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Simultaneous Determination of Organic Acids in Commercial Vinegar by Photometric Ion Chromatography

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A simultaneous determination method for five organic acids in vinegar has been established by photometric ion chromatography. Both trace components (lactic, gluconic, succinic and pyroglutamic acids) and the major component (acetic acid) in vinegar were separated on a TSK gel IC-Anion-PW column with 0.45 mM *o*-aminobenzenesulfonic acid aqueous solution as an eluent. Their elutions were detected as positive peaks before the system peak, and a negative peak after that by a UV monitor. The sensitivities of trace components were enhanced by adjusting their elutions close to the system peak. The lowest detection limits were 10 μM lactic acid, 5 μM gluconic and succinic acids and 3 μM pyroglutamic acid, respectively. The only pretreatment necessary was dilution of vinegar 100-fold with the eluent. The results obtained by the proposed method were in good agreement with those by the enzymatic one.

Keywords —— Photometric ion chromatography; simultaneous determination; organic acid; vinegar

Introduction

Brewery vinegar is utilized as a seasoning and which is made from cereals by alcoholic and acetic fermentations. Although acetic acid is the major organic acid in vinegar, a small quantity of other organic acids is also produced during fermentation or maturation. The production of these acids is useful as indicator of production control and enrichment of the flavor in vinegar. The simultaneous determination of these orgamic acids requires accurate and facile analytical methods.

Enzymatic methods have commonly been used in the determination of organic acids in foods. Although selective and sensitive, these cannot determine several organic acids simultaneously. On the other hand, various high-

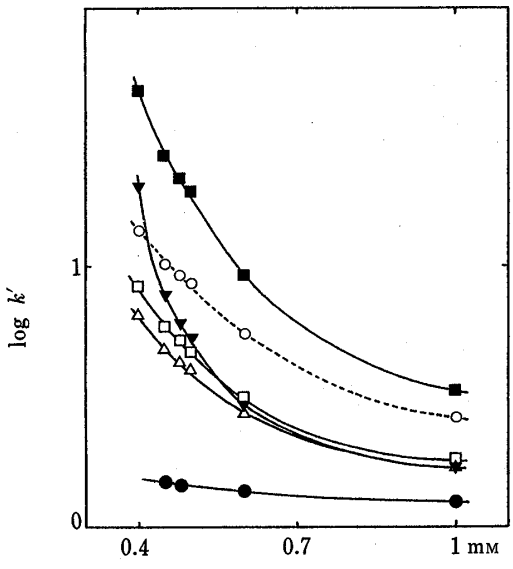

Fig. 1. Capacity Factors of Organic Acids as a Function of OABS Eluent Concentration

Symbols: ■; pyroglutamic acid, ▼; succinic acid, ○; system peak, □; gluconic acid, △; lactic acid, ●; acetic acid.

performance liquid chromatographic (HPLC) methods developed for such simultaneous determination and utilizing partition,[1,2] ion-exclusion[3-5] and ion-exchange[2,6,7] are frequently employed, but their application to vinegar was impossible for determination of the trace amounts of organic acids and acetic acid simultaneously because of the excessive amount of acetic acid present. Removal of acetic acid was necessary to determine the non-volatile organic acids in vinegar.[8,9]

Photometric ion chromatography (PIC) with light-absorbing eluent has become a popular type of ion chromatography (IC) because of the following advantages: it can be performed on a conventional HPLC system equipped with a UV detector and no derivatization procedures are required.[10,11] The authors reported the simultaneous determination of organic and inorganic anions by PIC[12] and its applications for several foods,[13,14] and suggested[15] that the control of sensitivity in PIC was that the signal intensity of analyte was enhanced when its elution was close to that of the system peak. This paper deals with the application of this technique for the simultaneous determination of small amounts of organic acids and the large amount of acetic acid in vinegar.

Experimental

1. Sample —— Seventeen commercial vinegars from nine manufacturers were used. For organic acid analysis, vinegar samples were diluted 100-fold with 0.45 mM *o*-aminobenzenesulfonic acid (OABS) and filtered through a 0.45 μm membrane. Test solution was kept at room temperature overnight.

2. Reagents —— Sodium acetate, succinic acid (guaranteed reagent, Wako Pure Chem. Ind., Ltd.), pyroglutamic acid (guaranteed reagent, Tokyo Kasei Kogyo Co., Ltd.), lithium lactate and sodium gluconate (reagent, Wako) were used as standard compounds. Other reagents were of guaranteed grade. All standard solutions of the organic acids were prepared by dissolving them in 0.45 mM OABS.

Test-Combinations (Boehringer Mannheim Yamanouchi) were used for the enzymatic determination of organic acids.

