



Chemometric classification of Chinese lager beers according to manufacturer based on data fusion of fluorescence, UV and visible spectroscopies



Jin Tan, Rong Li, Zi-Tao Jiang*

Tianjin Key Laboratory of Food Biotechnology, College of Biotechnology and Food Science, Tianjin University of Commerce, Tianjin 300134, People's Republic of China

ARTICLE INFO

Article history:

Received 22 January 2015

Received in revised form 18 March 2015

Accepted 19 March 2015

Available online 28 March 2015

Keywords:

Beers

Manufacturer

Data fusion

Synchronous fluorescence

UV and visible

Principal component analysis (PCA)

Linear discriminant analysis (LDA)

ABSTRACT

We report an application of data fusion for chemometric classification of 135 canned samples of Chinese lager beers by manufacturer based on the combination of fluorescence, UV and visible spectroscopies. Right-angle synchronous fluorescence spectra (SFS) at three wavelength difference $\Delta\lambda = 30, 60$ and 80 nm and visible spectra in the range $380\text{--}700$ nm of undiluted beers were recorded. UV spectra in the range $240\text{--}400$ nm of diluted beers were measured. A classification model was built using principal component analysis (PCA) and linear discriminant analysis (LDA). LDA with cross-validation showed that the data fusion could achieve $78.5\text{--}86.7\%$ correct classification (sensitivity), while those rates using individual spectroscopies ranged from 42.2% to 70.4% . The results demonstrated that the fluorescence, UV and visible spectroscopies complemented each other, yielding higher synergic effect.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Beer is the most widely and largest consumed alcoholic beverage in the world, and the third most popular drink overall, ranking after water and tea (Nelson, 2008). It is produced by the brewing and fermentation of starches, mainly derived from malted barley, while certain adjunct crops such as wheat, maize and rice maybe used. Beer is flavored with hops, which contribute bitterness and act as a natural preservative. China owns the largest beer production and consumption in the world (Li, Xu, Schwarz, & Gu, 2007). The most popular type of beer in China is American style lagers. Numbers of manufacturers produce tens of brands of lager beers with varied price and quality in Chinese market, however, little effort has been spent on the authentication of Chinese beers, especially the differentiation of manufacturers (Li et al., 2007).

The classification of beers, according to different types, brands and factories has aroused continuous interest, based on the determination of characteristic compounds in beers by various analytical instruments (Alcázar, Jurado, Palacios-Morillo, de Pablos, & Martín, 2012; Cajka, Riddellova, Tomaniova, & Hajslova, 2010; da Silva, Augusto, & Poppi, 2008; Li et al., 2007; Mahmood, Petraco, & He, 2012; Siebert, 2005; Siebert & Stenroos, 1989), or

the utilization of electronic tongues (Cetó, Gutiérrez-Capitán, Calvo, & del Valle, 2013; Ghasemi-Varnamkhasti et al., 2012; Gutiérrez et al., 2013) and sensor arrays (Zhang, Bailey, & Suslick, 2006). Recently, there was a notable growth for various spectroscopies including near-infrared (NIR) (Biancolillo, Bucci, Magri, Magri, & Marini, 2014; Di Egidio, Oliveri, Woodcock, & Downey, 2011; Li et al., 2009), mid-infrared (MIR) (Biancolillo et al., 2014; Engel, Blanchet, Buydens, & Downey, 2012; Vera et al., 2011), and UV–visible (Biancolillo et al., 2014; Vera et al., 2011; Weeranantanaphan & Downey, 2010) in beers authentication application.

To discriminate a unique target from other types of beers, for example, a Trappist beer from non-Trappist beers, an individual spectroscopy may be competent (Engel et al., 2012; Weeranantanaphan & Downey, 2010). However, regarding to a group of highly similar beers of a same type, a single spectroscopy solely may not be sufficient. In such circumstance, data fusion of different techniques with improved classification ability as compared with the use of individual techniques provides an alternative solution (Gutiérrez et al., 2013; Vera et al., 2011). A recent excellent example was the differentiation of a high quality Italian craft beer (Reale) from other beers based on the data fusion of thermogravimetry, NIR, MIR, UV and visible spectroscopies (Biancolillo et al., 2014). Fluorescence (Karoui & Blecker, 2011; Poryvkina, Tsvetkova, & Sobolev, 2014; Sergiel, Pohl, Biesaga, &

* Corresponding author. Tel.: +86 22 26669671; fax: +86 22 26669670.

E-mail addresses: ztjiang@tjcu.edu.cn, ztjiang@yahoo.com (Z.-T. Jiang).

Mironczyk, 2014; Sikorska, Górecki, Khmelinskii, Sikorski, & De Keukeleire, 2006; Sikorska, Górecki, Khmelinskii, Sikorski, & Koziół, 2005; Sádecká & Tóthová, 2007; Sádecká, Tóthová, & Májek, 2009) and UV–visible (Acevedo, Jiménez, Maldonado, Domínguez, & Narváez, 2007; Azcarate, Cantarelli, Pellerano, Marchevsky, & Camiña, 2013; Di Anibal, Rodríguez, & Albertengo, 2014; Di Anibal et al., 2012; Souto et al., 2010) spectroscopies have shown their great capability and convenience in classification of diverse foods and beverages. Fluorescence spectroscopy is of high sensitivity and selectivity, however, only a small portion of compounds exhibit fluorescence. Correspondingly, though UV–visible spectroscopy is limited to its relative low sensitivity and spectra overlap, many organic compounds can absorb UV or visible light. Thus, they are complementary to each other on certain occasions, for example, when used as detectors for liquid chromatography. The data fusion of fluorescence and UV–visible spectroscopies is anticipated to be effective. However, there is little attention paid to such kind of data fusion for food authentication.

