# Analysis of volatile organic compounds from Chinese vinegar substrate during solid-state fermentation using colorimetric sensor array

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## Abstract

Aroma is a significant index to reflect the quality of vinegar. This paper intends to investigate the volatile organic compounds (VOCs) of vinegar's substrate during solid-state fermentation. Gas chromatography and mass spectrometry (GC-MS), as well as colorimetric sensor array, was used comparatively to characterize the VOCs in the different stages of acetic acid fermentation. The chemical components of ethanol, 3-Methyl-1-butanol, acetic acid and ethyl acetate obtained from GC-MS were remarkably changed during solid-state fermentation. What's more, the technique of colorimetric sensor array was also used to characterize the VOCs of solid-state fermentation. The color changes of colorimetric sensor array before and after exposure to the vinegar's substrate samples were obtained by a Charge Coupled Device (CCD) camera. The digital data (i.e. RGB components of the image) representing the color change profiles for the vinegar samples were analyzed. Principle components analysis (PCA) was employed to present the trend of fermentation process through analyzing the signals obtained from colorimetric sensor array. Linear discriminant analysis (LDA) model based on PCA scores was used to distinguish vinegar's substrate samples per day during the whole process of fermentation. The result shows that, round to 60 per cent samples were correctly identified corresponding to their fermenting day. And 92.3 per cent samples were correctly identified with the error range of 3 days. The technique of colorimetric

sensor array was considered as an excellent method for VOCs measurement based on its advantages of non pretreatment requirement, fast, and costless.

**Key words:** volatile organic compounds (VOCs), colorimetric sensor array, pattern recognition, solid-state fermentation, substrate of vinegar;

## 1. Introduction

Vinegar is a famous traditional condiment with long history in China, [1] and the brewing technology is an important part of Chinese traditional culture. 'Zhenjiang' aromatic vinegar, as one of the most famous vinegars in China, [2] is produced by solid-state delaminating fermentation. A large number of studies showed that vinegar not only could be used as acid seasoning, but it is beneficial for health such as [3] preventing the colds, [4] treating the gastropathy, [5] keeping the tumors at bay and so on. What's more, with the improvement of people's living standard, [6] the requirement for the quality of vinegar is increasingly higher. However, the process of solid-state fermentation is built on manual operation, and the quality control of vinegar mostly relies on experience identification. In this case, the quality of the products is easily affected by the changing environment, which leads to a great variety of products of diverse quality.

Aroma is an essential index of vinegar quality; [7] aromatic components evaluation plays an important role in the identification and quality control of vinegar. [8] At present, sensory evaluation and chemical analysis are most used in analyzing vinegar quality. However, sensory evaluation is limited due to the subjectivity of human sense and pungency of acetic acid. Chemical analysis is time consuming, reagents demanding, costly, and cannot be assessed with the whole information.

Though the traditional electronic nose can characterize the odor of food and agro-products comprehensively, quickly and objectively [9], it is sensitive to temperature and humidity in the environment. [10] In addition, the acetic acid in vinegar can make the sensors of traditional electronic nose "poisoning". [11] Olfactory visualization is a normal electronic nose system which is based on the colorimetric sensor array. [12-14] Compared with the customary electronic nose technology, colorimetric sensor array has a stronger recognition ability, higher precision, wider range and [15] free of ambient humidity effect and more intuitive and vivid. Some relevant studies reported that colorimetric sensor array was used to [16] determine alcohol, [17] acetate and chloride, [18] hydrogen sulfide and [19] aldehydes.

This paper intends to investigate the volatile organic compounds (VOCs) of vinegar's substrate during solid-state fermentation. Gas chromatography and mass spectrometry (GC-MS), as well as colorimetric sensor array, was used comparatively to characterize the VOCs in the different stages of acetic acid fermentation. Principle components analysis combined with linear discrimination analysis were employed to present the trend of fermentation process through analyzing the signals obtained from colorimetric sensor array. Further more, effective colorimetric materials which were potential for characterization of solid-state fermentation would be selected.

## 2. Materials and methods

### *2.1. Acetic acid solid-state fermentation process and sample preparation*

The solid-state fermentation substrate of vinegar samples were obtained from 'Hengshun' brand, Jiangsu province Zhenjiang city. [20] 'Zhenjiang' aromatic vinegar, produced from solid-state fermentation methods of acetification, is a sticky rice-derived product of high reputation, much appreciated by the custom of China and Southeast Asian countries. The solid-state delaminating fermentation process of 'Hengshun' vinegar takes about 19 days.

Acetic acid fermentation process is a metabolic process that converts ethanol to acids; depend mainly on the effect of acetic acid bacteria fermentation to ethanol oxidation into acetic acid process. In the solid-state fermentation, that is also a process to broaden the active region of acetic bacteria. In conventional solid-state fermentation, the process is divided into five stages, which is also a process to broaden the active region of acetic bacteria, as shown in Fig.1. The green dots in the figure represent the distribution of acetic acid bacteria. Along with the fermentation, the acetic acid bacteria's density increased and the acetic acid bacteria's range expanded. On the first day of fermentation, fermented substrate of vinegar inoculated on the surface of fresh substrate; this process is named inoculation. The second and the third days are called heat extraction process: the rice hulls were paved on the surface of the substrate and then well-mixed. The rice hull can increase the contact area of the substrate and oxygen, which ensured the growth and metabolism of aerobic microbial in the substrate. During this time, upper acetic acid bacteria reproduced quickly. The third stage of the process lasted about 5 days; it mainly requires turning over the fermenting substrate layer by layer, expanding the solid-state fermentation substrate. The fourth stage of the process was turning over the fermenting substrate till the bottom of ponds, which aimed to provide enough oxygen and avoid overheating of the local substrate. After about 19 days, the substrate matured.

