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Note

Determination of organic acids in foods by high-performance liquid chromatography: lactic acid

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Lactic acid is a widely used food acidulant. It is also a major component of some foods such as fermented meat sausages, fermented dairy products and pickles. The lactic acid content of such food products influences their flavor, stability and keeping quality. The quantitative determination of lactic acid in these food products is therefore required for quality control purposes, meeting various laws and regulations and for labeling requirements.

The present analytical methods for the determination of lactic acid in foods include colorimetric methods[1,2], gas chromatographic (GC) methods[2,3], and liquid chromatographic methods[4,5]. Colorimetric methods are time consuming and lack specificity, GC methods require derivatization of the acid before analysis and the present liquid chromatographic methods were applied to only a few of the fermented food products.

In this paper, a simple, specific and more versatile high-performance liquid chromatographic (HPLC) method for the quantitative determination of lactic acid in a wide variety of food products is described.

EXPERIMENTAL

Apparatus

The high-performance liquid chromatographic (HPLC) system used in this study consisted of a Waters Assoc. liquid chromatograph equipped with Model 6000 A pump, Model U6K injector, and a data module (Waters Assoc., Milford, MA, U.S.A.). A Gilson Model 222 multiple-wavelength detector (Gilson Medical Electronics, Middleton, WI, U.S.A.) set at 210 nm and a sensitivity unit of 0.1 was also used. The column was 300 × 7.8 mm Aminex HPX-87 with a Micro-Guard Ion Exclusion Cartridge (Bio-Rad Labs., Richmond, CA, U.S.A.).

A Sorval Model RC-5 centrifuge with rotor no. SS-34 (DuPont, Newton, CT, U.S.A.) was used for centrifugation.

Reagents

HPLC mobile phase: 0.009 *N* sulfuric acid prepared from reagent-grade sulfuric acid and HPLC water (double distilled passed through 0.45-μm filter membrane). The mobile phase was used at a flow-rate of 0.7 ml/min.

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Disodium ethylenediaminetetracetate (EDTA) stock solution: 1% solution prepared from reagent-grade EDTA and HPLC water. The proper volume of the EDTA stock solution was added to lactic acid standard solution and sample solutions to give a final EDTA concentration of 0.05%.

Lactic acid standard solutions: five standard solutions were prepared using analytical-grade L-lactic acid (lithium salt) (Sigma, St. Louis, MO, U.S.A.) and HPLC water. EDTA was added to each solution to a final concentration of 0.05%. The lactic acid concentration of the standard solutions ranged from 0.250 mg/ml to 2.5 mg/ml.

Sample preparation

Liquids. The proper volume (10–20 ml) of the filtered sample (through Whatman No. 1 paper) was pipetted into a 100-ml volumetric flask, 5 ml of 1% EDTA solution were added and the volume was made to mark with HPLC water. The sample preparation was refrigerated until HPLC analysis.

Semi-solids and solids. The appropriate weight (10–25 g) was blended with 50 ml distilled water for 3 min. The sample blend was filtered or centrifuged at 12,100 *g* for 10 min. The supernatant was separated and the sediment was washed with 10–20 ml distilled water, recentrifuged and the washing was combined with the supernatant. The filtrate or the supernatant was then transferred to a 100-ml volumetric flask, 5 ml of 1% EDTA solution were added and the volume was made to mark with HPLC water. The sample preparation was refrigerated until HPLC analysis.

Lactic acid calibration curve

To test the response to various amounts of lactic acid, a calibration curve was constructed by injecting 10 μl of each of the lactic acid standard solutions. The corresponding data module area units (average of 3 runs) were plotted against the amounts of lactic acid injected.

Quantification of lactic acid in samples

The data module was first calibrated by injecting a known volume (10–25 μl) of a lactic acid standard solution (external standard method). A volume of 10 μl of the sample preparation was then injected and the amount of lactic acid was obtained directly from the calibrated data module. The data module calibration was checked regularly using lactic acid standard solutions.

Recovery of lactic acid

The lactic acid content of selected samples was approximately doubled by spiking with known amounts lactic acid. The spiked samples were prepared for lactic acid determination as described in the sample preparation section.

RESULTS AND DISCUSSION

The addition of EDTA to sample preparations to a final oncentration of 0.05% maintained the column efficiency and yielded reproducible results throughout the study. EDTA was chosen as a good chelating agent to mask any metal ions present in the sample preparations. According to the manufacturer of the column used in

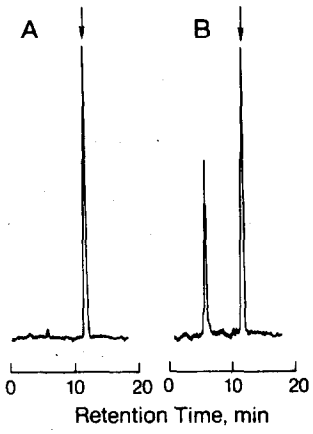

Fig. 1. HPLC chromatogram of lactic acid (A) and lactic acid in 0.05% EDTA (B). The lactic acid peak represents 15.5 μg.

this study, free metal ions should be avoided since they decrease the column efficiency[6]. EDTA did not increase the response to lactic acid as it did with ascorbic acid[7]. The EDTA peak has a shorter retention time (5.6 min) than that of lactic acid (11.4 min) and does not interfere with the quantitative determination of lactic acid as shown in Fig. 1.

