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Determination of total acid content and moisture content during solid-state fermentation process using hyperspectral imaging

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Abstract

The aim of this study was to demonstrate the capability of hyperspectral imaging (HSI) in rapidly predicting uniformity of vinegar cultural (fermentation substrate) during acetic solid-state fermentation (ASSF). The potential of HSI for predicting the total acid content (TAC), pH and moisture content (MC), whose distribution can characterize the uniformity of vinegar cultural, was investigated during ASSF. Spectral and image information of vinegar cultural was extracted from the region of interest (ROI), followed by standard Normal variate (SNV) to reduce the noise. Synergy interval PLS (siPLS) was used to select optimal wavelengths interval from the full spectral, and genetic algorithm (GA) was used to select optimal wavelength in the optimal interval. Besides, principal component analysis (PCA) was applied to select optimum characteristic images, and the first three principal component (PC) images were selected because PC1, PC2 and PC3 explained over 99.34% of variances of all spectral. Finally, PLS, LS-support vector machine (LS-SVM) and genetic algorithm (GA-PLS) were used to establish prediction model of uniformity indicators of vinegar cultural. The best model indicated high predictive capability with coefficient of determination (R^2) of 0.78, 0.8 and 0.92 of TAC, pH and MC for the testing data set, respectively. TAC, pH and MC were used to calculate every pixel points on the vinegar cultural by the best model. The results indicate that HSI has considerable promise for predicting uniformity of the vinegar cultural during ASSF.

Key words: Hyperspectral imaging technology; Solid-state fermentation; Uniformity; Synergy interval PLS; LS-support vector machine; Distribution

1. Introduction

Solid-state fermentation (SSF) is one of the typical representatives of the fermentation industry. It has continued to build up credibility in recent years in biotech industries due to its potential applications in the production of biologically active secondary metabolites, apart from feed, fuel, food, industrial chemicals and pharmaceutical products and has emerged as an attractive alternative to submerged fermentation [1]. SSF of traditional Chinese vinegar is a mixed-culture refreshment process that proceeds for many centuries without spoilage [2]. Traditional Chinese vinegars, also called cereal vinegars, are important seasoning in Chinese daily life through ages [3]. They are typically fermented from cereals via multiple steps including starch saccharification, alcohol fermentation, and acetic solid-state fermentation (ASSF) [4]. Among all the steps mentioned above, the last step (ASSF) is considered the most important one, because it is responsible for oxidizing ethanol into acetic acid, and also crucial for flavor compound formation.

Zhenjiang aromatic vinegar is the representative product of this technique. It is made from glutinous rice, and the starch saccharification and alcohol fermentation steps are similar to the technique of rice wine in China [3]. The ASSF is conducted by mixing alcohol mash with the wheat bran and rice hull in fermentation pots after saccharification and alcohol fermentation. The ASSF process is as followed: (1) the fermentation vinegar cultural (fermented substrate) of the 8th day from the last batch was taken as seed for the next round inoculation at the mass ratio of 1:13. (2) The rice hull is mixed with fermentation culture, which is loose and has very large interspace, can hold enough air for the aerobic microbial growth and metabolic activities. (3) Finally, the wheat bran was mixed with the rice hull and alcohol mash in fermentation pots. This step generally lasts about 20 days, and the vinegar cultural was stirred by the machine every day in order that the temperature and oxygen content is in the appropriate range. The uniformity of the vinegar cultural is very important during ASSF. Recently studies have mainly focused on the microbiology diversity, dynamics of the bacterial community and the change of the physical and chemical indicators in the ASSF of Chinese vinegars using sensors or chemical methods [1, 2, 5, 6]. However, the uniformity of the vinegar cultural is still poorly researched. In the ASSF process, the workers often judge the uniformity of the vinegar cultural by experience and sense organ, which are the lack of scientific basis. If the uniformity of the vinegar cultural can’t be judged in time, the vinegar cultural will easily agglomerate or harden in the fermentation pots. The main reason is that the temperature can’t be released timely so that the local temperature is too high [7]. Thus this study will focus on dissecting the uniformity about the formation and function of metabolites in vinegar cultural during SSF.

