DYNAMIC RAMAN FUSION SPECTROSCOPY FOR RAPID QUALITY DISCRIMINANT ANALYSIS OF RED WINE

Zheng-Yong Zhang*, Jun Liu

School of Management Science and Engineering, Nanjing University of Finance and Economics, Nanjing Jiangsu 210023, China; e-mail zyzhang@nufe.edu.cn

The dynamic Raman spectra of a brand of red wine (aa) as the main research object were collected over a range of laser integration times (1–5 s) to observe the changing trends of molecules in the wine under experimental conditions. The three-dimensional Raman characteristic spectrum of this wine was then constructed further by two-dimensional correlation fusion analysis. The fluctuations of the three-dimensional Raman spectra were also evaluated using a similarity algorithm. The correlation coefficients were 0.977 ± 0.011 and 0.990 ± 0.006 based on synchronous and asynchronous two-dimensional correlation Raman spectroscopy, respectively. These results suggested that the samples of wine aa were highly self-similar and could be effectively distinguished from two different brands of red wine (bb and cc) based on their different spectral responses. Therefore, this method has the potential to supplement existing methods for the classification analysis of red wine.

Keywords: *Raman spectroscopy, two-dimensional correlation analysis, fusion analysis, rapid discrimination, red wine.*

ДИНАМИЧЕСКАЯ СПЕКТРОСКОПИЯ КОМБИНАЦИОННОГО РАССЕЯНИЯ ДЛЯ ДИСКРИМИНАНТНОГО АНАЛИЗА КРАСНОГО ВИНА

Zh.-Y. Zhang*, J. Liu

УДК 535.375.5

Нанкинский университет финансов и экономики, Нанкин, Цзянсу 210023, Китай; e-mail zyzhang@nufe.edu.cn

(Поступила 24 сентября 2018)

В диапазоне времен лазерного излучения (1–5 с) получены динамические спектры КР красного вина марки (aa) как основного объекта исследования для наблюдения поведения молекул в вине в условиях эксперимента. Путем двумерного корреляционного анализа совмещенных данных построен трехмерный характеристический спектр КР вина. С использованием алгоритма подобия оценены флуктуации трехмерных спектров КР. На основе синхронной и асинхронной двумерной корреляционной спектроскопии КР получены коэффициенты корреляции 0.977±0.011 и 0.990±0.006. Показано, что образцы вина аа схожи и их можно эффективно отличить от двух других марок красного вина (bb и cc) на основании различия спектральных характеристик.

Ключевые слова: *спектроскопия комбинационного рассеяния света, двумерный корреляционный анализ, синтез данных, быстрое распознавание, красное вино.*

Introduction. Red wine is made from grapes as the main raw material. As a popular consumer product, its quality control is very important to producers. At present, there are two main ways to control its quality: one is to employ sensory evaluation experts, who can judge the quality level of red wine using their sense of taste and smell. However, this strategy is subjective and so can easily be influenced by the external environment [1]. Another way is to use instrumental analysis, including bionic identification devices such as

the electronic tongue or nose, as well as separation analysis methods such as chromatography, and mass spectrometry, but these strategies are often time-consuming [2, 3].

In recent years, spectral analysis has developed rapidly to become a fast analysis technology, in particular Raman spectroscopy, which has aroused great attention from scientists because of its advantages of fast analysis speed and easy portability [4]. However, traditional Raman spectral analysis focuses mainly on the acquisition and analysis of two-dimensional spectral data, and the utilization rate of information is relatively low. Fusion analysis is a new idea to improve the efficiency of data analysis, but traditional data fusion analysis mainly focuses on static data fusion. The general process comprises collecting sample information using the same instrument with different acquisition modes or different instruments, and then providing discriminant information using a direct spectral splicing or feature extraction fusion strategy. The internal connections between the molecules of the sample can often be ignored [5, 6]. Dynamic data fusion is more effective at extracting dynamic information from the target sample, a new trend in the development of analytical methods.

