

# Determination of Organic Acids in Beer After Extraction with an Anion-Exchange Resin

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## ABSTRACT

The organic acids in beer were recovered with an anion-exchange resin and then were converted to their methyl esters by treatment of the resin with boron trifluoride-methanol. More than 50 compounds recognizable as methyl esters were found by mass spectrometric analysis of the extract from 50 ml of beer. Satisfactory calibration lines were obtained for a number of volatile and nonvolatile acids via the method of standard additions and gas chromatographic analysis. The method is limited by the overlap of some methyl ester peaks in the gas chromatogram.

Keywords: Analysis, Beer, Gas chromatography, Ion-exchange resin, Methyl esters, Organic acids

The constituents of beer include a large number of organic acids. Besides contributing to the beer's flavor, many of these acids are derived from yeast and their concentrations provide an indicator of consistent fermentation performance. However, the quantitative analysis of these compounds in beer has generally proven to be troublesome. Paper partition chromatography (5), thin-layer chromatography (13), packed column gas chromatography (3,7), and liquid chromatography (4) are among the methods that have been used. These methods are characterized by long analysis times, and each of them usually detects only certain types of acids. Liquid ion chromatography using either single-column (1,8) or dual-column (11) technology has shown promise, but its usefulness appears to be restricted to a few of the more concentrated hydrophilic acids.

Therefore, a more general method was sought that would allow rapid isolation and quantitation of both hydrophobic and hydrophilic acids in beer. It appeared probable that an anion-exchange resin could be used to concentrate and isolate the acids from beer, as this technique has been applied to the analysis of organic acids in serum (9) and urine (14). In addition, it was recently shown that organic acids bound to an anion-exchange resin could be derivatized directly to their corresponding methyl esters by boron trifluoride in methanol without their prior removal from

the resin (15). As the following results will show, these techniques allowed a convenient method to be developed for the analysis of a large number of volatile and nonvolatile organic acids in beer.

## EXPERIMENTAL

### Reagents

Amberlyst A-26, Amberlite IRA-400, Amberlite IRA-904, 50% boron trifluoride in methanol, nonanoic acid (98%), and methylundecanoate (99%) were obtained from the Aldrich Chemical Co. (Milwaukee, WI). Dowex 1-X8 was supplied by the J. T. Baker Chemical Co. (Phillipsburg, NJ). Methylene chloride and methanol were obtained from the BDH Chemical Co. (Toronto, Ontario) and distilled before use. Sodium sulfate (reagent grade) was extracted with methylene chloride, dried, and roasted at 600°C. Sodium hydroxide and all of the organic acids were obtained from various sources and were of ACS grade.

### Resin Preparation

All anion-exchange resins were sequentially batch-washed at a rate of 25 g of resin per 100 ml of solvent with the following solvents: methylene chloride, methanol, distilled water, 1M sodium hydroxide, distilled water, and acetone. The resin then was air-dried.

### Analysis of Organic Acids in Beer

About 60 ml of beer was degassed by filtration through Whatman No. 1 filter paper and then made alkaline to pH 8 with 40% sodium hydroxide solution. Using a graduated cylinder, 50 ml of this beer was transferred into a beaker and 100 µg of nonanoic acid dissolved in 100 µl of methanol was added as an internal standard. One gram of resin was poured into the beer, and the mixture was stirred with a bar magnet for 30 min.

After allowing the beads to settle, the supernatant was carefully decanted and the resin was washed with 20 ml of distilled water, 30 ml of methanol, and 30 ml of absolute methanol. Without allowing the beads to dry, they were transferred to a 10-ml serum vial, and 2 ml of absolute methanol and 0.5 ml of 50% boron trifluoride-methanol were added. The vial was sealed and incubated at 55°C for 1 hr with shaking at 150 rpm.

The contents of the serum vial then were filtered through a 40-ml sintered glass funnel into a 30-ml separatory funnel con-

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taining 3 ml of a 4% sodium bicarbonate solution. Six milliliters of methylene chloride was poured over the beads and allowed to flow completely into the separatory funnel. After an initial gentle shaking, which produced carbon dioxide, the aqueous layer was extracted with methylene chloride by vigorous shaking for 1 min. The organic layer was poured into a 15-ml centrifuge tube, and the aqueous layer was extracted again with 3 ml of methylene chloride. The methylene chloride extracts were combined in the centrifuge tube, shaken briefly with 3 ml of distilled water, and then centrifuged at  $1,000 \times g$  for 5 min in a bench top centrifuge (Mistral 1000).

