Improved Method for the Analysis of Organic Acids and New Derivatization of Alcohols in Complex Natural Aqueous Matrixes: Application to Wine and Apple Vinegar

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An improvement in the procedure for investigation of organic acids and a new derivatization method, amenable to gas chromatographic−mass spectrometric detection of alcohols, are presented. The latter is based on the formation of phenacetyl esters. The simultaneous application of the two methods also allows data to be obtained on some volatile neutrals present in complex natural aqueous fluids. Application to wine and cider vinegars allowed detection of a number of previously unreported components, among which are interesting partially esterified polycarboxylic esters.

Keywords: *Organic acids; alcohols; phenylacetyl chloride; diazomethane; methyl esters; wine vinegar; apple vinegar*

INTRODUCTION

Identification and quantitation of organic acids and alcohols in complex natural aqueous matrixes is an important and general problem of primary interest in food sciences, research, quality assessment, and regulatory matters. Activity of workers in this field alone is growing fast with an ever widening market of natural products, often emerging from local production, but receiving fresh interest by the industry. A good knowledge of these systems is therefore mandatory. Most of the recently published work, e.g., in the study of vinegars and wines, was essentially based on established procedures (*1*−*11*) and often designed to monitor specific substances (*12*−*22*). Monitoring the production of esters is used to follow the aging of wines (*23*−*30*).

The analysis of alcohols is generally performed as their trimethylsilyl ether derivatives, especially for positive identification by gas chromatography/mass spectrometry. An alternative method of derivatization of lower molecular substances, which has longer retention times and good elution properties and is also amenable to sensitive selective single ion mass spectrometry, is highly desirable. Carboxylic acids pools are usually best identified as their methyl esters; other substances, such as esters present in the matrixes, have been the subject of a large number of analytical chemical procedures; but gas chromatographic/mass spectrometric identification is usually the most rapid, stringent, and informative, whenever applicable (*31, 32*).

In this study we have developed an improved procedure to produce a pool of carboxylic acids, with the aim of minimizing any type of material loss, and a pool of other solvent-extractable substances which have been subjected to a new derivatization method of the alcohols present. The latter can be identified in the gas chromatography−single ion mass spectrometry profile, simultaneously allowing the separation of other components in a different elution range.

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MATERIALS AND METHODS

Safety. Special conditions must be exercised in handling toxic and potentially explosive solutions of diazomethane.

Instruments. GC−MS analyses were performed with a Fisons TRIO 2000 gas chromatograph−mass spectrometer, working in the positive ion 70 eV electron impact mode and using He as carrier gas. Spectra were recorded in the range 35−450 u. The five most intense peaks, in addition to that of the parent ion, are reported for the least common compounds. Injector temperature was kept at 250 °C and the column (Supelco MDN-5S, 30 m long, 0.25 mm i.d., 0.50 μm film thickness) temperature was 50 °C for 5 min, increased to 220 °C with a gradient of 10 °C/min, then from 220 °C to 310 °C with a gradient of 30 °C/min and maintained at 310 °C for 5 min.

Materials. All commercially available chemicals were purchased from Aldrich, Italy. Ethyl ether (99.8%) was washed with deionized water, dried over anhydrous Na_2SO_4, and distilled in order to free it from the added stabilizer (BHT), and kept under nitrogen. Triethylamine (99%) was freed from traces of lower amines by refluxing it with some benzoic anhydride for 1h and subsequently distilling it. All of these chemicals were carefully checked by GC for impurities. All of the other reagents were found not to yield spurious artifacts in the GC analysis by testing them with suitable authentic specimens.

Wine and apple vinegars were Italian commercial products.

Derivatization of Alcohols with Phenylacetyl Chloride. A mixture of pure alcohols (methanol 0.73 mg/mL, ethanol 0.29 mg/mL, 2-propanol 0.27 mg/mL, 2-methyl-2-propanol 0.42 mg/mL, 1-butanol 0.42 mg/mL, 3-methyl-1-butanol 0.34 mg/mL, 1-pentanol 0.48 mg/mL, and 1-methyl-1-cyclohexanol 0.49 mg/mL) dissolved in ethyl ether was used to find the best experimental conditions for their derivatization. The best results were obtained by stirring the mixture (2 mL) at 50 °C for 1 h in a sealed vial with phenylacetyl chloride (0.3 mL) and triethylamine (0.2 mL) and finally quenching the reaction by addition of water (3 mL) at room temperature. After dilution with ethyl ether, the organic phase was extracted in sequence with 5% aqueous HCl, water, and 10% aqueous sodium carbonate. GC analysis of the organic phase showed that primary and secondary alcohols were completely converted to their corresponding esters, but that the tertiary alcohols reacted only partially. 1-Methyl-1-cyclohexanol was found to be particularly unreactive even in

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separate tests using anhydrous conditions on the sodium alcoxide prepared from sodium and the alcohol. The same resistance to esterification was found when acetyl chloride was employed, a result indicating that the cause for the low reactivity resides mainly in the steric bulk about the alcohol function. No stringent reproducibility and quantitative data were collected, but preliminary rough estimates indicated that the procedure may be extended to make quantitative determinations.

Following are the mass spectra (70 eV) of phenylacetates: 2-Methyl-2-propanol (r.t. 16.4 min): 192(M^+, 7), 177(5), 119-(8), 91(38), 65(11), 57(100), 41(39); 3-methyl-1-butanol (r.t. 19.1 min): 206(M^+, 3), 136(18), 119(6), 91(100), 71(18), 70(52), 65-(13), 55(10), 43(84); 1-pentanol (r.t. 19.6 min): 206(M^+, 15), 136(54), 119(4), 115(13), 91(58), 71(12), 65(15), 55(7), 43(100); and 1-methyl-1-cyclohexanol (r.t. 21.6 min): 232(M^+, 1), 217-(2), 137(3), 136(4), 119(3), 118(4), 97(100), 91(48), 55(92).