Herein, we report the chemometric classification of Chinese lager beers according to manufacturer based on the data fusion of fluorescence, UV and visible spectroscopies. The objective of this study is not only to establish a model to differentiate Chinese beer manufacturers, but also to utilize the synergy between different spectroscopies, to show that fluorescence, UV and visible spectroscopies provide complementary information, and to reveal the extent the data fusion can improve the discrimination performance.

2. Materials and methods

2.1. Beer samples

A total of 135 canned (330 mL) samples of 27 beer brands (each represented by five cans) from eleven Chinese manufacturers were purchased in three different local supermarkets. Three cans of each brand were bought in Jun 2014 (all the production dates on the cans were prior to Jun 2014) and the other two in Nov 2014 (the production dates were between Jun and Oct 2014). Thus the five cans of each brand are believed to be able to represent the natural variability of beer products to some degree. As all the samples are America style lagers, their colors are pale yellow with tiny difference between them which is hard to discern by naked-eye. The % ABV (alcohol by volume) and original gravity (°P) of these samples ranges in 2.5–4.3% and 8.0–12.0 °P, respectively, as shown on the labels of the beer cans. The detailed sample information is listed in Table 1. Eleven manufacturers are identified by letters from A to K. All the beers were analyzed after collection immediately. Prior to spectroscopy measurement, beers were ultrasonicated for 15 min to eliminate the interference from dissolved carbon dioxide and then filtrated via 0.45 µm membrane filters to circumvent the influence of bacteria and large insoluble particles as much as possible.

2.2. Fluorescence spectroscopy

Fluorescence spectra were obtained with a 970CRT spectrofluorometer (INESA, China) with a 150 W xenon lamp source for excitation. The slit widths for excitation and emission were both 5 nm. The acquisition interval and the integration time were set at 1 nm and 0.1 s, respectively. The right-angle geometry was used for the spectra acquisition with a 1 cm fused-quartz cuvette. All the fluorescent spectra were measured on undiluted beer samples except for additional statement. Excitation–emission matrices (EEMs) were obtained by recording the emission spectra in the range from 290 to 700 nm, excited from 250 to 550 nm with 10 nm intervals. For synchronous fluorescence spectra (SFS), the

Table 1

Detailed information of the investigated Chinese lager beers.

Manufacturer	Sample code ^a	Draft style ^b	Original gravity (°P) ^c	% ABV (alcohol by volume) ^c
A	A1	No	10.0	3.6
	A2	No	9.0	3.3
	A3	No	9.1	3.6
	A4	No	9.7	3.6
	A5	Yes	8.0	3.1
B	B1	Yes	8.0	2.5
	B2	No	11.0	3.7
	B3	No	12.0	4.1
	B4	No	8.0	2.5
	B5	No	9.0	3.3
	B6	No	10.0	3.3
	B7	No	10.0	3.3
C	C1	No	11.0	4.3
	C2	No	10.0	4.0
D	D1	No	11.0	4.3
	D2	No	10.0	3.6
E	E1	No	11.0	4.3
	E2	No	11.0	4.3
F	F1	No	11.1	4.3
	F2	No	10.3	4.0
G	G	No	11.5	4.5
H	H	No	8.0	2.5
I	I	Yes	10.0	3.6
J	J1	No	10.0	3.3
	J2	Yes	10.5	3.7
K	K1	Yes	10.0	3.6
	K2	No	10.0	3.6

^a Codes “A–K” refer to the beer manufacturers. Subscript values “1, 2, ...” indicate the different brands of the same manufacturer and are randomly distributed to each brand. Samples from different manufacturers with the same subscript value have no connection with each other.

^b Means unpasteurized.

^c Indicated on the labels of the beer cans.

excitation and emission in the 300–600 nm range with a wavelength interval of 1 nm were scanned simultaneously with a constant wavelength difference $\Delta\lambda$ between them. Spectra were recorded at the $\Delta\lambda$ values of 30, 60 and 80 nm for each sample. Fluorescence intensities were plotted as a function of the excitation wavelength. No pretreatment was applied to the fluorescence spectra prior to statistical analysis other than auto-scaling of fluorescence intensities at each excited wavelength separately toward all the samples. Auto-scaling was executed to give variables with zero means and unit standard deviation, that is, each entry was subtracted by column means, and then divided by column standard deviations.

2.3. UV–visible spectroscopy

UV–visible spectra were recorded on a U-3900 spectrophotometer (Hitachi, Japan). The scan speed and slit width were 240 nm min^{−1} and 2 nm, respectively. Due to the different molar extinction coefficients of beers in UV and visible spectra, UV signals in the range 240–400 nm were acquired after diluting each beer sample with ultrapure water by 1:25 (v/v), while visible spectra in the range 380–700 nm were collected directly on undiluted beer samples. UV and visible spectra were pretreated with first-derivative to correct for baseline shifts. The first-derivative of the spectra was taken using the Savitzky and Golay (1964) method with second-order smoothing polynomials through seven smoothing points.

2.4. Statistical analysis

Principal component analysis (PCA) was initially employed for exploratory spectral analysis, and subsequently, Fisher-linear

discriminant analysis (Fisher-LDA, Belhumeur, Hespanha, & Kriegman, 1996) was used to derive a classification rule according to manufacturer based on PCs scores (Berrueta, Alonso-Salces, & Héberger, 2007). In low-level data fusion, all the data of SF, UV and visible spectroscopies were grouped into a new matrix, and then PCA and LDA were executed sequentially (Vera et al., 2011). In mid-level data fusion, PCA was firstly carried out toward SF, UV and visible spectra separately, and then LDA was run on the combination of the scores of the yielded PCs (Vera et al., 2011). In all the analysis, PCs with eigenvalues higher than one explaining more variance than average were considered to be significant and were used as variables for LDA. It is one of the criteria that have been used to select significant PCs (Berrueta et al., 2007; Cajka et al., 2010; Li et al., 2007; Siebert, 2005). All the statistical analysis was performed using IBM SPSS, version 19.0 (IBM, USA).