[**Figure 1** about here]

The solid fermentation substrates of vinegar were sampled every day in the whole process (2012.12.19-2013.1.7). All samples were kept at a temperature around 0 $^{o}$C before the trial and a daily detection was performed in order to avoid interruptions by storage time.

***2.2. SPME-GC/MS analyses***

Analyses were performed on gas chromatograph-mass spectrometer HP6890-5973 (Agilent, USA) with a nonpolar column (30 m$\times$0.25 mm ID$\times$0.25 μm film).The initial temperature was 35 $^{o}$C, the column was heated to 100 $^{o}$C at a rate of 5 $^{o}$C /min, and then heated at a rate of 3 $^{o}$C /min and 10 $^{o}$C /min to 200 $^{o}$C and 220 $^{o}$C respectively, and held at 220 $^{o}$C for 15 min. The inlet temperature and ion source temperature were 230 $^{o}$C and 220 $^{o}$C. Mass spectra were obtained at an electron impact potential of 70 eV with a range of 30–500 amu. The data were processed through the

HP-Chemstation System workstation. In this work, the selection of fibers, the extraction time and the extraction temperature were optimized previously. The optimal SPME methodology conditions were as follows. The optimal fiber was 75 μm Carboxen–PDMS (CAR–PDMS), the extraction time was 40 min and the extraction temperature was 40 ℃.

The solid fermentation substrate of vinegar at 5 stages were detected by GC-MS. Five stages were inoculation (first day), heat extraction (2nd day), scoop (7th day), grinning (9th day) and maturing (19th day), the experiments were repeated three times. 3.0 g samples and 2.5 g solid sodium chloride were placed in 15 mL extraction flask in a 40 $^{o}$C thermostatic water bath. SPME fiber collected the volatiles of samples. After 40 mins, the extracted head was inserted into the injection port of the GC-MS and desorpted for 3min at 250 $^{o}$C.

### *2.3. Artificial olfaction system*

The portable artificial olfaction system aimed at detecting the smell of liquid such as wine and vinegar was developed by our laboratory. It was used to characterize and discriminate the odor of solid fermentation substrate of vinegar in the fermentation process. A schematic diagram of the system is presented in Fig.2. This artificial olfaction system includes gas volatile system, gas detection system, power system and control system. Images of the sensor array were captured by a CCD camera (CMLN-13S2M/C, Sony, Japan). The parameter of Gamma was set at 1.26; and exposure time was 0.38 s.

[**Figure 2** about here]

### *2.4. Colorimetric sensor array analyses*

According to our pre-experiment, 10g solid fermentation substrate of vinegar was put in gas collecting chamber, and the colorimetric sensor array was placed inside the reaction chamber. The camera recorded the image of the sensor array before it exposed to the gas. 15mins later, the vacuum pump was turned on, and the volatile gas was put into reaction chamber by vacuum pump. The color change of colorimetric sensor was recorded by CCD camera immediately. Sampling interval is alterable under the intervention of the computer, and the minimum time of sampling interval in the artificial olfaction system is 2 second (which means the system collects the images every 2 seconds at the fastest), hence the changes of colorimetric sensor array could be monitored in real time. Since each sensor array contains 15 colorimetric dyes (nine metalloporphyrins materials and six pH indicators), which printed on a silica gel plates, the response signal of each dye in an array could be expressed by the red (R), green (G), and blue (B) values, which resulted in 45 variables (15 dyes ×3 color components, RGB). All variables were utilized in statistical and quantitative analyses and subsequent pattern recognition. The whole device was arranged in the chamber of constant temperature about 25 $^{o}$C.

### *2.5. Multivariate statistical analysis*

Multivariate analysis methods play a key role in characterizing the odor of vinegar's solid fermentation substrate based on the colorimetric sensor array. All algorithms were implemented in Matlab R2010a (Mathworks, USA) under Windows XP.

## 3. Results and discussion

### *3.1 Fermentation temperature*

Fermentation temperature is an important factor during the vinegar production. The temperature of solid fermentation substrates of vinegar is raised by the reproduction and metabolic of acetic acid bacteria. If the environment is suitable for breeding, the acetic acid bacteria will reproduce actively and have a high rate of metabolism.[21] The raw materials present in a solid-state during the fermentation process, which makes the heat consumption difficult and causes overheating. The overheating of solid fermentation substrates brings the poor quality of vinegar. Hence, the fermentation temperature needs to be controlled in the process of traditional solid-state fermentation.

The experiment was performed at December and January, the environmental temperature was ranged from 4~11 $^{o}$C. The temperatures of acetic acid fermentation were monitored to ensure the acetic acid fermentation process performed normally. The temperature of acetic acid fermentation is showed in Table 1.The optimum temperature of acetic acid bacteria is 38 $^{o}$C [22]. In general, the fermentation temperature could not be over 45 $^{o}$C, and the fermentation substrates were turned over before the temperature reached 45 $^{o}$C.

