ELSEVIER

Food Chemistry 75 (2001) 101–108

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods Section

A rapid gas chromatographic method for direct determination of short-chain (C_2–C_{12}) volatile organic acids in foods

Ming-Hua Yang[a], Youk-Meng Choong[b,*]

[a]*Department of Food and Nutrition, Hung-Kuang Institute of Technology, 34 Chung-Chi Road, Shalu, Taichung Hsien, Taiwan, Republic of China*

[b]*Department of Food Sanitation, Ta-Jen Institute of Technology, 20 Wei-Shin Road, Yan-Puu Hsiang, Pintung Hsien, Taiwan, Republic of China*

Received 25 November 2000; received in revised form 10 May 2001; accepted 10 May 2001

Abstract

A simple, rapid and accurate GC analytical method for direct quantification of short-chain volatile organic acids in liquid foods was established. Hydrophilic 1,3-butanediol was selected as the internal standard. Thirteen volatile organic acids including acetic, propionic, isobutyric, butyric, isovaleric, valeric, caproic, heptanoic, caprylic, capric, lauric, lactic and levulinic acids were simultaneously determined with detection limits 0.025–1 ng . The recovery rates of tested acids from fruit juice and vinegar were 92–109% with coefficients of variation below 9.4%. The contents of volatile organic acids in 37 commercial liquid food samples were determined. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Short-chain volatile acids; Liquid foods; Gas chromatography; Direct injection; Quantitative determination

1. Introduction

Short-chain volatile organic acids (VOAs) with carbon numbers ranging from two to 12 significantly affect the flavor and quality of food (Alur, Doke, Warrier, & Nair, 1995). These volatile acids, mainly acetic acid and less often propionic acid and butyric acid, may originate from raw materials or be generated by fermentation during processing and storage. The levels of volatile acids in a variety of foods have special indications. For instance, in 100 ml of wine the volatile acid content as represented by acetic acid should be <0.05 g (Anonymous, 1992). However, in cases of unqualified materials, contaminated utensils, inadequate sterilization and microbial contamination during storage, sugar fermentation results in the formation of volatile acids and impairs the quality of products. Therefore, in some foods volatile acid content is an index to quality assurance.

The determination of volatile acids can be traditionally conducted by either indirect or direct ways. The former is to measure residual non-volatile acids after evaporation of volatile acids and then subtract the amount of non-volatile acids from that of total acids. On the other hand, the direct method is to titrate distilled volatile acids with standard alkaline solutions (AOAC, 1984; Blanco Gomis & Mangas Alonso, 1996). Nevertheless, both methods determine the total amount rather than the composition of volatile acids.

There are many ways to determine organic acids in foods (Blanco Gomis & Mangas Alonso, 1996). Among which gas chromatography (GC) with split injection is unsatisfactory for the analysis of free C_1–C_{12} short-chain organic acids, since their high polarity retards the separation of these compounds from food and the response of flame ionization detector (FID) to them is quite low (Larsson & Roos, 1983). Therefore, these short-chain organic acids usually need to be derivatized before analysis (Van Huyssteen, 1970). But the derivatization process is time-consuming. In addition, the volatility of the derivatized compounds is so high as to reduce the recovery and affect the accuracy and repeatability of GC quantification (Burke & Halpern, 1983; Mccalley, Thomas, & Leveson, 1984; Moffat, McGiill, & Anderson, 1991). To solve these problems, headspace analysis was considered (Mulligan, 1995). Alternatively,

* Corresponding author. Tel.: +886-8-7624002 ext. 352; fax: +886-8-7621972.

E-mail address: ymchoong@ccsun.tajen.edu.tw (Y.-M. Choong).

0308-8146/01/$ - see front matter © 2001 Elsevier Science Ltd. All rights reserved.
PII: S0308-8146(01)00211-4

the short-chain organic acids were prepared into less volatile derivatives for GC analysis (Bahre & Maier, 1996; Chauhan & Darbre, 1982; Kim, Zlatkis, Horning, & Middleditch, 1987; Molnar-Perl & Szakacs-Pinter, 1986; Shaw & Bickling, 1986). However, the procedure is still tedious and not applicable to routine analysis.

Gas chromatography, which provides high resolution as well as excellent sensitivity, is one of the most important modern analytical techniques. In our experience, direct injection gave higher detection response than the usually used split injection mode, and the insertion of glass wool into the glass liner of the injection port helped to prevent the analytical column from being contaminated by non-volatiles as to reduce the interference from contaminants (Choong, Ku, Wang, & Lee, 1997; Lee, Su, Yang, Wang, & Choong, 1998; Wang, Lee, & Choong, 1997; Lin & Choong, 1999). We also found the commercial megapore GC columns were quite resistant to water (Lin & Choong, 1999). Even when the water solution was directly injected into the column, the separation effect and the retention time remained the same as of the new column. That means aqueous samples without any pretreatment were eligible for direct injection into GC columns and being quantitatively analyzed.