2.5. Method validation

The PCA-LDA classification model was validated by cross-validation with five cross-validation groups ($k = 5$). All the samples were randomly split into five subsets of approximately the same size, where a given subset was left out to be used as an evaluation set while the remaining four subsets were used to yield the classification rule. The process was repeated for five times until each group was left out once.

3. Results and discussion

3.1. Fluorescence spectroscopy

In application of fluorescence spectroscopy in food analysis, EEMs and SFS are often depicted (Guilbault, 1999; Poryvkina et al., 2014; Sergiel et al., 2014; Sikorska, Górecki, Khmelinskii, Sikorski, & De Keukeleire, 2004; Sikorska, Górecki, et al., 2006; Sikorska et al., 2005; Sádecká & Tóthová, 2007; Sádecká et al., 2009). They contain more useful information about the fluorescent species than conventional excitation and emission spectra. Regarding to the fluorescence of beers, it was reported that right-angle geometry for diluted beer samples and front-face geometry for bulk beer samples enabled the observation of an intense short-wavelength fluorescence in diluted samples and a weak emission at longer-wavelength in bulk samples (Sikorska, Górecki, et al., 2006).

In the present study, the right-angle geometry for both the bulk and diluted (3% v/v, beer:water) beer samples were measured. The right-angle fluorescence of the undiluted samples did not show significantly decreased and distorted fluorescence spectra which may be possibly encountered when applying right-angle fluorescence to concentrated solutions (Karoui & Blecker, 2011). Thus, to maintain the original organization of the sample matrix and enable the obtained results to be extrapolated to the native bulk samples, the right-angle geometry of undiluted beer samples was recorded. The tested samples showed similar shape of fluorescence spectra. As illustrated in Fig. 1a, an intense emission band with excitation at 370 nm and emission at 440 nm, and a less intense emission band with excitation at 450 nm and emission at 520 nm are observed, which can be ascribed to iso- α -acids, phenolic compounds and vitamin B group (Christensen, Ladefoged, & Norgaard, 2005; Sikorska, Górecki, et al., 2006; Sikorska et al., 2008). The less intense emission band with maximal excitation at 450 nm and emission in the range 500–600 nm was reported to be attributed to riboflavin (vitamin B₂) (Sikorska, Gliszczyńska-Świąto, et al., 2008).

The fluorescence of diluted beers (3% v/v, beer:water) showed similar but less intense fluorescence. Surprisingly, the

characteristic band belonging to aromatic amino acids (Sikorska, Górecki, et al., 2006), which could not be observed in the bulk beer, was also not found in the diluted beers. This may be caused by the fact that the beers were not diluted enough, or the likely lack of aromatic amino acids in the studied Chinese lager beers. As it has already been able to offer useful information, the right-angle geometry of undiluted beers was directly recorded and the further dilution was not performed. The benefits of doing this are the easy sample preparation without dilution and avoidance of additional device for front-face geometry which has not been widely equipped in many laboratories.

Although containing less information than EEMs, SFS is simpler and faster to collect, and has been widely employed in classification of various foods and beverages. It has been shown that the SFS of beers obtained at $\Delta\lambda = 30$ and 60 nm reflect the major fluorophores in beers and hence yield stronger fluorescence intensities (Sikorska, Gliszczyńska-Świąto, et al., 2008). As can be seen from Fig. 1b, for SFS at $\Delta\lambda = 30$ nm, three overlapping bands with maxima at 395, 420 and 475 nm are observed. The fluorescence maxima shifts to shorter wavelengths with increasing $\Delta\lambda$, and the SFS at $\Delta\lambda = 60$ and 80 nm show higher intensity, which is related to the Stokes shift for vitamin B group (3500 cm^{-1} for riboflavin) (Sikorska, Górecki, et al., 2006). For $\Delta\lambda = 60$ nm, an obvious band broadening results in only two bands with maxima at 393 and 466 nm, and along with the further increase of the $\Delta\lambda$ to 80 nm, only one band at 393 nm is observed and the peak at 466 nm changes to a shoulder peak.

PCA was firstly applied to the SFS of the beers (Figs. 2, S1 and S2). As can be seen from Fig. 2b, the beers from different manufacturers are totally intermingled with each other. Replicate samples of the same brand are grouped in cluster, some of which are overlapped and others not, indicating the batch-to-batch variation. Although the SFS at different $\Delta\lambda$ reflect various fluorescent species in beers to differed degrees, the PCA plots of $\Delta\lambda = 30, 60$ and 80 nm show similar location patterns for all the samples. The selection of $\Delta\lambda$ here does not affect the PCA results significantly.

The PCA loading plot expresses the extent to which the generated PCs correlate with the original variables. As shown in Fig. 2c, the most relevant variables to the first PC are the fluorescence intensities at excitation from 400 to 500 nm, which is in agreement with the excitation of riboflavin (Sikorska, Górecki, et al., 2006), while those to the second PC are the fluorescence intensities from 320 to 380 nm, which is closely to the excitation of iso- α -acids (Christensen et al., 2005). Thus it could be presumably concluded that the differentiation of the beers by the PCA derived from the SFS relies on the disparity of the contents of riboflavin and iso- α -acids in different beer samples, while the former plays a more dominant role than the latter. Such diversity of these organic compounds in beers may stem from different raw materials such as yeasts and hops and processing technology employed by different manufacturers.