The two-dimensional correlation analytical strategy is a kind of dynamic data analysis technology and has developed rapidly in recent years. It comprises obtaining a series of dynamic spectral data from the test sample under a perturbation then calculating a three-dimensional spectrum using two-dimensional correlation analysis. This method can improve spectral resolution and discriminant analysis ability [7–10]. However, the traditional perturbation strategies are suitable for temperature, pressure, and chemical reaction, but, in general, are very time-consuming. In the present study, a laser perturbation strategy is adopted, with acquisition times of only a few seconds, which should greatly improve the efficiency of signal acquisition. Further, to the best of our knowledge, this will be the first time that a two-dimensional correlation of the Raman spectra of red wine has been provided. The potential application of this new dynamic Raman spectroscopy fusion analytical method will be demonstrated using red wine classification discriminant analysis as an example.

Experimental. *Samples and instruments.* Samples of three brands of dry red wine were purchased from Suning supermarket (Nanjing, China). The Raman spectra were obtained using a portable laser Raman spectrometer (Prott-ezRaman-d3, Enwave Optronics Inc., Irvine, CA, USA). The excitation wavelength of the laser was 785 nm, the laser power about 450 mW, the integration time 1–5 s, and the average number of scans 2. The spectrometer operated over a spectral range from 250 to 2339 cm^{-1} with a resolution of 1 cm^{-1}.

Data processing. The calculation of the two-dimensional correlation Raman spectra was carried out using 2D-shige software (Shigeaki Morita, 2004-2005, Kwansei-Gakuin University, Nishinomiya, Japan). The generalized two-dimensional correlation spectrum can be calculated as described by Noda [11, 12]:

$$X(\nu_1, \nu_2) = \Phi(\nu_1, \nu_2) + i\Psi(\nu_1, \nu_2).$$

The intensity of the two-dimensional correlation Raman spectrum $X(\nu_1, \nu_2)$represents the quantitative measure of a comparative similarity ($\Phi(\nu_1, \nu_2)$, synchronous correlation) or dissimilarity ($\Psi(\nu_1, \nu_2)$, asynchronous correlation) of two different Raman spectral variables, ν_1 and ν_2, with an ordered interval.

The correlation coefficients of the red wine samples were determined using the formula function CORREL in Excel software (Microsoft Corp., Redmond, WA, USA).

$$R = \frac{\mathrm{cov}(F_a, F_b)}{\sqrt{\mathrm{cov}(F_a, F_a)\,\mathrm{cov}(F_b, F_b)}},$$

where R is the correlation coefficient, F_a is a two-dimensional correlation Raman spectrum matrix of one sample, and F_b is the other sample.

Results and discussion. *Analysis of red wine by Raman spectroscopy.* Red wine is rich in nutrients. Information on the contents of some chemical components can be obtained by traditional chromatography and mass spectrometry, but it is difficult to identify red wine brands. Raman spectroscopy is a new type of molecular vibration spectroscopy, which can obtain the whole characteristic information of the sample molecules, although they are sometimes disturbed by fluorescence. One brand of red wine, aa, was used as the main research object in the present study. Its Raman spectra under laser perturbation (Fig. 1) show an obvious changing trend under the different laser integration times of the experimental sample. Using a laser integration time of 1 s, there was one fluorescent package and no obvious Raman signal, but for a laser time of 2 s, there were two Raman peaks: one peak at ~885 cm^{-1} could be attributed to the C-C stretching vibration, and the other peak at ~1005 cm^{-1} to the ring vibration of phenylalanine, according to [13–17]. At the same time, the fluorescence peak signal was also enhanced. For a laser time of 3 s, the fluorescence signal was beyond the upper limit of the acquisition equipment. For laser integration times of 4 and 5 s, the fluorescence

peaks exhibited an increased overflow area. During the experiment, the laser intensity was maintained at around 450 mW. As the laser integration time increased, the time of laser irradiation lengthened, and the spectral information of the material molecules was collected. Meanwhile, the thermal and fluorescence effects were also enhanced [18]. The content of fluorescent substances in red wines is rich and is closely related to its quality. Thus, the characteristic information of red wine samples can be obtained in a very short time using Raman spectroscopy.