As we can see from Table 1, in the inoculation process, the initial temperature of the acetic acid fermentation is low because of the limited metabolic heat from the less quantity of acetic acid bacteria. The rice hulls were mixed with solid fermentation substrates of vinegar in heat extraction process; this increased the oxygen and the contact area with raw materials. With the rapid reproduction of acetic acid bacteria, the temperature raised rapidly from 26 $^{o}$C to 44 $^{o}$C during the heat extraction process. In scoop process, the solid-state fermentation substrate was expanded by turning over, acetic acid bacteria still reproduced actively and the temperature was maintained at 40 $^{o}$C. During the grinning process, the substrates which were necessary for acetic acid bacteria reduced, then the temperature gradually declined till the fermented substrate matured.

[**Table 1** about here]

### *3.2 GC - MS detection of solid fermentation substrates of vinegar at different fermentation stages*

Aroma components of solid fermentation substrates of vinegar at different fermentation stages were detected by GC-MS, the result is listed in Table 2. As shown in Table 2, the contents of lipids such as ethyl acetate, isoamyl acetate and ethyl palmitate generally increased at the beginning and then decreased. At the same time, the result shows that the alcohols declined along with the fermentation and the acids increased slowly at initial stage, along with the fermentation, then it increased quickly. [20] The acetone transferred to propanol in later stage, reducing its content. As a result of Maillard reaction, Strecker degradation and bacterial autolysis, tetramethylpyrazine, 2, 4-Di-tert-butylphenol and other aromatic compounds were generated in the later stage of the fermentation [23].

[**Table 2** about here]

In could be concluded from Table 2, the chemical components of ethanol, 3-Methyl-1-butanol, acetic acid and ethyl acetate remarkably changed during solid-state fermentation. Content changes of ethanol, 3-Methyl-1-butanol, acetic acid and ethyl acetate are representative; the results are shown in Fig.3. The acetic acid fermentation is a process oxidizing alcohol to acetic acid by acetic acid bacterium. As a result, the contents of ethanol and 3-Methyl-1-butanol gradually reduced. Acetic acid increased slowly in the initial stage of fermentation while increased sharply in the later stage of fermentation. This can be explained by the reason that the acetic acid bacteria were not the dominant bacteria in the initial stage of fermentation, and a part of generated acid had an esterification with the ethanol. Later, thanks to the breeding of a large number of acetic acid bacteria, acetic acid content raised sharply.

The content of the ethyl acetate achieved maximum in about 7 days, then began to decline. Due to pre-glycosylated, liquefaction and alcohol fermentation process, the content of ethyl acetate was high at inoculation stage. A large amount of acid generated along with the acetic acid fermentation, meanwhile, the daily "turn over" helped the underlying ethanol esterificated with acetic acid and generated ethyl acetate. The continuing increase of ethyl acetate content reached its maximum value after a week. At the last stage, the content of the ethyl acetate declined, as we inferred, was volatized with "turn over".

[**Figure 3** about here]

***3.3 Sensor responses optimization***

The sampling interval of the CCD camera was set as 1min, and the reaction time was 24min. Fig.4 shows the difference maps of sensor array exposed to the solid fermentation substrate at different reaction time. [24] This implied changes in the nature of volatiles emitted over time, some sensor returned to their baseline values. As can be seen from the Fig.4, the difference maps were changing all the time from 2 min to 20 min. When the reaction time reached 20 min, the reaction reached equilibrium with the sensor array and the difference map tended to be stable. Therefore, the reaction time was set at 20 min finally.

[**Figure 4** about here]

### *3.4 PCA analysis*

The data of colorimetric sensor variables containing overlapped information which bring great difficulty in the research. Principal components analysis (PCA) makes it possible to extract useful information from original data, and to present the trend of solid-state fermentation process with an intuitive way.

[**Figure 5** about here]

Fig.5a shows a 2-dimensional (2D) space of all vinegar samples in fermentation represented by PC1, PC2. Geometrical exploration based on PCA score plots shows the clusters trend in the 2-dimension space. In Fig.5a, clusters of most vinegar samples continued spreading with fermentation time increased. The vinegar samples belong to heat extraction (1-2d) and maturing stage of fermentation (17-19d) could be separated directly by PCA. However, the separation of other samples was not clear, and especially some overlapped could be observed from vinegar samples of scoop and grinning stage. This can be explained by continuous and uneven characteristic of solid-state fermentation. Fig.5b shows a 2-dimensional (2D) space of vinegar samples in a certain day of each stage of fermentation (1st, 2nd, 7th, 9th, 19th day, respectively). In this figure, the clusters trend of vinegar samples is clearer. It could be concluded that there are inherent compositional differences among the VOCs of vinegar samples during fermentation process.

In order to further investigate characterization of samples using colorimetric sensor array, the algorithm of LDA is employed to discriminate vinegar samples with different days of fermentation. PCA scores were used as vectors inputted into the LDA classifiers as latent variables.

### *3.5 Characterization of solid fermentation substrate based on the colorimetric sensor array*

Solid-state fermentation of Zhenjiang vinegar is a process of poly-bacteria fermentation. Not only does it generate the acetic acid from the raw materials through acetic acid bacteria but it also turns the protein, fat from the raw materials into flavoring substances. Besides, the autolytic activities of bacteria generate a variety of flavor substances. Different fermentation stages produce substances differently.