The GC separations obtained from analysis of the derivatization of the standard mixture of alcohols as well as separated fractions containing alcohols (see infra) from natural matrixes, with derivatives prepared either with trifluoroacetic anhydride or trimethylchlorosilane−hexamethyldisilazane in pyridine, were definitively inferior to those using phenylacetyl chloride, especially owing to the interference of side products and compounds derived from the derivatization agents.

Derivatization of Hydroxypolycarboxylic Acids with Diazomethane. When either a racemic mixture of tartaric acid, or the *meso*-isomer, were heated with ethereal diazomethane, monomethylation to dimethyl *O*-methyltartrates occurred to a small extent, as previously reported (*33*). The reaction of the corresponding dimethyl esters with diazomethane, though, did not produce *O*-methylation, which must therefore precede the fast methyl esterification. Under the same conditions, malic, 2-hydroxyglutaric, and citric acids also underwent *O*-methyl ether formation, although to a much lesser extent.

Sample Preparation for the Analysis of Acidic Components in Vinegars (Method A). Vinegar (10 mL), made slightly basic (pH 8.5−9.0) by addition of a 6 M aqueous solution of sodium hydroxide at 0 °C, was concentrated under reduced pressure (ca. 20 Torr) on a water bath kept at ca. 40 °C. The solid residue was extracted twice with ether, treated with a small quantity of methanol (0.1 mL), and acidified with ethereal hydrogen chloride at 1.5× the amount of the total acidity initially present in the sample. After the mixture was stirred for 15 min at room temperature, excess HCl was eliminated, together with some solvent, at ca. 40 °C. The sample was diluted with ether (5 mL) and a measured volume of a diphenylmethane (internal standard) solution in ether of known concentration was added. Free acids in the solution were transformed into their methyl esters by addition of an ether solution of diazomethane (from Aldrich Diazald) that had been obtained by distillation and made alkali free (*34*). The solution obtained was analyzed by GC−MS.

Sample Preparation for the Analysis of Acidic and Neutral Components in Vinegar (Method B). After neutralization of the sample (vide infra), the solution, saturated with solid sodium chloride (1.5 g), was thoroughly extracted with ether (5 × 10 mL). The combined extracts, dried over Na_2SO_4, were submitted to careful high-efficiency fractionation with Spaltrohr equipment using an MMS155 column (Fischer) in order to distill off enough solvent (ca. 40 mL). The composition of the distillate was continuously monitored by GC in order to rule out loss of analytes. The residual basified vinegar was treated as previously described in order to analyze the acidic components.

Free Acids. Diphenylmethane was selected as the internal standard for the quantitation of methyl esterified acids. This compound is not present in vinegars. To obtain absolute values we calculated the calibration factor for each component. In all cases here-reported, the integral ion current is that obtained by an EI-ion source operating at 70 eV. Owing to the limitation of the scope of this work, no extensive evaluation of the quantitation procedure was carried out, but we tested the recovery of our procedures by assaying the six most abundant acids present in vinegar simulating the two methods (Method A and B) used for vinegar. The results are shown in Table 1.

Table 1. Recovery of a Standard Solution of the Six Most Abundant Acids Present in Simulated Vinegar Analyzed by GC as Their Methyl Esters

acid	Method A[a] mg/L (% recovery)	Method B[a] mg/L (% recovery)
lactic	845 (99.4)	847 (99.6)
phosphoric	348 (93.5)	357 (96.0)
succinic	1138 (99.8)	1120 (98.2)
malic	489 (99.8)	483 (98.6)
tartaric	1290 (89.0)	1250 (86.2)
citric	187 (98.4)	189 (99.5)

[a] Average of 3 determinations.

Table 2. Quantitation as Methyl Esters of Acids Present in a Sample of Vinegar

acid	method A[a] (mg/L)	method B[a] (mg/L)
lactic	469.0	432.0
phosphoric	54.8	65.6
succinic	197.0	162.0
succinic monoethyl ester	32.5	32.0
2-methyl-2-hydroxysuccinic	7.2	8.9
malic	16.2	18.4
glutaric	3.5	1.3
2-hydroxysuccinic 1-ethyl ester	0.6	0.8
2-hydroxysuccinic 4-ethy ester	2.9	3.5
tartaric	560.0	581.0
2-ketoglutaric	10.8	12.5
2-hydroxyglutaric	45.8	54.5
phenyllactic	0.3	0.4
citric	19.2	19.5
isocitric	1.4	1.6
3-carboxyethyl-3-hydroxyglutaric	0.7	0.9
3-carboxyethyl-3-hydroxyglutaric 1-ethyl ester	0.5	0.7

[a] Average of 3 determinations.

The tests of Table 1 were repeated on a sample of vinegar with the average results of 3 determinations shown in Table 2.

Volatile Components. The neutral esters and the alcohols contained in the neutral extract obtained with method B, after suitable concentration, were analyzed by GC−MS. Derivatization with phenylacetyl chloride moved the volatile alcohols from the early-eluting part of the GC profile, directly indicating which peaks had to be attributed to alcohols and yielding further structural confirmation.

Treatment of the above extract with 3 M aqueous sodium hydroxide for 2 h at 45 °C in a sealed vial caused the full hydrolysis of the esters contained in the neutral extract. Ether extraction of the hydrolyzed mixture, followed by suitable concentration, gave a mixture of volatiles from which the ester peaks in the GC profile have been subtracted, yielding further confirmation of any preliminary direct identification and simplifying the GC profile. Selective ion detection at 91 amu ($C_7H_7^+$) made possible the sensitive detection solely of the alcohols as their phenylacetyl esters from any mixture.

