



Effects of aroma quality on the biotransformation of natural 2-phenylethanol produced using ascorbic acid

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ABSTRACT

Background: Natural 2-phenylethanol (2-PE) is an important flavoring that emits the aroma of roses. During biotransformation, the aroma quality of natural 2-PE is affected by its main by-products, which include butanol, isobutyric acid, butyric acid, and isovaleric acid. Thus, controlling undesirable by-product formation can reduce the effect of odor on 2-PE aroma quality.

Results: 2-PE was produced through biotransformation using L-phenylalanine as a substrate and glucose as a carbon source. Ascorbic acid was added to the system to improve the redox reaction and suppress the generation of by-products. Principal component analysis of the aroma quality of 2-PE was performed using an electronic nose. Similarity analysis revealed that the effects of four by-products on 2-PE aroma quality may be ranked in the following order: isovaleric acid > butyric acid > isobutyric acid > butanol. The sample that exhibited the best similarity to the standard 2-PE sample (99.19%) was the sample to which ascorbic acid had been added during glucose metabolism.

Conclusions: 2-PE produced through the addition of ascorbic acid exhibited the closest aroma similarity to the standard 2-PE sample.

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1. Introduction

2-Phenylethanol (2-PE), an aromatic alcohol, emits a fresh rose scent. Natural 2-PE, especially that in rose oil, is present in a number of plants [1,2,3]. As a result of its delicate aroma, 2-PE is the second most-often used flavor after vanillin. The compound and its derivatives can be used in food, tobacco, and cosmetics. The global annual output of 2-PE is nearly 10,000 t. Chemical synthesis is the basic method used to produce 2-PE. However, benzene or styrene, the raw materials used to synthesize 2-PE, are regulated chemicals [3, 4]. Methods to produce natural 2-PE include plant extraction and microbial transformation. Essential oils extracted from roses to produce 2-PE account for only a small proportion of the annual output of the compound [5,6,7]. As such, the production of 2-PE through biotransformation presents broad application prospects.

Aroma quality is an important consideration in the production of 2-PE through biotransformation. By-products such as butanol, isobutyric acid, butyric acid, and isovaleric acid are produced during biotransformation; these by-products are adsorbed, together with 2-PE, during post-processing, thereby affecting the aroma quality of natural 2-PE. High-temperature vacuum distillation is often used to

separate impurities from 2-PE [8]. However, high temperature and vacuum conditions can change the aroma quality of 2-PE. To address this problem, reduction of the concentration of by-products during biotransformation and separation of these compounds by atmospheric distillation has been proposed. Fusel alcohol (e.g., butanol) and miscellaneous acid (e.g., isobutyric acid, butyric acid, and isovaleric acid) generation during an alternative strategy of glucose metabolism and ethanol oxidation has been discussed [9]. However, little information on the control of impurities through addition of exogenous substances has been reported.

An electronic nose is an instrument that can discriminate and recognize various gases and odors [10]. Electronic noses consist of a headspace sampler, sensor array, and pattern recognition modules. These devices are capable of performing the following functions: generating volatile compounds of a sample, reacting on contact, and recording data as a fingerprint. Today, electronic noses are used to monitor food quality. These devices have also shown promising application potential in determination of aroma quality [11].

Taking our previous studies [12,13] as bases, a 2-PE biotransformation system was developed in the present work using L-phenylalanine (L-Phe) as the substrate. An alternative strategy of glucose metabolism and ethanol oxidation was applied to this system, and a post-processing procedure using macroporous resin was performed. Ascorbic acid was added during 2-PE biotransformation, and the effect of by-products on the aroma quality of the 2-PE obtained was investigated.

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2. Materials and methods

2.1. Strains and reagents

Saccharomyces cerevisiae AS 2.1182 was used for 2-PE biotransformation. Macroporous adsorption resin (HZ816) was obtained from Shanghai Huazhen Science and Technology Co., Ltd. (Shanghai, China), and 99.5% L-Phe was acquired from Wuxi Jinghai Amino Acid Co., Ltd. (Wuxi, China). A standard 2-PE sample was provided by the Shanghai Research Institute of Fragrance and Flavor Industry (Shanghai, China). Analytical-grade ascorbic acid and other chemicals were purchased from Sigma (St. Louis, MO, USA) or Sinopharm Chemical Reagent Co., Ltd. (Beijing, China) unless otherwise indicated.

Two buffers, namely, buffers A and B, were used during high-performance liquid chromatography (HPLC) analysis. Buffer A contained water, while buffer B contained methanol.

2.2. Medium and biotransformation

The biotransformation medium consisted of 30 g/L glucose, 8 g/L L-Phe, 5 g/L MgSO₄, 5 g/L K₂HPO₄, and 1 g/L NaCl.

Biotransformation was conducted in a fermentor. Briefly, 3.5 L of 10 g/L *S. cerevisiae* AS 2.1182 was inoculated directly to a 5 L fermentor (BioFlo3000, New Brunswick Scientific Co., Inc., Edison, NJ, USA). The bioreactor was equipped with pH, temperature, and dissolved oxygen monitoring and control systems. The dissolved oxygen tension level was controlled under cascade mode with a constant air flow rate of 3 L/min. Biotransformation was conducted at normal pressure and 30°C with an agitation speed of 400 rpm. The concentrations of glucose, L-Phe, ethanol, and 2-PE were monitored during biotransformation. Glucose, L-Phe, and resin HZ816 were added batch wise according to the requirements of the reaction system.