In the present study, C_2–C_{12} volatile organic acids in aqueous samples such as vinegar, fruit juice, lactic acid drink, fermented milk and soy sauce were quantitatively analyzed by the direct injection method. All samples were mixed with an appropriate amount of aqueous internal standard solution and then directly injected into a gas chromatograph without derivatization. The purpose of this study is to establish a simple, rapid and accurate GC analytical method for direct quantification of short-chain volatile organic acids in liquid foods.

2. Materials and methods

2.1. Materials

Thirty-seven test samples, including six kinds of fruit juice (orange, plum, mango, cherry, grape and apple juice), five samples each of vinegar, vinegar beverage and soy sauce, four samples of fresh milk, two samples of spoiled milk (as control), five samples of fermented milk, three samples of yogurt drink and two samples of lactic acid beverage, were purchased from the local supermakets in Tainan and Pintung areas. The authentic compounds such as acetic acid (2C), propionic acid (3C), isobutyric acid (iso 4C), butyric acid (4C), isovaleric acid (iso 5C), valeric acid (5C), caproic acid (6C), heptanoic acid (7C), caprylic acid (8C), capric acid (10C), lauric acid (12C), lactic acid (Lac), levulinic acid (Lev), 1,3-butanediol (1,3-BuOH), 2,3-butanediol (2,3-BuOH), 1,5-pentanediol (1,5-POH) and 1,6-hexanediol (1,6-HOH) were obtained from TCI (Tokyo, Japan) and of purity >98%.

2.2. Preparation of standard solutions

Five hundred milligrams of acetic, propionic, isobutyric, butyric, isovaleric, valeric, caproic, heptanoic, caprylic, capric, lauric, lactic and levulinic acids as well as 1,3-butanediol were separately weighed into 100-ml volumetric flasks and dissolved with distilled water or methanol.

2.3. Determination of the relative response factors of short-chain organic acids to 1,3-butanediol

The solutions of 0.5% (w/v) volatile organic acids including acetic, propionic, isobutyric, butyric, isovaleric, valeric, caproic, heptanoic, caprylic, capric, lauric, lactic and levulinic acids were individually mixed with 0.5% (w/v) 1,3-butanediol aqueous solution (as the internal standard, abbreviated IS) in three different ratios, i.e. VOAs: 1,3-butanediol = 2:1, 1:1 and 1:2. Each mixture (0.1 μl) was directly injected into a Hitachi G-3000 gas chomatograph (Tokyo, Japan) equipped with a flame ionization detector (H_2 flow rate 30 ml/min, air flow rate 300 ml/min) and a Chrompack CP-Wax 52 CB fused silica column (30m×0.53 mm i.d., 1.0 μm film thickness, Netherlands). Glass wool was inserted in the glass liner of the injection port. Detector temperature and injector port temperature were 280 and 240 °C, respectively. Helium was used as the carrier gas at flow rate 3 ml/min. The oven temperature was programmed at 75 °C for 1 min, raised to 180 °C at 6 °C/min, then increased to 230 °C at 10 °C/min, and finally held at 230 °C for 5 min.

The relative response factor (RRF_{VOA}) was calculated by dividing the peak area ratio of each VOA to 1,3-butanediol with their weight ratio as shown in the following equation, where A represents peak area and W represents weight in milligrams.

$$RRF_{VOA} = (A_{VOA}/A_{IS}) \div (W_{VOA}/W_{IS})$$

$$= (A_{VOA}/W_{VOA}) \div (A_{IS}/W_{IS})$$

2.4. Determination of the detection limit of each short-chain volatile organic acid

The standard solutions of C_2–C_{12} volatile organic acids (5 mg/ml) were separately diluted with distilled water or methanol to obtain final concentrations of 20, 10, 5, 2.5, 1, 0.5, 0.25 and 0.1 μg/ml. The diluted solutions (0.5 μl) were directly injected into GC and the detection limit of each volatile organic acid was determined at peak/noise ratio >2.

2.5. Recovery tests

In a 7-ml vial, 50 μl or 200 μl of 0.5% short-chain volatile organic acid solution was mixed with 1 ml of vinegar or fruit juice. After the addition of 0.5% internal standard solution (50 μl), 0.1 μl of the mixture was directly injected for GC analysis. The recovery rate of each volatile organic acid was shown as the mean of three replicates.