Identification of the Components of the Obtained Fractions by GC−MS. Each peak was carefully investigated across its profile in order to establish its homogeneity. After preliminary identification with the help of the available mass-spectral library, each recorded spectrum was carefully examined in order to securely establish identification or determine the nature of the eluted component. Usually, to obtain a definitive positive identification, the GC retention time of each component was checked against the retention time of an authentic sample that had been obtained from commercial sources or prepared synthetically.

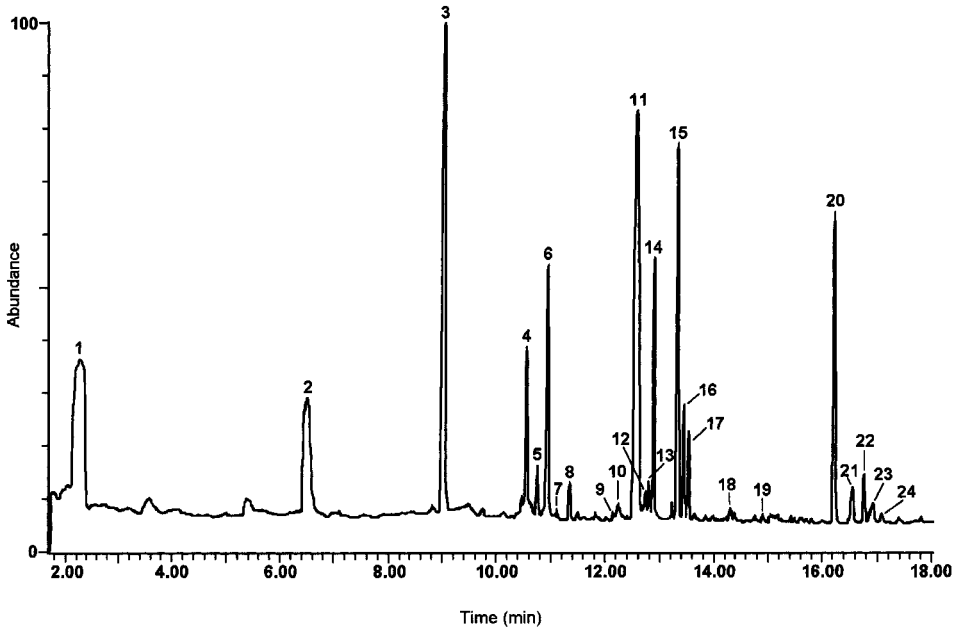

Figure 1. GC−Profile (MS total ion current) of the methylated free acids present in a sample of wine vinegar (see Table 3 for peak identifications).

Table 3. Free Acids Detected and Identified as Methyl Esters in Wine Vinegar

GC[a] peak number and retention time (min)	acid	GC[a] peak number and retention time (min)	acid
1 (2.2)	lactic	**13** (12.8)	4-methoxycarbonyl-γ-butyrolactone
2 (6.4)	phosphoric	**14** (12.9)	2-hydroxyglutaric
3 (9.0)	succinic	**15** (13.3)	2-methoxy-3-hydroxysuccinic
4 (10.5)	succinic monoethyl ester	**16** (13.4)	2-methoxyglutaric
5 (10.7)	2-methyl-2-hydroxysuccinic	**17** (13.5)	2,3-dimethoxysuccinic
6 (10.9)	malic	**18** (14.3)	2-methoxy-2-pentendioic
7 (11.1)	glutaric	**19** (14.9)	3-phenyllactic
8 (11.3)	2-methoxysuccinic	**20** (16.1)	citric
9 (12.1)	malic 1-ethyl ester	**21** (16.5)	3-methoxy-3-carboxyglutaric
10 (12.2)	malic 4-ethyl ester	**22** (16.7)	isocitric
11 (12.6)	tartaric	**23** (16.9)	3-carboxyethyl-3-hydroxyglutaric
12 (12.7)	2-ketoglutaric	**24** (17.1)	3-carboxyethyl-3-hydroxyglutaric 1-ethyl ester

[a] See Figure 1. Elution conditions cut off esters of very volatile nature.

Table 4. Quantitation of Free Acids as Methyl Esters in Different Samples of Vinegar

GC[a] peak	acid	white wine vinegar (A)	white wine vinegar (B)	red wine vinegar (C)	apple vinegar (D)
1	lactic	910.0	673.1	200.6	887.3
2	phosphoric	79.2	56.7	40.7	38.5
3	succinic	230.6	304.2	219.8	146.8
4	succinic monoethyl ester	21.2	100.6	48.6	18.7
5	2-methyl-2-hydroxysuccinic	7.0	10.6	6.8	20.1
6	malic	12.6	8.8	127.9	535.3
7	glutaric	1.6	1.7	n.d.	n.d.
9	malic 1-ethyl ester	n.d.	n.d.	0.3	1.0
10	malic 4-ethyl ester	n.d.	n.d.	1.8	2.4
11	tartaric	285.2	191.6	596.2	n.d.
12	2-ketoglutaric	4.5	2.9	2.2	9.6
14	2-hydroxyglutaric	37.5	65.1	37.2	32.2
19	3-phenyllactic	0.3	1.1	0.1	1.5
20	citric	20.4	10.5	140.4	9.2
22	isocitric	0.7	3.6	2.2	1.0
23	3-carboxyethyl-3-hydroxyglutaric	0.3	0.4	0.5	0.9
24	3-carboxyethyl-3-hydroxyglutaric 1-ethyl ester	0.6	0.2	1.0	0.2

[a] See Figure 2.

Following are mass spectra of partially esterified polycarboxylic acids which were present as methyl ethyl esters in the diazomethane derivatized pool of acids.