The upper water layer was removed, and the methylene chloride layer was dried by passing it through a 12-cm Pasteur pipet half filled with sodium sulfate. The dried methylene chloride was transferred into a 5-ml Reacti-Vial (Pierce, Rockford, IL), and its volume was reduced to 100  $\mu$ l under a nitrogen stream. A volume of 1.0  $\mu$ l was injected into the gas chromatograph.

### Gas Chromatography

Gas chromatography was performed on a Hewlett-Packard (Palo Alto, CA) 5890 gas chromatograph (GC) fitted with an on-column injector, a flame ionization detector, and a Hewlett-Packard 3396A integrator. The column was a Supelcowax 10 fused silica capillary (0.32 mm  $\times$  30 m) with a deactivated fused silica guard column (0.32 mm  $\times$  1 m). The carrier and makeup gas was helium. The oven program conditions were as follows: initial temperature = 50°C, initial time = 7 min, rate = 5°C per minute, final temperature = 245°C, final time = 10 min, total time = 56 min.

Gas chromatography and mass spectrometry were performed on a Hewlett-Packard model 5790 GC directly interfaced to a VG Instruments 12-250 mass spectrometer (MS) in the electron impact mode. Column and conditions for the GC/MS system were similar to the HP 5890 system. GC peaks were identified by comparison of their mass spectra with those of the National Bureau of Standards mass spectral library.

### Effects of pH on Recovery of Nonanoic Acid

Four 150-ml samples of distilled water were adjusted to pH 4, 6, 8, and 10 with dilute hydrochloric acid and dilute sodium hydroxide. One-gram samples of Dowex 1-X8 (OH<sup>-</sup> form) were equilibrated in 100 ml of each solution, and each resin sample then was removed and placed in a 15-ml beaker containing 5 ml of the pH-adjusted water. Nonanoic acid (100  $\mu$ g) dissolved in 100  $\mu$ l of methanol was added to each mixture, which then was stirred magnetically for 15 min. The resin samples then were treated as described above. At the end of the derivatization step, 100  $\mu$ g of methylundecanoate was added as internal standard. The percent recovery of nonanoic acid in each sample was calculated by peak area comparison of the methyl nonanoate and methyl undecanoate peaks.

### Effects of Temperature and Time on the Methylation Reaction

Distilled water (150 ml) containing nonanoic acid (2 mg/L) and succinic acid (2 mg/L) was adjusted to pH 8 with dilute sodium hydroxide. This solution was then stirred with 3.0 g of Amberlyst A-26 resin for 15 min, and the resin was divided into 3-  $\times$  1-g portions. The three portions were treated with 0.5 ml of 50% boron trifluoride-methanol in a 10-ml serum vial and incubated at either 55°C for 30 min, 55°C for 3 hr, or 70°C for 30 min. The reaction solutions were analyzed as described above after the addition of 2 mg/L of toluene as the internal standard at the end of the reaction.

### Effect of Resin Type on Recovery of Organic Acids

Four 50-ml aliquots of a degassed commercial beer containing 2 mg/L of nonanoic acid as the internal standard were analyzed as described above with 1 g of either Dowex 1-X8, Amberlyst A-26, Amberlite IRA-400, or Amberlite IRA-904 resin. The rela-

tive peak areas for oxalic, malonic, decanoic, pimelic (heptanedioic), malic, azelaic (nonanedioic), and citric acid methyl esters were calculated with respect to the recovered nonanoic acid methyl ester.

### Effect of Resin Amount on Recovery of Organic Acids

Three 50-ml aliquots of degassed beer containing nonanoic acid (2 mg/L) were analyzed as described above with either 0.5, 1.0, or 2.0 g of Amberlyst A-26. The relative peak areas for hexanoic, pyruvic, octanoic, oxalic, malonic, fumaric, phenylacetic, and malic acid methyl esters were calculated with respect to the recovered nonanoic acid methyl ester.

