Sensors and Actuators B 183 (2013) 608–616

Contents lists available at SciVerse ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Monitoring vinegar acetic fermentation using a colorimetric sensor array

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ARTICLE INFO

Article history:
Received 24 October 2012
Received in revised form 15 March 2013
Accepted 9 April 2013
Available online 17 April 2013

Keywords:
Colorimetric sensors array
Multivariate calibration
Monitor
Vinegar
Acetic fermentation

ABSTRACT

This paper attempted a portable colorimetric sensor array to monitor vinegar acetic fermentation. We fabricated a colorimetric sensor array by printing 15 chemically responsive dyes (i.e. 9 porphyrins/metalloporphyrins and 6 pH indicators) on a C2 reverse silica-gel flat plate. The colorimetric sensor array, with specific colorific fingerprint to the VOCs in vinegar, was successfully used to monitor vinegar acetic fermentation with the help of multivariate calibration. Principal component analysis (PCA) and linear discriminant analysis (LDA), as two multivariate calibration tools, were used to classify vinegar samples at different acetic fermentation stages. Experimental results show that LDA achieved a better result and its classification rate is 100% by leave-one-out cross-validation (LOOCV). Besides, we systemically studied the change of VOCs during vinegar acetic fermentation with the help of hierarchical cluster analysis (HCA). This research work shows that the colorimetric sensor technique has a potential in monitoring vinegar acetic fermentation.

1. Introduction

Vinegar is consumed worldwide as a food condiment and preservative. Vinegar is traditionally produced by cereal with a long history of more than 3000 years in China [1]. At present, there are a variety of popular vinegars around the world and each region possesses its own typical vinegar [2]. Different from submerged fermentation technique for vinegar in Europe, solid or semi-solid fermentation technique for vinegar is commonly used in Asia [3]. Vinegar fermentation usually has three steps including starch saccharification, alcohol fermentation, and oxidation of ethanol to acetic acid. In solid or semi-solid fermentation, the coexistence of different microbes could provide numerous enzymes for synthesis of flavor and functional materials, such as organic acids and volatile components [4,5]. Chinese vinegars are mostly produced by a typical aerobic solid-state fermentation technique. After saccharification and alcohol fermentation through semi-solid culturing, solid-state acetic acid fermentation is conducted by mixing alcohol mash with the wheat bran and rice hull in different pots or ponds. During this process, the content of acetic acid rises whereas the content of ethanol drops. Generally, this process is maintained around 20 days, and is crucial to the final product quality. Real-time PCR results suggested that the biomass showed a “system microbes self-domestication” process in the first 5 days, then reached a higher level at the 7th day before gradually decreasing until completing the acetic fermentation process at the 20th day [6].

The microbial diversity and its dynamic change are of great significance to the quality and characteristics of the fermentation products. Although the basic objective of the process is the transformation of ethanol into acetic acid by the metabolism of the acetic bacteria, several other chemical and biochemical processes, which will characterize the final product, take place in parallel [7]. Monitoring the vinegar fermentation, therefore, is of importance to obtain a high-quality vinegar product. And nowadays, a few of methods of monitoring by gas chromatography (GC) [8,9], HPLC [10,11], GC–MS [12] can be employed for this. However, these methods mentioned above are laborious and time-consuming, and would not be available for the real-time monitoring.

The gas sensor technique, also called electronic nose (E-nose), is a very promising technique for real-time quality monitoring in food processing [13,14]. Most of gas sensor systems commercially available at present are almost equipped with metal oxide semiconductor (MOS) sensors, whose responses are quite reproducible [14,15]. However, MOS gas sensor is sensitive to humidity, and water vapor affects measurements by the sensor technique, and the varying temperature and humidity can lead the baseline shift of sensor response signal [16]. So the traditional MOS sensor system often equipped with the extra temperature-controlled and humidity-controlled accessories, when it was applied in liquid food. Moreover, vinegar contains lots of weak acids (e.g. acetic acid) that can poison MOS sensors very rapidly, even when kept at high temperature. So it is challenging to rapid achieve process monitoring of vinegar acetic fermentation using the traditional MOS sensor technique.

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http://dx.doi.org/10.1016/j.snb.2013.04.033

Recently, a novel and portable colorimetric sensor is being probed, which is not sensitive to humidity due to the hydrophobicity of the sensor materials and sensor plate. This observation was also supported by our previous study, in which the detection results did not change with humidity varying from 10% to 50% [17,18]. The basic principle of this technique is to utilize the color change induced by reaction between volatile materials and an array of chemical dyes upon ligand binding for chemical vapor detection and differentiation. Chemical dyes are often selected according to their sensitivity to the specific volatile organic compounds (VOCs). For instance, metalloporphyrin is a natural choice for the detection of volatile organic vapors [19,20]. Fabricating a colorimetric sensor array is usually by printing the chemical responsive dyes on a reverse phase silica gel plate. The array's responses originate from partially selective and specific interactions between the VOCs of interest and the chemically responsive dyes. A color change profile for each object can be obtained by differentiating the images of the sensor array before and after exposure to the VOCs of objects [20–23]. The digital data representing the color change profiles were analyzed with the help of multivariate calibration. In recent years, some relevant studies on the application of this novel sensor technique in food quality control have been reported [24–26]. Our previous research also reported the application of this novel sensor technique to evaluate fish freshness [17,18]. Researches mentioned above show that the colorimetric sensor technique has a huge potential in the analysis of VOCs of foodstuff, especially for liquid samples. In this paper, we presented an approach to the application of the portable colorimetric sensor array in monitoring vinegar acetic fermentation. With the help of multivariate calibration, we also systemically studied the change of VOCs during the vinegar acetic fermentation.

