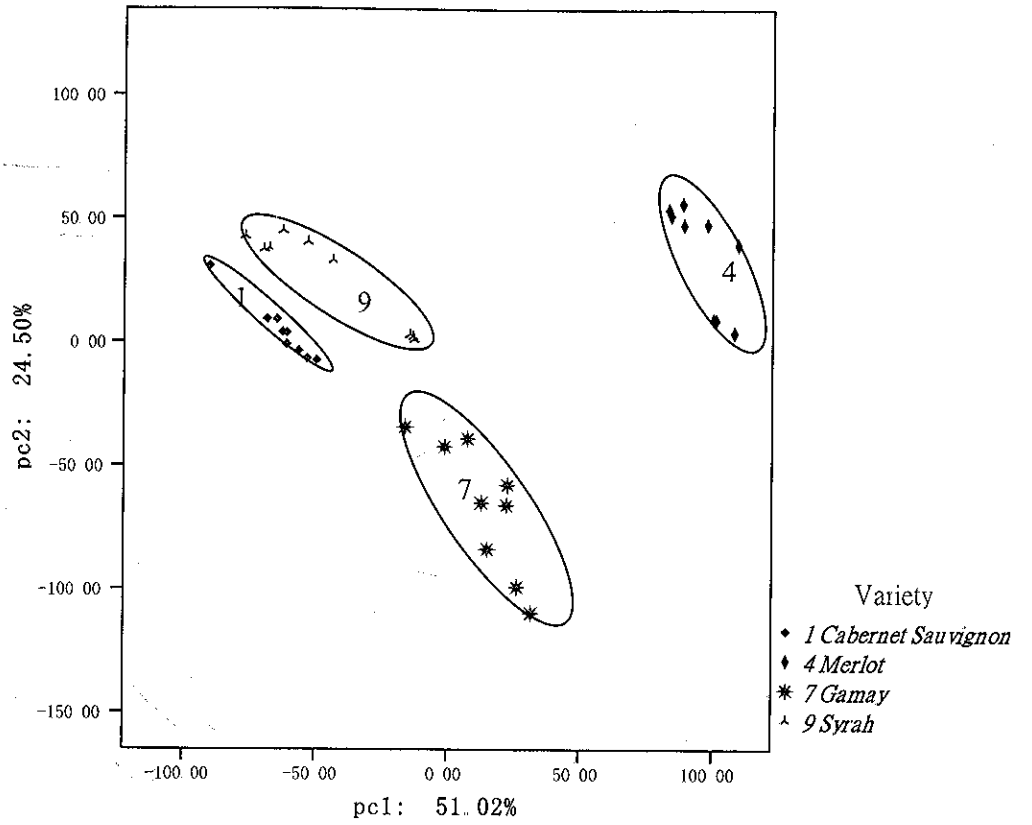

Fig. 2. PCA score map determined by principal components 1 and 2 for three-dimensional fluorescence spectra of different variety wines in 2004.