### Calibration Lines

Individual stock solutions of a number of organic acids were prepared at nominal concentrations of 1 mg/ml in methanol. Aliquots of each were combined to provide a standard organic acid mixture. One liter of beer was degassed, adjusted to pH 8, and extracted with 45 g of Amberlite IRA-904 resin to remove endogenous organic acids and, thereby, provide a beer blank. An aliquot of the standard organic acid mixture and 100  $\mu$ g of nonanoic acid were added to 50 ml of the beer blank, which was then analyzed as described above with 1 g of Amberlite IRA-904 resin. The concentration range of each acid over five spiked beer samples corresponded with the range expected for that acid in beer. The peak area ratio of the internal standard to the analyte then was plotted against the concentration of added analyte to obtain the calibration line for each analyte.

## RESULTS AND DISCUSSION

Previous workers (15) had shown that organic acids could be recovered from aqueous solutions with anion-exchange resins and subsequently converted to their methyl esters in situ on the resin with a boron trifluoride-methanol reagent. Although this reagent is sensitive to traces of water remaining from the extraction step, washing the resin with methanol and the use of excess reagent were effective in allowing efficient derivatization to occur. The extent of extraction of organic acids by the resin was reported to be dependent on the pH of the analyte solution, and this was confirmed for recovery of nonanoic acid from a distilled water solution using Dowex 1-X8 resin (Fig. 1). Because any attempt to raise the pH of beer above pH 8 caused haze development, all subsequent analyses of beer were carried out at this pH to avoid possible losses of acids by adsorption on the precipitate.

A number of different anion-exchange resins were selected for evaluation. These included Dowex 1-X8, Amberlyst A-26, Amberlite IRA-400, and Amberlite IRA-904, all of which are strongly basic and were expected to bind organic acids tightly. One-gram samples of each of these resins were used to remove the organic acids from 50 ml of a production beer. Methylation and subsequent gas chromatographic analysis typically yielded a chromatogram such as that shown in Figure 2. More than 30 of the

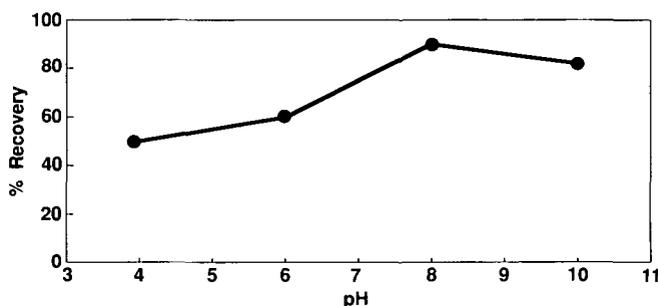


Fig. 1. Effect of pH on recovery of nonanoic acid with Dowex 1-X8 resin.

peaks were identified by mass spectral comparison with the NBS library of mass spectra, and most of the unidentified peaks were also methyl esters, as evidenced by the presence of a significant proportion of ions with a mass-to-charge ratio of 74 in their

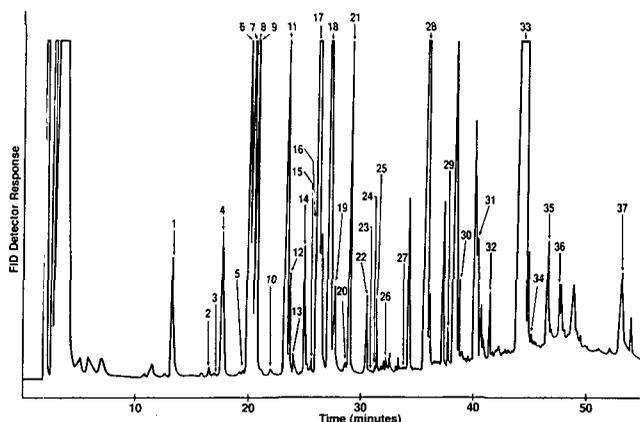


Fig. 2. Gas chromatogram of the methyl esters of organic acids recovered from a commercial beer using Amberlite IRA-904 resin. Peak numbers correspond with those listed in Table I.