2.6. Quantitative determination of C_2–C_{12} volatile organic acids in foods

2.6.1. The GC method with a direct injection mode

One milliliter of the sample was taken separately from each fruit juice (300 ml), vinegar (300 ml), vinegar drink (200 ml), soy sauce (1000 ml), fresh milk (900 ml), spoiled milk (200 ml), fermented milk (900 ml), yogurt drink (125 ml), and lactic acid drink (1000 ml) and then mixed with 50 μl of 0.5% 1,3-butanediol aqueous solution in 7-ml vials. Then, 0.1 μl of the mixture was directly injected for GC analysis. The content of each volatile organic acid was calculated as follows:

$$\text{VOA (mg/ml)} = (A_{\text{VOA}} / A_{\text{IS}}) \times (W_{\text{IS}} / RRF_{\text{VOA}}) \times 1/V$$

where V represents the volume of food sample in milliliters.

2.6.2. Determination of acetic acid with AOAC methods

Based on AOAC official methods 950.35 (AOAC, 1998a) and 945.52 (AOAC, 1998b) with modification, the volatile acid in 10 ml of the aqueous sample was distilled by steam and collected into 100 ml distillate. The content of volatile acids was determined by titration with 0.1N standardized NaOH solution and expressed as acetic acid content.

2.6.3. Determination of lactic acid with a spectrophotometric method

Fifty milliliters of the aqueous sample was continuously extracted with 100 ml ethyl ether for 3 h based on AOAC Official Method 937.05 (AOAC, 1998c). The extract was then reacted with ferric chloride under acidic pH and the absorbance of the yellow product was measured at 440 nm.

3. Results and discussion

3.1. Conditions of GC analysis

For selection of the analytical column, polar Chrompack CP-Wax column (30 m×0.53 mm) as well as CP-Sil 24 CB and CP Sil 8 CB columns (30 m×0.53 mm) of medium polarity was considered. Results showed the polar CP-Wax column was the best choice for the analysis of polar volatile organic acids (data not shown).

The column temperature gradient for this direct injection method was described in Section 2. Fig. 1 shows the chromatogram of authentic compounds. The retention times of acetic, propionic, isobutyric, butyric, isovaleric, valeric, caproic, heptanoic, caprylic, lactic, capric, levulinic and lauric acids are listed in Table 1. In general, the retention of short-chain organic acids is directly proportional to their carbon numbers. The higher polarity of lactic and levulinic acids greatly increased their retention as compared with propionic and heptanoic acids, respectively. Clark and Bunch (1997) analyzed volatile acids in tobacco, tea and coffee using derivatization-purge and trap gas chromatography. They found the retention times of acetic acid, isobutyric, butyric, isovaleric, valeric, 3-methylvaleric, hexanoic, heptanoic and octanoic acids were 2.33, 4.11, 4.68, 5.63, 6.54, 7.69, 8.48, 10.36 and 10.71 min, respectively. Besides the carbon number and molecular polarity, the steric conformation is another factor which affects the retention of short-chain organic acids regardless of derivatization.

To find the appropriate internal standard, 2,3-butanediol, 1,3-butanediol, 1,5-pentanediol and 1,6-hexanediol were separately added into the mixture of authentic compounds and analyzed accordingly. The retention time (RT) of 1,5-pentanediol (23.12 min) was close to that of caprylic acid (23.24 min). Similarly, 1,6-hexanediol (RT = 28.29 min) was so close to lauric acid (RT = 28.25 min) as not to be selected. In addition, by comparing the chromatograms of the other two

Table 1
Relative response factors (RRF), retention times (RT) and detection limits of various volatile organic acids determined as described in Section 2

Compound	RRF[a]	RT[b]	Detection limit (ng)
Acetic acid (2C)	0.52	10.70	1.25
Propionic acid (3C)	0.93	12.63	0.25
Iso-butyric acid (iso4C)	1.20	13.29	0.25
Butyric acid (4C)	1.08	14.63	0.50
Iso-valeric acid (iso5C)	1.33	15.53	0.125
Valeric acid (5C)	1.22	17.01	0.25
1,3-Butanediol (1,3-BuOH)[c]	1.00	17.23	ND
Caproic acid (6C)	1.34	19.24	0.50
Heptanoic acid (7C)	1.44	21.35	0.50
Caprylic acid (8C)	1.45	23.24	0.50
Lactic acid (Lac)	0.21	25.07	5.0
Capric acid (10C)	1.41	25.95	1.0
Levulinic acid (Lev)	0.43	26.70	2.5
Lauric acid (12C)	1.43	28.25	1.0

[a] RRF of various volatile acid to 1,3-butanediol.
[b] CP-wax column (0.53 mm×30 m, DF = 1 μm) was used.
[c] Internal standard. ND, not determined.

compounds with those of the liquid foods such as vinegar, fruit juice, lactic acid drink, fermented milk and soy sauce, 1,3-butanediol did not interfere with the separation of original ingredients at all. Therefore, 1,3-butanediol was selected as the internal standard in this study.