The total acid content (TAC), pH value and moisture content (MC) are very important indicators during ASSF. Especially, it is worth noting that in physicochemical analysis the evaluation of TAC, pH values and MC have raised great attentions for both researchers. TAC plays a key role in determining the sensory properties and affects microbial growth [8]. The pH value is regarded the one of the most important condition affecting microorganism growth, and microbial protein is synthesized during the SSF process. So this process variable must be monitored in time so that the process of SSF can be effectively optimized and controlled [8]. For MC, if excessive levels are observed, water accumulates within the void spaces of the solid matrix resulting in oxygen limitation. In contrast, if the MC is insufficient, microorganism growth will be hindered [9]. Their distribution can reflect the uniformity of the vinegar cultural during ASSF. If the solid form culture utilized in the production of vinegar is unevenly distributed this can influence the culture breed and metabolism. However, in ASSF, no existing electrode can record TAC, pH and MC on-line in a solid medium because of the lack of free water, thus its determination is time-consuming and labor-intensive. Therefore, a rapid and accurate analytical method is essential for monitoring these process variables to guarantee the quality and visualize the uniformity of the vinegar cultural during ASSF. A hyperspectral imaging (HSI) system, which consists of both a digital camera and a spectrograph, can acquire images with both high spatial and spectral resolution contents. Therefore, this system could be considered an extension of a video image analysis system with hundreds of narrow spectral bands along the spectral axis. With HSI, a spectrum for each pixel can be obtained and a gray scale or tonal image for each narrow band can be obtained [10]. HSI may capture both spatial and biochemical information simultaneously so that the likelihood of predicting TAC, pH and MC accurately could be much greater. Furthermore, HSI could give the distribution of TAC, pH and MC in vinegar cultural.

This study aimed at exploring the feasibility of HSI technique for predicting TAC, pH and MC of vinegar cultural. In addition, distribution maps were visualized using image processing algorithms to show spatial variation of TAC, pH and MC within vinegar cultural. The specific objectives were to (1) acquire hyperspectral images of examined vinegar cultural samples in the spectral range of 400-960 nm; (2) extract spectral information in the region of interest (ROI); (3) synergic interval PLS (siPLS) and genetic algorithm (GA) were used to select the optimum spectral variables; (4) principal component analysis (PCA) was applied to select optimum characteristic images; (5) build quantitative relationships using Partial least square (PLS), least squares support vector machine (LS-SVM) and GA-PLS; (6) develop image processing algorithms to generate prediction map for visualizing the spatial distribution of TAC, pH and MC within vinegar cultural to show the uniformity of the vinegar cultural.

2. Materials and methods

2.1 vinegar cultural samples

Vinegar cultural samples were collected in June, 2015, from Jiangsu Hengshun Vinegar Industry Co., Ltd (Jiangsu, People's Republic of China) located in the eastern coastal province of Jiangsu (31º 37´-32º 12´ northern latitude, 118º 58´-119º 27´ east longitude). Samples were prepared from three fermentation runs conducted at the factory site. For each fermentation trial, sampling was conducted in triplicate, with samples selected from the same depth in the fermentation vinegar culture.

2.2 Image acquisition and pre-processing

A hyperspectral imaging system (Fig. 1(a)) was used to acquire hyperspectral images of tested vinegar cultural in the wavelength range of 400-1000 nm. A scanning rate was selected to achieve a square pixel. The horizontal motorized platform whose movement speed was preset for 1.25mm/s automatically moved the sample to the pre-determined initial position. The number of scan lines was set at 618 lines /picture and the number of scans in each line was 1628 covering the spectral region of 430–960 nm using a 618 pixel camera. Thus, a spatial block of a 1628 × 618 ×618 image was created, which was represented by a 3-D image with x-axis, y-axis and λ-axis coordinate information. A single HSI for each sample was stored in a raw format before being processed. During data acquisition, the HSI system is sensitive to changes in ambient conditions such as temperature and humidity, accordingly, room temperature was kept at approximately 25 °C and 55% R.H.