[**Figure 6** about here]

The results of colorimetric sensor array reflect the color changes on all sensors. This technology transferred olfactory information to visual information and made the odor "visible". Fig.6 shows the difference maps of solid fermentation substrate of vinegar at different fermentation stages. Compared to traditional electronic nose, colorimetric sensor array is more visualized and vivid. As it shown in this figure, during the reaction process, some changes were becoming more and more evident (the colors were getting brighter in the fig.), that is, the VOCs which reacted with these sensors had gained their contents. At the same time, changes in some sensors were getting smaller (the colors were getting bleaker in the fig.), which means, contents of some VOCs were reducing. In addition, colors of some sensors were turning brighter first and bleaker later. These changes actually coordinated with the aroma components changes analyzed by GC-MS. However, [25] aroma components measured by GC-MS were the molecular components, which were difficult to be consistent with VOCs messages obtained by human. Nevertheless, [26-27] the colorimetric sensor array could express the complete information of target substance.

[**Figure 7** about here]

The color changes of bromocresol green (a) and neutral red (b) during 19 days are shown in Fig.7. Bromocresol Green is a pH indicator, which was sensitive to acid [28-29]. Our tests indicate that the response value of bromocresol green which is exposed to acid is 4-8 times as large as other chemically responsive dyes. The color changes from the figure demonstrate acetic acid bacteria were not the dominant bacteria from the 2nd day to the 9th day; meanwhile, the limited generated acetic acid mainly reacted with alcohols (i.e. esterification). Therefore, changes of bromocresol green were not obvious during the pre-fermentation. Thereafter, acetic acid bacterium gradually became took the dominance that they generated amounts of acetic acid. Hence why, the changes of sensor were particularly evident in the latter period. Neutral red was sensitive to alcohol. As we can see from Fig.7(b), the color changes decreased at heat extraction stage, because of the alcohol consume by acetic acid bacteria; then the color became brighter, this is because that the operation of "turn over", which bring the alcohol from the bottom to the top. After the stage of grinning, there was no alcohol supply, so the content of alcohol became less, which presents bleaker colors in the figure. To conclude, it is possible to have a real-time monitor on the vinegar fermentation when establishing an aroma data base for acetic acid solid-state fermentation based on colorimetric sensor array technique.

### *3.4 Sensor responses*

The algorithm of LDA was employed to discriminate vinegar samples with different days of fermentation. By maximizing the ratio of the inter-class distance to the intra-class distance, LDA aims to find a linear transformation to achieve the maximum class discrimination, [30] such that the feature clusters are most separable after the transformation which can be achieved through scatter matrix analysis. By means of searching for a group of basis vectors, which make different class samples, have the largest between-class distance and the smallest within-class distance, and contemporaneously to get optimal transformation in classical LDA.

[**Table 3** about here]

The results of LDA model to identify the fermented substrate of vinegar from different days can be seen from Table 3, the principal components number is 15. Table 3 shows the recognition rate of every day and provides the sources of errors. The result shows that, round to 60 per cent samples were correctly identified corresponding to their fermenting day. And 92.3 per cent samples were correctly identified with the error range of 3 days. This is because the continuity and inhomogeneity of solid-state fermentation has made the classification process difficult. Although there were some wrong classifications, the colorimetric sensor array still showed good characterization capability, for the error sources were mostly in the close days.

## Conclusions

A portable artificial olfaction system based on colorimetric sensor array was developed to characterize the flavor of vinegar's solid fermentation substrate in different fermentation stages. The colorimetric sensor array was made from nine metalloporphyrins materials and six pH indicators printed on a silica gel plates. The color changes of colorimetric sensor array before and after exposure to the vinegar's substrate samples were obtained by a CCD camera. The response data was analyzed with PCA and LDA models which showed good characterization skills. At the same time, aroma components of substrates of vinegar at different fermentation stages were detected by GC-MS. This work has demonstrated the potential of colorimetric sensor array, which was not only convenient for detection but also characterized the overall aroma information of vinegar's solid fermentation substrate during acetic acid fermentation process.

Therefore, colorimetric sensor array provides an effective and convenient platform for real-time monitoring during the vinegar fermentation process.

## Acknowledgements

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## Compliance with Ethics Requirements

No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication. I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part.

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## Figure captions

Fig.1 Acetic acid fermentation process diagram

Fig.2 The diagram of artificial olfaction system

Fig.3 Content changes of Ethanol, 3-Methyl-1-butanol, Acetic acid and Ethyl acetate (%)

Fig.4 The response value of sensor at different reaction time

Fig.5 Classification result achieved by PCA (a) 5 stages (b) 5 days

Fig.6 Classification the substrate of vinegar in different fermentation stages by colorimetric sensor array

Fig. 7 The color changes of bromcresol green (a) and neutral red (b) during 19 days

## Table captions

Table 1 The change of temperature through the acetic acid fermentation

Table 2 Aroma components of substrate of vinegar in different fermentation stages (%)

Table 3 Results of LDA model to identify the different days of fermented substrate of vinegar

