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Traditional Vinegars Identification by Colorimetric Sensor

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Abstract

An expanded colorimetric array detector that was capable of the highly sensitive and highly selective discrimination of vinegars was developed. The system of olfaction visualization and operational approach were discussed. Thirty dyes were selected from natural dyes, chemoresponsive dyes and pH dyes. A 5×6 colorimetric sensor array was created by printing the dyes on reverse phase silica gel plates. Four traditional vinegars were measured by the colorimetric sensor array. With cluster analysis, all samples were assembled 'Zhenjiang Vinegar', 'Shanxi Vinegar', 'Sichuan Vinegar', 'Jiangzhe Vinegar' when the similarity was 12. This work showed the potential applications of the olfaction visualization technology for visual analyzing and fingerprint identifying the aroma of food.

Key words: traditional vinegar; identification; colorimetric sensor; principle component analysis; hierarchical cluster analysis

1. Introduction

Vinegar is defined as a liquid fit for human consumption and contains a specified amount of acetic acid. China has more than 5000 year's history of producing vinegar. Every year over 26 million hectoliters of vinegar is produced in China. Chinese people treat the vinegars as favorite condiments, health products and even medicines. More than 3.2 million liters of vinegar is consumed every day in China [1]. Because the quality of vinegar directly affects the people's health, Chinese governments have paid more and more attention on the quality control of vinegar. The China State Bureau of Quality and Technical Supervision had issued the market allowance policy on vinegar quality and safety in 2002 in China. From then on, the vinegar enterprises must take out the vinegar producing license and pass the on-the-spot check of the producing necessary conditions. At the same time, the China State Bureau of Standards has issued the

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related standards to specify the classification, requirement, labeling, packaging, storage, transport and inspecting of vinegar to ensure the quality of vinegar and the people's health. In China, studies on Chinese vinegar quality evaluation are scarce.

The quality control of vinegar is widely investigated by using sensory analysis and chemical analysis methods. Sensory analysis requires a well trained testing panel and limited vinegar samples are examined at each tasting session, in order not to excessively tire the assessors. At the same time, the accuracy and objectivity cannot always be ensured because the assessors are influenced by their healthy conditions, emotions and environment. Since vinegars contain more than 100 compounds, chemical analysis methods are complicated and expensive [2-5]. Also, they are not convenient for real-time, on-line and in situ detection. Therefore, there is a need for new reliable methods for the assessment of characteristics of Chinese vinegar products.

Normal electronic nose systems generally allow for distinction between analytes of different chemical functionality, the discrimination of compounds within similar chemical compounds remains a challenging goal [6,7]. Professor Kenneth S. Suslick has previously reported the colorimetric array detection of a wide range of odorants using a family of metalloporphyrins immobilized on reverse-phase silica and on hydrophobic membranes[8, 9]. Here we report the use of an expanded colorimetric array detector that is capable of the highly sensitive and highly selective discrimination of vinegars. This work was the first step to develop a commercial electronic nose on the Chinese vinegar characterization.

2. Materials and Methods

2.1. Four traditional vinegars

Table 1. The details of the vinegar samples utilized in the experiment

Sample name	Type	Raw materials	Total acidity (g/100 ml)[a]	Fermentation method	Production area
Zhenjiang vinegars	Aromatic vinegar	Water, sticky rice, wheat bran, sugar, salt	5.0	Solid fermentation	Jiangsu province
Shanxi vinegars	Mature vinegar	Water, broomcorn, barley, pea, wheat bran, sodium	5.0	Solid fermentation	Shanxi province
Jiangzhe vinegars	Rice vinegar	Water, rice, wheat bran, sugar, salt, sodium	5.0	Solid fermentation	Jiangzhe province
Sichuan Vinegars	Herbal medicine vinegar	Water, rice, wheat bran, Chinese herbal medicine	5.0	Solid fermentation	Sichuan province

[a] As total acidity expressed in % acetic acid.

Four famous traditional vinegars, which are ‘Zhenjiang Vinegar’, ‘Shanxi Vinegar’, ‘Sichuan Vinegar’, ‘Jiangzhe Vinegar’, were used as testing samples. The name, type, raw materials, total acidity, fermentation method and production area were copied from the vinegar bottle labels and listed in Table 1. The four traditional vinegars are all based on solid-state fermentation. In the National Industrial Standard of Vinegar, vinegar is classified into three grades, depending on its concentration of acetic acid (3.5 − 4.5%, 4.5 − 6%, and >6%, respectively). Therefore, these four vinegars used in this study were belonged to the same class with the same concentration of acetic acid (5.0% g/100ml) as shown in Table 1. However, each type of vinegar has its own taste, flavor characteristic, and market in China. Ten samples from each type of vinegar were collected from supermarket.

