

Determination of Citric Acid and Its Decomposed Products in Edible Oils by Gas Liquid Chromatography

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ABSTRACT AND SUMMARY

A quantitative gas liquid chromatographic (GLC) method for four organic acids—citric, aconitic, itaconic, and citraconic acids—in refined oils and fats was developed. After the organic acids are extracted with water from soybean oils, the extract is esterified with *n*-butanol and an aliquot of the ester is subjected to GLC on a temperature-programmed 10% DEGS column equipped with a flame ionization detector. At 20 and 30 ppm levels, recoveries of the acids from the oil varied from 95 to 100% except for itaconic acid. Citric acid content in commercial edible oils in Japan was from trace to 16 ppm. However, aconitic, itaconic, and citraconic acids were not detected.

INTRODUCTION

For many years, citric acid has been used and studied as a synergist of antioxidants for the edible oils and fats.

In general, it has been considered that, in oils, citric acid is dehydrated to aconitic acid at a temperature above 175 C. Aconitic acid is further decomposed to itaconic acid and then to citraconic acid. However, it is possible that mesaconic acid is produced by decarbonization of *trans*-aconitic acid. And it is also possible that 3-ketoglutaric acid is produced by simultaneous decarbonization and dehydration of citric acid (1).

The majority of methods for the analysis of citric acid in oils and fats are colorimetric depending on the Furth and Hermann reaction (2-4), in which color is formed in the presence of pyridine and acetic anhydride. Recently, Ohlson et al. (5) developed a direct titrimetric method for small amounts of citric acid in refined glyceride oils and fats. However, these methods are not applicable for simultaneous determination of citric acid and its thermally decomposed products.

In recent years, gas liquid chromatography (GLC) has been the technique for the separation and identification of organic acids. A quantitative gas chromatographic method for organic acids in food, via their *n*-butyl esters, has been reported by Yamashita et al. (6).

This paper describes a method for the quantitative GLC determination of citric, aconitic, itaconic, and citraconic acids in refined oils and fats as *n*-butyl esters.

EXPERIMENTAL PROCEDURE

Reagents and Materials

The following reagents were purchased from commercial sources: citric acid (Kanto Chemical Co.); *trans*-aconitic acid, itaconic acid, citraconic acid, citric acid tri-*n*-butyl ester, itaconic acid di-*n*-butyl ester, and *o*-terphenyl (Tokyo Kasei Co.) *trans*-Aconitic acid tri-*n*-butyl ester and citraconic acid di-*n*-butyl ester were synthesized from aconitic and citraconic acids (6).

The commercially bleached soybean oil from Hohnen Oil Co. was deodorized in our pilot plant. Antioxidants were not added. General characteristics were as follows: Lovibond color, Red 1.0, Yellow 10, Blue 0 (5 1/4 in. cell), Acid Value: 0.23, Peroxide Value: 0.4, Iodine Value: 132.5; fatty acid compositions—palmitic 11.6%, stearic 3.1%, oleic 23.3%, linoleic 54.5%, and linolenic 7.2%.

The characteristics of commercial salad and frying oils are shown in Table I.

Gas Chromatography

The following conditions were used:

Instrument:	Shimazu GC-5A Gas Chromatograph with a dual flame ionization detector.
Column:	Glass, 3 mm ID, 2 m long, packed with 10% DEGS, Chromosorb WAW, 80-100 mesh.
Flow Rate:	Nitrogen carrier gas, 60 ml/min to each column, hydrogen 50 ml/min to each flame, air 100 ml/min.
Temperatures:	Inlet and detector temperature were 200 C. Column oven was programmed between 150 C and 200 C, initial hold at 150 C for 10 min, programmed at 5 C/min for 10 min, final hold at 200 C for 30 min.
Sensitivity:	10 ² .
Range:	8.
Chart Speed:	10 mm/min.

Measurements of the Reagent Purity

Purity of citric, aconitic, itaconic, and citraconic acids was estimated by titration with 0.1 N sodium hydroxide (7). Purity of four standard *n*-butyl esters of the organic acids was estimated by the saponification method (8).

TABLE I

Organic Acid Contents in Commercial Edible Oils^a, as ppm

Manu- facturer	Trade name	Components	Acid value	Peroxide value	Iodine value	Citric acid
A	Salad oil	Rapeseed, Soybean	0.04	0.5	122.7	13.9
A	Salad oil	Rapeseed, Cottonseed	0.05	0.7	108.1	16.4
B	Frying oil	Soybean, Rapeseed	0.04	1.6	127.5	11.2
B	Salad oil	Rapeseed, Soybean	0.04	0.6	121.9	13.5
C	Salad oil	Rapeseed, Soybean	0.05	0.5	122.0	trace
C	Frying oil	Soybean, Rapeseed	0.04	1.4	126.7	9.0
D	Salad oil	Rapeseed, Soybean, Corn	0.05	0.5	116.3	14.8
D	Frying oil	Soybean, Rapeseed	0.04	0.6	127.4	trace
E	Frying oil	Rice bran	0.09	1.0	105.7	11.6

^a All the oils are retail store products. No aconitic, itaconic, or citraconic acids were detected in any of the oils.