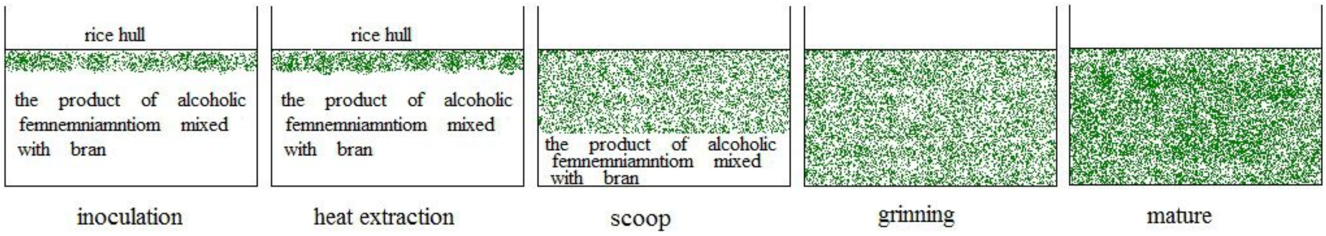


**Fig.1** Acetic acid fermentation process diagram

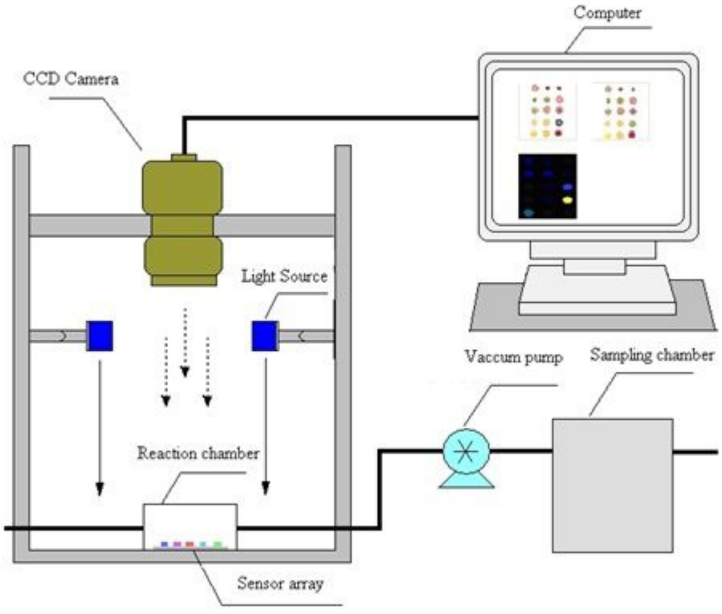


**Fig.2** The diagram of artificial olfaction system

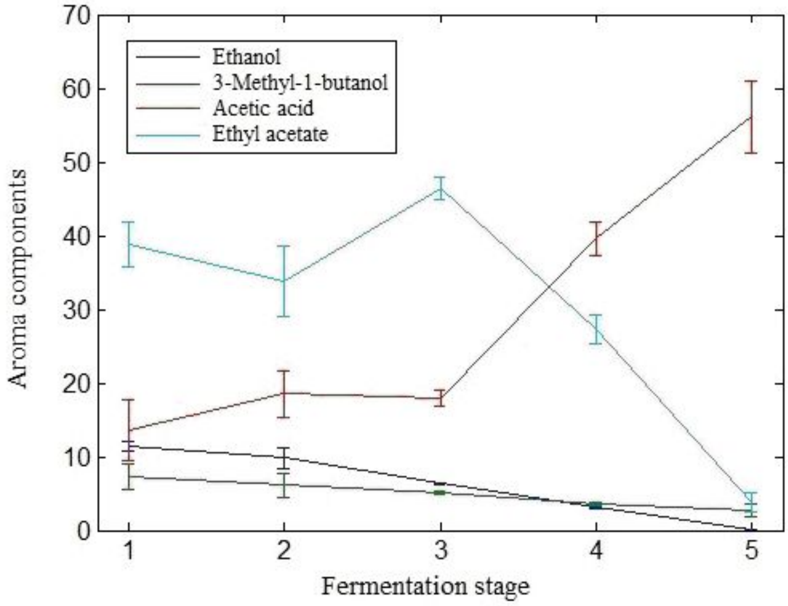


**Fig.3** Content changes of Ethanol, 3-Methyl-1-butanol, Acetic acid and Ethyl acetate (%)

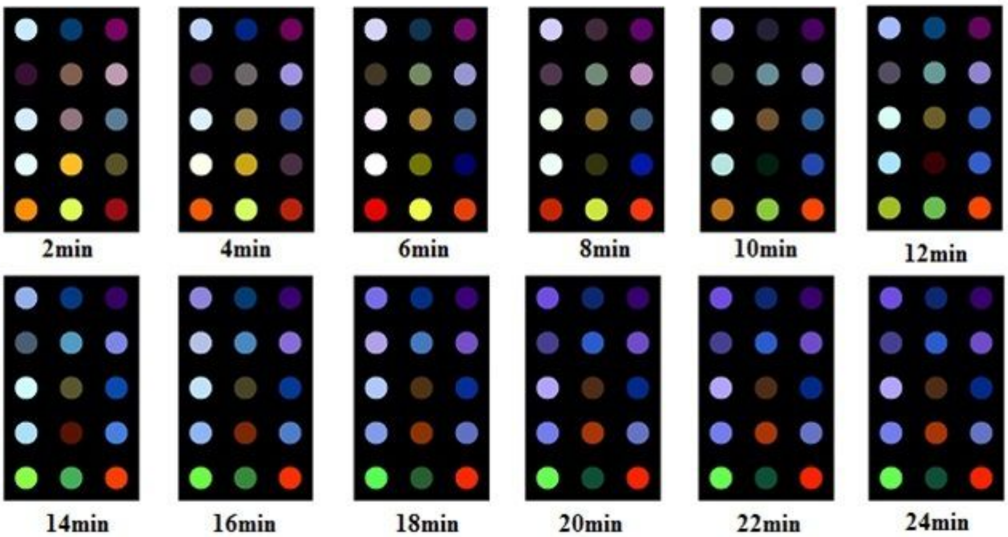


**Fig.4** The response value of sensor at different reaction time

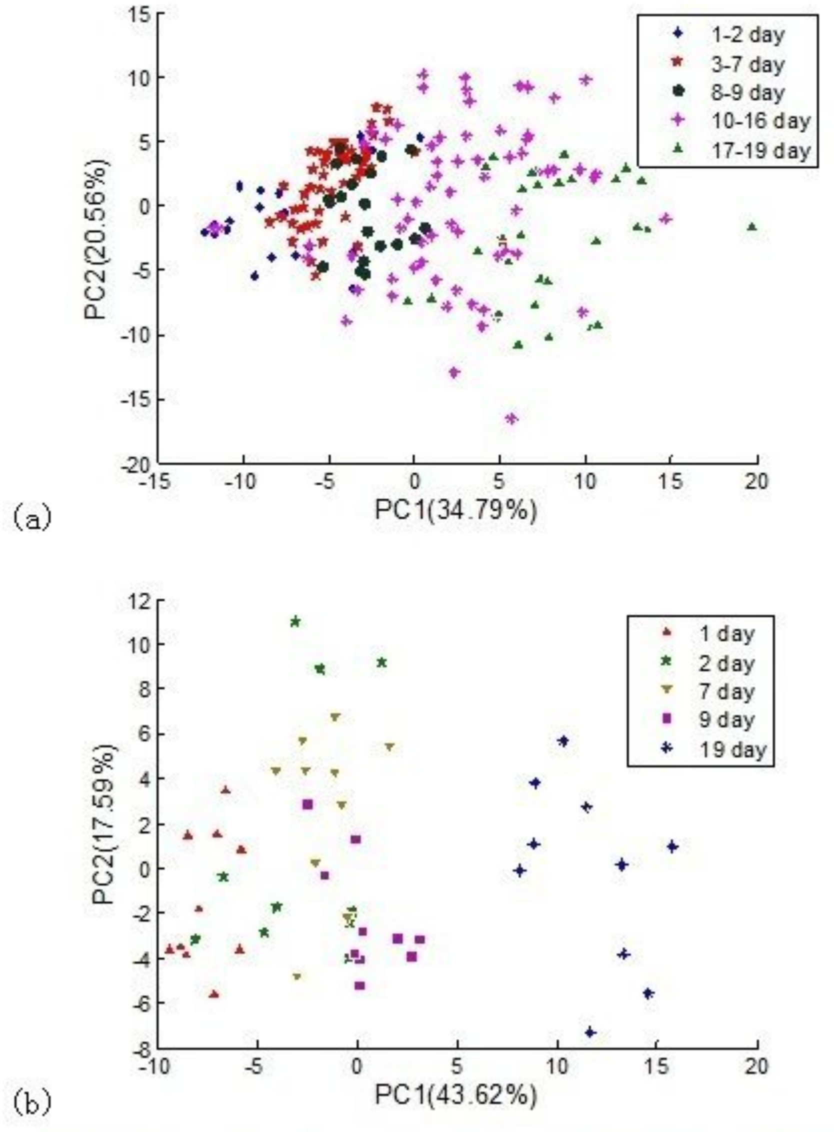


**Fig.5** Classification result achieved by PCA (a) 5 stages (b) 5 days

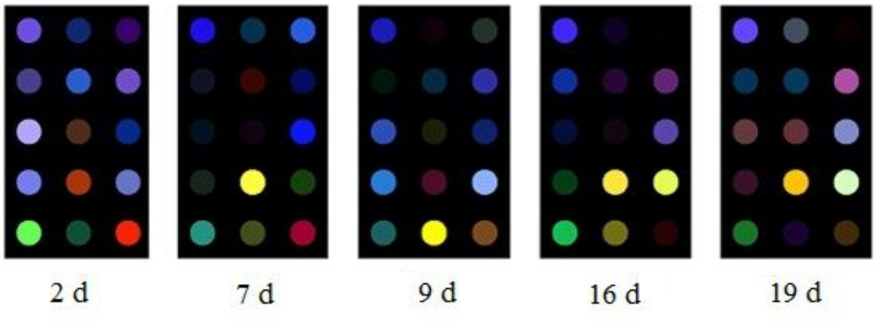


**Fig.6** Classification the substrate of vinegar in different fermentation stages by colorimetric sensor array

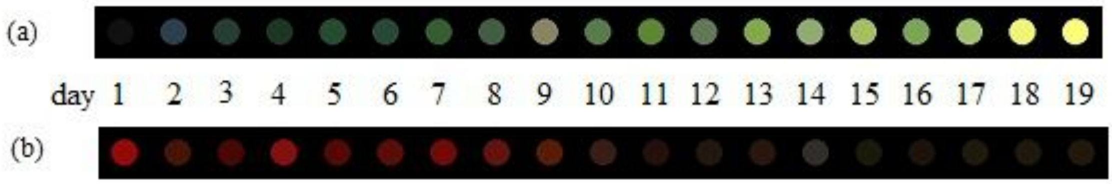


**Fig. 7** The color changes of bromcresol green (a) and neutral red (b) during 19 days

**Table 1** The change of temperature through the acetic acid fermentation

| Days | mean temperature [a] | maximum temperature | minimum temperature |
|---|---|---|---|
| 1 | 26.16±0.81 | 28.30 | 21.00 |
| 2 | 38.85±6.01 | 45.70 | 28.30 |
| 3 | 44.43±0.36 | 44.90 | 41.80 |
| 4 | 39.02±1.02 | 41.90 | 37.00 |
| 5 | 41.74±3.74 | 48.10 | 36.40 |
| 6 | 40.50±2.50 | 44.30 | 34.60 |
| 7 | 36.01±1.50 | 38.50 | 33.20 |
| 8 | 41.34±1.82 | 44.50 | 34.80 |
| 9 | 42.25±2.79 | 47.30 | 38.60 |
| 10 | 39.31±2.41 | 43.40 | 33.70 |
| 11 | 38.69±1.94 | 41.70 | 35.10 |
| 12 | 36.99±1.28 | 39.00 | 33.80 |
| 13 | 36.01±1.83 | 38.50 | 32.00 |
| 14 | 32.27±0.14 | 32.40 | 31.90 |
| 15 | 31.90±0.96 | 32.90 | 29.10 |
| 16 | 30.26±0.56 | 30.80 | 28.00 |
| 17 | 25.03±1.79 | 29.10 | 21.50 |