In Fig.1(c), to allow error correction and to obtain a relative reflectance, a dark image and a white image were obtained by covering the lens with a cap and taking an image from a white reference as described in reference [11].

A rectangle region of interest (ROI) as 100 × 100 pixels was selected for each sample. The relative reflectance for each image was calculated by averaging the spectral responses of each pixel in the ROI. After averaging, 60 samples were obtained in this work. In Fig. 1(d), each spectrum was smoothed with Standard Normal Variate (SNV) to eliminate variations in the baseline promoted by light scattering of each spectrum. SNV is a very common pre-treatment in spectroscopy [10].

Fig.1

2.3 TAC, pH value and MC determination

2.3.1 TAC measurement

Ten gram vinegar cultural from ROI of each sample then was used for TAC determination. The reference measurement of TAC (in terms of acetic acid, g/100 g vinegar culture) was in accordance with the official analytical methods for vinegar in China (GB/T 5009.41-2003). First, each sample should hydrate in distilled water for 2 hours before measuring. Then 20 m L of the diluents was mixed with 60 m L distilled water and titrated with 0.01 M Na OH standard to end point pH = 8.2. Finally, the volume of the consumed Na OH was recorded and TAC was computed according to the equation provided in GB/T 5009.41-2003. All chemical reagents used in the chemical analyses were of analytical grade.

2.3.2 The pH value measurement

Ten gram vinegar cultural from ROI of each sample then was used for pH determination. Distilled water (50mL) was added to 10 g sample and the mixture was agitated vigorously. After 30 min, the pH of the vinegar cultural was determined with a pH meter (PHS-3 C, Shanghai Precision and Scientific Instrument, China).

2.3.3 MC measurement

The thermogravimetric method was used to measure the MC of the vinegar culture. A thermogravimetric balance (HB 43S Halogen balance, Mettler Toledo, Greifensee, Switzerland) was used as a reference method. Vinegar culture samples of approximately 10 g were heated at a 105 °C desiccation temperature that remained constant during the analysis. The measurement stopped as soon as the mean weight loss per 90 s was lower than 1 mg. The thermogravimetric method precision is ± 0.1% of moisture. The samples were cooled in a dryer, weighed and moisture loss was calculated as a percentage.

2.4. Multivariate data analysis

After pre-process, the large spectral data contains lots of hidden information, which has a strong relationship with TAC, pH and MC prediction. Therefore, it is important to select a reliable modeling method to build a calibration model for quantitative analysis. Currently, there are a variety of modeling methods, such as PLS [12], Least Squares-support vector machine (LS-SVM) [13, 14], multiple linear regression (MLR) [15], artificial neural network (ANN) [16], Genetic algorithm-PLS (GA-PLS) [17] and other [18]. In this study, PLS, LS-SVM and GA-PLS were applied to correlate spectra with reference values for quantitative determination of TAC, pH and MC in vinegar cultural, respectively. Besides, the selected most model was used to estimate the concentration of TAC, pH and MC in each pixel of vinegar cultural. The results could show the TAC, pH and MC distribution in vinegar cultural, which could reflect the uniformity of vinegar cultural during ASSF.

2.4.1 Optimal wavelength interval selection

To further extract the spectral information related to TAC, pH and MC in the vinegar culture, siPLS was used to select the optimum spectral variables in comparison with a reference [18]. The siPLS algorithm is an all-possible interval-combinations procedure test based on all possible PLS of all subsets of intervals. The principle of this algorithm is to split the data set into a number of intervals (variable-wise) and to calculate all possible PLS model combinations of two, three or four intervals. The optimal combination of intervals and number of PCs were optimized by cross-validation and determined by the lowest root mean square error of cross validation (*RMSECV*).

