



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



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## ABSTRACT

Total acid content (TAC) and moisture content (MC) are very important parameters during Solid-State Fermentation (SSF) processes. The feasibility of using hyperspectral imaging (HSI) technology for predicting TAC and MC in vinegar cultures during SSF processes was investigated. Prediction models were constructed using variables selected from spectral and spatial data from associated 3-D hyperspectral datacubes to predict the relative content of TAC and MC for each pixel in the hyperspectral image. Models were developed using genetic algorithm (GA) optimization combined with partial least squares regression (PLS) dependent on the spectral variables yielded good prediction results for both TAC and MC. The determination coefficients ( $R_p^2$ ) for TAC and MC were 0.8565 and 0.8162, respectively. Finally, the distribution maps of TAC and MC for a vinegar culture sample were obtained. These distribution maps could be implemented to estimate the uniformity of fermentation products during SSF.

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## 1. Introduction

Solid-State Fermentation (SSF) has become a very attractive alternative to submerged fermentation for specific applications (Pandey, 2003). In China, the SSF is maintained empirically (Solieri and Giudici, 2009) since the process is not fully understood and some main variables have yet to be quantified unlike to liquid fermentation have been deeply studied (Maldonado et al., 1975; Jamal et al., 2014). So far, comparatively fewer studies were on rapidly detecting the main variables and the uniformity of the fermentation substrate during SSF.

In this study, we consider acetic acid fermentation of Zhenjiang balsamic vinegar (ZJBV) as a typical representative example of SSF in a traditional Chinese fermentation process. The process of SSF

requires the establishment of a solid base culture of SSF produced primarily from several kinds of cereal including glutinous rice, wheat bran and rice hull, as shown in Fig. 1. Combined with a unique cycling-inoculation, a fermentation culture extracted from the 8th day is selected as a seed (starter) for the subsequent inoculation stages of the SSF process. Since no additional microbe is supplied during the fermentation process, the microbial community and quality of each fermentation circle is supposed to be comparatively stable (Xu et al., 2011). In this process, total acid content (TAC) and moisture content (MC) are important indicators of the status of the fermentation process (Zongbao et al., 2010). TAC plays a key role in determining the sensory properties (Liu D et al., 2004) and affects microbial growth (Qian, 2000). 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. If the solid form culture utilized in the production of vinegar is unevenly distributed this can influence the culture breed and metabolism. Therefore, TAC and MC must be periodically monitored to effectively optimize and control the process of SSF. Furthermore, there is currently no existing device that can measure TAC and MC on-line in a solid medium due to the lack of free water. Current methods to

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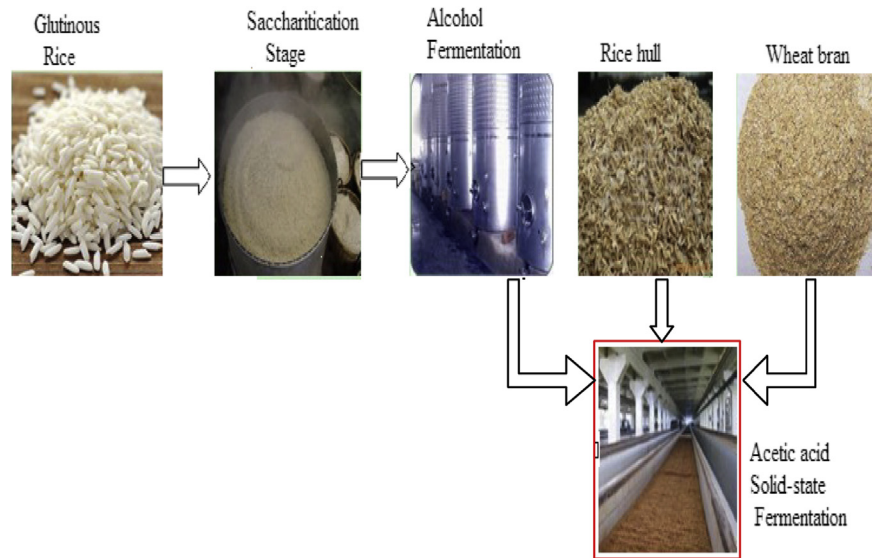


Fig. 1. A flow chart for the culture formation of Zhenjiang vinegar acetic acid solid-state fermentation.

determine the levels of TAC and MC are by wet-analytical chemistry methods and sensory evaluation and, consequently, are time-consuming and labor-intensive. The above all raise the impendency to develop more efficient techniques.

As a relatively novel non-destructive technology, hyperspectral imaging (HSI) is an emerging technique that integrates the advantages of machine vision and visible infrared spectroscopy to attain spatial and spectral information from an object (Sun and Brosnan, 2003; Zheng et al., 2006). Furthermore, during data acquisition, the HSI system records the spatial information in a line-wise manner; simultaneously the spectrum for each pixel is recorded with the spatial dimensions making the implementation of this technique feasible for on-line applications and to permit automation of a variety of routine inspection tasks (Alomar et al., 2003; ElMasry et al., 2013). SSF is often accompanied with the changes in the external attributes (e.g. color, texture) and the internal attributes (e.g. chemical compositions, metabolites). Therefore, the aim of this work was to explore the feasibility of HSI technique for predicting TAC and MC contents and their distributions in a vinegar culture during SSF (Fig. 1).

## 2. Materials and methods

### 2.1. Vinegar culture samples

The original vinegar culture samples were collected in May and June 2014, from Jiangsu Hengshun Vinegar Industry Co., Ltd (Jiangsu, People's Republic of China) located in the eastern coastal province of Jiangsu (31°37'–32°12' northern latitude, 118°58'–119°27' east longitude). After saccharification and alcohol fermentation, SSF is conducted by mixing alcohol mash with the wheat bran and rice hull as shown in Fig. 1. Rice hull and wheat bran are mixed with fermentation culture, which is loose in consistency, and has very large interspaces, and could hold sufficient air to enable aerobic microbial growth and the associated metabolic activities. 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. After averaging, 60 samples were obtained in this work. To develop the TAC and MC

prediction models, all samples were divided into two sets, one sample taken out from every three samples were assigned to prediction set, and the other two samples were assigned to a calibration set. Thus, the calibration set contains 40 samples; the prediction set contains 20 samples.

### 2.2. Chemical analysis of the samples

#### 2.2.1. Total acid content measurement

In this study, 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 h before measuring. Then 20 mL of the solution was mixed with 60 mL 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.

#### 2.2.2. The moisture content 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  $\pm 0.1\%$  of moisture. The samples were cooled in a dryer, weighed and moisture loss was calculated as a percentage.

### 2.3. Hyperspectral imaging analysis of vinegar culture

A flow chart of the HSI analysis is demonstrated in Fig. 2. The process consisted of nine steps, namely: (1) Acquisition of hyperspectral images; (2) Spectral variable extraction and preprocess; (3) Characteristic picture extraction by principal component analysis (PCA); (4) Extraction of optimum intervals from spectral information by synergy interval partial least square (siPLS); (5) Optimum

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