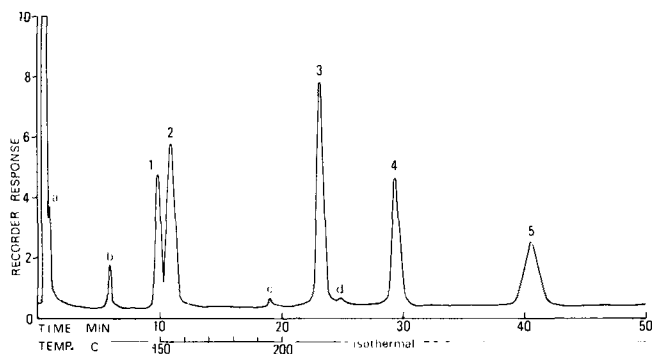


FIG. 1. Gas chromatogram of n-butyl esters of organic acids, esterified by sulfuric acid and n-butanol. Peak identification: 1 = itaconic acid di-n-butyl ester, 2 = citraconic acid di-n-butyl ester, 3 = o-terphenyl (internal standard), 4 = aconitic acid tri-n-butyl ester, 5 = citric acid tri-n-butyl ester, and peaks a,b,c, and d = unknown compounds.

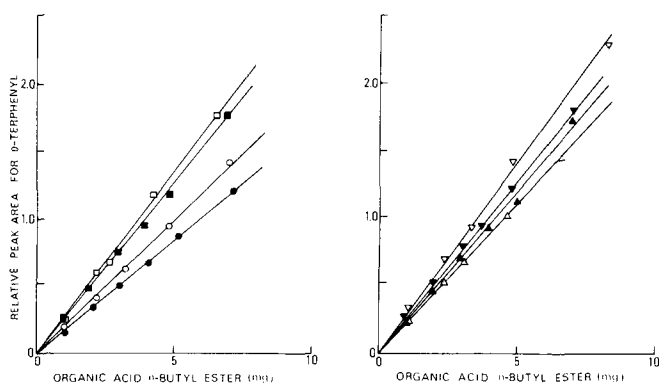


FIG. 2. Calibration curves of citric, aconitic, itaconic, and citraconic acids. Citric acid: \square — \square — standard ester, \bullet — \bullet — esterified; itaconic acid: \square — \square — standard ester, \blacksquare — \blacksquare — esterified; aconitic acid: \triangle — \triangle — standard ester, \blacktriangle — \blacktriangle — esterified; citraconic acid: ∇ — ∇ — standard ester, \blacktriangledown — \blacktriangledown — esterified.

Determination of Esterification Rate

An aqueous solution (25 ml) containing 0.5~3.5 mg of citric acid was pipetted to a 50 ml round bottom flask. The solution was neutralized with 0.1 N sodium hydroxide, since without this neutralization, esterification of the four organic acids was incomplete. The water was removed by the rotary evaporator at 50 C and the residue was dried at 60 C, 1 mmHg for 30 min to remove the moisture. 2 ml of butanol, 0.2 ml of sulfuric acid, and 2 g of anhydrous sodium sulfate were added to the flask. The flask was connected to a reflux condenser and boiled on a mantle heater for 30 min. After being cooled, the solution was transferred into a separatory funnel with 50 ml of n-hexane, and 50 ml of water was added. After the funnel was shaken, the reaction products went into the n-hexane layer. After the water layer was discarded, the n-hexane layer was dried with anhydrous sodium sulfate to remove the moisture and trace amounts of sulfuric acid, and collected through filter paper into the Kuderna-Darnish apparatus equipped with a 5 ml ground glass test tube. The n-hexane was removed by vacuum distillation and about 1 ml of light yellow product remained in the tube.

n-Hexane solution (2 ml) containing 2 mg of o-terphenyl internal standard was added to the tube and filled up to 5 ml with n-hexane. Two microliters of the sample solution were then chromatographed on the DEGS column two times for each concentration of citric acid. From the chromatograms obtained, relative peak areas of the internal standard and citric acid n-butyl esters were calculated.

TABLE II

Slope Factors from Calibration Curves, and Esterification Rates

Acid	Standard ester	Esterified acid	Esterification rate (%)
Citric	0.200	0.170	85.0
Aconitic	0.222	0.226	101.8
Itaconic	0.267	0.253	94.8
Citraconic	0.270	0.248	91.9

Similar experiments were also carried out for aconitic, itaconic, and citraconic acids. The solution of standard n-butyl esters of the four organic acids containing 1~7 mg per 5 ml n-hexane were prepared and chromatographed respectively. From the chromatograms obtained, relative peak areas between the internal standard and standard n-butyl esters were calculated. From the results, esterification rates of the four organic acids were determined.