In all the three PCA score plots derived from the SFS, it is noteworthy that the draft style beers (samples 'A5', 'B1', 'J2' and 'K2') are distinguished from the common pasteurized ones from the same manufacturers. The scores of the draft style beers are located in the right side in the first PC compared with the corresponding pasteurized ones. Such result can be explained by their relative higher fluorescence which may be related to the 'cold pasteurization' (filtration) in the production of canned draft style beers that would leave them with more vitamin B than the pasteurized ones.

3.2. UV and visible spectroscopies

The investigated Chinese lager beers showed in general similar UV and visible spectra to other types of beers (Biancolillo et al., 2014; Weeranantanaphan & Downey, 2010). For UV spectra

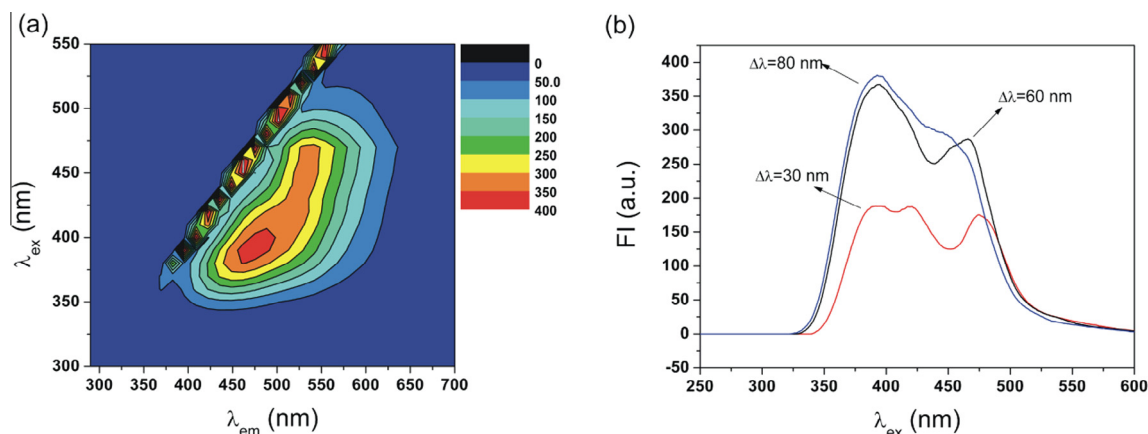


Fig. 1. (a) Contour map for the excitation–emission matrix (EEM) of sample ‘A1’. The wavelength interval was 10 nm. (b) Synchronous fluorescence spectra (SFS) of sample ‘A1’. The wavelength interval was 1 nm. Right-angle geometry of undiluted beers was recorded for both EEM and SFS.

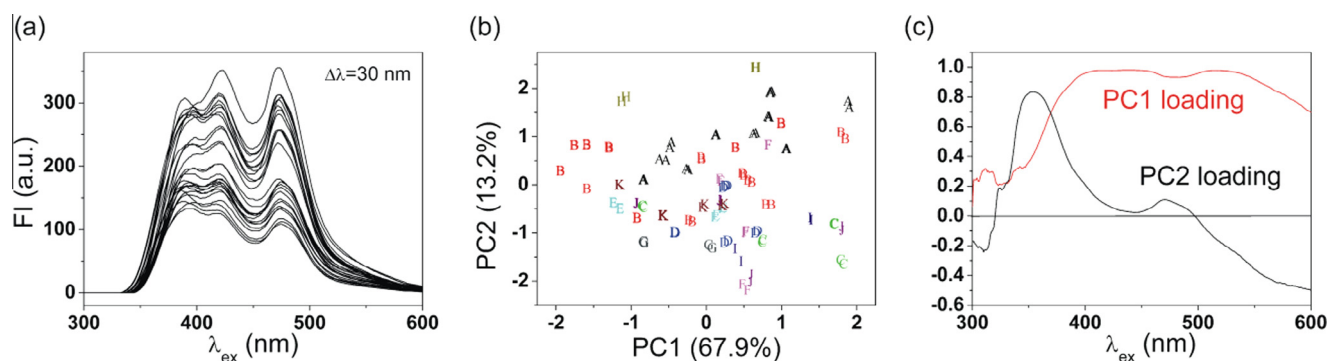


Fig. 2. (a) Synchronous fluorescence spectra (SFS) at $\Delta\lambda = 30$ nm of the beers. The wavelength interval was 1 nm. Right-angle geometry of undiluted beers was recorded. (b) The corresponding principal component analysis (PCA) score plot toward 135 samples using the SFS data at $\Delta\lambda = 30$ nm as variables (27 brands \times 5 cans \times 301 wavelengths). Eleven manufacturers are identified by letters from A to K. (c) The corresponding plot shows the loadings to the first two PCs.

(Fig. 3a), a strong band from 250 to 300 nm and a weak band from 300 to 350 nm are observed. The typical compounds that can absorb the light in these wavelength regions are iso- α -acids, vitamin B group and phenolic compounds (Christensen et al., 2005). For visible spectra (Fig. 3d), the beers show lower absorbance owing to smaller molar extinction coefficients, and the absorbance decreases with the increase of wavelength. The representative components absorbing visible light in beers include riboflavin and phenolic compounds such as flavonols (Vera et al., 2011). Although the peak assignments in UV and visible spectra to specific compounds are hard to achieve due to the peak overlap, they can provide comprehensive information which may be complementary to fluorescence spectra.

PCA was employed to the obtained UV and visible spectra, respectively. As presented in Fig. 3, the beers from different manufacturers are still severely overlapped with each other. The variables highly associated with the first PC are the absorbance from 300 to 400 nm in UV section, which is partially related to riboflavin, and from 400 to 600 nm in visible region, which refers to the beer color. The absorbance in such broad wavelength ranges is the composition of numbers of organic compounds which are all responsible for the discrimination but to varied degrees.