2.4.2 Optimal wavelength selection

The acquired hyperspectral images are high dimensional, and thus suffer from the problem of multicollinearity during multivariate analysis. However, some wavelengths in the whole spectrum are irrelevant to TAC, pH and MC prediction, and removing these irrelevant wavelengths can promote the computation speed and make the model easier to be interpreted. There is no standard method to identify the optimal wavelengths from the full wavelengths, and many selection methods, such as independent component analysis (ICA) [19], principal component analysis (PCA) [20] and genetic algorithm (GA) [21], have been proposed in previous studies.

In a simplified GA, there are at least five components: encoding, population initialization, individual selection, crossover, and mutation. Input spectral variables will be encoded with binary data: zeros and ones as chromosomes. GA requires a number of possible candidate solutions to start with. For instance, at first step, in the selected optimal wavelength interval, all spectral variables will be selected. Fitness of every chromosome will be evaluated using a predefined fitness function to determine whether it satisfies constraints. If it is satisfied, its output will be the selected results; if not, chromosomes with better fitness will be selected to “survive”. Then, from crossover and mutation, offspring will be generated (similar to the combination of bands). The fitness of every chromosome will be evaluated again. This step will be repeated until the fitness satisfies the predefined constraints [22]. A subset of spectral variables sensitive to TAC, pH and MC will be obtained.

2.4.3 Prediction model

After selected the most wavelength, the PLS, LS-SVM and GA-PLS was used to rapidly predict TAC, pH and MC of the vinegar cultural, respectively.

Among them, LS-SVM is a powerful tool and supervised learning method that can be used to classification or regression in nonlinear models. The SVM algorithm is based on the statistical learning theory and the structural risk minimization and typically achieves the convex optimization problems by solving the quadratic programs. The theory and more details of SVM and LS-SVM can be found in the literature [23]. LS-SVM was used to rapidly predict TAC, pH and MC of the vinegar cultural during ASSF.

GA-PLS [24] was proved that a large absolute PLS regression coefficient indicates an important variable in a model obtained for auto scaled data. The algorithm we have used for this study is specifically devoted to the problem of variable selection. Define the parameters of the GA-PLS: Maximum number of PLS components, 15, Number of runs, 100, the amount of evaluations, 200. The parameters were defined according to the Ref [17, 25]. The details of the settings required are fully covered in the references.

2.5. Image analysis

2.5.1 Image segmentation

After the hyperspectral images black and white corrected, the image was segmented by threshold value method. Image segmentation aimed to isolate vinegar cultural sample from its background as well as from undesirable pixels which exhibit abnormal reflectance values. Representative single-band reflectance images of the vinegar culture at nine selected wavelengths from 430 to 910 nm are shown in Fig. 2(a) to illustrate the general pattern of the hyperspectral images and differences between the different spectral regions. The gray value difference is the biggest at the 900 nm, the background can be isolated. To isolate vinegar cultural sample from the background, the two-peak method was used to determine the threshold size. The histogram of HSI was shown in Fig.2 (b). The image at wavelength 900 nm was segmented using a simple threshold at a level of 60. All pixels above this threshold were distinctly assigned to the background as shown in Fig.2 (c). The resulting segmented image was used as a mask to identify all pixels belonging to the vinegar cultural sample.

Fig.2

2.5.2 Extraction of optimum feature pictures by PCA

Each sample has 618 images at every wavelength. PCA was used to reduce the hyperspectral data dimensions. The top three PCs (i.e. PC1, PC2, and PC3) issued from PCA were taken into account in the further analysis. The total accumulative contribution rate of variance of the raw spectral data for the 60 samples using the top three PCs was 99.34%. PC1, PC2 and PC3 images are shown in Figure 3, obtained by PCA. It also was found that the PC1 image provided the best representation of the original sample, because the variance contribution rate explained by PC1 image is the highest, reaching 95.68%. Thus, the dominant bands are determined according to the PC1 image in this work, and the three dominant bands with highest weight coefficients were selected by investigating all weighting coefficients. The three characteristic images at 598 nm, 684 nm and 858 nm are shown in Fig. 3(d–f).