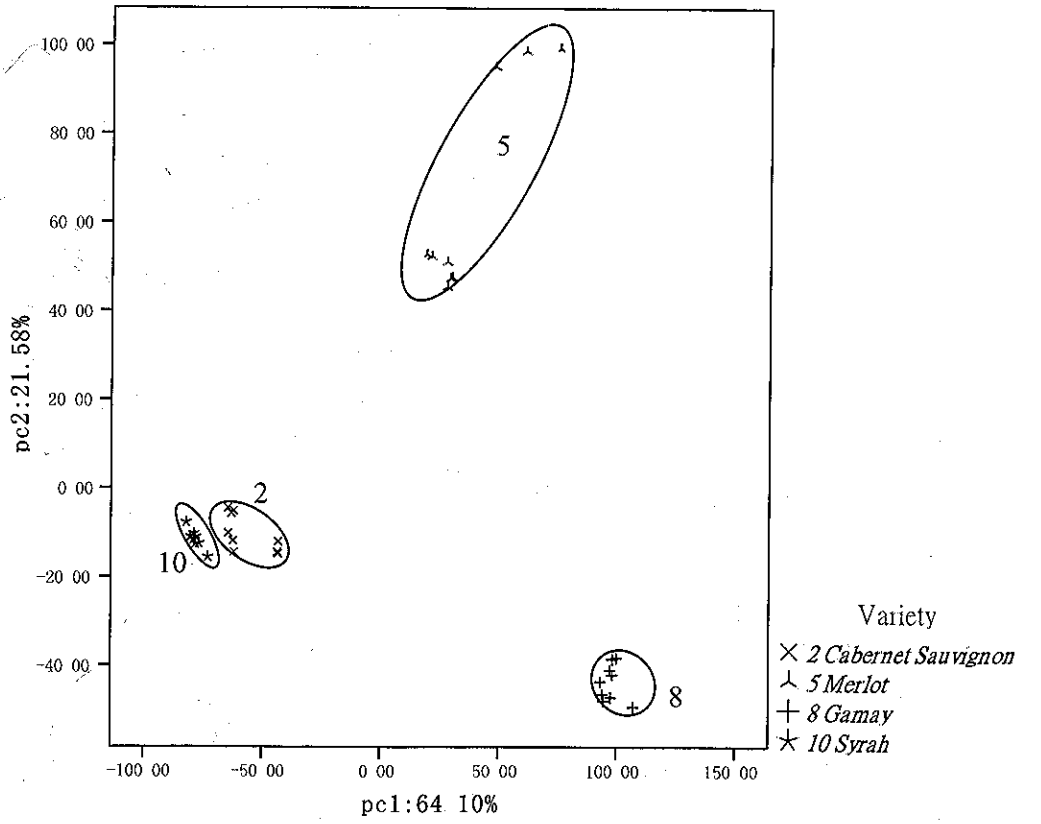

Fig. 3. PCA score map determined by principal components 1 and 2 for three-dimensional fluorescence spectra of different variety wines in 2005.