With the present method, it took only 30 min or so to analyze the commercial liquid foods. The GC chromatograms of vinegar, soy sauce, fruit juice and fermented milk are shown in Fig. 2A–D.

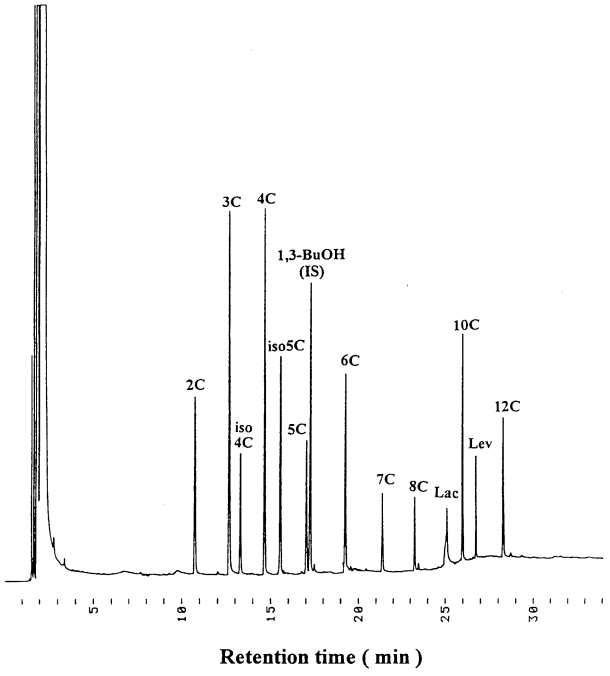

Fig. 1. The gas chromatogram of short-chain (C_2–C_{12}) volatile acids and the internal standard (IS) 1,3-butanediol obtained with the direct injection method. Peaks: 2C, acetic acid; 3C, propionic acid; iso4C, isobutyric acid; 4C, butyric acid; iso5C, isovaleric acid; 5C, valeric acid; 1,3-BuOH (IS), 1,3-butanediol; 6C, caproic acid; 8C, caprylic acid; Lac, lactic acid; 10C, capric acid; Lev, levulinic acid; 12C, lauric acid.

3.2. The relative response factors of short-chain organic acids to 1,3-butanediol

After hydrophilic 1,3-butanediol was selected as the internal standard, the relative response factor of each VOA was determined as described in Section 2 (Table 1). The concentration ratio of each VOA to 1,3-butanediol was plotted against their peak area ratio and the coefficients of determination (R^2) thereafter obtained were all higher than 0.92.

3.3. The detection limits

To understand the lowest detectable level of each VOA, standard solutions of eight concentrations were prepared and analyzed as described in Section 2. FID signal and attenuation were both set to one to ensure highest sensitivity. The detection limits of acetic, propionic, isobutyric, butyric, isovaleric, valeric, caproic, heptanoic, caprylic, lactic, capric, levulinic and lauric acids at an injection volume of 0.5 μl are listed in Table 1.

Roorda, Geonnet, and Rocca (1982) reported the detection limits of the p-nitrobenzyl derivatives of lactic and acetic acids were 1.8 and 1.1 ng, respectively. Meanwhile, the detection limit obtained with UV method (254 nm) was the same as that obtained with electrochemical method (1.1 V). With enzymatic methods, acetic acid could be determined at concentrations as low as 0.4 ppm and lactic acid at 1 ppm or so (Anonymous, 1975). Blanco Gomis and Mangas Alonso (1996) used an HPLC method with a capillary C18 column

Table 2
Recovery rates of the spiked volatile acids from vinegar determined by the direct injection method

Compound[a]	Blank (μg) (A)[b]	Amount added(μg)(B)	Amount found (μg) (C)[c]	Recovery(%)[d]	CV(%)[e]
2C	26932	1000	27981	104.9	7.9
3C	0	250	261	104.4	6.5
Iso4C	0	250	239	95.6	4.8
4C	0	250	258	103.2	6.7
Iso5C	203	250	263	105.2	7.3
5C	0	250	229	91.6	4.2
6C	0	250	262	104.8	5.6
7C	0	250	231	92.4	3.8
8C	0	250	272	108.8	8.3
Lac	3194	1000	4254	106.0	7.8
10C	207	250	451	99.2	3.6
Lev	356	250	623	106.8	8.7
12C	431	250	699	107.2	6.5

[a] Abbreviations: 2C, acetic acid; 3C, propionic acid; iso4C, isobutyric acid; 4C, butyric acid; iso5C, isovaleric acid; 5C, valeric acid; 6C, caproic acid; 8C, caprylic acid; Lac, lactic acid; 10C, capric acid; Lev, levulinic acid; 12C, lauric acid.

[b] The content of each acid in 1 ml of vinegar.