Fig.3

2.6 Visualization of TAC, pH and MC of the vinegar cultural

Compared with traditional spectroscopy, HIS has the advantage of providing spatial information, which can be used to visualize TAC, pH and MC in vinegar cultural by creating concentration images or maps. In the current study, an image processing algorithm was developed to transfer the optimized model to each pixel of the hyperspectral images for creating TAC, pH and MC distribution maps. The best model was used to calculate the TAC, pH and MC in each pixel of the vinegar cultural. The resulting distribution maps were displayed with a linear color scale (from blue to red), in which high TAC, pH and MC values were displayed in yellow/red while low TAC, pH and MC values were displayed in blue. By checking the color variation in the distribution maps, the predicted distribution of TAC, pH and MC within a vinegar cultural sample can be easily assessed.

2.7. Software

All image processing and data analysis procedures described above were executed using programs developed in Matlab 7.1 (MathWorks, Natick, MA, USA). Extraction of reflectance spectra from the hyperspectral images was finished using ENVI 4.3 (ITT Visual Information Solutions, Boulder, CO, USA).

3. Results and discussion

3.1. Measured TAC, pH and MC

The descriptive statistics for the TAC, pH and MC of 60 vinegar cultural samples determined by reference methods above are summarized in Table.1. The mean, standard deviation (SD) and the range of TAC, pH and MC are shown in Table.1. All samples were divided into two sets. To avoid bias in subset selection, a 2:1 calibration/ prediction division was adopted. All samples were sorted according to their respective y-value (viz. the reference measurement value of TAC, pH and MC). Then two spectra of every three samples were selected into the calibration set, so that finally the calibration set contains 40 spectra and 20 in prediction set.

Table.1

3.2 The result of the selected optimum interval and wave points

After SNV preprocessing, the responses were further used to select the optimum spectral variables by siPLS algorithm. First, selected spectral regions were divided into equidistant subintervals, such as the 11, 12 … 29 and 30 intervals with the selected number of intervals being optimized by cross-validation. For TAC, the lowest *RMSECV* was achieved when the full spectra were split into 21 intervals and the optimum combinations of intervals were [6 11 14 16], the lowest *RMSECV* was 0.574 mg /g, as shown in Table.2. For pH value, the combined intervals selected by siPLS are presented in Table.2, where four intervals [8 12 15 24] were selected, and the lowest *RMSECV* was 0.043. For MC, the combined intervals selected by siPLS were four intervals [10 13 17 19] were selected, and the lowest *RMSECV* was 4.61 g/g. The global optimum siPLS model was achieved with 15 subintervals and 4 PLS factors. Spectral region of specific interest, related to the absorption of certain components can improve the predictive ability compared to regressions using the whole spectral variables. GA was employed to select the most informative variables. All the parameters were set as follows: the number of iteration: 100, population initialization: 50, probability of cross-over: 0.95, probability of mutation: 0.01. The selected optimum wavelength was as followed in Table.2. The eight wavelengths (850.95、440.14、453.41、609.58、435.18、624.15、625.01、848.32、442.63 and 610.44 nm) were defined as the most relevant wavelengths in predicting TAC. Similarly, twelve wavelengths (731.29、722.60、555.02、729.55、726.08、604.44、568.61、595.04、717.39、719.13、734.77 and 723.47) were selected for pH value and twelve wavelengths (544、546、531、535、575、832、835、840、849、895、896 and 901 nm) were selected for efficient prediction of MC. By using only these particular wavelengths in building optimized PLS, LS-SVM and GA-PLS models in predicting the same constituents, the results as shown in Table. 3 revealed that the predictability of these models is still good, indicating the robustness of the developed models.