Ethyl methyl succinate (**4**): 160(M^+, 3), 133(8), 129(28), 128(20), 115(100), 114(17), 101(30), 88(7), 87(12), 59(12), 55(27).

Malic acid 1-ethyl-4-methyl ester (**9**): 176(M^+, 1), 145(13), 144(7), 131(4), 127(5), 117(21), 116(8), 103(100), 71(63), 61-(19), 43(54).

Malic acid 1-methyl-4-ethyl ester (**10**): 176(M^+, 1), 145(2), 144(3), 131(16), 130(8), 117(100), 103(25), 89(14), 71(38), 61-(4), 43(17).

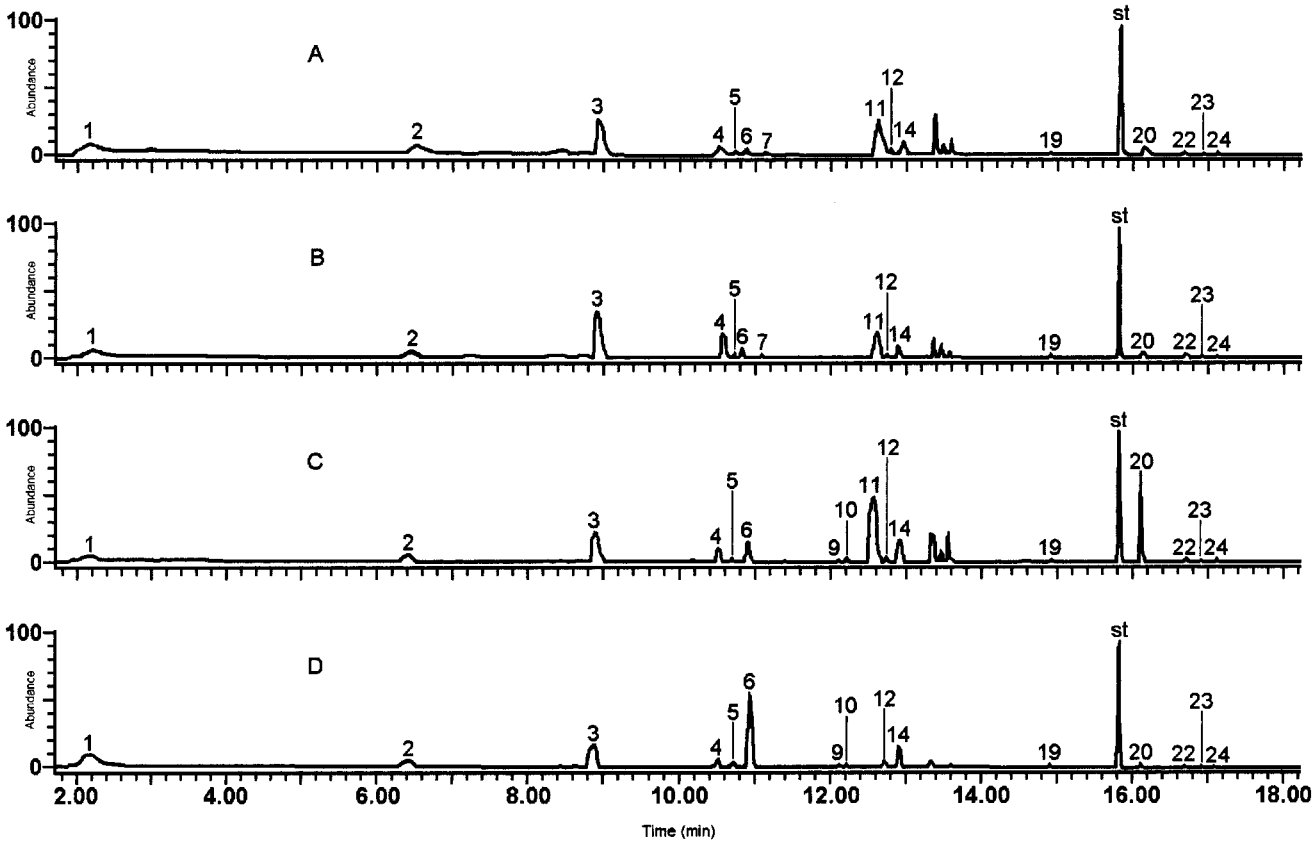

Figure 2. GC−Profile (MS total ion current) of the methylated free acids of four different vinegars: (A) and (B) white wine vinegars, (C) red wine vinegar, and (D) apple vinegar; st, diphenylmethane (Table 4).