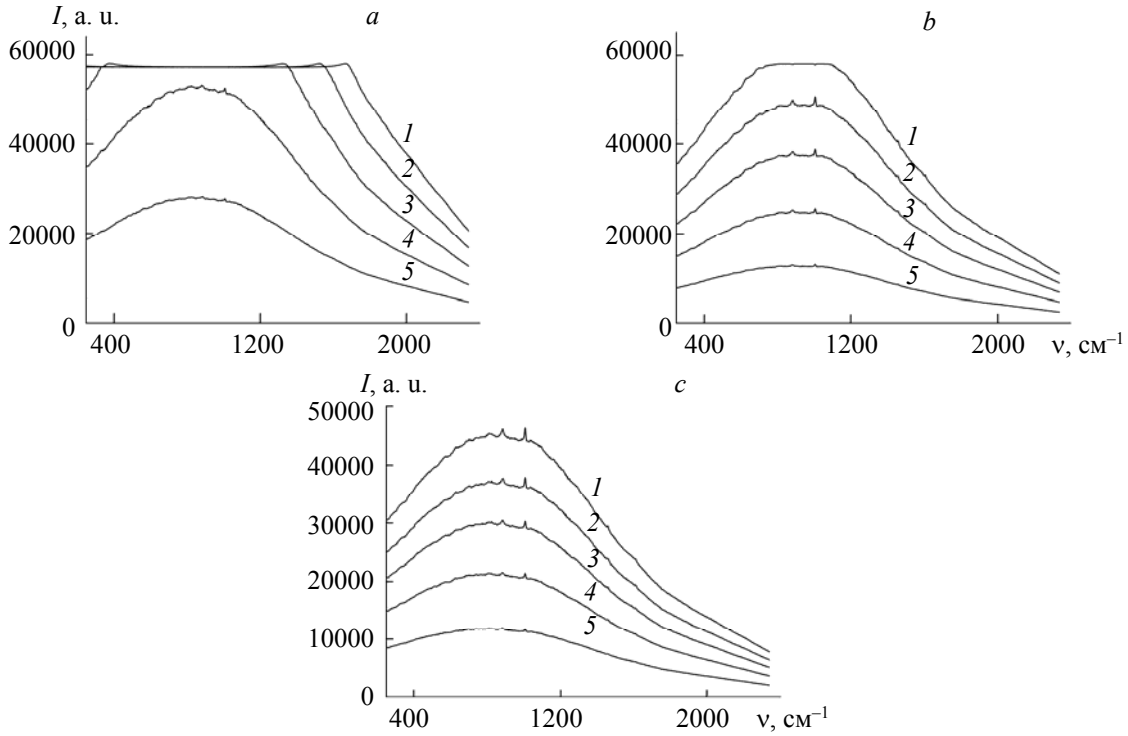

Fig. 1. Raman spectra at different laser integration time (5 (1), 4 (2), 3 (3), 2 (4), and 1 s (5)) of wine aa (a), wine bb (b), and wine cc (c).

Analysis of red wine by two-dimensional correlation Raman spectroscopy. The analysis above is a routine analytical method in spectrographic analysis, which is suitable for obtaining two-dimensional feature information from red wine samples. However, the relationship between the various molecules of the sample can be further investigated and analyzed. In recent years, Noda has proposed a creative two-dimensional correlation spectroscopy: a series of spectral data is first collected when the sample system responds to an external perturbation, then a spectrum or the average spectrum is used as a reference. Finally, the spectral information is obtained in three-dimensional space after calculating the correlation between these spectra and the reference spectrum. In the present study, to the best of our knowledge, the two-dimensional correlation Raman spectra of red wine has been obtained for the first time, using the average spectrum as the reference and the laser integration time as a perturbation. Figure 2a shows the synchronous two-dimensional correlation Raman spectrum of wine aa, with two obvious auto peaks and some cross-peak lines. The auto peaks were located at around the coordinates 1580, 1580 and 860, 860 cm^{-1} with positive intensities. These auto peaks reflect the susceptibility of the Raman spectral intensity to the external perturbation, and the cross-peak lines represent the interaction of two different spectral variables. The positive and negative correlation signals are denoted by the unshaded and shaded regions. Figure 2b shows the asynchronous two-dimensional correlation Raman spectrum of wine aa, exhibiting two obvious cross peaks and some other cross peak lines. One cross peak was located at around the coordinates 1690, 885 cm^{-1} with negative intensities, and the other at around 885, 1690 cm^{-1} with positive intensities. The figure depicts the red wine sample from the three-dimensional feature space. Compared with the traditional two-dimensional spectrum, it has made full use of many two-dimensional spectra. Therefore, the spectral information was richer, and the information utilization rate was improved, as well as the resolution of the spectroscopy.