3. Apparatus —— The IC system included a Shimadzu LC-6A pump, a Rheodyne 7125 injector (loop of 10 μl), a Shimadzu SPD-6AV detector and a Shimadzu C-R3A Chromatopac calculator. Two anion-exchange columns of TSK gel IC-Anion-PW (4.6 mm i.d.×5 cm, Tosoh) were connected in series and kept at 40 °C. The eluent was 0.45 mM OABS (pH 3.5) and was kept at a flow rate of 1 ml/min. The detection wavelength was 302 nm.

Results and Discussion

1. IC Conditions

Vinegar contains gluconic, lactic, succinic and pyroglutamic acids as trace organic acids.[16,17] The signal intensity of the sample ion, as mentioned above, can be enhanced by adjusting its elution close to the system peak in PIC. This phenomenon is expressed in the formula, $PA \propto k'_s/(k'_e - k'_s)$, where PA is peak area of sample and k'_e and k'_s are the capacity factors of system peak and sample, respectively.[15] These results suggest that acetic acid should be eluted close to solvent front and other acids should be eluted near the system peak to determine all organic acids in vinegar simultaneously.

Figure 1 shows the relationship between the capacity factor and the eluent concentration when an IC-Anion-PW column and OABS solution as an eluent were used. It was reported that the concentration of succinic or pyroglutamic acid was generally the lowest in the four trace organic acids.[16,17] Therefore, the elution of the system peak should be adjusted between succinic and pyroglutamic acids and that of acetic acid should be close to the solvent front to enhance the sensitivities of succinic and pyroglutamic acids and to suppress the sensitivity of acetic acid. An eluent concentration around 0.45 mM was most suitable for this as shown in Fig. 1. In this case,

two analytical columns in series were necessary to separate the peaks completely. The column temperature should be kept constant because this chromatography is based on the phenomenon in which the adsorption of the eluent occurs on the column packing material.[15]

Figure 2 shows a typical chromatogram of organic acids in a 100-fold diluted standard mixture. The concentrations of organic acids used in the mixture were those given in the literature.[16,17] This chromatogram had two extraneous peaks: the first peak (*ca.* 2 min) was generally attributed to be the "injection peak",[18] and the second one (*ca.* 14 min) "system peak", as used in our derived formula. The peak directions of acetic, lactic, gluconic and succinic acids, which were eluted before the system peak, were positive, while that of pyroglutamic acid, which was eluted after the system peak, was negative. The coefficient of variation of peak areas ($n=6$) was 1.0% for acetic acid, 2.6% for lactic acid, 2.8% for gluconic acid, 5.5% for succinic acid and 1.7% for pyroglutamic acid, respectively, by the injection of the same standard mixture as given in Fig. 2. Consequently, the great advantage of this method over other HPLC methods is the simultaneous determination of small amounts of organic acids and a large amount of acetic acid in vinegar with single injection.

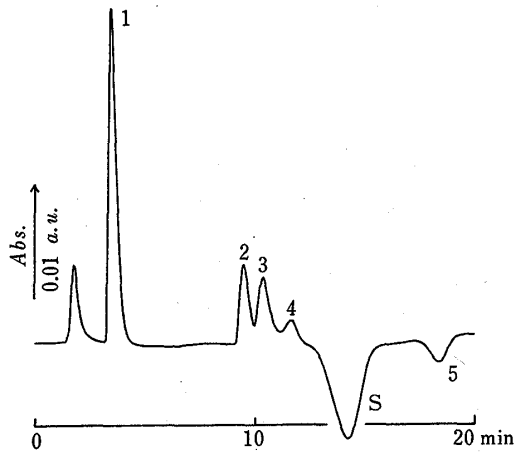

Fig. 2. Chromatogram of Organic Acid Standard Mixture

Peaks: 1; acetic acid (7 mM), 2; lactic acid (0.2 mM), 3; gluconic acid (0.2 mM), 4; succinic acid (50 μM), 5; pyroglutamic acid (50 μM), S; system peak.

2. Detection Limits and Calibration Curves

The detection limits and correlation coefficient of calibration curves for the five organic acids are summarized in Table I. Detection limits of the four trace organic acids were significantly lower than that of acetic acid, since the reduction in denominators in our derived formula to elute the trace acids near the system peak caused enlargement of their peak areas. Straight calibration curves were obtained for the peak areas against the concentrations in a range of almost two orders. When concentrations of the analytes were above these ranges, their retention times decreased slightly, perhaps due to overloading of the eluting agent (OABS) in the adsorption mechanism. The ranges listed in Table I were low and wide enough for the determination of organic acids in vinegar.