[c] Each data was represented as the mean of three replicates.

[d] Recovery(%) = [(C−A)/B] ×100%.

[e] Coefficient of variation.

(30×320 μm i.d.) and UV detection (206 nm) to quantify several organic acids in apple juice. They could detect citric acid at 2.9 ng with an injection volume of 60 nl.

3.4. Recovery test

The recovery of 13 VOAs from vinegar and fruit juice was examined by separately adding 1000 μg of acetic acid and lactic acid as well as 250 μg of propionic, isobutyric, butyric, isovaleric, valeric, caproic, heptanoic, caprylic, capric, levulinic and lauric acids into 1 ml of the food samples. About 92–109% of the added VOA was recovered from vinegar as shown in Table 2. Similarly, there were 93–108% of the added VOA recovered from orange juice (Table 3). In addition, the coefficients of variation (CV) were all below 9.4%. These data indicated the great accuracy of the present method.

3.5. Comparison with AOAC methods

Table 4 shows VOA contents of vinegar, yogurt drink and soy sauce determined by the present GC method in

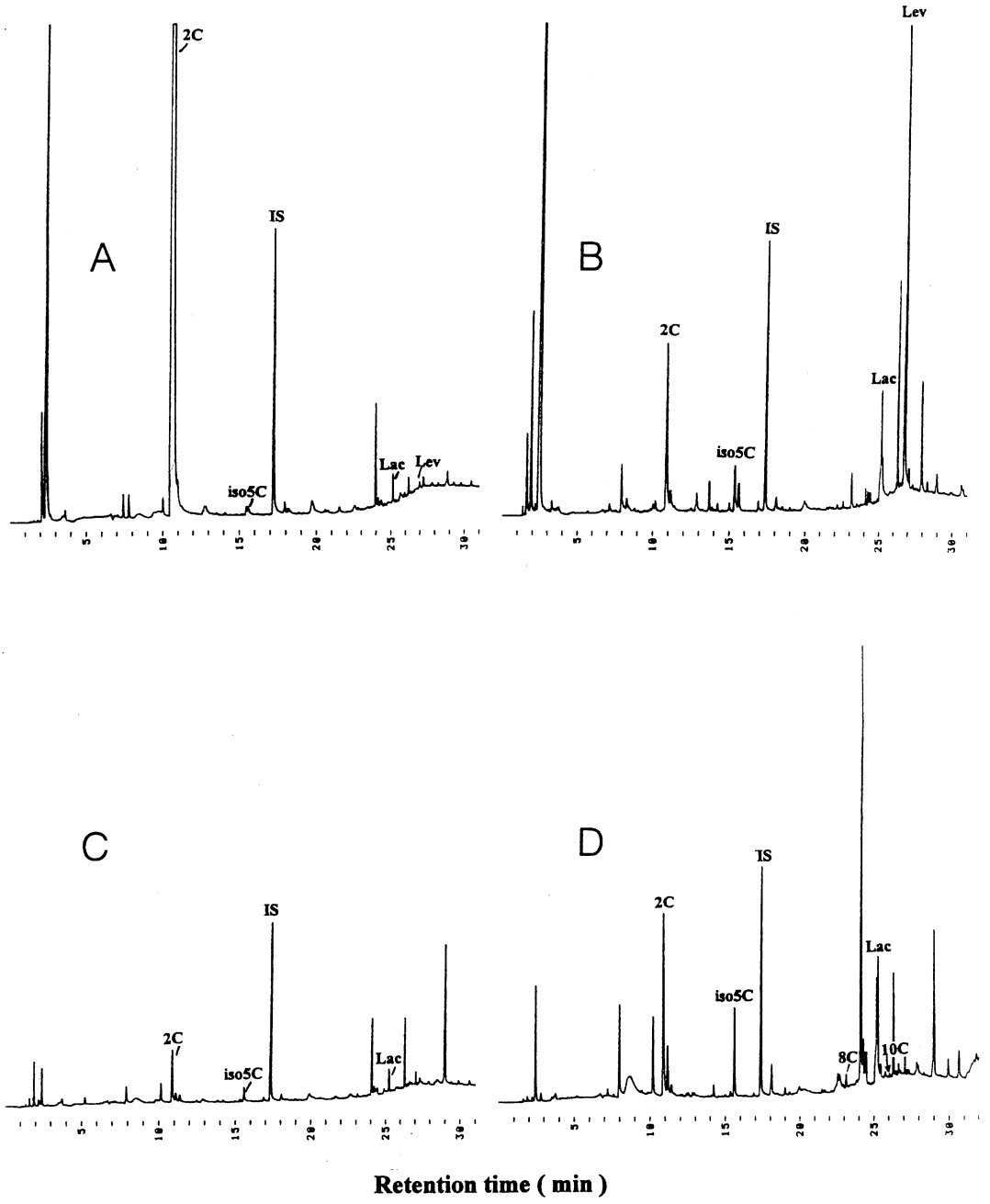

Fig. 2. Gas chromatograms of short-chain (C_2–C_{12}) volatile acids in commercial vinegar (A), soy sauce (B), grape juice (C) and yogurt drink (D) obtained with the direct injection method. Peak identification is the same as in Fig. 1.