2.2. The colorimetric sensor array and olfaction visualization equipment

The design of the colorimetric sensor array is based on two fundamental requirements: (1) the chemo-responsive dye must contain a center to interact strongly with analytes, and (2) this interaction center must be strongly coupled to an intense chromophore. The first requirement implies that the interaction must not be simple physical adsorption, but rather must involve other, stronger chemical interactions. Chemoresponsive dyes are those dyes that change color, in either reflected or absorbed light, upon changes in their chemical environment [10].

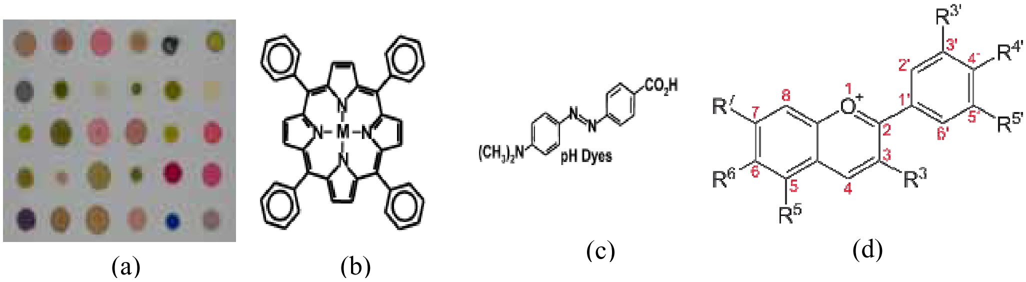

Fig. 1. Colorimetric sensor array, 5×6 colorimetric sensor array printed on a hydrophobic surface, 30 mm×25 mm (a). Examples of metalloporphyrin (b), pH indicator (phenol red (c)) and natural dyes (anthocyanin) are illustrated.

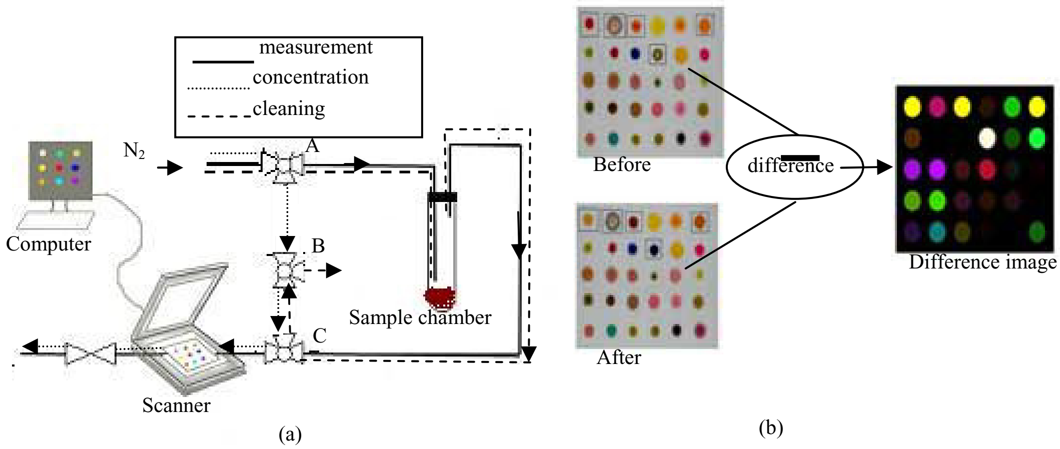

Fig. 2. The diagram of colorimetric measurement (a) and the difference image (b). A, B, C: three-way valve, During the measurement, three di erent phases could be distinguished: concentration, measurement and cleaning. The electro-valves, controlled by a computer program, guided the N2 through di erent circuits depending on the measurement phase.

Disposable colorimetric arrays of chemoresponsive dyes have been created by printing the dyes on reverse phase silica gel plates [11]. Thirty dyes were selected from natural dyes, chemoresponsive dyes and pH dyes. The chemoresponsive dyes and pH dyes were selected and purchased from Sinopharm Chemical Reagent Co.Ltd China. A 5×6 colorimetric sensor array used in this work (Fig. 1) was spotted on C2 reverse phase silica gel plates using 0.1 mL microcapillary pipettes.

The equipment of colorimetric sensor array system (Fig.2 a) was developed to analysis according to colorimetric arrays. Gas streams (N2) containing the vapors of interests were generated by flowing nitrogen through the vinegar in a thermo-stated, glass-fritted bubbler. Digital mass-flow controllers were utilized to control nitrogen flow speed. The ‘before’ image was first acquired on the flatbed scanner; an array was then exposed to a flowing stream of N2 containing the analytes of interest, and the array was then scanned again after equilibration. Images were obtained at 1200 dots per inch in RGB color mode using an ordinary flatbed scanner (Epson Perfection 1200S). Color-difference maps (Fig.2 b) were obtained from the scanned RGB images by digitally subtracting the image before exposure to analytes from the image after exposure, using a 314-pixel average from the center of each dye spot (thus avoiding subtraction artifacts at the periphery of the spots) [10].