3. Interfering Compounds and Sample Preparation

Vinegar contains carbohydrates, salt, free amino acids and other ingredients in addition to organic acids. Among these, chloride and amino acids were considered to be retained on the ion-exchange column and to interfere with the analysis. Chlorine ion in the vinegars diluted 100-fold, however, gave no peak because of its delayed elution (*ca.* 52 min) and low concentration. Glutamic and

TABLE I. Calibration Curves and detection Limits of Organic acids

Organic acid	Detection limit (μM)	Calibration curve	
		Linear range (mM)	Correlation coefficient
Acetic	50	0.1—15	0.9997
Lactic	10	0.01—0.5	0.9996
Gluconic	5	0.01—0.5	0.9998
Succinic	5	0.01—0.5	0.9993
Pyroglutamic	3	0.005—0.5	0.9997

aspartic acids, both of which are acidic amino acids, showed peaks at the retention times of 4.5 min and 7.5 min, respectively. But their contents were very small and their retention times differed from those of the organic acids of interest.

In an aqueous solution, gluconic acid is in equilibrium with gluconolactone which is not retained on the ion-exchange column. This equilibrium lies on the side of gluconic acid at high pH. To prevent a baseline disorder, however, it is preferable that the test solution be prepared by dissolving it in the eluent. Gluconic acid requires more than 5 h to reach equilibrium at the pH of the eluent. Consequently, the test solution was prepared by diluting the vinegar 100-fold with the eluent and keeping it overnight at room temperature as described in Experimental. This simple preparation is an advantage of the present method.

4. Determination of Organic Acids in Vinegars

Using the method described above, organic acids in 9 samples of rice vinegars (made from rice alone) and in 8 alcoholic vinegars (made from cereals and alcohol) were determined. Figure 3 shows an example of a chromatogram for rice vinegar; as the baseline became stable 30 min after injection, successive injections were possible at 30 min intervals. Our data for rice vinegar samples

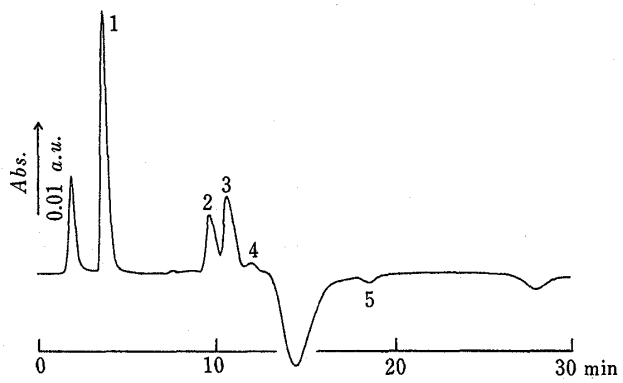

Fig. 3. Chromatogram of Organic Acids in Rice Vinegar

Peaks: 1; acetic acid (6.49 mM), 2; lactic acid (0.19 mM), 3; gluconic acid (0.25 mM), 4; succinic acid (20 μM), 5; pyroglutamic acid (17 μM).

TABLE II. Contents of Organic Acids in Commercial Vinegar

Sample		Organic acids (mg/100 ml)				
		Acetic	Lactic	Gluconic	Succinic	Pyroglutamic
Rice vinegar	1	4280	nd	725	37	6.5
	2	4390	nd	274	13	58
	3	4330	nd	470	8.3	nd
	4	4430	86	51	24	3.9
	5	3900	171	490	24	22
	6	4240	270	39	28	30
	7	4310	nd	412	18	nd
	8	4490	nd	108	17	nd
	9	4510	17	314	9.5	5.2
Average		4320	60.3	320	19.7	14.0
Alcoholic vinegar	1	4190	nd	706	14	12
	2	4160	18	nd	nd	nd
	3	4260	126	549	5.9	5.2
	4	4200	94	196	nd	12
	5	4420	84	220	7.1	nd
	6	4290	12	182	nd	nd
	7	3900	nd	922	nd	6.5
	8	4100	nd	nd	nd	nd
Average		4190	41.9	347	3.4	4.4