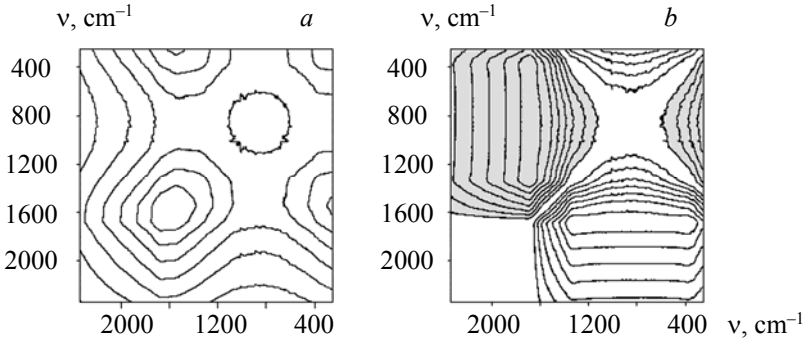

Fig. 2. Synchronous (a) and asynchronous (b) two-dimensional correlation Raman spectra of wine aa.

Analysis of the quality fluctuation of red wine. The correlation coefficient is a commonly used algorithm for describing similarities between samples [19]. The correlation coefficient should be close to 1 for similar samples and close to 0 for different samples. The correlation coefficient method was used to study the fluctuation of the two-dimensional correlation spectra of the samples of wine aa (Fig. 3). Correlation coefficient of the control group was 1, meaning that the red wine samples were consistently similar. The six randomly selected samples were used as the experimental group (wine aa), and the average value of these samples was taken as the theoretically true value, then the correlation coefficients between multiple samples and their mean values were calculated. Figure 3 shows that the average value and standard deviation calculated from the synchronous two-dimensional correlation Raman spectra were 0.977 ± 0.011. The corresponding results for asynchronous two-dimensional correlation Raman spectra were 0.990 ± 0.006. Therefore, the fluctuation of the two-dimensional correlation spectroscopy of the wine samples was not large, and the similarities between the same samples were great. The standard deviation values indicated that the stability of the asynchronous two-dimensional correlation Raman spectroscopy was higher than that of the synchronous two-dimensional correlation Raman spectroscopy. The mean values showed that the similarities among these samples were close to 1.

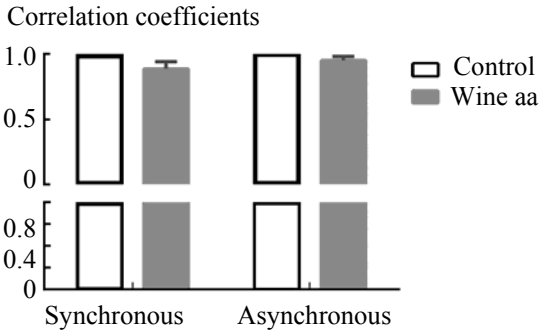

Fig. 3. Correlation coefficients between the different samples of wine aa based on synchronous and asynchronous two-dimensional correlation Raman spectroscopy.