Table.2

3.3. Prediction of chemical constituents

HSI systems acquire abundant spatial information while collecting spectral information. After siPLS and GA selected the variables, the independent variables of the model decreased from full spectral of 618 to below 12 variables. The prediction of the TAC, pH and MC of vinegar cultural samples were carried out by using PLS, LS-SVM and GA-PLS, respectively. The results as shown in Table.3 revealed that the predictability of these models. By comparing, TAC was predicted by the optimal model of GA-PLS with determination coefficient (*R*) of 0.86 with *RMSEP* of 0.713 mg /g, pH and MC were predicted by the optimal model of the LS-SVM with determination coefficient of 0.803, 0.851 with *RMSEP* of 0.056 and 4.53 g/g, respectively. These results suggest that the prediction models optimized with leave-one-out cross-validation are representative and the models work accurately in unknown samples. Despite the accurate chemical reference methods used in this study, the current results indicate how the proposed method was suited as a practical replacement for the conventional chemical method with reasonable accuracy. The critical advantage of this method is that it is quite accurate and highly reproducible. However, the inhomogeneity of the vinegar cultural, which is of great importance with respect to the method of sampling, must be put into consideration.

Table 3

3.4. Distribution map of TAC, pH and MC in vinegar cultural

As the major chemical indicators were successfully predicted from spectral data extracted from the images by employing multivariate analyses, HSI system provides another possibility to work in the spatial dimensions of the same images. Besides, HSI provides a good alternative with a profound consideration of the spatial dimensions of the examined sampled by considering the structure heterogeneity of the sample. In this way, the spectrum of any point in the sample can be used for calculating the concentrations of chemical constituents (e.g. TAC, pH or MC) because each pixel in the HSI has its own corresponding spectrum. Each constituent is displayed and mapped in different visual appearance according to its concentration. In this study, each constituent has different the optimal prediction model, which can be used to calculate each pixels point concentration.

Fig. 4 shows the distribution on the vinegar cultural based on the optimum model, and is colored according to the band intensity indicating the relative content of the TAC, pH and MC of the vinegar cultural. The color bar was extended from a low content (in blue) to a high content (in red). The resulting false color mapping with intensity scaling was then used to display compositional contrast between pixels in the image. Obviously, the images can clearly visualize the TAC, pH and MC in the vinegar cultural. It was easy to recognize the uniformity of the vinegar cultural during ASSF.

Fig.4 has higher concentration of TAC (7.72 mg/g) in the red region and has lower concentration (3.34 mg/g) in the blue region. The pH and MC have different distribution in different region. Therefore, the distribution map can rapidly reflect the uniformity of the vinegar cultural based on hyperspectral imaging technology and different algorithm. In fact, this result is extremely significant in evaluation of the uniformity of vinegar culture during SSF if implemented in a large scale production. It may be used to expose the hidden compositional information that other optical methods are not able to differentiate. Depending on the sample, this technique enables identification and characterization of the relative content of various main metabolites that are distributed within the vinegar cultural. Moreover, hyperspectral imaging produces detailed maps showing the TAC, pH and MC distribution in vinegar cultural sample. Study of this map of the sample can provide data on spatial localization of metabolites accumulation. Thus, HSI allows monitoring of the metabolites distribution and its changes in vinegar cultural. This may also be valuable for investigation of the uniformity of the vinegar cultural where metabolites are involved, such as the vinegar cultural fermentation state and the uniformity.

Fig. 4

4. Conclusion

The results presented illustrate that HSI is a powerful tool for TAC, pH and MC analysis in vinegar cultural. These indicators can be detected in vinegar cultural samples non-destructively. After hyperspectral image acquisition and pre-processing, average spectral obtained from the ROI of vinegar cultural were used for model development. HIS technique has been successfully applied to rapidly predict the physical and chemical indicators of the vinegar cultural for simultaneous estimation of TAC, pH and MC during ASSF. The distribution map of the vinegar cultural has very good response the uniformity of the vinegar cultural. Thus, HSI allows monitoring of the main metabolite distribution and their changes during ASSF, which can reflect the uniformity of the vinegar culture. This may also be valuable for investigating many biological processes where TAC, pH and moisture are involved.

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