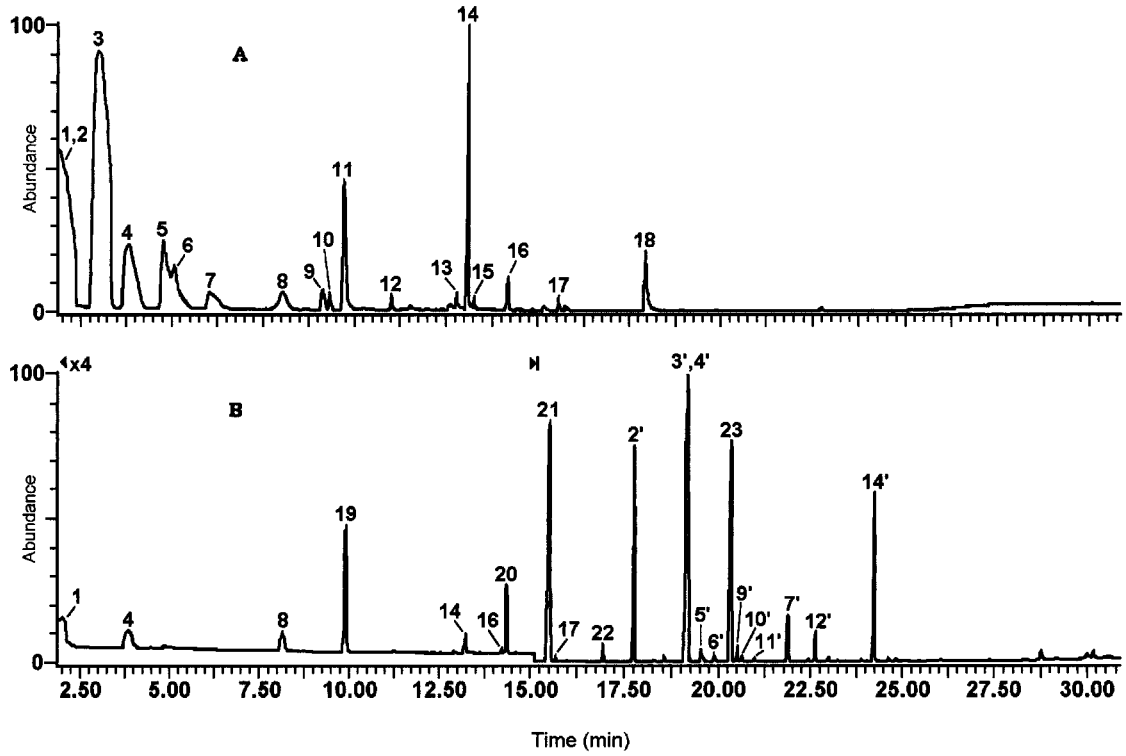

Figure 3. GC−Profile (MS total ion monitor) of underivatized (A; Table 5) and phenylacetyl chloride derivatized (B; Table 6) neutral extracts from a wine vinegar.

3-Carboxyethyl-3-hydroxyglutaric acid 1,5-dimethyl ester (**23**): 189(2), 175(16), 157(10), 143(100), 139(4), 115(6), 111-(7), 101(56), 74(8), 69(9), 59(19), 57(8), 43(19).

3-Carboxyethyl-3-hydroxyglutaric acid 1-ethyl-5-methyl ester (**24**): 203(3), 189(26), 175(3), 157(100), 143(74), 139(7), 115-(30), 111(11), 101(37), 87(7), 74(5), 59(9), 43(19).

Table 5. Identified Components of the Total Neutral Extract

GC[a] peak number and retention time (min)	compound	GC[a] peak number and retention time (min)	compound
1 and **2** (2.0)	ethyl acetate + 2-methyl-1-propanol	**11** (9.7)	3-acetoxy-1-butanol
3 (2.9)	3-hydroxy-2-butanone	**12** (11.5)	benzyl alcohol
4 (3.8)	3-methyl-1-butanol	**13** (12.8)	1,3-propanediol diacetate
5 (4.7)	2,3-butanediol	**14** (13.1)	2-phenylethanol
6 (5.0)	1,3-butanediol	**15** (13.3)	*N*-(3-methylbutyl)acetamide
7 (6.0)	ethyl lactate	**16** (14.0)	diethyl succinate
8 (8.1)	3-methylbutyl acetate	**17** (15.6)	2-phenethyl acetate
9 (9.1)	2-acetoxy-3-hydroxybutane	**18** (17.9)	4′-hydroxyphenethyl alcohol
10 (9.3)	1-acetoxy-3-hydroxybutane		

[a] See Figure 3A.

Table 6. Identified Components of the Total Neutral Extract after Derivatization with Phenylacetyl Chloride

GC[a] peak number and retention time (min)	compound	GC[a] peak number and retention time (min)	compound
1 (2.0)	ethyl acetate	**3′** and **4′** (19.1)	3-methylbutyl phenylacetate + 3-phenylacetoxy-2-butanone
4 (3.8)	3-methyl-1-butanol	**5′** (19.5)	3-phenylacetoxy-2-butanol
8 (8.1)	3-methylbutyl acetate	**6′** (19.7)	1-phenylacetoxy-3-butanol
19 (9.8)	benzaldehyde	**23** (20.3)	*N*,*N*-diethylphenylacetamide[b]
14 (13.1)	phenylethanol	**9′** (20.8)	2-phenylacetoxy-3-acetoxybutane
16 (14.0)	diethyl succinate	**10′** (20.9)	1-acetoxy-3-phenylacetoxybutane
20 (14.2)	methyl phenylacetate	**11′** (21.2)	1-phenylacetoxy-3-acetoxybutane
21 (15.4)	ethyl phenylacetate	**7′** (21.8)	ethyl lactate-2-phenylacetoxy
17 (15.6)	2-phenethyl acetate	**12′** (23.0)	benzyl phenylacetate
22 (16.8)	propyl phenylacetate	**14′** (24.2)	2-phenylethyl phenylacetate
2′ (17.7)	2-methylpropyl phenylacetate		

[a] See Figure 3B. [b] From derivatization procedure.

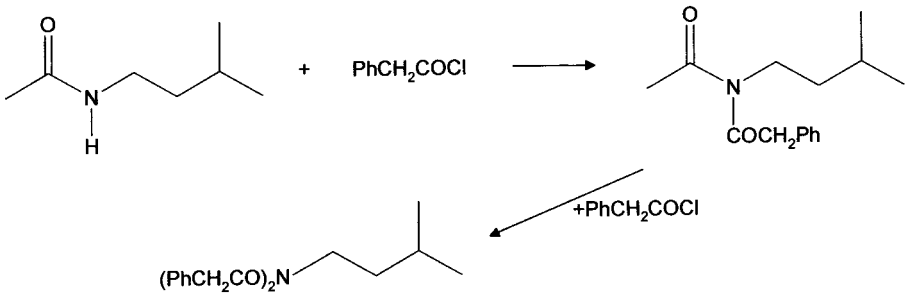

Figure 4. Phenylacetyl derivatization.