comparison with the data obtained by AOAC methods. The present method provided the information of 13 volatile organic acids simultaneously, whereas AOAC methods just focused on one major VOA at a time. Except for lactic acid content in soy sauce, the contents of acetic and lactic acids obtained by AOAC methods were obviously lower than those determined by the GC method. These lower results may be caused by sample loss during distillation and solvent extraction.

3.6. The contents of short-chain organic acids in commercial liquid foods

Volatile organic acid contents of six fruit juice samples, five vinegar samples, five vinegar beverage samples and five soy sauce samples are shown in Table 5. Acetic, isovaleric, lactic and levulinic acids were detected in orange juice, plum juice and all vinegar beverages. In addition, capric acid was also found in grape juice and one soy sauce sample. Besides, one more acid, lauric acid, was detected in mango juice, cherry juice, apple juice, all vinegar samples, and four soy sauce samples. Among 13 target VOAs, acetic acid appeared to be the most abundant acid in vinegar (56.93–105.83 mg/ml) and vinegar beverages (14.44–36.44 mg/ml). Meanwhile, levulinic acid was much more than other acids in soy sauce (24.29–31.36 mg/ml). Acetic and lactic acids were the major VOAs found in fruit juice.

The major difference between the VOA spectra of 16 dairy samples and those of fruit juice, vinegar and soy sauce is the disappearance of levulinic acid (Table 6). Due to bacteria fermentation, lactic acid content increased dramatically in fermented milk (8.31–24.55 mg/ml) and yogurt drink (10.94–20.51 mg/ml) as compared with the content of fresh milk (0.73–1.89 mg/ml).

Table 3
Recovery rates of the spiked volatile acids from orange juice determined by the direct injection method

Compound[a]	Blank(μg) (A)[b]	Amount added(μg)(B)	Amount found (μg) (C)[c]	Recovery(%)[d]	CV(%)[e]
2C	2296	1000	3347	105.1	6.8
3C	0	250	239	95.6	5.7
Iso4C	0	250	262	104.8	4.9
4C	0	250	263	105.2	7.3
Iso5C	276	250	542	106.4	7.3
5C	0	250	271	108.4	8.2
6C	0	250	267	106.8	7.9
7C	0	250	243	97.2	6.8
8C	0	250	254	101.6	3.6
Lac	4251	1000	5321	107.0	9.4
10C	0	250	262	104.8	5.7
Lev	784	250	1021	94.8	8.9
12C	0	250	232	92.8	6.7

[a] Abbreviations are the same as in Table 2.
[b] The content of each acid in 1 ml of orange juice.
[c] Each data was represented as the mean of three replicates.
[d] Recovery (%) = [(C−A)/B]×100%.
[e] Coefficient of variation.

Table 4
Comparison of the GC method with AOAC methods in the determination of volatile acid contents of vinegar, yogurt drink and soy sauce

Samples[a]	Valatile acids (mg/ml)[b]												
	2C	3C	iso4C	4C	iso5C	5C	6C	7C	8C	Lac	10C	Lev	12C
GC Method													
V	26.93	–[c]	–	–	0.20	–	–	–	–	3.19	0.11	0.15	0.23
Y	6.89	0.09	–	–	1.15	–	–	–	–	19.88	0.16	–	0.12
S	3.59	–	–	–	0.13	–	–	–	–	7.03	0.03	27.20	0.08
AOAC Methods													
V	23.16									2.84			
Y	5.14									18.49			
S	3.42									7.45			

[a] Abbreviations: V, vinegar; Y, yogurt drink; S, soy sauce.
[b] Abbreviations are the same as in Table 2.
[c] Minus sign means not detectable.