2.3. Statistical method

In order to discrimination between these four vinegars, principle component analysis (PCA) [6,7] and hierarchical cluster analysis (HCA)[10] were performed.

3. Results and discussion

3.1. Four traditional vinegars

China and European has a long history for brewing vinegar. The main difference between Chinese vinegars and European vinegars is the raw materials. Chinese vinegars are mainly produced from rice, sticky rice and wheat bran, while European vinegars are usually fermented from wine, cider, fruit juices, malted barley, honey or pure alcohol. The types of Chinese vinegars mainly rely on their raw materials, traditional technologies and production areas. Different types of vinegars have the same main components, water and acetic acid. Though vinegars contain hundreds of trace components, which contribute to smell, taste and the responses of the gas sensors, their mass is no more than 10%. The four vinegars measured in this study are introduced as following.

Shanxi old mature vinegar is the most famous vinegar in northern China. Shanxi old vinegars use sorghum as the main raw material with a very large dosage of great koji (about 60% of the raw materials). Production of Shanxi old mature vinegar takes about 18 months, although the acid production itself takes less than 10 days. The most time-consuming stage is the maturation process. Upon fermentation, half of the solid Pei is ‘‘fumed’’ (heated in a jar with a lid at 70℃ for 4 days) and then mixed with the remaining half and leached. Flavor compounds are formed chemically during fuming. The filtrate is transferred to a big jar and is exposed to the sun (solarized); in winter, surface ice is removed. Solarization and ice removal entail a concentration increase of acetic acid and flavor substances formed by chemical and enzymatic reactions [2, 15].

Zhenjiang aromatic vinegar is most famous in Southern China. Its main raw materials are sticky rice and wheat koji. Henshun Group Co., Ltd., is the largest manufacturer and its product is sold in more than 43 countries. Zhenjiang aromatic vinegar is a typical example of the so-called ‘‘cook method’’ for ethanol production. However, the acetic-acid stage is a unique multilayer SSF with a fed-batch fermentation process in a series of two open containers (big jar or basin). First, a certain amount of rice wine (about 14% ethanol, v/v) is mixed with wheat bran, rice hull, and so-called vinegar seeds (i.e., starter culture) to form a semi-solid substrate in the first container (half volume). The substrate is divided in 10 layers; every 24 h, the top layer is mixed with fresh rice hull to increase porosity and transferred to the second container; after 10 days, the first container is thus empty and the second one full. This process is called ‘‘fed-batch fermentation by layers’’. Ethanol oxidation and acetic-acid formation mainly take place in the top layer of the second container, which is exposed to the air.

Sichuan is a hilly province in Southwest China. The raw materials for Sichuan bran vinegar production process are herb koji (including as many as 108 medicinal herbs), which is the liquid extract of smartweed leaves (a marsh plant, Polygonum hydropiper, Laliao in Chinese) used to initiate vinegar fermentation; and wheat bran, used both as carrier and substrate in the fermentation process. Production of herb koji is rather selective for specific microorganisms.

The name Jiangzhe is a combination of the names of two Chinese provinces (i.e., Jiangsu and Zhejiang). Production of Jiangzhe rose vinegar is a combination of SSF and SmF. First, microorganisms grow spontaneously on steamed rice. After about 10 days, pigmented microorganisms (mainly Monascus) predominate (‘‘rice blossoming’’), performing a simultaneous saccharification and alcohol fermentation. Water is then added to ‘‘blossoming rice,’’ and within 20 days, acid-producing microorganisms, such as Acetobacter, proliferate and form a bacterial film at the surface. From that moment on, the broth is stirred every 2 days. It takes another 3 to 4 months to complete the fermentation. The filtrate of the fermented broth is then bottled and pasteurized without further maturation.

3.2. Principle component analysis (PCA) and hierarchical cluster analysis (HCA)

Metalloporphyrins are nearly ideal for the detection of metal-ligating vapors because of their open coordination sites for axial ligation, their large spectral shifts upon ligand binding, and their intense coloration. A series of metalated tetraphenylporphyrins (TPP) was used to provide differentiation based on metal-selective coordination[12-14]. Common pH indicator dyes change color in response to changes in the proton acidity or basicity of their environment. Among the most responsive is Nile Red. Natural dyes were extracted from Rhododendron, red radish, Jasminum nudiflorum, black rice by alcohol and refined by chromatogram method. The natural dyes are anthocyanins, a group of natural phenolic compounds changed their color according to the environment. Each analyte response is represented as the red, green, and blue values of each of the 30 dyes, i.e. a 90 dimensional vector. Ten samples for each type of vinegar which has been analyzed by the panel were then evaluated by the colorimetric sensor array system. PCA was used to analyze the 90 dimensional vector of the RGB response of the 30 dyes used in these arrays. PCA shows our 30-dye array has an extraordinarily high level of dispersion: 11,14, and 21 dimensions are required to define 90%, 95% and 99% of the total variance, respectively. From the PCA, not all of the 90 dimensions are equally important. In fact, roughly 95% of all information is contained in 14 specific dimensions (i.e. linear combinations of the 72 different R, G, and B values). The four traditional vinegars could be distinguished by PC1 (61.3%) and PC2 (9.3%) as shown in Fig 3.