| 18 | 25.58±1.41 | 28.50 | 23.60 |
| 19 | 22.70±0.34 | 23.60 | 20.70 |

a mean±std

**Table 2** Aroma components of substrate of vinegar in different fermentation stages (%)

| | compounds | inoculation | heat extraction | scoop | grinning | mature |
|---|---|---|---|---|---|---|
| | ***Etanol*** | ***11.44±0.70$^{a}$*** | ***9.87±1.41$^{b}$*** | ***6.36±0.14$^{c}$*** | ***3.07±0.14$^{d}$*** | ***0.1±0$^{e}$*** |
| | 2-Methyl-1-propanol | 0.59±0.09 | 0.52±0.10 | 0.41±0.02 | 0.25±0.02 | 0.17±0.08 |
| | Trimethyl-Silanol | | | | | 0.77±0.52 |
| | 2-Methyl-1-butanol | | 1.14±0.01 | | | 0.89±0.63 |
| | (S)-(-)-2-Methyl-1-butanol | 1.75±0 | | | | |
| | ***3-Methyl-1-butanol*** | ***7.21±1.72$^{a}$*** | ***6.15±1.60$^{ab}$*** | ***5.05±0.26$^{bc}$*** | ***3.59±0.25$^{cd}$*** | ***2.75±0.86$^{d}$*** |
| alcohols | 1-Pentanol | 0.15±0.04 | 0.14±0.01 | 0.08±0.01 | 0.04±0 | |
| | Hexyl alcohol | 0.53±0.14 | 0.54±0.06 | 0.29±0.01 | 0.18±0.02 | |
| | Diisobutylcarbinol | 0.93±0.21 | 0.81±0 | | 0.41±0.07 | 0.36±0.19 |
| | 2,3-Butanediol | 0.24±0.23 | 0.19±0.17 | 0.1±0.01 | 0.51±0.12 | 0.39±0.12 |
| | (R,R)-2,3-Butanediol | | | | | 0.65±0.55 |
| | Phenethyl alcohol | 2.60±0.41 | 2.46±0.23 | 1.64±0.09 | 2.36±0.12 | 2.18±1.72 |
| | Acetic acid,2-(trimethylsilyl)- | | | | | 0.39±0.30 |
| | ***Acetic acid*** | ***13.59±4.16$^{a}$*** | ***18.48±3.23$^{a}$*** | ***17.97±1.10$^{a}$*** | ***39.61±2.22$^{b}$*** | ***56.12±4.84$^{c}$*** |
| acids | Isobutyric acid | 0.2±0.04 | 0.25±0.06 | 0.18±0.03 | 0.25±0.03 | 0.55±0.10 |
| | Isovaleric acid | 0.72±0.13 | 0.7±0.13 | 0.57±0.04 | 0.81±0.13 | 1.54±0.61 |