RESULTS AND DISCUSSION

After the reliability of these new procedures and their potential effectiveness were established, we tested them on natural aqueous matrixes. Wine and apple vinegars of different commercial origins were chosen. Interest in these "poor quality" products is rapidly increasing, and attempts are being made to improve quality, establish standards, and characterize production. Many of the above-cited publications have dealt with the detection and quantitation of specific components. Our procedure promises to be complementary or substitutive for those already known. The chosen matrixes also posed the challenge of the presence of a single water-soluble organic acid (acetic) in high concentrations (4−10%).

Figure 1 shows the GC−MS profile of the free acids contained in a wine vinegar sample: twenty four components were identified, and the artifacts generated by the action of diazomethane on the hydroxy polyacids were revealed. Analysis of the GC−MS data observed for the acidic fraction using our methodology allowed 20 different acids to be identified, many of which have never been recorded previously. On the other hand, a number of components reported in some studies, as well as in other certified wine vinegars, were not present in our sample (Table 3).

The most interesting result from our procedure, which carefully avoided hydrolysis of esters present, was the detection of a number of partially esterified polycarboxylic acids which showed up as methyl ethyl ester in the diazomethane derivatized pool of acids. They correspond to the compounds **4**, **9**, **10**, **23,** and **24** of Table 4 and are intermediates to the diesters formed in the aging of alcoholic-aqueous matrixes (*35, 36*).

Typically, the abundant acids of the Krebs cycle, i.e., succinic and citric, showed up unexpectedly as both mono- and di-ethyl derivatives. Our method did not recognize partially methylated polycarboxylic acids, but if this information is needed, it might be readily obtained by derivatizing the acids pool with CD_2N_2 (*34*) prior to GC−MS analysis. The retention times would remain unchanged, but the parent ions and/or some other fragment would show the mixed presence of deuterated and undeuterated methyl esters. Recent reports, using different extraction procedures, demonstrated the presence of significant concentrations of the C_6 and C_8 linear fatty acids (*8, 11*) which were not detected in the present work.

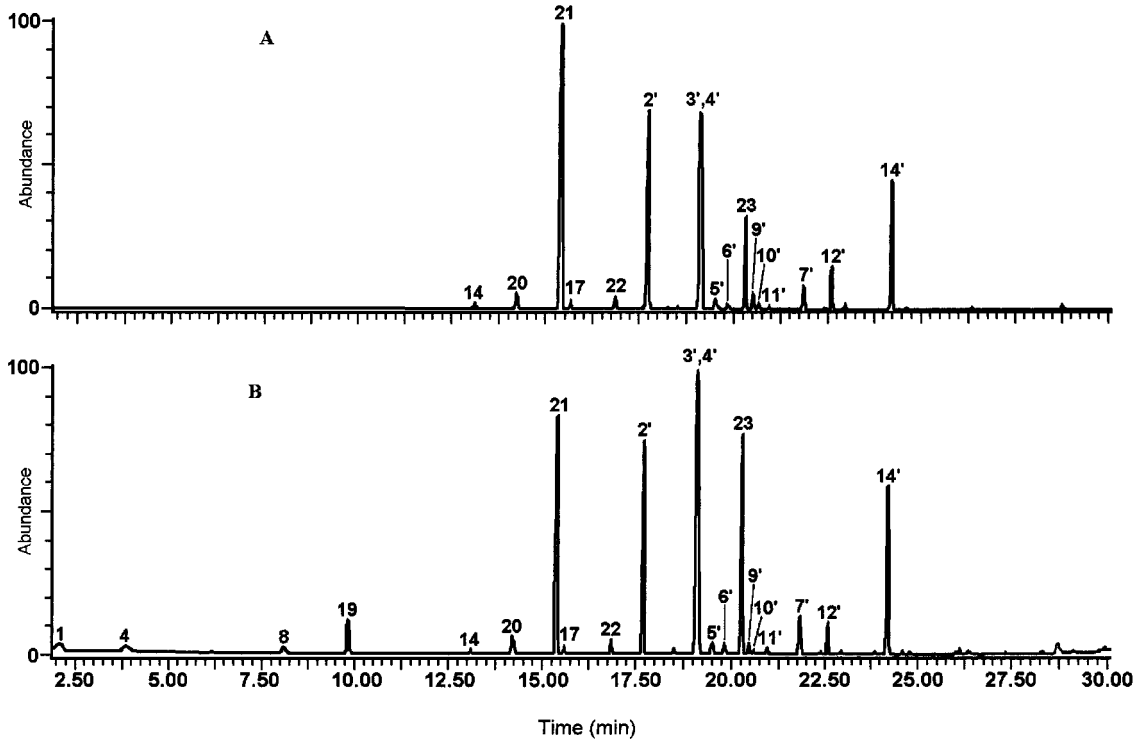

Figure 5. Single ion (*m*/*z* 91; A) and total ion (B) profiles of a phenylacetyl chloride derivatized neutral extract after GC separation (Table 6).

The reproducibility of the extraction of the free acids has been tested and found very satisfactory with the vinegar samples. We also compared the present procedure with some alternatives and determined that the former yielded the best results. Quantitative analyses of four different samples from different firms, one of which was apple vinegar, are reported in Table 4. GC data for the acid pool must be amended for artifacts arising principally in the methylation with diazomethane and possibly in thermal reactions on the free derivative occurring in the gas chromatograph as reported in the Materials and Methods Section.