Table 5
The contents of volatile acids in fruit juice, vinegar, vinegar beverage and soy sauce samples

Samples[a]	Valatile acids(mg/ml)[b]											
	2C	3C	iso4C	4C	iso5C	5C	6C	8C	Lac	10C	Lev	12C
Orange juice	4.67	–	–[c]	–	0.51	–	–	–	6.33	–	0.45	–
Plum juice	2.84	–	–	–	0.51	–	–	–	5.12	–	2.07	–
Mango juice	3.95	–	–	–	0.49	–	–	–	3.35	0.16	0.78	0.56
Cherry juice	3.07	–	–	–	0.52	–	–	–	5.39	0.15	0.84	0.11
Grape juice	2.89	–	–	–	0.48	–	–	–	5.01	0.21	0.33	–
Apple juice	2.93	–	–	–	0.48	–	–	–	4.77	0.25	1.07	0.08
Vinegar-1	56.93	–	–	–	0.20	–	–	–	3.19	0.21	0.35	0.43
Vinegar-2	84.98	–	–	–	0.11	–	–	–	2.18	0.14	0.46	0.13
Vinegar-3	105.83	–	–	–	0.16	–	–	–	2.27	0.13	1.54	0.19
Vinegar-4	70.96	–	–	–	0.11	–	–	–	1.04	0.10	1.92	0.25
Vinegar-5	69.01	–	–	–	1.65	–	–	–	9.21	0.14	3.23	0.43
VB-1	15.45	–	–	–	0.34	–	–	–	6.03	–	1.16	–
VB-2	14.46	–	–	–	0.24	–	–	–	5.66	–	1.65	–
VB-3	14.44	–	–	–	0.23	–	–	–	4.09	–	0.94	–
VB-4	14.93	–	–	–	0.25	–	–	–	4.79	–	1.13	–
VB-5	36.44	–	–	–	1.44	–	–	–	8.73	–	0.95	–
S-1	3.59	–	–	–	0.13	–	–	–	7.03	0.03	27.20	0.08
S-2	5.98	–	–	–	0.41	–	–	–	15.37	0.04	31.36	0.37
S-3	7.37	–	–	–	0.11	–	–	–	17.98	0.11	29.53	0.10
S-4	4.22	–	–	–	0.35	–	–	–	12.32	0.10	24.29	0.24
S-5	1.05	–	–	–	0.13	–	–	–	2.51	0.07	26.07	–

[a] Abbreviations: VB, vinegar beverage; S, soy sauce.
[b] Abbreviations are the same as in Table 2.
[c] Minus sign means not detectable.

Table 6
The contents of volatile acids in dairy products

Samples[a]	Valatite acids (mg/ml)[b]											
	2C	3C	iso4C	4C	iso5C	5C	6C	8C	Lac	10C	Lev	12C
FM1	1.09	–[c]	–	–	0.77	–	0.11	–	1.89	0.21	–	1.06
FM2	1.35	–	–	–	1.02	–	0.07	–	0.73	0.19	–	0.39
FM3	0.97	–	–	–	0.76	–	0.05	–	1.09	0.13	–	0.42
FM4	0.79	0.47	–	–	1.09	–	–	0.08	1.40	0.26	–	0.28
SM1	2.01	–	–	–	0.51	–	–	–	3.54	0.15	–	0.37
SM2	3.17	–	–	–	0.42	–	–	–	4.41	0.14	–	0.23
FerM1	1.21	–	–	–	0.36	–	–	–	9.93	0.11	–	0.32
FerM2	2.83	–	–	–	0.85	–	–	–	20.63	0.23	–	0.24
FerM3	1.08	–	–	–	0.44	–	–	–	8.31	0.27	–	0.79
FerM4	3.64	–	–	–	1.11	–	–	–	24.02	0.33	–	0.19
FerM5	2.72	–	–	–	1.37	–	–	–	24.55	0.26	–	0.17
Y-1	6.89	0.09	–	–	1.15	–	0.05	–	19.88	0.20	–	0.22
Y-2	2.46	–	–	–	0.22	–	–	–	10.94	0.17	–	0.08
Y-3	6.25	0.06	–	–	1.13	–	0.08	–	20.51	0.29	–	0.20
LB-1	5.02	–	–	–	0.54	–	–	–	4.38	0.18	–	0.66
LB-2	4.89	–	–	–	0.73	–	–	–	5.48	0.19	–	0.21

[a] Abbreviations: FM, fresh milk; SM, spoiled millk; FerM, fermented milk; Y, yogurt drink; LB, lactic beverage.
[b] Abbreviations are the same as in Table 2.
[c] Minus sign means not detectable.

4. Conclusions

A simple, rapid and accurate GC method for direct determination of 13 short-chain volatile organic acids in liquid foods was established. With the current method, tedious pretreatment of samples such as distillation and extraction was omitted. Therefore, it took only 40 min to simultaneously determine the contents of 13 VOAs in comparison with 4 h to determine acetic and lactic acids with AOAC methods. Furthermore, the recoveries of

VOAs, 92–109% from vinegar and 93–108% from orange juice, and coefficients of variation below 9.4% indicated the accuracy of the present method.

References

Alur, M. D., Doke, S. N., Warrier, S. B., & Nair, P. M. (1995). Biochemical methods for determination of spoilage of foods of animal origin: a critical evaluation. *Journal of Food Science and Technology (India)*, *32*, 181–188.