| | Valeric acid | | | | | 0.09±0 |
| | Hexanoic acid | | 0.09±0.08 | 0.05±0 | 0.12±0.05 | 0.41±0.30 |
| | ***Ethyl acetate*** | ***38.85±3.07$^{a}$*** | ***33.77±4.82$^{a}$*** | ***46.43±1.56$^{b}$*** | ***27.28±1.96$^{c}$*** | ***3.84±1.25$^{d}$*** |
| | Isobutyl acetate | 0.92±0.16 | 0.79±0.13 | 1.19±0.10 | 0.51±0.11 | 0.55±0.06 |
| | Isoamyl acetate | 5.57±1.08 | 4.27±1.28 | 5.54±0.58 | 3.41±0.61 | 2.22±0.70 |
| | Ethyl caproate | 0.73±0 | 0.85±0 | 0.45±0 | 0.56±0 | 0.29±0 |
| | Hexyl acetate | 0.06±0.01 | 0.08±0.05 | 0.07±0.01 | 0.09±0.02 | 0.04±0.01 |
| | Ethyl heptanoate | | 0.06±0.01 | 0.05±0 | 0.05±0.03 | 0.02±0 |
| | Ethyl lactate | 2.27±0.75 | 1.05±0.09 | 1.84±0.04 | 2.00±0.05 | 1.19±0.52 |
| esters | Ethyl caprylate | 0.30±0.12 | 0.46±0.09 | 0.58±0.12 | 0.85±0.17 | 0.19±0 |
| | Ethyl nonanoate | 0.07±0 | 0.08±0.03 | 0.10±0.03 | 0.16±0 | 0.04±0 |
| | Ethyl caprate | 0.08±0.04 | 0.08±0.03 | 0.18±0.04 | 0.26±0.03 | 0.07±0 |
| | Diethyl succinate | 0.71±0.16 | 0.56±0.22 | 0.46±0.02 | 0.45±0.03 | 0.37±0.29 |
| | Ethyl phenylacetate | 0.12±0.01 | 0.13±0.04 | 0.07±0.01 | 0.06±0.01 | 0.07±0.05 |
| | Phenethyl acetate | 1.22±0.07 | 0.9±0.13 | 1.07±0.01 | 1.60±0.17 | 1.53±1.01 |
| | gamma-Nonanolactone | | | | | 0.07±0.06 |
| | Ethyl palmitate | 0.19±0.04 | 0.19±0.04 | 0.34±0.04 | 0.30±0.02 | 0.13±0.04 |
| | Acetaldehyde | | | | 0.12±0.03 | 0.09±0.02 |
| | Isovaleraldehyde | | | | | 0.09±0.06 |
| aldehydes | Hexanal | | | 0.01±0 | | 0.02±0 |
| | 3-Furaldehyde | | 0.07±0 | 0.05±0 | | |
| | Furfural | 0.21±0.04 | 0.17±0.02 | 0.15±0 | 0.40±0.08 | 1.61±1.86 |