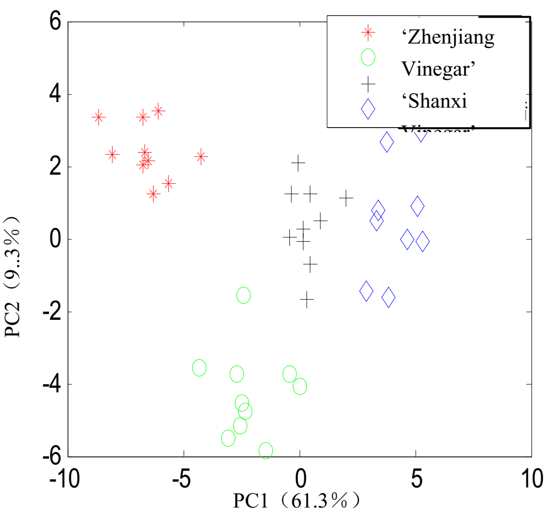

Fig. 3. The results of principal component analysis colorimetric sensor array measurements for four vinegars

To examine the multivariate distances between the analyte responses in this 90-dim. RGB color space, a hierarchical cluster analysis (HCA) was performed; this analysis makes use of only the digital data representing the observed difference maps—no chemical information is included. The resulting dendrogram for the responses to saturated analyte vapors is shown in Fig. 4. Remarkably, the clusters formed are in keeping with the type of vinegars and electronic properties of the VOCs. HCA of the response of 4 vinegars reveals a clustering of different tradition vinegars into distinct branches assembled as 'Zhenjiang Vinegar', 'Shanxi Vinegar', 'Sichuan Vinegar', 'Jiangzhe Vinegar' when the similarity was 12 (Fig. 4).

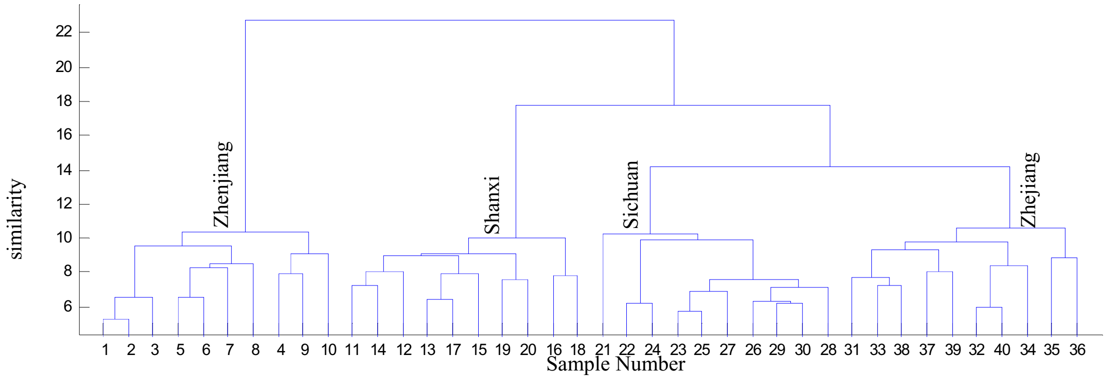

Fig. 4. Plot dendrogram graphs of all samples data from colorimetric sensor array measurement

4. Conclusions

Vinegar is favorite food and a large amount of vinegar is produced and consumed every year in China. There is no effective method or instrument for real-time, on-line and in situ detecting the vinegars yet, though Chinese government has paid more and more attentions on the quality control of vinegar. An expanded colorimetric array detector that was capable of the highly sensitive and highly selective discrimination of vinegars was developed. The system of olfaction visualization and operational approach were discussed. Thirty dyes were selected from natural dyes, chemoresponsive dyes and pH dyes.

The responses of the array of 30 colorimetric sensors array to 4 vinegars were studied by principal component analysis (PCA) and cluster analysis (CA) to investigate the presence of classes inside the sample population. This work showed the potential applications of the olfaction visualization technology for visual analyzing and fingerprint identifying the aroma of food. It was shown that characterizing the Chinese vinegars by the colorimetric sensors array was highly related to their type.

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