Although our study concentrated on separation and identification methods, we have also examined the acids of four other certified vinegars, one of which was made from cider, whereas one of the wine vinegars was red and two were white. It was apparent that apple vinegar is characterized by elevated contents of lactic and malic acid, the latter present in amounts some 40−60 times as much as those in the white wine vinegars examined. The sample of red wine vinegar appeared very rich in citric, malic, and tartaric acid, but poor in lactic acid. The GC profiles obtained showed notable variations among the samples (Figure 2).

The total neutral extract GC−MS profile is shown in Figure 3A, and the GC−MS profile after derivatization with phenylacetyl chloride is shown for comparison in Figure 3B. Table 5 reports the identified components of the full neutral extract; the phenylacetyl esters are listed in Table 6. Low molecular alcohols and polyols may be missed in the extraction because of their strong affinity with the salts of the organic and inorganic acids or owing to the low volatility of their derivatives. The GC profile, on the other hand, may not reveal the low volatility polyols after complete derivatization with phenylacetyl chloride. An example of such disappearance is shown by the 4′-hydroxy-2-phenethyl alcohol (peak **18**, Figure 3A).

Inspection of the two GC profiles of Figure 3 shows the advantages of this derivatization: all of the alcohols were moved away from the early-eluting region to a later region permitting easier detection and identification of the ester peaks. This is true also for the alcohols which are identified with the more stable (thermally and in the parent ion decomposition) phenylacetates in an essentially free region. Profile A showed the presence of *N*-(3-methylbutyl)acetamide, which disappeared after derivatization with phenylacetyl chloride, possibly due to the reaction shown in Figure 4, yielding a product which was not eluted. On the other hand *N*,*N*-diethylphenylacetamide is derived from the side reaction of phenylacetyl chloride with triethylamine.

Some alcohols did not react quantitatively with phenylacetyl chloride and could, in part, be found in the ester region of profile B. Two of these were diols wherein, after the first derivatization, the second reaction might be sterically hindered. Two alcohols never previously detected were found, i.e., 2-hydroxybutanone and 1,3-butandiol, but many of those previously observed were not present in our sample. 1,3-Propandiol diacetate was present in profile A, but could not be detected after reaction of the mixture with phenylacetyl chloride, perhaps due to a double exchange of the acyl group with phenylacetyl chloride. The interesting 4′-hydroxy-2-phenylethanol has never been reported as a vinegar component.

Other non-hydroxy esters were ethyl acetate, diethyl succinate, and 2-phenethyl acetate. Overall, the acyl moiety of the present esters was the acetyl group, although a lactoyl group was also observed. The number of significant peaks was very limited, if compared to the large number in Table 5. We found 3-methylbutyl acetate and three isomeric acetoxy butanols which were not found in that list.

Selective ion monitoring at 91 amu, corresponding to the search for a common feature of the decomposition

of the parent ions of phenylacetyl chloride derivatives, allows the GC profile to be dramatically simplified by eliminating any other component. Figure 5 compares the total ion current GC profile (Figure 5B) and the selected ion monitoring of the ion at 91 amu (Figure 5A).

This improved procedure for the separation of the organic acid material contained in biological fluids, typified here by wine vinegar, and the new derivatization of alcoholic components by phenylacetyl chloride allows these fractions to be separated, their components to be identified, and quantitative determinations to be made. The new derivatization method permits both the selective detection and positive identification of alcohols and the simplification of the region of the GC(−MS) profile where other neutral components, especially ethers, may be present. Application to the vinegar test system has allowed a number of previously undetected components to be identified. The general applicability of these procedures to biological fluids offers a new tool for the study of their components.

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LITERATURE CITED

(1) Stahl, E.; Laub, E.; Woller, R. Liquid chromatography of wines and fruit juices. 2. Preparation of samples, quantitative determination of acids and further practical applications. *Z. Lebensm.-Unters. Forsch.* **1974**, *156*, 321−328.

(2) Bertolini, L.; Castagnetti, G. B.; Gambini, G. Organic acids in vinegar: HPLC identification. *Ind. Bevande* **1994**, *23*, 324−326.

(3) Sasakawa, K.; Kato, T. Analysis of vinegars. *Kanzei Chuo Bunsekishoho* **1987**, *27*, 43−48.

(4) Yamamoto, A.; Matsunaga, A.; Mizukami, E.; Hayakawa, K.; Miyazaki, M. Simultaneous determination of organic acids in commercial vinegar by photometric ion chromatography. *Eisei Kagaku* **1990**, *36*, 332−337.

(5) Sakata, K.; Kawai, S.; Yagi, A.; Ina, K.; Kawamura, Y. ^{13}C NMR Spectroscopic analysis of vinegar. *Nippon Shokuhin Kogyo Gakkaishi* **1991**, *38*, 765−769.

(6) Blanch, G. P.; Tabera, J.; Sanz, J.; Herraiz, M.; Reglero, G. Volatile composition of vinegar. Simultaneous distillation−extraction and gas chromatographic−mass spectrometric analysis. *J. Agric. Food Chem.* **1992**, *40*, 1046−1049.

(7) Carnacini, A.; Gerbi, V. Wine vinegar, a product to protect and exploit. *Ind. Bevande* **1992**, *21*, 465−478.

(8) Gerbi, V.; Zeppa, G.; Carnacini, A. Rapid extraction of volatile compounds in wine and vinegar using Extrelut resin. *Ital. J. Food Sci.* **1992**, *4*, 259−267.

(9) Hur, S.; Lee, W. Volatile flavor components in pumpkin vinegar. *J. Food Sci. Nutr.* **1998**, *3*, 119−122.

(10) Hur, S.; Lee, H.; Lee, T.; Lee, W.; Hong, J. Microbiological characteristics and volatile components of deastringent persimmon vinegar. *J. Food Sci. Nutr.* **1998**, *3*, 230−233.