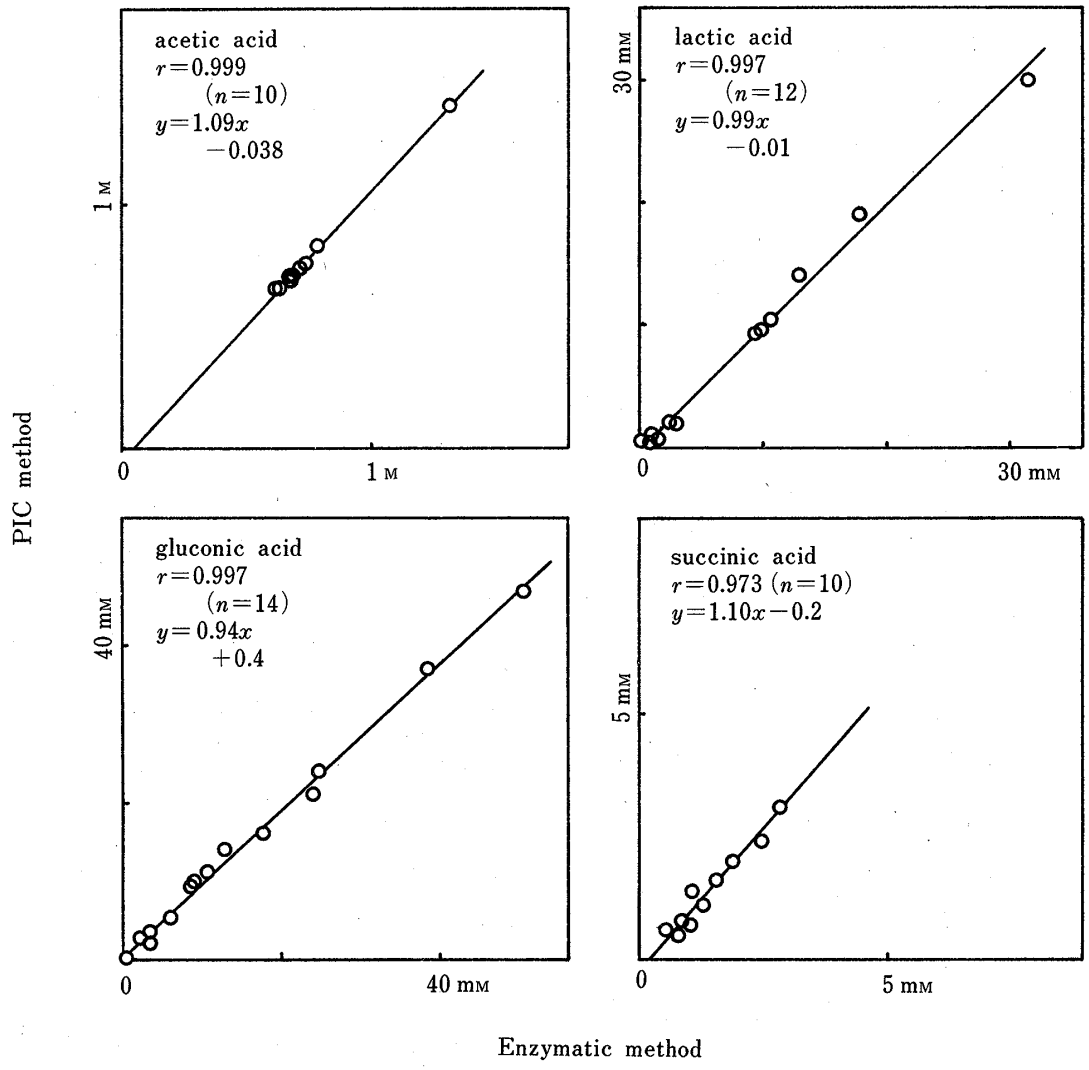

Fig. 4. Comparison of the Results Obtained for Organic Acids in Vinegar Using the PIC and Enzymatic Methods

(Table II) were very similar to those of Masai[17] with a carboxylic acid analyzer. The difference in the results between rice and alcoholic vinegar samples was regarded as significant ($p<0.001$) for succinic and pyroglutamic acids, but not for lactic acid. The fact that lactic acid was detected as D,L-isomers in most vinegars by enzymatic determination suggested the artificial addition of lactic acid to stimulate the fermentation. On the other hand, the high concentration of succinic acid in rice vinegar may be due to the longer period of fermentation compared to alcoholic vinegar. The difference in the content of pyroglutamic acid, which is a decomposed amino acid product, may reflect the amount of glutamic acid in the raw material.

5. Comparison of the Proposed Method with the Enzymatic Method

The enzymatic method is frequently employed to analyze accurately a particular organic acid in a great number of samples. Figure 4 shows a comparison of the results obtained for organic acids in vinegars by the proposed and the enzymatic methods. The correlation coefficient for succinic acid was 0.973 because of its low concentration close to the determination limit by the proposed

method, but the results for others agreed well with the correlation coefficient being ≧0.997. This method using a conventional HPLC system seems very suitable and practical for the simultaneous determination of organic acids in vinegar.

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