3.3. Data fusion

The comparison between the LDA results according to manufacturer using individual procedures and data fusion is listed in Table 2. The classification results for each class expressed in terms

of sensitivity (true positive rate) and specificity (true negative rate) are shown in Supplementary data (Tables S1–S22). Neither SF nor UV and visible spectra could individually afford highly correct classification of the studied beers by manufacturer. The sensitivity of cross-validation is 42.2–47.4%, 70.4% and 58.5% for SFS, UV and visible spectra, respectively. The LDA score plots (Figs. 4a and S3) show that beers from different manufacturers are totally intermingled, rendering the accurate classification by manufacturer impossible.

In low-level data fusion, the individual spectra obtained from the three spectroscopies were combined into a new matrix composed of 135 samples and 783 variables. As Fisher-LDA cannot work with such a large number of variables, PCA was firstly applied to the matrix and Fisher-LDA according to manufacturer was then executed on the scores of the PCs with eigenvalues higher than one. The PCA loadings (Figs. S4–S6) show that the variables relevant to the first PC are iso- α -acids for SFS, riboflavin for UV spectra and beer color for visible spectra. It is noteworthy that the first two PCs of SFS are interchanged compared with using the individual observations which is shown in Fig. 2. The low-level data fusion combines all the data and re-generates new orthogonal PCs.

Compared with those using the individual observations, the LDA in low-level data fusion using the new PCs offers better results as the sensitivity of cross-validation significantly enhances to 83–86.7%. As can be seen from Fig. 4b, although not completely separated, the samples of each manufacturer are concentrated into different clusters, with the partial overlap across several manufacturers, for example, ‘B’ mixed with ‘K’, and ‘J’ mingled with ‘C’ and

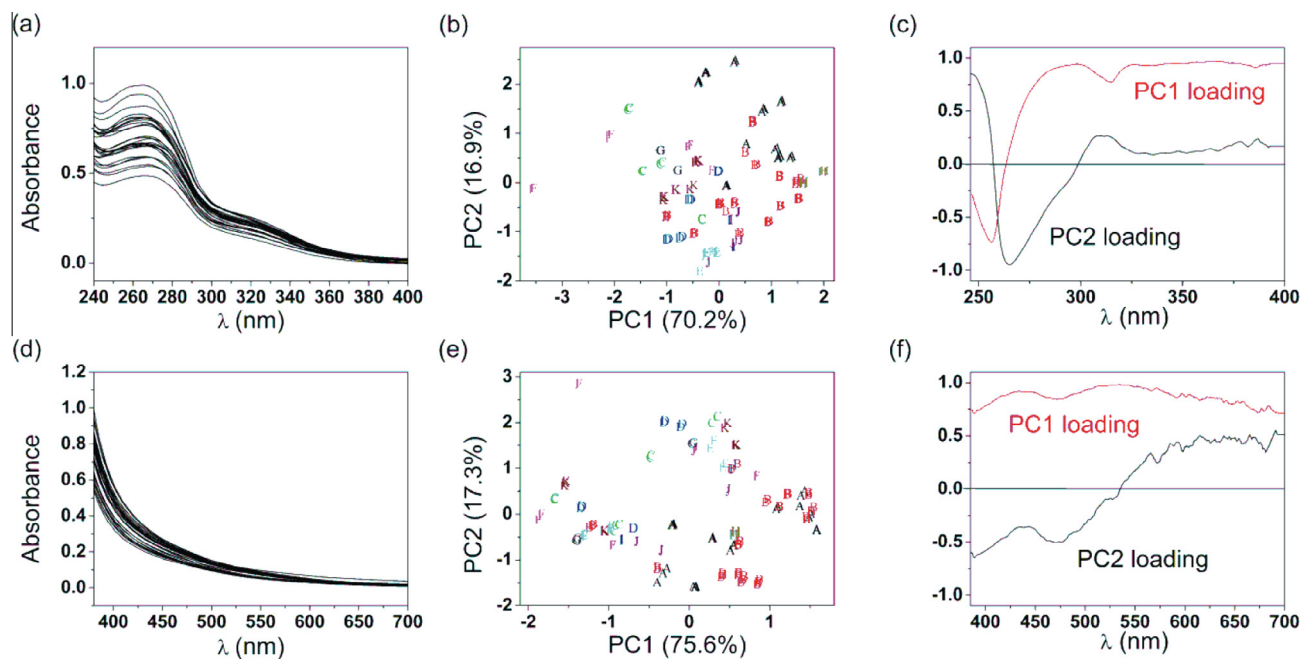


Fig. 3. UV (a) and visible (d) spectra of the beers. Diluted (1:25, v/v) and undiluted beers were test for UV and visible spectroscopies, respectively. The wavelength interval was 1 nm. The corresponding principal component analysis (PCA) score plots using the UV (b, 27 brands \times 5 cans \times 161 wavelengths) and visible (e, 27 brands \times 5 cans \times 321 wavelengths) spectra. Eleven manufacturers are identified by letters from A to K. The corresponding plots show the loadings to the first two PCs of UV (c) and visible (f) spectra.

Table 2

Results of LDA classification according to manufacturer from individual procedures and data fusion.