Relative Recoveries of Organic Acids from Oil

Soybean oil (200 g) was taken into a 300 ml three-necked flask, and 2 ml of ethanol solution containing 4 or 6 mg of each of the four organic acids were added: 4 mg corresponds to 20 ppm and 6 mg corresponds to 30 ppm. The mixture was stirred under vacuum, 10 mmHg, at 80 C for 10 min to dissolve the organic acids in the soybean oil and to remove ethanol. After being cooled, the soybean oil was completely transferred to 1 liter separatory funnel using 300 ml of n-hexane. Distilled water (200 ml) was added to extract the organic acids. The separatory funnel was automatically shaken for 1 hr.

The water layer containing the organic acids was collected through a filter paper. The aqueous extract (100 ml) was neutralized with 0.1 N sodium hydroxide, evaporated, esterified with n-butanol, and chromatographed. These experimental procedures were similar to those described above. From the chromatograms obtained, relative recoveries of the organic acids from the soybean oil were determined.

RESULTS AND DISCUSSION

Purity of the Reagents

Purity of the organic acids was calculated from neutralization value and each acid proved to be pure (98.8-100%).

Purity of the standard n-butyl esters of the organic acids was calculated from saponification values. The purities of the n-butyl esters of the citric, aconitic, and citraconic acid were satisfactory. The purity of itaconic n-butyl ester was 87.0%, as determined by gas chromatography.

Esterification Rates

Gas chromatography of n-butyl esters of four organic acids is illustrated in Figure 1. The peaks a, b, c, and d were not identified. They were probably formed by esterification and b may be attributed to the dibutyl ether (6). When the chromatography was performed isothermally, the peaks of the butyl esters of the organic acids were not separated.

Calibration curves of each organic acid are shown in Figure 2. Slope factors calculated from the calibration curves are shown in Table II. Esterification rates of the organic acids utilized in these experiments are as follows: citric acid 85.0%, aconitic acid 101.8%, itaconic acid 94.8%, and citraconic acid 91.9%, respectively.

Recoveries

The recoveries of the organic acids from the oil are shown in Table III. The experiments were carried out six times for each acid. The recoveries of citric, aconitic, and citraconic acids were above 95% and the lowest recovery,

TABLE III
Recoveries of Individual Organic Acids from the Soybean Oil, as % w/w

Acid	Acid added (ppm)				Total mean Recoveries
	20		30		
	Mean ^a	Range	Mean ^a	Range	
Citric	97.8 (2.18) ^b	94.9 ~ 101.3	97.9 (1.17)	96.2 ~ 99.5	97.9 (2.47)
Aconitic	100.1 (0.81)	98.7 ~ 101.5	99.4 (1.82)	98.2 ~ 102.4	99.8 (2.06)
Itaconic	80.4 (2.10)	78.0 ~ 84.6	79.9 (5.87)	73.5 ~ 88.4	80.1 (6.24)
Citraconic	92.3 (3.23)	87.6 ~ 96.1	98.4 (1.49)	96.5 ~ 100.5	95.3 (6.03)

^aMean of six determinations

^bStandard deviations in parentheses

80%, was obtained for itaconic acid.

From the results, the mean recoveries and standard deviations were calculated. By the t-test (level of significance α 5%) there were no differences between the means of 20 and 30 ppm for citric, aconitic, and itaconic acids. However, for citraconic acid considerable difference existed.

Gas Chromatography

As shown in Figure 2, 1 mg of the organic acid n-butyl ester can be determined by gas liquid chromatography. This means that, although experimental data is shown for only 20 and 30 ppm, 5 ppm of organic acid in oils can be determined precisely. However, from the GLC the lower limit of detection proved empirically to be 1 ppm or less.

Application of the GLC Method

The method was applied to determine the organic acids in commercial edible oils on the Japanese market. Table I shows the results from five series of determinations. On the average 10~15 ppm of citric acid were found in salad and frying oils. However, only trace amounts of citric acid were found in C salad oil and D frying oil. The results suggest that the conditions for adding citric acid on the deodorizing tower are a somewhat delicate. Since the normal deodorization temperature (above 220 C) is sufficiently above the decomposition temperature of citric acid (175 C), in the cases of C salad oil and D frying oil it may be that the citric acid was added at the beginning stage of deodorization or was added at the temperature above 175 C during the cooling phase of deodorization. The temperature at which citric acid is added is consequently very important, for when the citric acid is added at above 175 C, even during

the cooling phase of deodorization, citric acid is decomposed. The degree of decomposition of citric acid depends on the temperature and time. Therefore, further study of these problems is necessary.

Aconitic, itaconic, and citraconic acids were not found in any of the samples of the salad and frying oils. It is generally said that aconitic, itaconic, and citraconic acids are formed by decomposition of citric acid (1). However, contrary to this idea, they were not found in the commercial edible oils at all. It may be that at the usual conditions under which citric acid is added in deodorizing operations, these organic acids are not formed or are distilled out if formed. So research is needed on the possibilities of producing mesaconic acid and other miscellaneous substances during deodorization.

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