TABLE I  
Methyl Esters of Organic Acids Identified in the Anion Exchange Resin Extract of Commercial Beer

Peak <sup>a</sup>	Compound
1	Methyl hexanoate (caproate) C6
2	Methyl heptanoate (enanthate) C7
3	Methyl 3-methyl-2-oxobutanoate
4	Methyl 2-hydroxypropanoate (lactate)
5	Methyl 3-methyl-2-oxopentanoate
6	Methyl 2-oxopropanoate (pyruvate)
7	Methyl octanoate (caprylate) C8
8	Methyl 2-hydroxy-3-methylbutanoate
9	Dimethyl ethanedioate (oxalate)
10	Dimethyl 2-methylpropanedioate
11	Methyl nonanoate (pelargonate) C9 <sup>b</sup>
12	Dimethyl propanedioate (malonate)
13	Methyl 2-hydroxy-4-methylpentanoate
14	Dimethyl 2-butenedioate (fumarate)
15	Methyl 2-furancarboxylate (furoate)
16	Dimethyl butanedioate (succinate)
17	Methyl decanoate (caproate) C10
18	Methyl benzoate
19	Methyl decanoate
20	Dimethyl pentanedioate (glutarate)
21	Methyl undecanoate (undecylate) C11 <sup>b</sup>
22	Methyl benzenethanoate (phenylacetate)
23	Methyl 2-hydroxybenzoate (salicylate)
24	Methyl dodecanoate (laurate) C12
25	Dimethyl hexanedioate (adipate)
26	Methyl benzenepropanoate (hydrocinnamate)
27	Dimethyl heptanedioate (pimelate)
28	Dimethyl 2-hydroxybutanedioate (malate)
29	Methyl 3-phenyl-2-propenoate (cinnamate)
30	Dimethyl nonanedioate (azelate)
31	Methyl hexadecanoate (palmitate) C16
32	Methyl 2-hydroxybenzenepropanoate (melilotate)
33	Trimethyl 2-hydroxy-1,2,3-propane-tricarboxylate (citrate)
34	Methyl 9-octadecanoate (oleate) C18
35	Trimethyl 1-hydroxy-1,2,3-propane-tricarboxylate (isocitrate)
36	Methyl 4-hydroxy-3-methoxybenzoate (vanillate)
37	Methyl 3-[4-hydroxy-3-methoxyphenyl]-2-propenoate (ferulate)

<sup>a</sup> Peak numbers correspond with those in Figure 2.

<sup>b</sup> Internal standard.

mass spectra. All of the identified methyl esters are listed in Table I and have been reported previously in beer (6). The technique is quite effective at removing and chromatographing a wide range of hydrophobic and hydrophilic organic acids from beer. In fact, so many acids are extracted that several significant overlaps occur, including methyl pyruvate with methyl octanoate and dimethyl succinate with methyl decanoate.

Although comparison of the relative recoveries of a number of organic acids with the four resins showed some differences, no one resin was systematically more efficient than the others at recovering all of the acids (Fig. 3). However, the IRA-904 resin was easiest to decant and it seemed to be the most resistant to mechanical breakdown during stirring. All of the resins generated a significant artifact peak identified as methyl benzoate by mass spectrometry. Both the IRA-904 and Dowex 1-X8 resins generated less of this artifact than the other two resins.

The effect of resin amount on the recovery of a number of acids was investigated at treatment rates of 0.5, 1.0, and 2.0 g of resin per 100 ml of beer (Fig. 4). Although the highest treatment rate resulted in a higher recovery of certain acids, this level was undesirable for the final method because of the larger amounts of benzoate artifact generated during the workup. The final treat-

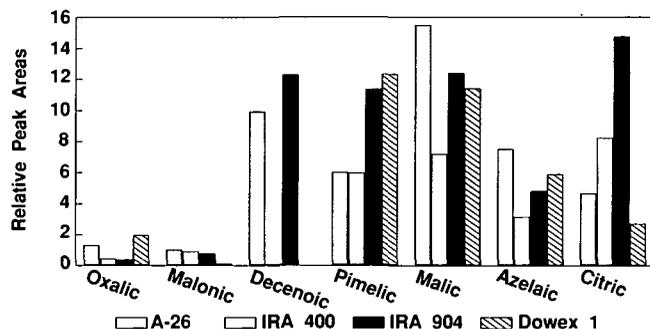


Fig. 3. Effect of resin type on recovery of individual organic acids from beer. A-26 = Amberlyst A-26, IRA-400 = Amberlite IRA-400, IRA-904 = Amberlite IRA-904, and Dowex 1 = Dowex 1-X8.