(11) Charles, M.; Martin, B.; Ginies, C.; Etievant, P.; Coste, G.; Guichard, E. Potent aroma compounds of two red wine vinegars. *J. Agric. Food Chem.* **2000**, *48*, 70−77.

(12) Diaz de Bethencourt, C.; Gomez-Cordoves, C.; Blanco, M.; De la Calera, J. G. Phenolic compounds of low molecular weight. Their effect on the quality of vinegar. *Rev. Agroquim. Tecnol. Aliment.* **1980**, *20*, 247−256.

(13) Antonelli, A.; Carnacini, A.; Versari, A. Liquid−liquid extraction of silylated polyalcohols from vinegar, and their determination by capillary GC. *J. High Resolut. Chromatogr.* **1994**, *17*, 553−555.

(14) Garcia-Parrilla, M. C.; Camacho, M. L.; Heredia, F. J.; Troncoso, A. M. Separation and identification of phenolic acids in wine vinegar. *Food Chem.* **1994**, *50*, 313−315.

(15) Galvez, M. C.; Barroso, C. G.; Perez-Bustamante, J. A. Influence of the origin of wine vinegar in their low molecular weight phenolic content. *Acta Hortic.* **1995**, *388*, 269−272.

(16) Garcia-Perrilla, M. C. Polyphenolic compounds in wine vinegar. *Aliment., Equipos Tecnol.* **1996**, *15*, 79−81.

(17) Garcia-Perrilla, M. C.; Heredia, F. J.; Troncoso, A. M. Phenols HPLC analysis by direct injection of sherry wine vinegar. *J. Liq. Chromatogr. Relat. Technol.* **1996**, *19*, 247−258.

(18) Igler, A.; Rauter, W. Capillary gas chromatography determination of synthetic DL-malic acid in products of fruit. *Z. Lebensm.-Unters. Forsch.* **1996**, *203*, 283−286.

(19) Okamoto, A.; Sugi, E.; Koizumi, Y.; Yanagida, F.; Udaka, S. Polyamine content of ordinary foodstuffs and various fermented foods. *Biosci., Biotechnol., Biochem.* **1997**, *61*, 1582−1584.

(20) Erbe, T.; Brueckner, H. Chiral amino acid analysis of vinegars using gas chromatography. Selected ion monitoring mass spectrometry. *Z. Lebensm.-Unters. Forsch. A* **1998**, *207*, 400−409.

(21) Garcia-Parrilla, M. C.; Heredia, F. J.; Troncoso, A. M. Sherry wine vinegars: phenolic composition changes during aging. *Food Res. Int.* **1999**, *32*, 433−440.

(22) Andlauer, W.; Stumpf, C.; Fuerst, P. Influence of the acetification process on phenolic compounds. *J. Agric. Food Chem.* **2000**, *48*, 3533−3536.

(23) Simpson, R. F. Aroma and compositional changes in wine with oxidation, storage and aging. *Vitis* **1978**, *17*, 274−287.

(24) Rodopulo, A. K.; Bezzubov, A. A.; Egorov, I. A.; Nechaev, L. N. Study of aromatic substances in dry table wines. *Prikl. Biokhim. Mikrobiol.* **1979**, *15*, 309−313.

(25) Shinohara, T.; Shimizu, J.; Shimazu, Y. Esterification rates of main organic acids in wines. *Agric. Biol. Chem.* **1979**, *43*, 2351−2358.

(26) Marais, J.; Pool, H. J. Effect of storage time and temperature on the volatile composition and quality of dry white table wines. *Vitis* **1980**, *19*, 151−164.

(27) Shinohara, T.; Shimizu, J. Formation of ethyl esters of main organic acids during aging of wine and indications of aging. *Nippon Nogei Kagaku Kaishi* **1981**, *55*, 679−687.

(28) Rapp, A.; Guentert, M.; Ullemeyer, H. Changes in aroma substances during bottle aging of white wines from Riesling grapes. *Z. Lebensm.-Unters. Forsch.* **1985**, *180*, 109−116.

(29) Martinez de la Ossa, E.; Perez, L.; Caro, I. Variations of the major volatiles through aging of sherry. *Am. J. Enol. Vitic.* **1987**, *38*, 293−297.

(30) Francioli, S.; Guerra, M.; Lopez Tamames, E.; Guadayoi, J. M.; Caixach, J. Aroma of sparkling wines by headspace/solid-phase microextraction and gas chromatography/mass spectrometry. *Am. J. Enol. Vitic.* **1999**, *50*, 404−408.

(31) Legendre, M. G.; Fisher, G. S.; Schuller, W. H.; Dupui, H. P.; Rainer, E. T. Novel technique for the analysis of volatiles in aqueous and nonaqueous systems. *J. Am. Chem. Soc.* **1978**, *100*, 552−555.

(32) Kubota, T.; Oki, Y.; Hiroko, U.; Haramaki, Y. Phenylacetic acid in vinegar. *Nippon Nogei Kagaku Kaishi* **1989**, *63*, 49−50.

(33) Schmidt, O. T.; Zeiser, H. Methylation of trihydoxyglutaric acids and tartaric acid with diazomethane. *Ber. Dtsch. Chem. Ges.* **1934**, *67B*, 2120−2127.

(34) Strazzolini, P.; Giumanini, A. G.; Verardo, G. Low-water-contents diazomethane-d_2 and its isotopic assay. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 2943−2946.
(35) Svejcar, V. Aging of Tokay wines. *Polnohospodarstvo* **1985**, *31*, 762−767.
(36) Colagrande, O.; Silva, A. Technology of sparkling wine productions: scientific interpretation of some traditional aspects. *Ind. Bevande* **1986**, *15*, 257−262.

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