Amperometric Detection in High-Performance Liquid Chromatography for Determining Organic Acids in Fermented Foods

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Introduction
Various organic acids, such as tartaric, malic, citric and lactic acid, present in fermented fruit juice and dairy products condition good taste and aroma of the products. The acid determination in such foods and foods is thus essential for their process and quality control. For determining these acids, gas chromatography and high-performance liquid chromatography (HPLC) with UV or fluorescent detection are commonly employed. In these methods, derivatization of acids with an appropriate labeling reagent is often required before separation for detecting acids in high sensitivity, however, such procedure is rather troublesome and time-consuming.

We previously developed a new method for determining acids by means of voltammetric reduction of quinone [1, 2]. In the present study, an HPLC system with electrochemical detection (HPLC-ECD) was fabricated for determining acids in the fermented foods, since the acid content can be determined by measuring the current signal of the reduction peak of quinone caused by the eluted acids. Assessment of the method was made for determining organic acids in fruit juices during fermentation by yeasts.

Experimental
A two-channel HPLC-ECD system was fabricated using an ion-exclusion column and an electrochemical detector with a glassy carbon working electrode (Fig. 1). An aqueous solution of 0.1 mM HClO$_4$ and an ethanol solution containing 6 mM 2-methyl-1,4-naphtho-quinone (vitamin K$_3$, VK$_3$) and 76 mM LiClO$_4$ served as a mobile phase and a quinone solution, respectively. Both solutions were deaerated and made to flow at 1.0 mL/min through the separate channels.

Fruit juice sample of commercial origin was incubated with yeasts at 21ºC for several days. During the fermentation, 1 mL aliquots of the sample was withdrawn and diluted with 0.1 mM HClO$_4$ containing 1 mM 3-methylglutaric acid (internal standard) and served as a test solution. A 20 µL of the test solution was injected into the column maintained at 45ºC, and the eluate was allowed to merge with the quinone solution in the mixing coil. Each acid in the eluate was detected with ECD at –0.7 V vs. SCE.

Result and Discussion
A typical chromatogram for a standard acid mixture of citric, tartaric, malic, lactic, succinic, 3-methylglutaric and acetic acid at 20 nmol each appears in Fig. 2. The acids were well separated within 20 min. Peak area was linearly related to the amount of acid injected, ranging from 1.0 to 40 nmol (r > 0.998) in all cases. Relative standard deviation (RSD) of the acids at 5.0 nmol was less than 4.1% (n = 10), and the acid detection limit (S/N = 3) for a single injection was 1.0 nmol.

The changes of each acid content were monitored by this method during the fermentation of grape juice with yeasts. The chromatograms obtained before and after the fermentation are shown in Figs. 3A and 3B, respectively. As seen in Fig. 3B, citric, tartaric and malic acid tend to decrease in contrast to the increase in lactic, succinic and acetic acid following the fermentation for 4 days. Such features differ depending on the kinds of sample fruit juices and the fermentation conditions.

Because of the simple and rapid procedure without the derivatization, the present HPLC-ECD method was shown as an effective method for determining organic acids in various fermented foods such as wines, dairy products and pickled vegetables.

Fig. 1. HPLC-ECD system.
Column: Ion-exclusion column, Rpak KC-811
(300 mm x 8 mm i.d., Temp. 45ºC).
Mobile phase: 0.1 mM HClO$_4$.
Quinone solution: 6 mM VK$_3$ + 76 mM LiClO$_4$ in ethanol.

Fig. 2. Chromatogram of standard acid mixture.
Peaks: 1, citric and tartaric acid; 2, malic acid; 3, lactic acid; 4, succinic acid; 5, 3-methylglutaric acid (I.S.); 6, acetic acid.

Fig. 3. Chromatograms of grape juice obtained before (A) and after fermentation with yeasts for 4 days (B). Peaks: 1, citric and tartaric acid; 2, malic acid; 3, lactic acid; 4, succinic acid; 5, 3-methylglutaric acid (I.S.); 6, acetic acid.

References