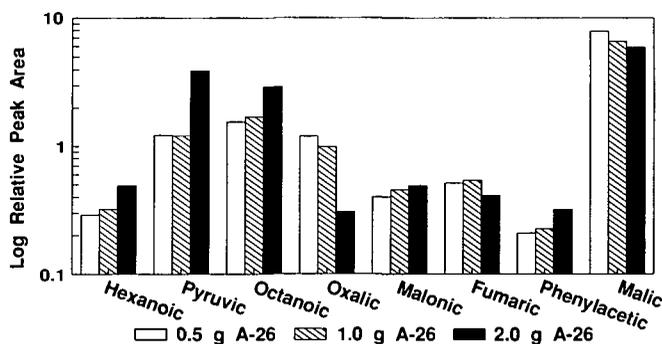


Fig. 4. Effect of resin amount on recovery of individual organic acids from beer with Amberlyst A-26 resin.

TABLE II  
Effect of Incubation Temperature and Time on the Methylation Reaction

Component	Relative Area Ratio <sup>a</sup>		
	55°C/30 min	55°C/3 hr	70°C/30 min
Methyl nonanoate	7.6	6.6	4.9
Dimethyl succinate	4.1	5.1	4.0
Methyl benzoate	18.5	54.5	37.9

<sup>a</sup> Peak area ratio of the toluene internal standard to that of the component.

ment level chosen for establishing the calibration lines was 1 g of resin per 50 ml of beer.

Finally, the effects of incubation temperature and time on the methylation step also were investigated using nonanoic and succinic acids as representatives of the mono- and dicarboxylic acids, respectively, in beer. The results in Table II suggest that an incubation time of 30 min at 55°C is sufficient to derivatize both types of acid, with longer reaction times and higher temperatures merely leading to increased amounts of methyl benzoate artifact.

Calibration lines were obtained for a number of acids by the standard additions method using a beer blank from which endogenous organic acids had been removed by treatment with an anion-exchange resin. The calibration line results are listed in Table III. Whereas about half of the acids analyzed gave satisfactory calibration lines, the remainder gave less than desirable correlation coefficients. This was usually attributable to overlap with neighboring peaks in the chromatogram. Lactic acid, however, was simply not recovered to any significant extent, and its analysis is not feasible with this method. Whether this was a result of poor binding to the resin or poor conversion during the methylation step was not investigated.

The reproducibility of the method was examined by six repetitive analyses of a commercial lager beer (Table IV). Although the relative standard deviation for the malonic acid was rather

high, those of the remaining acids are low enough to allow some confidence in their mean concentrations.

A series of commercial lager and ale brands then were analyzed, and the results are reported in Table V. Although there are significant differences between some of the organic acid concentrations in these beers, in most cases, these differences probably have little impact on the flavors. Hexanoic acid is a possible exception, as its concentration in Ale 2 is approaching its reported taste threshold of 8 mg/L (10) and, consequently, this compound may make some contribution to the brand's flavor. The values for oxalic acid concentration correspond with those reported in the literature (2,12), with Lager 1 and Ale 2 having values at the upper end of the expected range.

## SUMMARY

Removal of organic acids from beer with an anion-exchange resin and in situ methylation with boron trifluoride-methanol has been shown to be a convenient method of analysis for both volatile and nonvolatile acids. In some cases, peak overlaps lead to inaccuracies, which could probably be overcome by further optimization of the chromatographic procedure.