Procedure	$\Delta\lambda^c$	UV	Vis	Sensitivity (%)		Specificity (%)		Number of PCs used (cumulative proportion of variability they account for, %)		
				Calibration	Cross-validation ^d	Calibration	Cross-validation ^d	SF ^e	UV	Vis
Individual spectroscopy	30	–	–	57	42.2	95.7	94.2	7 (98.5)	–	–
	60	–	–	57.8	43.7	95.8	94.4	5 (99.1)	–	–
	80	–	–	57	47.4	95.7	94.7	5 (99.3)	–	–
	–	✓	–	82.2	70.4	98.2	97	–	7 (98.6)	–
	–	–	✓	71.1	58.5	97.1	95.9	–	–	9 (97.3)
Low-level data fusion ^a	30	✓	✓	98.5	86.7	99.9	98.7	20 (98.3) ^f	–	–
	60	✓	✓	99.3	84.4	99.9	98.4	19 (98.6) ^f	–	–
	80	✓	✓	99.3	83	99.9	98.3	19 (98.6) ^f	–	–
Mid-level data fusion ^b	30	✓	✓	100	83	100	98.3	7 (98.5)	7 (98.6)	9 (97.3)
	60	✓	✓	98.5	80.7	99.9	98.1	5 (99.1)	7 (98.6)	9 (97.3)
	80	✓	✓	98.5	78.5	99.9	97.9	5 (99.3)	7 (98.6)	9 (97.3)

^a Combination of individual spectroscopies.

^b Combination of PCs of individual observations.

^c Wavelength interval in synchronous fluorescence spectra (nm).

^d Divided into five cross-validation groups.

^e Synchronous fluorescence.

^f New PCs of the combined data of synchronous fluorescence, UV and visible spectra.

'I'. Although still not perfect, the improved performance of the low-level data fusion shows that combining results from different procedures adds more useful information, leading to better classification.

Unlike low-level data fusion, feature reduction in mid-level data fusion is usually performed on the PCs obtained individually for each original data matrix. In the present study, taking the mid-level data fusion of the SFS at $\Delta\lambda = 30$ nm, UV and visible spectra as an example, the PCs used in the fusion are the first seven PCs of each SFS and UV spectra and the first eight PCs of the visible spectra. All the selected PCs cumulatively account for at least 97% of the total variance for each spectroscopy. Fig. 4c shows the obtained LDA score plot. The partial overlap of samples from different manufacturers still persists as in the low-level data

fusion. Samples 'B' and 'K' are still severely intermingled, and two of the sample 'G' fall in the region of class 'F'. The sensitivity of cross-validation ranges from 78.5% to 83% and decrease a bit compared with those in the low-level data fusion. It is somewhat surprising since low-level data fusion is normally less competent than the mid-level one (Biancolillo et al., 2014). Such result could be explained by the fact that the first PCs of SFS, UV and visible spectra are all relevant to riboflavin, and the second PCs of SFS and UV spectra are both associated with iso- α -acids. Thus the LDA based on the combination of these PCs in mid-level data fusion suffers from information redundancy. However, in low-level data fusion, PCA is applied to the combined data and new orthogonal PCs are generated, and hence such redundancy is eliminated.

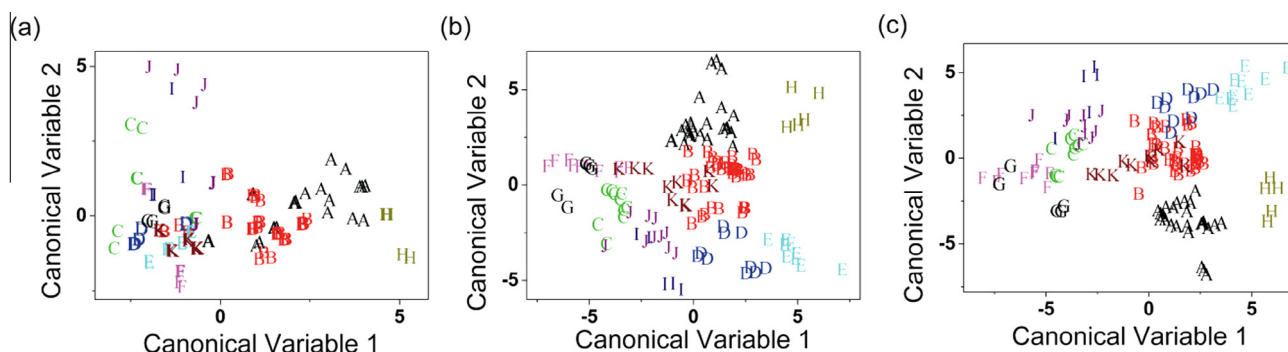


Fig. 4. Linear discriminant analysis (LDA) score plots for classification of the beers according to manufacturer based on the synchronous fluorescence spectra (SFS) at $\Delta\lambda = 30$ nm (a), the low-level (b) and mid-level (c) data fusion of SFS at $\Delta\lambda = 30$ nm, UV and visible spectra. Eleven manufacturers are identified by letters from A to K.

The LDA score plots for the low-level and mid-level data fusion involving SFS at $\Delta\lambda = 60$ and 80 nm (Fig. S7) exhibit similar distribution patterns to those at $\Delta\lambda = 30$ nm. However, it should be noted that although the SFS at $\Delta\lambda = 80$ nm offers the highest sensitivity (47.4%) among the LDA of an individual SFS, the data fusions involving it obtain the lowest rate (83% and 78.5%) among the three $\Delta\lambda$. Such observation may be attributed to the fact that the SFS at $\lambda = 80$ nm would neglect some fluorescent species with small Stokes shift and hence could not provide as much information as those at $\lambda = 30$ and 60 nm. Such omission may just be needed for UV–visible spectra and hence reduces the synergic effect in data fusion. Contrarily, the SFS at $\Delta\lambda = 30$ and 60 nm could reflect more fluorescent compounds in beers, although maybe to a lower degree for some species with larger Stokes shift, for example, riboflavin (Sikorska, Górecki, et al., 2006). Such comprehensive reflection of fluorescent species makes the SFS at $\Delta\lambda = 30$ and 60 nm preferred in data fusion. The exact nature and extent of the effect of selection of $\Delta\lambda$ in SFS on classification in data fusion deserve further investigation.