Quality discriminant analysis of red wine. Samples of the other two brands of red wine were also studied using the above methods. Figure 1c shows that for red wine bb, the intensity of Raman spectroscopy increased as the laser integration time increased, especially the two most obvious Raman peaks, for a laser integration time of 4 s. The signal overflow of the instrumental signal limit or the integration time of 5 s was due to the enhanced signal response of the fluorophore. Only red wine cc (Fig. 1c) showed that the Raman signal intensity increased as the laser integration time increased. For an integration time of 5 s, the signal was clear with no signal overflow. These dynamic Raman spectroscopic signals showed that the three different brands of red wine had different components and different spectral responses. This is the basis for the further two-dimensional correlation discriminant analysis. Figure 4 shows the synchronous and asynchronous two-dimensional correlation Raman spectroscopy for wines bb and cc, and that their spectra were obvi-

ously different from the target wine aa. The synchronous graph for wine bb had one clear auto peak at around the coordinates 900, 900 with positive intensities. Its asynchronous graph had two cross peaks and some cross-peak lines. There was one cross peak located at around coordinates 1600, 900 cm^{-1} with negative intensities, and another located at around 900, 1600 cm^{-1} with a positive signal intensity. The synchronous graph for wine cc had one obvious auto peak at around 880, 880 cm^{-1}, with a positive signal intensity and many fine structures in the diagram. Its asynchronous graph had two cross peaks with rich spectral lines. One of the cross peaks was located at around coordinates 1600, 400 cm^{-1} with negative intensities, and the other at around 400, 1600 cm^{-1} with positive intensities. The correlation coefficients (Fig. 5) between red wines bb and aa based on synchronous and asynchronous two-dimensional correlation Raman spectroscopy were 0.512 and 0.457, respectively. The correlation coefficients between red wines cc and aa based on synchronous and asynchronous two-dimensional correlation Raman spectroscopy were 0.522 and 0.548, respectively. These values were much lower than the self-similarities between the red wine aa samples. Therefore, this method of three-dimensional representation of red wine has the potential for application in the quality discrimination of red wine.

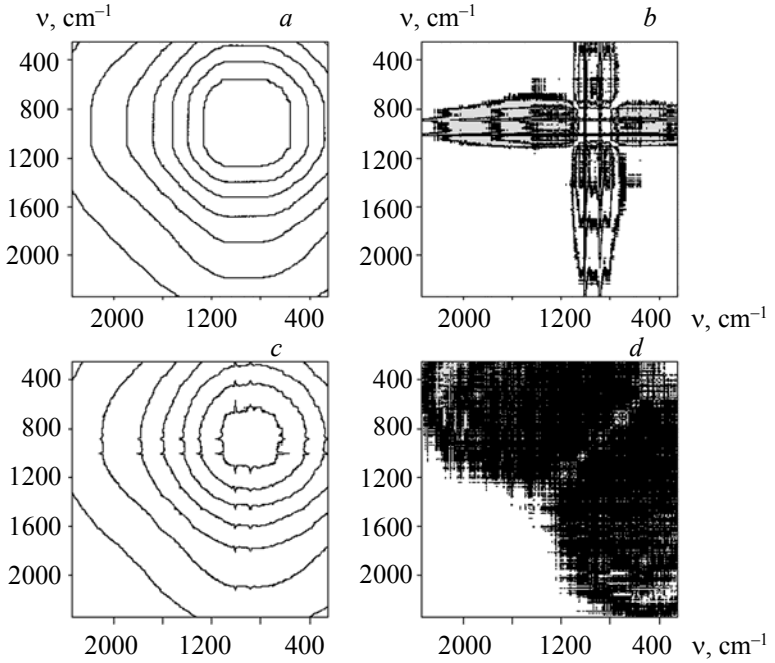

Fig. 4. Synchronous (a, c) and asynchronous (b, d) two-dimensional correlation Raman spectra of wine bb (a, b) and cc (c, d).

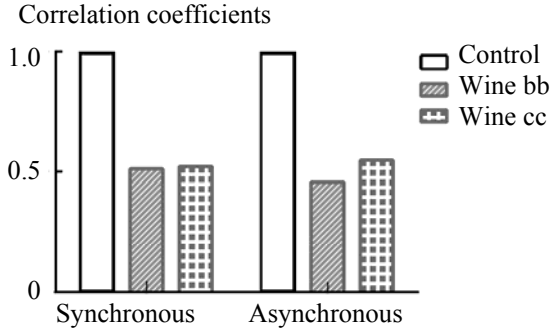

Fig. 5. Correlation coefficient result between wine bb with wine aa, and wine cc with wine aa, based on synchronous and asynchronous two-dimensional correlation Raman spectroscopy.