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## LITERATURE CITED

- Barber, L. E. The analysis of organic acids by ion chromatography in beer and wort. *J. Am. Soc. Brew. Chem.* 48:44-46, 1990.
- Brenner, M. W. Gushing beer II: Causes and some means of prevention. *Proc. Congr. Eur. Brew. Conv.* 6:349-362, 1957.
- Clark, B. J., Harold, F. V., Hildebrand, R. P., and Morrieson, A. S. Trace volatile constituents of beer IV. Volatile acids. *J. Inst. Brew.* 68:179-187, 1962.
- Coote, N., and Kirsop, B. H. The content of some organic acids in beer and other fermented media. *J. Inst. Brew.* 80:474-483, 1974.
- Enebo, L., Blomgren, G., and Johnsson, E. Low molecular weight non-volatile organic acids in wort and beer. *J. Inst. Brew.* 61:408-411, 1955.
- Engan, S. Beer composition: Volatile substances. In *Brewing Science*. J. R. A. Pollack, Ed. Academic Press, New York, Vol 2, pp. 93-165, 1981.
- Harrison, G. A. F., and Collins, E. Gas chromatographic determination of individual alpha-keto acids in beer. *Proc. Am. Soc. Brew. Chem.* pp. 101-104, 1968.
- Knudsen, E. J., and Siebert, K. J. Application of ion chromatography to beer, wort and brewing water. *J. Am. Soc. Brew. Chem.* 42:65-70, 1984.
- Liebich, H. M., Pickert, A., and Woll, J. Analysis of the oxocarboxylic acid fraction in serum and urine as *O*-methyloximes by thermionic specific detection. *J. Chromatogr.* 217:255-262, 1981.
- Meilgaard, M. Flavor and chemistry of beer. Part II: Flavor and threshold of 239 aroma volatiles. *Tech. Q. Master Brew. Assoc. Am.* 12:151-168, 1975.
- O'Donnell, D. C., McIntosh, J., Fernando, J. S. P., Sue, J. M., and Blenkinship, B. K. Use of ion chromatography to study organic acids in beer. *Proc. Congr. Inst. Brew. Aust. N.Z. Sect.* 19:221-234, 1986.
- Schildbach, R., and Greif, P. Detection of oxalic acid and its effect on haze development in beer. *Proc. Congr. Eur. Brew. Conv.* 16:709-721, 1977.
- Suomalainen, H., and Ronkainen, P. J. Keto acids in bakers yeast and in fermentation solution. *J. Inst. Brew.* 69:478-482, 1963.
- Thompson, J. A., and Markey, S. P. Quantitative metabolic profiling of urinary organic acids by gas chromatography-mass spectrometry: Comparison of isolation methods. *Anal. Chem.* 47:1313-1321, 1975.
- Zhu, A., and Xu, G.-Y. Derivatization at the liquid-solid interface: Application to trace analysis of organic acids in aqueous samples. *J. Chrom.* 314:421-428, 1984.

TABLE III  
Summary of Calibration Line Constants for Analysis  
of Organic Acids in Beer

Component	Calibration		Correlation Coefficient
	Range (mg/L)	Slope	
Hexanoic acid	0.2-5.0	0.121	0.999
Lactic acid	0.2-5.0	0.007	0.442
Pyruvic acid	0.2-5.0	0.629	0.959
Octanoic acid	1-20	0.199	0.977
Oxalic acid	1-20	0.022	0.990
Malonic acid	0.1-2.5	0.123	0.990
Fumaric acid	0.2-5.0	0.148	0.999
Decanoic acid	0.2-5.0	0.373	0.996
Phenylacetic acid	0.2-5.0	0.275	0.994
Malic acid	1-20	0.040	0.965
Citric acid	2-50	0.062	0.969

TABLE IV  
Six Replicate Analyses of a Lager Beer

Component	Mean	Standard Deviation	Relative Standard Deviation (%)
	Concentration (mg/L)		
Hexanoic acid	2.05	0.27	13.0
Octanoic acid	6.46	0.32	5.0
Oxalic acid	12.11	1.03	8.5
Malonic acid	1.36	0.25	18.0
Fumaric acid	2.74	0.33	12.0
Phenylacetic acid	0.17	0.02	12.0

TABLE V  
Organic Acid Content of Commercial Lagers and Ales

Component	Concentration (mg/L)				
	Lager 1	Lager 2	Lager 3	Ale 1	Ale 2
Hexanoic acid	2.5	3.2	2.1	2.9	5.4
Octanoic acid	4.5	4.9	4.2	5.2	6.3
Oxalic acid	27.0	9.5	15.1	8.0	27.0
Malonic acid	1.6	1.4	1.3	1.3	1.6
Fumaric acid	3.6	3.1	1.6	1.9	2.4
Phenylacetic acid	0.15	0.19	0.17	0.14	0.31