Comparing the LDA results between an individual spectroscopy and the data fusion of three spectroscopies, it can also be found that the UV and visible spectroscopies contribute complementary information to fluorescence spectroscopy. In the LDA of individual data of SFS, sample 'B1' was wrongly categorized into class 'A'. This incorrect recognition could be ascribed to higher content of vitamin B in sample 'B1' as it is a draft style beer. However, the sample wrongly classified was correctly identified in data fusion. The high similarity of the beers from the same manufacturer in UV–visible spectra compensates for their difference in SFS.

Except the classification by manufacturer, the discrimination of different brands of the same manufacturer was also attempted. As there are only one or two brands commercially available for manufacturer C–K each, the discrimination of beer brands was limited to manufacturer A and B. No obvious separation between samples related to brand was observed. Several brands of beers made by the same manufacturer could not be differentiated in either PCA or LDA score plot. Such observation may be related to the high similarity in raw materials and processing technology employed by the same factory.

4. Conclusions

In conclusion, this study achieved satisfactory discrimination among Chinese lager beers made by different manufacturers based on the data fusion of fluorescence, UV and visible spectroscopies. The PCA preliminarily separated the samples and revealed the PCs and relevant variables to the discrimination, while the LDA with cross-validation allowed the qualitative classification by manufacturer with relatively high accuracy. The main compounds

responsible for the classification were presumed to be iso- α -acids, phenolic compounds, and vitamin B group such as riboflavin. Compared with individual spectroscopy, the data fusion showed better discrimination ability. The coupling of fluorescence and UV–visible spectroscopies overcame the constraints when used individually. The selection of $\Delta\lambda$ in SFS did not have significant influence on the PCA results; however, it affected the PCA–LDA results in data fusion. The data fusion involving the SFS at a certain $\Delta\lambda$ which can reflect more fluorescent compounds may yield higher synergic effects. The data fusion of fluorescence, UV and visible spectroscopies, and maybe with the addition of other complementary spectroscopy or other kinds of technique, is promising for food authentication application and is worth further investigation.

Acknowledgements

The present research was financially supported by the National Natural Science Foundation of China (Grant 21405111), Tianjin Natural Science Foundation (Grant 12JCZDJC34100 and 13JCYBJC18700), Innovation Team Training Program of Tianjin Universities (Grant TD12-5049), and Tianjin Funding Project for Excellent Young College Teachers (Grant 507-125RCPY0317).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2015.03.085>.

References

- Acevedo, F. J., Jiménez, J., Maldonado, S., Domínguez, E., & Narváez, A. (2007). Classification of wines produced in specific regions by UV–visible spectroscopy combined with support vector machines. *Journal of Agricultural and Food Chemistry*, 55, 6842–6849.
- Alcázar, A., Jurado, J. M., Palacios-Morillo, A., de Pablos, F., & Martín, M. J. (2012). Differentiation of blonde beers according to chemical quality indicators by means of pattern recognition techniques. *Food Analytical Methods*, 5, 795–799.
- Azcarate, S. M., Cantarelli, M. A., Pellerano, R. G., Marchevsky, E. J., & Camiña, J. M. (2013). Classification of Argentinean Sauvignon blanc wines by UV spectroscopy and chemometric methods. *Journal of Food Science*, 78, C432–C436.
- Belhumeur, P., Hespanha, J., & Kriegman, D. (1996). Eigenfaces vs Fisherfaces: Recognition using class specific linear projection. *European Conference on Computer Vision*, 45–58.
- Berrueta, L. A., Alonso-Salces, R. M., & Héberger, K. (2007). Supervised pattern recognition in food analysis. *Journal of Chromatography A*, 1158, 196–214.
- Biancolillo, A., Bucci, R., Magri, A. L., Magri, A. D., & Marini, F. (2014). Data-fusion for multiparameter characterization of an Italian craft beer aimed at its authentication. *Analytica Chimica Acta*, 820, 23–31.
- Cajka, T., Ridelova, K., Tomaniova, M., & Hajslova, J. (2010). Recognition of beer brand based on multivariate analysis of volatile fingerprint. *Journal of Chromatography A*, 1217, 4195–4203.

