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Note

Determination of tyramine in cheese by reversed-phase high-performance liquid chromatography with amperometric detection

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Tyramine, the biogenic amine derivative of tyrosine, is an active pressor amine which occurs naturally in many foods[1,2], especially fermented food products. Tyramine is formed during the cheese-making process by the bacterial degradation of milk protein and subsequent decarboxylation of tyrosine. Tyramine generally does not represent any hazard to individuals unless large amounts are ingested or the normal routes of catabolism are inhibited or are genetically deficient. Usually tyramine is metabolized to *p*-hydroxyacetic acid by monoamine oxidase in the human intestine, liver and kidneys and excreted via the urine. However, tyramine can cause a hypertensive crisis[3–6] in patients treated with monoamine oxidase inhibitor drugs such as antidepressants and antitubercular drugs. Particularly with cheese, it is well known as the so-called cheese effect or cheese reaction.

The tyramine content of cheese is known to be variable not only in different types of cheese but also in the same type of cheese. Therefore, the determination of the tyramine content of cheese is required for pharmaceutical, therapeutic and food hygiene purposes. Tyramine in cheese has been determined by fluorometric methods[7–10] and with amino acid analyzers[11,12]. However, these methods are time-consuming and require relatively large amounts of sample.

In this paper, we describe a method for the detection of tyramine in cheese by reversed-phase high-performance liquid chromatography (HPLC) with amperometric detection (AMD), which provides a sensitive and specific method for compounds that possess intrinsic electrochemical activity[13].

EXPERIMENTAL

Apparatus

The liquid chromatograph was a Trirotor-VI (Jasco, Tokyo, Japan) with a

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LiChrosorb RP-Select B (7 μm) column (250 × 4 mm I.D.) (Cica-Merck, Tokyo, Japan). The detector was an E-558 amperometer (IRICA, Kyoto, Japan) with a glassy carbon electrode. The applied potential was maintained at 0.7 V *versus* an Ag/AgCl reference electrode with a sensitivity setting of 4 nA full-scale. An F-1000 fluorescence spectrometer (Hitachi, Tokyo, Japan) with an excitation wavelength of 225 nm and an emission wavelength of 305 nm was used.

Other equipment consisted of an Ultra-Turrax homogenizer (IKA-Werk, F.R.G.), an H-107 type B centrifuge (Kokusanenshinki, Tokyo, Japan) and a 0.45-μm Chromatodisc 13A membrane filter (Kurashikiboseki, Osaka, Japan).

Reagents

A standard solution was prepared by dissolving 10 mg of tyramine (Sigma, St. Louis, MO, U.S.A.) in 10 ml of mobile phase and diluting to 1 μg/ml with mobile phase.

The mobile phase consisted of 0.05 *M* phosphate buffer (pH 7.5) containing 1 m*M* EDTA. This solution was passed through the 0.45-μm membrane filter and acetonitrile added to make a final concentration 10%. The flow-rate was 1.0 ml/min at ambient temperature.

Perchloric acid (5%) was prepared by diluting 70% perchloric acid (Kanto Chemical, Tokyo, Japan).

Sample preparation

Weigh 5 g of cheese into a 50-ml centrifuge tube. Add 10 ml of 5% perchloric acid and 10 ml of dichloromethane and homogenize for 2 min in the Ultra-Turrax homogenizer. Decant the aqueous phase into another flask and discard the organic phase. Repeat the procedure twice, then collect all the aqueous phases. Neutralize a 1-ml aliquot of aqueous phase with 0.1 *M* sodium hydroxide solution and dilute to 5 ml with distilled water. Filter through the 0.45-μm membrane filter and inject 10 μl of the eluate into the HPLC system.

RESULTS AND DISCUSSION

Fig. 1 shows a typical chromatogram of tyramine obtained from a cheese extract. The retention time of tyramine was *ca.* 10 min and the detection limit was 0.2 ng at the a signal-to-noise ratio of 3. The detection limit of tyramine in cheese with this method was 0.3 μg/g.

Fig. 2 shows the relationship between the applied potential of the working electrode and the recorder response. The onset potential of tyramine oxidation is about 0.55 V. The response of the detector to tyramine depends on the applied voltage. Higher potentials were impractical because of the background noise generated. Therefore, the detector operating potential was set at 0.7 V. The calibration graph was checked by measuring peak areas. Linearity of the detector response for tyramine was obtained by injecting 1–5 ng.

A recovery study using five tyramine-spiked Camembert cheeses at levels of 1, 10 and 100 μg/g showed recoveries of 92.3–95.3% with a coefficient of variation of 2.4–3.1%.

Fluorimetric detection was used for comparison with the proposed method. A

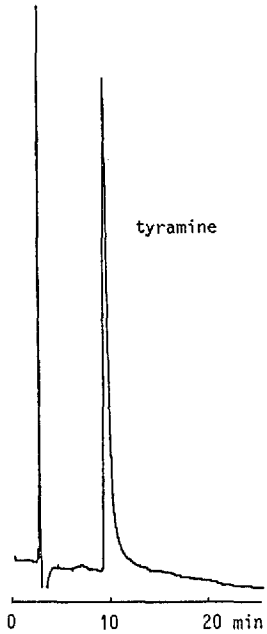
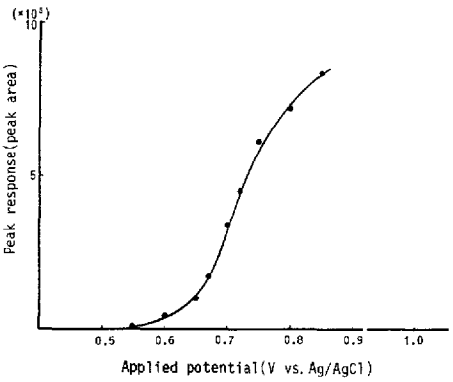


Fig. 1. Typical high-performance liquid chromatogram of tyramine obtained from cheese extract. Chromatographic conditions: stationary phase, LiChrosorb RP-Select B prepacked in a 25 cm × 4.6 mm I.D. stainless-steel column; mobile phase, 0.05 *M* phosphate buffer (pH 7.5) containing 10% acetonitrile; flow-rate, 1 ml/min at ambient temperature; detector applied voltage, 0.7 V *vs.* Ag/AgCl.



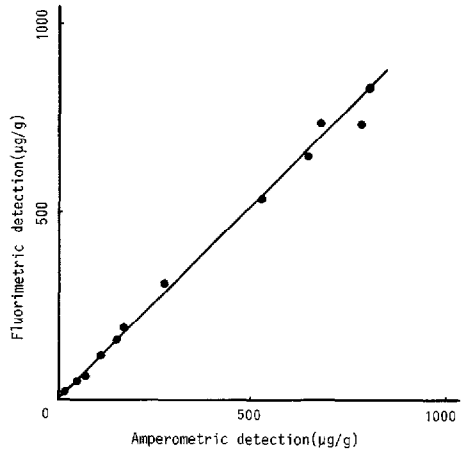


Fig. 2. Effect of applied potential on detector response to tyramine with amperometric detection.

Fig. 3. Correlation of tyramine contents in various cheeses measured with amperometric and fluorimetric detection. $y = 0.999x + 8.393$; $r = 0.995$ ($n = 12$).

good correlation between the two methods was obtained, as shown as in Fig. 3 using twelve different cheeses. The minimum detectable amount with the method is 0.2 ng; this represents a *ca.* 25-fold increase over the fluorimetric detection sensitivity.

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