Butanol, isobutyric acid, butyric acid, and isovaleric acid were added to the standard 2-PE sample at final concentrations of 10–50 mg/L. Samples were then analyzed by an electronic nose to determine the effects of the different by-products on the aroma quality of natural 2-PE.

Ascorbic acid (0.2 g/L) was added to the bioreactor at the start of glucose metabolism (Group II) and 4 h after ethanol oxidative degradation began (Group III). The sample without ascorbic acid (Group I) was designated as the control. This procedure was conducted to determine the effect of ascorbic acid on the produced by-products.

For post-processing, 30 g/L macroporous adsorption resin (HZ816) was added to the bioreactor after completion of fermentation. Adsorption was conducted for 0.5 h at 400 rpm with an aeration rate of 3 L/min.

2.3. Analytical methods

Samples obtained from the biotransformation solution were centrifuged for 10 min at 4000 rpm and 4°C. The supernatant was then used to determine the concentrations of 2-PE, ethanol, L-Phe, and glucose. After biotransformation, resin HZ816 was used to separate the products in the fermentation broth. The resin was loaded into a glass chromatographic column and washed with 95% ethanol. The resin was desorbed by ethanol flowing at a rate of 2 mL/min until the 2-PE concentration in the eluate was <10 mg/L. Macromolecules were removed through microfiltration membranes (0.22 µm), and ethanol was removed by distillation to obtain the pure 2-PE sample.

Residual sugar contents were determined using an SBA-90 Biosensor (Shandong Academy of Sciences, Shandong, China). A 6890A gas chromatograph (Agilent Technologies, Ltd., Santa Clara, CA, USA) was used to determine the concentrations of ethanol, 2-PE, butanol, butyric acid, isobutyric acid, and isovaleric

acid under the following conditions: chromatographic column, 19091J-413 HP-5 (30 m × 0.32 mm × 0.25 µm); injection amount, 1 µL; injection port temperature, 250°C; detector temperature, 250°C; and temperature gradient of 50°C for 3 min, 250°C for 10°C/min, and maintenance at 250°C for 5 min.

L-Phe contents were determined using an Agilent 1260 HPLC separation module fitted with a C18 column (250 mm × 4.5 mm; XDB-C18). Around 50 µL of the samples was injected into the column and eluted at a flow rate of 1 mL/min with 60% buffer B at the start of elution. The concentration of buffer B was gradually increased to 80% after 20 min, and the concentration of L-Phe was determined at 254 nm. Standard curves of the metabolites were obtained to achieve accurate quantification.

All of the 2-PE samples were analyzed using a FOX 4000 (Alpha MOS, Toulouse, France) electronic nose. This device included 18 metal oxide sensors that measure changes in electrical resistance in the presence of volatile compounds. The detection conditions were as follows: carrier gas, synthetic dry air; flow rate, 150 mL/min; 0.01 g of sample added to 1 mL of distilled water in a 10 mL vial; generation time, 600 s; temperature, 40°C; stirring speed, 500 rpm; injection volume, 500 µL; injection speed, 1000 µL/s; needle total volume, 2.5 mL; needle temperature, 60°C; acquisition time, 120 s; and lag time, 360 s [14]. The similarity (S, %) of the two perfume ingredients was calculated using the following formula:

$$S = \frac{1 - \sqrt{\sum_{i=1}^{18} (a_i - b_i)^2}}{\sqrt{\sum_{i=1}^{18} (a_i + b_i)^2}} \times 100\% \quad [\text{Equation 1}]$$

where *a* and *b* are the values of the corresponding fragrance sensor of the prepared 2-PE sample and the standard 2-PE sample, respectively.

2.4. Statistical analysis

One-way ANOVA was performed to estimate the overall significance of the data. A probability level of 5% (*p* < 0.05) was considered statistically significant. Correlation data analysis was conducted through principal component analysis (PCA) by using Xlstat. All experiments were performed in triplicate.

3. Results and discussion

3.1. Effects of the main by-products on the aroma quality of 2-PE

During 2-PE production by biotransformation, by-products, such as butanol, isobutyric acid, butyric acid, and isovaleric acid, produce a distinct and unpleasant odor that destroys the aroma quality of natural 2-PE. The present experiment was conducted to study the effect of different concentrations of by-products on 2-PE aroma quality. Different concentrations of the four by-products were added to the standard 2-PE sample, and the effects of these compounds on the aroma quality of 2-PE were analyzed. The effects of main by-products on the aroma quality of 2-PE showed the following order: isovaleric acid > butyric acid > isobutyric acid > butanol (Fig. 1). The similarity in aroma quality of the produced 2-PE sample and the standard 2-PE sample was apparent when the concentration of butanol was >40 mg/L. The aroma quality similarity between the 2-PE samples was <99.00% when the concentrations of isobutyric, butyric, and isovaleric acids were >30 mg/L. Moreover, the similarity of the products was >99.00% when equal concentrations of the four by-products were mixed provided that the concentrations of individual by-products were less than 20 mg/L. The similarity in aroma quality between 2-PE samples was <98.00% when the concentration of the four by-products exceeded 30 mg/L. Under this condition, a distinct smell that affected the aroma quality of 2-PE was detected.

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