- Cetó, X., Gutiérrez-Capitán, M., Calvo, D., & del Valle, M. (2013). Beer classification by means of a potentiometric electronic tongue. *Food Chemistry*, 141, 2533–2540.
- Christensen, J., Ladefoged, A. M., & Norgaard, L. (2005). Rapid determination of bitterness in beer using fluorescence spectroscopy and chemometrics. *Journal of the Institute of Brewing*, 111(1), 3–10.
- da Silva, G. A., Augusto, F., & Poppi, R. J. (2008). Exploratory analysis of the volatile profile of beers by HS-SPME-GC. *Food Chemistry*, 111, 1057–1063.
- Di Anibal, C., Rodriguez, M. S., & Albertengo, L. (2014). UV-visible spectroscopy and multivariate classification as a screening tool to identify adulteration of culinary spices with Sudan I and blends of Sudan I + IV dyes. *Food Analytical Methods*, 7, 1090–1096.
- Di Anibal, C., Ruisánchez, I., Fernández, M., Forteza, R., Cerdà, V., & Callao, M. P. (2012). Standardization of UV-visible data in a food adulteration classification problem. *Food Chemistry*, 134, 2326–2331.
- Di Egidio, V., Oliveri, P., Woodcock, T., & Downey, G. (2011). Confirmation of brand identity in foods by near infrared transreflectance spectroscopy using classification and class-modelling chemometric techniques – The example of a Belgian beer. *Food Research International*, 44, 544–549.
- Engel, J., Blanchet, L., Buydens, L. M. C., & Downey, G. (2012). Confirmation of brand identity of a Trappist beer by mid-infrared spectroscopy coupled with multivariate data analysis. *Talanta*, 99, 426–432.
- Ghasemi-Varnamkhasti, M., Rodríguez-Méndez, M. L., Mohtasebi, S. S., Apetrei, C., Lozano, J., Ahmadi, H., et al. (2012). Monitoring the aging of beers using a bioelectronic tongue. *Food Control*, 25, 216–224.
- Guilbault, G. G. (1999). *Practical Fluorescence*. New York: Marcel Dekker.
- Gutiérrez, J. M., Haddi, Z., Amari, A., Bouchikhi, B., Mimendia, A., Cetó, X., et al. (2013). Hybrid electronic tongue based on multisensor data fusion for discrimination of beers. *Sensors and Actuators B*, 177, 989–996.
- Karoui, R., & Blecker, C. (2011). Fluorescence spectroscopy measurement for quality assessment of food systems – A review. *Food and Bioprocess Technology*, 4, 364–386.
- Li, H., Takahashi, Y., Kumagai, M., Fujiwara, K., Kikuchi, R., Yoshimura, N., et al. (2009). A chemometrics approach for distinguishing between beers using near infrared spectroscopy. *Journal of Near Infrared Spectroscopy*, 17(2), 69–76.
- Li, Y., Xu, Y., Schwarz, P. B., & Gu, G. (2007). Organic acids of commercial beers in China: A chemometric study. *Journal of the American Society of Brewing Chemists*, 65(2), 86–91.
- Mahmood, N., Petraco, N., & He, Y. (2012). Elemental fingerprint profile of beer samples constructed using 14 elements determined by inductively coupled plasma-mass spectrometry (ICP-MS): Multivariation analysis and potential application to forensic sample comparison. *Analytical & Bioanalytical Chemistry*, 402, 861–869.
- Nelson, M. (2008). *The Barbarian's beverage: A history of beer in ancient Europe*. Abingdon, Oxon: Taylor and Francis.
- Poryvkina, L., Tsvetkova, N., & Sobolev, I. (2014). Evaluation of apple juice quality using spectral fluorescence signatures. *Food Chemistry*, 152, 573–577.
- Sádecká, J., & Tóthová, J. (2007). Fluorescence spectroscopy and chemometrics in the food classification – A review. *Czech Journal of Food Sciences*, 25, 159–173.
- Sádecká, J., Tóthová, J., & Májek, P. (2009). Classification of brandies and wine distillates using front face fluorescence spectroscopy. *Food Chemistry*, 117, 491–498.
- Savitzky, A., & Golay, M. J. E. (1964). Smoothing and differentiation of data by simplified least squares procedures. *Analytical Chemistry*, 36, 1627–1639.
- Sergiel, I., Pohl, P., Biesaga, M., & Mironczyk, A. (2014). Suitability of three-dimensional synchronous fluorescence spectroscopy for fingerprint analysis of honey samples with reference to their phenolic profiles. *Food Chemistry*, 145, 319–326.
- Siebert, K. J. (2005). Multivariate analysis of routine beer analysis results. *Journal of the American Society of Brewing Chemists*, 63(3), 113–120.
- Siebert, K. J., & Stenroos, L. E. (1989). The use of multivariate analysis of beer aroma volatile compound patterns to discern brand-to-brand and plant-to-plant differences. *Journal of the American Society of Brewing Chemists*, 47, 93–101.
- Sikorska, E., Gliszczyńska-Świgło, A., Insińska-Rak, M., Khmelinskii, I., De Keukeleire, D., & Sikorski, M. (2008). Simultaneous analysis of riboflavin and aromatic amino acids in beer using fluorescence and multivariate calibration methods. *Analytica Chimica Acta*, 613, 207–217.
- Sikorska, E., Górecki, T., Khmelinskii, I. V., Sikorski, M., & De Keukeleire, D. (2004). Fluorescence spectroscopy for characterization and differentiation of beers. *Journal of the Institute of Brewing*, 110(4), 267–275.
- Sikorska, E., Górecki, T., Khmelinskii, I. V., Sikorski, M., & De Keukeleire, D. (2006). Monitoring beer during storage by fluorescence spectroscopy. *Food Chemistry*, 96, 632–639.
- Sikorska, E., Górecki, T., Khmelinskii, I. V., Sikorski, M., & Koziol, J. (2005). Classification of edible oils using synchronous scanning fluorescence spectroscopy. *Food Chemistry*, 89, 217–225.
- Souto, U. T. C. P., Pontes, M. J. C., Silva, E. C., Galvão, R. K. H., Araújo, M. C. U., Sanches, F. A. C., et al. (2010). UV-vis spectrometric classification of coffees by SPA-LDA. *Food Chemistry*, 119, 368–371.
- Vera, L., Aceña, L., Guasch, J., Boqué, R., Mestresa, M., & Busto, O. (2011). Discrimination and sensory description of beers through data fusion. *Talanta*, 87, 136–142.
- Weeranantanaphan, J., & Downey, G. (2010). Identity confirmation of a branded, fermented cereal product by UV spectroscopy: A feasibility study involving a Trappist beer. *Journal of the Institute of Brewing*, 116(1), 56–61.
- Zhang, C., Bailey, D. P., & Suslick, K. S. (2006). Colorimetric sensor arrays for the analysis of beers: A feasibility study. *Journal of Agricultural and Food Chemistry*, 54, 4925–4931.