Conclusion. Traditional quality discrimination analysis of red wine can rely on evaluation by tasting experts and by analysis of its characteristic flavors. Raman spectroscopy can obtain information on the characteristic vibrations of molecules in red wine more quickly and simply than these traditional methods. In the present study, two-dimensional correlation spectroscopy was applied to study Raman spectra with different laser integration times (1–5 s) to establish a set of characteristic dynamic spectra of red wine samples and to achieve the fusion of the Raman dynamic spectrum of red wine in three dimensions. The discriminant analysis of samples of three brands of red wine was demonstrated based on their different components and spectral responses. The analytical time was short, no sample pretreatment was needed, the spectral acquisition could be completed within 15 s, and the three-dimensional spectral algorithm analysis was highly automated. Therefore, this procedure offers potential for rapidly discriminating the quality of different brands of red wine.

Acknowledgment. This research was financial support by National Natural Science Foundation of China (61602217, 61373058) and University Natural Science Research Project of Jiangsu Province (16KJB150015).

REFERENCES

1. I. L. Francis, P. O. Williamson, *Aust. J. Grape Wine Res.*, **21**, 554–567 (2015).
2. H. X. Yu, L. Q. Sun, J. Qi, *Chin. J. Nat. Med.*, **12**, 517–524 (2014).
3. D. W. Jeffery, M. D. Mercurio, M. J. Herderich, Y. Hayasaka, P. A. Smith, *J. Agric. Food Chem.*, **56**, 2571–2580 (2008).
4. C. A. T. Dos Santos, R. N. M. J. Páscoa, J. A. Lopes, *Trends Anal. Chem.*, **88**, 100–118 (2017).
5. M. Sha, Z. Y. Zhang, D. D. Gui, Y. B. Wang, L. L. Fu, H. Y. Wang, *Food Anal. Methods*, **10**, 3415–3423 (2017).
6. E. Borràs, J. Ferré, R. Boqué, M. Mestres, L. Aceña, O. Busto, *Anal. Chim. Acta*, **891**, 1–14 (2015).
7. I. Noda, *J. Mol. Struct.*, **1124**, 3–7 (2016).
8. I. Noda, *J. Mol. Struct.*, **1124**, 197–206 (2016).
9. I. Noda, *J. Mol. Struct.*, **1124**, 42–52 (2016).
10. I. Noda, *J. Mol. Struct.*, **1124**, 29–41 (2016).
11. I. Noda, Y. Ozaki, *Two-Dimensional Correlation Spectroscopy – Applications in Vibrational and Optical Spectroscopy*, John Wiley & Sons, Chichester, 1–195 (2005).
12. I. Noda, *J. Mol. Struct.*, **1124**, 53–60 (2016).
13. D. A. Magdas, F. Guyon, I. Feher, S. C. Pinzaru, *Food Control*, **85**, 385–391 (2018).
14. G. X. Wang, H. Y. Wang, H. Wang, Z. Y. Zhang, J. Liu, *Spectrosc. Spectral Anal.*, **36**, 729–735 (2016).
15. L. Mandrile, G. Zeppa, A. M. Giovannozzi, A. M. Rossi, *Food Chem.*, **211**, 260–267 (2016).
16. C. Martin, J. Bruneel, F. Guyon, B. Médina, M. Jourdes, P. Teissedre, F. Guillaume, *Food Chem.*, **181**, 235–240 (2015).
17. Q. Wang, Z. Li, Z. Ma, L. Liang, *Sens. Actuators, B*, **202**, 426–432 (2014).
18. Z. Y. Zhang, M. Sha, H. Y. Wang, *J. Raman Spectrosc.*, **48**, 1111–1115 (2017).
19. J. Chen, Q. Zhou, I. Noda, S. Sun, *Appl. Spectrosc.*, **63**, 920–925 (2009).