| | | | | | | |
|---|---|---|---|---|---|---|
| ketone compounds | Benzaldehyde | 0.76±0.26 | 1.15±0.08 | 0.37±0.11 | 0.26±0.03 | 0.16±0.12 |
| | Acetone | 1.3±0.44 | 1.84±0.94 | 0.90±0.20 | 0.71±0.09 | 0.28±0.14 |
| | 2,3-Butanedione | 1.10±0.18 | 1.59±0.11 | 1.12±0.08 | 1.31±0.08 | 1.41±1.17 |
| | 3-Hydroxy-2-butanone | 3.51±1.16 | 6.68±1.70 | 3.50±0.28 | 5.58±0.34 | 10.1±1.08 |
| phenols | Hydroxybenzene | | | | | 0.03±0.02 |
| | 2,6-Di-tert-butylphenol | 0.14±0 | | | | |
| | 2,4-Di-tert-butylphenol | | | | | 0.62±0.49 |
| Other organic compounds | Hexamethylcyclotrisiloxane | 0.08±0.01 | 0.07±0.01 | 0.08±0.01 | 0.07±0.02 | 0.08±0.03 |
| | Dimethyl disulfide | | | | | 0.03±0.01 |
| | Decamethylcyclopentasiloxane | 0.27±0.11 | 0.37±0.05 | 0.23±0.03 | 0.16±0.01 | 0.14±0 |
| | Tetramethylpyrazine | 0.04±0 | | | | 0.17±0.16 |
| | 2(2-Ethoxyethoxy)ethanol | 0.07±0.01 | 0.08±0 | | | 0.1±0.08 |
| | Azulene | | 0.04±0 | | 0.03±0 | |
| | Hexamethylcyclotrisiloxane | | | | | 0.16±0.02 |
| | 1-Methylnaphthalene | | 0.06±0.01 | 0.05±0 | 0.04±0.01 | |

P: Values in the same row followed by different letters (a-e) differ significantly at a $p < 0.05$ level.

**Table 3** Results of LDA model to identify the different days of fermented substrate of vinegar

| Days | Recognition rate | Error sources | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1 | 10/10 | | | | | | | |
| 2 | 7/10 | 2→1 | 2→3 | ***2→12*** | | | | |
| 3 | 7/10 | 3→5 | 3→6 | 3→6 | | | | |
| 4 | 9/10 | 4→5 | | | | | | |
| 5 | 6/10 | 5→3 | 5→3 | 5→3 | | | | |
| 6 | 5/10 | 6→3 | 6→5 | 6→7 | ***6→12*** | ***6→12*** | | |
| 7 | 7/10 | ***7→1*** | 7→4 | 7→8 | | | | |
| 8 | 5/10 | 8→6 | 8→7 | 8→7 | 8→9 | 8→9 | | |
| 9 | 6/10 | 9→8 | 9→11 | 9→12 | 9→12 | | | |
| 10 | 5/10 | ***10→3*** | 10→8 | 10→9 | 10→11 | 10→12 | | |
| 11 | 8/10 | 11→12 | ***11→16*** | | | | | |
| 12 | 7/10 | 12→9 | 12→13 | 12→14 | | | | |
| 13 | 3/10 | ***13→9*** | 13→11 | 13→14 | 13→15 | 13→16 | ***13→17*** | ***13→19*** |
| 14 | 5/10 | ***14→2*** | 14→11 | 14→13 | 14→13 | 14→15 | | |
| 15 | 4/10 | ***15→11*** | 15→16 | 15→17 | 15→17 | 15→18 | ***15→19*** | |
| 16 | 5/10 | 16→13 | 16→13 | 16→15 | 16→17 | 16→17 | | |
| 17 | 6/10 | 17→15 | 17→16 | 17→18 | 17→19 | | | |
| 18 | 5/10 | 18→15 | 18→15 | 18→17 | 18→19 | 18→19 | | |
| 19 | 4/10 | ***19→13*** | ***19→15*** | 19→17 | 19→18 | 19→18 | 19→18 | |
| Total | 114/190 | | | | | | | |