2. Materials and methods

2.1. Samples preparation

The vinegar samples used in this study were collected from Jiangsu Hengshun Vinegar-Industry Co., Ltd. in July 2012. Total 4 batches of samples were collected from four different time points in the acetic acid fermentation (i.e. 5th day, 12th day, 19th day, and raw vinegar). Each sample collection was done at the same depth of fermentation culture (about 20 cm from the upper surface). Once collected, each fresh sample (25 mL) was immediately stored using a sterile plastic bag at 4 °C until the next usage. Each batch has 15 samples amounting to a total of 60 samples. In order to ensure that all samples are of independences each other, all samples from the same batch were collected from different fermentation pots.

2.2. Colorimetric sensor array

The design of a colorimetric sensor array is often based on two fundamental requirements: (1) each chemical responsive dye must contain a center to interact strongly with analytes and (2) the interaction center must be strongly coupled to an intense chromophore. The required dye classes include: (1) Lewis acid/base dyes (i.e. metal ion containing dyes), (2) Brønsted acidic or basic dyes (i.e. pH indicators), and (3) dyes with large permanent dipoles (i.e. zwitterionic solvatochromic dyes) [19]. Porphyrins and their metal complexes are a natural choice for recognition of analytes with Lewis acid/base capabilities. 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. Common pH indicator dyes change color in response to changes in the proton (Brønsted) acidity or basicity of their environment [23,27].

To optimize array response, we tested lots of commercially available sensing materials in the experiment. Eventually, we found that the following nine metalloporphyrins materials and six pH indicators were the best choice in this work. All metalloporphyrins reagent (Analytical-Grade) were purchased from Sigma–Aldrich Chemical Co. (Shanghai, China), including:

(1) 5,10,15,20-Tetraphenyl-21H,23H-porphin,
(2) 5,10,15,20-Tetraphenyl-21H,23H-porphine manganese(III) chloride,
(3) 2,3,7,8,12,13,17,18-Octaethyl-21H,23H-porphine manganese(III) chloride,
(4) 5,10,15,20-Tetrakis(4-methoxyphenyl)-21H,23H-porphine iron(III) chloride,
(5) 5,10,15,20-Tetraphenyl-21H,23H-porphine iron(III) chloride,
(6) 5,10,15,20-Tetraphenyl-21H,23H-porphine copper(II),
(7) 5,10,15,20-Tetrakis(pentafluorophenyl)-21H,23H-porphyrin iron(III) chloride,
(8) 5,10,15,20-Tetrakis(4-methoxyhenyl)-21H,23H-porphine cobalt(II),
(9) 5,10,15,20-Tetraphenyl-21H,23H-porphine zinc, synthetic, low chlorine.

Six kinds of pH indicators were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), including: (1) Thymol Blue, (2) Bromothymol Blue, (3) Bromocreslo Green, (4) Cresol Red, (5) Bromocresol Purple, and (6) Neutral Red.

The steps of making colorimetric sensor were arranged as follows: (1) Each chemically responsive dye (20 mg) was dissolved in 10 mL of chloroform solution. The mixture was preprocessed for 2 h by ultrasound at room temperature, and eventually obtained 9 kinds of pigments solution. (2) Each pigment solution was spotted on the plate using 0.1 μL microcapillary tubes. (3) Once printed, the arrays were stored in a nitrogen-flushed glove bag before the further usage in this experiment. Eventually, we can get a colorimetric sensor array consisting of 15 chemically responsive dyes by the above method.

2.3. Data acquisition

A functional prototype of colorimetric sensor array system for data acquisition was constructed as shown in Fig. 1. Images of the sensor array were captured by an HP Scanjet 4890 flatbed scanner (Hewlett Packard Inc., Shanghai, China), and the scanner's resolution was set at 600 dpi. The initial image of sensors array was first captured by the flatbed scanner before exposed to the vinegar sample, and then the array was exposed to vinegar sample. In the experiment, 25 mL liquid sample was poured into a Petri dish (⌀ 9 cm) that was tightly closed by a lid of the Petri dish. Herein, the sensor array was inverted attached to the lid of Petri dish. The sample was stored at 4 °C before data acquisition but the ambient temperature is controlled at 25 °C when sampling. Headspace generation for sampling was achieved using the uniform arrangements including the ambient temperature, the volume of sample, the size of Petri dish, and the headspace time. Once reaching nearly complete equilibration, we took out the sensor array from the Petri dish to scan it again, and got a "final" image. We can get a colorful difference image by subtracting the "initial" image from the "final" image, and the difference image provided a color change profile that is a characteristic fingerprint to VOCs in sample. To avoid factitious non-uniformity, the center of each dye spot (a round area consisting of 800 pixels) was averaged. The difference image is a RGB color image consisting of 3 color components images (i.e. R image, G image, and B image). Thus, each dyes can provide 3 variables (R, G, and B gray value), and 15 dyes in the sensor array can provide 45 variables (15 dyes × 3 color components). Herein, the