The calibration curve constructed for lactic acid indicated a linear detector response over a range of 2–25 μg in the presence of 0.05% EDTA. The curve indicated also that as low as 2 μg of lactic acid can be quantified reliably by the HPLC method.

Chromatograms of selected samples are shown in Fig. 2. Lactic acid was re-

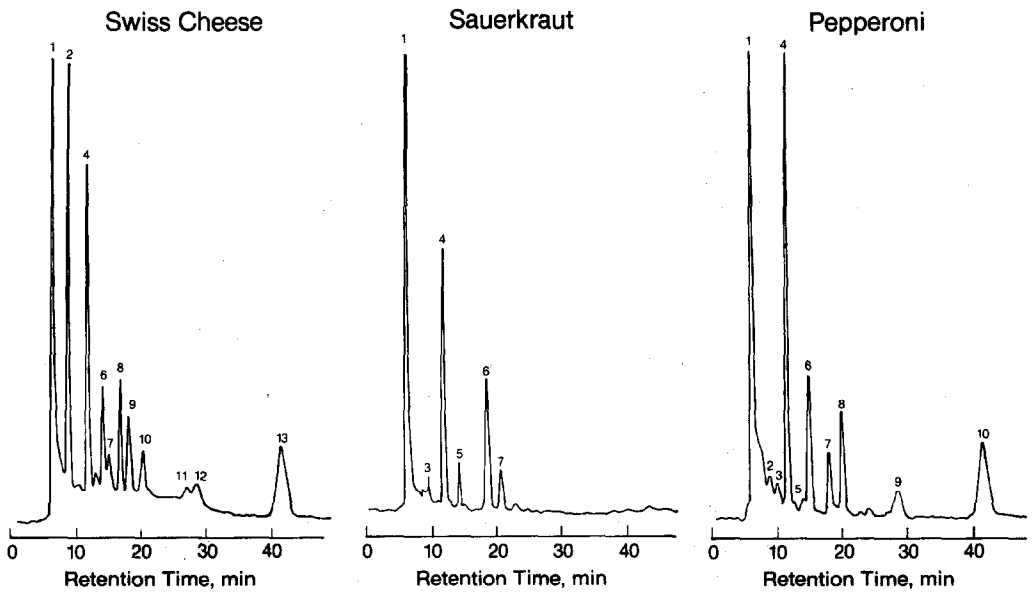

Fig. 2. HPLC chromatograms of selected food products. Peak no. 4 in all chromatograms is of lactic acid.

TABLE I

LACTIC ACID CONTENT OF VARIOUS FOOD PRODUCTS

Sample	*Lactic acid content* (g/100 g)*	*Coefficient of variation (%)*
Meat products		
Italian hot sausage	0.217 ± 0.008	3.7
Italian sweet sausage	0.247 ± 0.005	2.0
Bologna	0.389 ± 0.019	4.9
Summer sausage	0.782 ± 0.038	4.9
Beef summer sausage	1.076 ± 0.027	2.5
Pepperoni	1.173 ± 0.036	3.1
Hard salami	1.441 ± 0.050	3.5
Dairy products		
Buttermilk	0.911 ± 0.021	2.3
Plain yogurt	1.208 ± 0.050	4.1
Swiss cheese	0.671 ± 0.024	3.6
Blue cheese	1.275 ± 0.063	4.9
Pickles		
Spanish olives	0.340 ± 0.012	3.5
Spanish olives brine	0.636 ± 0.029	4.6
Sauerkraut	1.125 ± 0.033	2.9

* Average of 6 determinations: g/100 ml for brine.

solved as a single peak in all samples analyzed with no interference from other compounds. This indicated that the method is specific for lactic acid. The identity of the lactic acid peak was confirmed by two methods. Firstly, the relative retention time of the lactic acid peak was calculated by dividing its absolute retention time by that of the EDTA peak. In all samples analyzed, the relative retention time for the lactic acid peak was 2.03 ± 0.02. Secondly, various samples were spiked with a known amount of standard lactic acid and the area of each peak was compared before and

TABLE II

RECOVERY OF LACTIC ACID FROM FOOD PRODUCTS

Sample	*Recovery* (%)*	*Coefficient of variation (%)*
Summer sausage	93.4 ± 0.8	0.9
Pepperoni	93.9 ± 3.4	3.6
Hard salami	97.8 ± 3.2	3.3
Buttermilk	93.4 ± 2.2	2.4
Swiss cheese	95.0 ± 3.4	3.6
Plain yogurt	97.0 ± 2.9	3.0
Sauerkraut	95.9 ± 2.7	2.8
Spanish olives	95.4 ± 4.4	4.6
Spanish olives brine	95.4 ± 3.8	4.0

* Average of 6 determinations.

after spiking. In all spiked samples, the peak identified as the lactic acid peak was the only peak to have increased in area. The increase in the area of the lactic acid peak after spiking was always proportional to the amount of standard lactic acid used for spiking.

The lactic acid content of the various food products analyzed by the HPLC method is presented in Table I. The coefficients of variation resulting from six determinations were 2.0–4.9% indicating the precision of the method. The recoveries of lactic acid from various samples are presented in Table II. They ranged from 93.4 ± 0.8 to 97.8 ± 3.2% emphasizing the accuracy of the method.

In summary, the results obtained from this study indicated that the developed HPLC method for the quantitative determination of lactic acid in food products is simple, specific, precise and accurate. In addition, the method can be used for the identification and quantification of other organic acids in such food products.

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