Anonymous. (1975). Enzymic method for analysing milk products. Deutsche-Milchwirtschaft, 26 (39, Beil. Lebensmittel-Labor), II-VI.

Anonymous. (1992). Acidity and individual acids. In *Wine Analysis* (pp. 1–11). Taipei: Research Institute for Wines, Taiwan Tobacco & Wine Monopoly Bureau.

AOAC. (1984). Acidity of milk. In AOAC, 14th ed. (pp. 278–279), Washington DC.

AOAC. (1998a). Cereal Foods. AOAC Official Method 950.35. Acetic and propionic acids in bread.

AOAC. (1998b). Fish and Other Marine Products. AOAC Official Methods 945.52. Fatty acids (volatile) in seafood.

AOAC. (1998c). Dairy Products. AOAC Official Method 937.05. Lactic acid in milk and milk products.

Bahre, F., & Maier, H. G. (1996). Electrophoretic clean-up of organic acids from coffee for the GC/MS analysis. *Fresenius Journal of Analytical Chemistry*, *355*, 190–193.

Blanco Gomis, D., & Mangas Alonso, J. J. (1996). Analysis for organic acids. In M. Leo, & L. Nollet (Eds.), *Handbook of Food Analysis* (pp. 715–743). New York: Marcel Dekker.

Burke, D. G., & Halpern, B. (1983). Quaternary ammonium salts for butylation and mass spectral identification of volatile organic acids. *Analytical Chemistry*, *55*, 822–826.

Chauhan, J., & Darbre, A. (1982). Determination of acetyl and formyl groups as pentafluorobenzyl esters by means of gas-liquid chromatography with electron-capture detection. *Journal of Chromatography*, *240*, 107–115.

Choong, Y. M., Ku, K. L., Wang, M. L., & Lee, M. H. (1997). Simple and rapid method for the simultaneous determination of levulinic acid, sorbic acid and benzoic acid in foods. *Journal of the Chinese Agricultural Chemical Society*, *35*, 26–39.

Clark, T. J., & Bunch, J. E. (1997). Determination of volatile acids in tobacco, tea, and coffee using derivatization-purge and trap gas chromatography-selected ion monitoring mass spectrometry. *Journal of Chromatographic Science*, *35*, 206–208.

Kim, K., Zlatkis, A., Horning, E. C., & Middleditch, B. S. (1987). Gas chromatography of volatile fatty acids as tert-butyldimethylsilyl esters. *Journal of High Resolution Chromatography and Chromatography Communications*, *10*, 522–523.

Larsson, M., & Roos, C. (1983). Determination of C1-C4 fatty acids as p-bromoacyl esters using glass-capillary gas chromatography and electron-capture detection. *Chromatographia*, *17*, 185–190.

Lee, M. H., Su, N. W., Yang, M. H., Wang, M. L., & Choong, Y. M. (1998). A rapid method for direct determination of free cholesterol in lipids. *Journal of the Chinese Agricultural Chemical Society*, *36*, 123–133.

Lin, H. J., & Choong, Y. M. (1999). A simple method for the simultaneous determination of various preservatives in liquid foods. *Journal of Food and Drug Analysis*, *7*, 291–304.

Mccalley, D. G., Thomas, C. W., & Leveson, L. L. (1984). Analysis of carboxylic acids by gas chromatography. *Chromatographia*, *18*, 309–312.

Moffat, C. F., McGiill, A. S., & Anderson, R. S. (1991). The production of artifacts during preparation of fatty acid methyl esters from fish oil, food products and pathological samples. *Journal of High Resolution Chromatography and Chromatography Communications*, *14*, 322–326.

Molnar-Perl, I., & Szakacs-Pinter, M. (1986). Gas chromatographic analysis of C_1-C_{20} carboxylic acids esterified in aqueous solutions as isobutyl esters. *Chromatographia*, *17*, 493–496.

Mulligan, K. J. (1995). Aqueous alkylation of anions for static headspace sampling with analysis by capillary gas chromatography mass spectrometry. *Journal of Microcolumn Separations*, *7*, 567–573.

Roorda, Y., Geonnet, C., Rocca, J. L. (1982). Pre-column derivatisation of short-chain carboxylic acids. UV and electrochemical detections. *Analusis*, 10, 409.

Shaw, C. A. R., & Bickling, M. K. L. (1986). Direct alkylation of carboxylic acids in aqueous samples. *Chromatographia*, *21*, 157–160.

Van Huyssteen, J. J. (1970). The determination of short-chain fatty acids in aqueous solution by gas-liquid chromatography. *Water Research*, *4*, 645–659.

Wang, M. L., Lee, M. H., & Choong, Y. M. (1997). Simple method for determination of free fatty acids and total fatty acids in fats and oils. *Journal of the Chinese Agricultural Chemical Society*, *35*, 581–595.