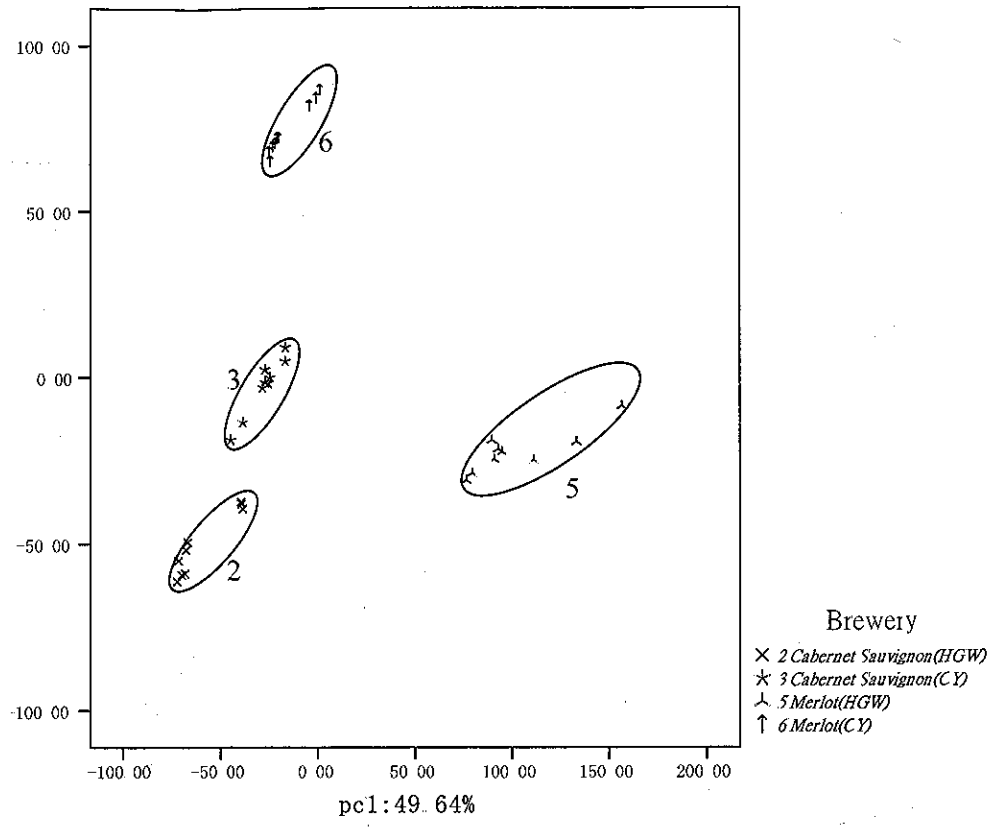

Fig. 4. PCA score map determined by principal components 1 and 2 for three- dimensional fluorescence spectra of different brewery wines in 2005

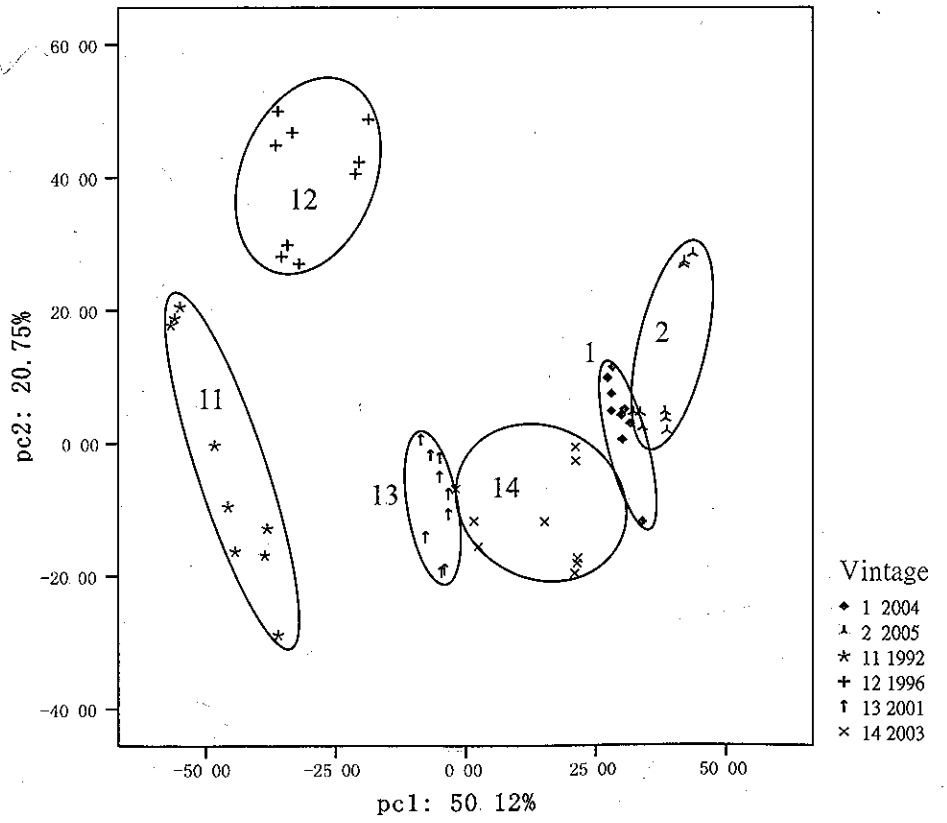

Fig. 5. PCA score map determined by principal components 1 and 2 for three- dimensional fluorescence spectra of Cabernet Sauvignon with different vintage

This preliminary study shows that the fluorescence peaks were found in excitation wavelength 260 , 290 and 329 nm. The number and location were different with variety, brewery and vintage. PCA performed on the whole collection of three-dimensional fluorescence spectra allowed for a good discrimination of wine samples. The technique is non-destructive, rapid, easy to use and inexpensive. It needs neither a particular sample preparation nor special qualification of the personnel. However, these preliminary findings should be confirmed with a larger set of samples and additional wine types. In addition, this wine collection should be analyzed by classical methods and a joint analysis of the fluorescence, Sensory and phenolic compounds data sets should allow to investigate the correlations between the different data sets.

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