Influence of Sample Preparation on the Composition of Quince (*Cydonia oblonga*, Mill.) Flavor

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The volatiles of quince fruit (*Cydonia oblonga*, Mill.) were studied by standard controlled capillary gas chromatography (HRGC) and combined capillary gas chromatography–mass spectrometry (HRGC–MS) using high-vacuum distillation–extraction (HVD/E) (I) and simultaneous distillation–extraction (SDE) (II). The fruit juice was investigated at natural pH (3.7) (A) and after neutralization (pH 7.0) and enzymic inhibition by adding glucono-δ-lactone (B). With I-A, HRGC and HRGC–MS revealed the two diastereoisomeric theaspiranes among the major volatile constituents (each 1 mg/kg), whereas with I-B only small amounts of these compounds (each <30 μg/kg) were determined. HRGC and HRGC–MS after SDE of juice volatiles (II-A/B) led to 3,4-didehydro-β-ionol and a number of bicyclo[4.3.0]nonanes. The potential chemical pathways yielding the different results are discussed.

One of the most important steps in flavor analysis is the isolation of volatiles, since the method used affects thoroughly the resulting flavor composition (Sugisawa, 1981, 1983). This statement has been recently supported by Ohloff et al. (1985) who pointed out the often-observed carelessness with which the isolation of volatiles is sometimes carried out, thus leading to misinterpretations of results. In the course of our studies on flavor precursors, inter alia, we came upon quince (*Cydonia oblonga*, Mill.) flavor, which was found to be a nice example to demonstrate the importance of the isolation technique used for the results later obtained by chromatographic and spectroscopic techniques. Quince flavor has been previously studied by Schreyen et al. (1979) and Tsuneya and Ishihara (1983), both presenting quite different findings.

EXPERIMENTAL SECTION

Fruits. Fresh ripe quince fruits (*C. oblonga*, Mill.) were available from the local market.

Sample Preparations. High-vacuum distillation–extraction (HVD/E) (I) and simultaneous distillation–extraction (SDE) (II) were used as flavor isolation techniques. Freshly prepared fruit juice was investigated at natural pH (3.7) (A) and after neutralization (pH 7.0) and enzymic inhibition by adding glucono-δ-lactone (B) (Heyworth and Walker, 1962). In Table I the experimental conditions are summarized, which were used after homogenizing the fruits (cut, seeds removed) with a Braun mixer.

High-Vacuum Distillation. The samples (cf. Table I) were high-vacuum distilled [40–50 °C (0.1 bar)] as previously described (Idstein and Schreier, 1985).

Liquid–Liquid Extraction. The aqueous distillates were each extracted with pentane–dichloromethane (2:1) over 24 h (Drawert and Rapp, 1968). The extracts were dried over anhydrous sodium sulfate and carefully concentrated to 1 mL on a Vigreux column (45 °C).

Simultaneous Distillation–Extraction. SDE was performed over 2 h with pentane–diethyl ether (1:1) using the SDE head described by Schultz et al. (1977). The extracts were dried over anhydrous sodium sulfate and carefully concentrated to 1 mL on a Vigreux column (45 °C).

Capillary Gas Chromatography (HRGC). A Carlo Erba Fractovap 4160 gas chromatograph equipped with an FID as well as a J & W fused silica DB-Wax capillary column (30-m length, 0.25-mm i.d.; d = 0.25 μm) and a 2-m uncoated fused silica precolumn (retention gap) was used. On-column injection with an air-cooled injection system was employed. The temperature program was 50–250 °C at 4°/min. The flow rates for the carrier gases were 2.5 mL/min He, for the make-up gas 30 mL/min N_2, and for the detector gases 30 mL/min H_2 and 300 mL/min air, respectively. The detector temperature was kept at 230 °C. Volumes of 0.3 μL were injected.

Table I. Sample Preparation of Quince Fruit Juice[a] at Natural pH (A) and after Neutralization and Enzymic Inhibition (B) for Subsequent High-Vacuum Distillation–Extraction (I) and Simultaneous Distillation–Extraction (II)

	A		B	
	I	II	I	II
fruits (cut, seeds removed), kg	1.4	0.7	1.4	0.7
addn of H_2O dist, L	1.0	0.5	–	–
addn of 0.2 M phosphate buffer (pH 7.0), L	–	–	1.0	0.5
addn of glucono-δ-lactone, g	–	–	35	17.8
total wt, kg	2.2	1.1	2.2	1.1
pH	3.7	3.7	5.2	5.6
pH adjustment with 2 N NaOH	–	–	+	+
addn of 2-heptanol (int std), μg/kg	272	600	277	547

[a] Obtained by using a Hafico press.

Results of qualitative analyses were verified by comparison of HRGC retention and mass spectral data with those of authentic reference compounds. Quantitative HRGC determinations were carried out by standard controlled calculations using a Hewlett-Packard 3388 A laboratory data system without consideration of distillation and extraction yields, i.e. calibration factors for all compounds F = 1.00.

Capillary Gas Chromatography–Mass Spectrometry. A Varian Aerograph 1440 gas chromatograph equipped with a Carlo Erba water-cooled on-column injection system was coupled by an open-split interface to a Finnigan MAT 44 mass spectrometer. A J & W fused silica DB-Wax capillary column (30-m length, 0.31-mm i.d.; d = 0.25 μm) connected to a 3-m uncoated piece of fused silica capillary column (retention gap) was used. The conditions were as follows: temperature, isothermal for 2.5 min at 40 °C and then from 40 to 210 °C at 5°/min; carrier gas flow rate, 2.5 mL/min He; temperature of ion source and all connection parts, 200 °C; electron energy, 70 eV; cathodic current, 0.8 mA; injection volumes, 0.3 μL.

RESULTS AND DISCUSSION

The HRGC separations of our four sample preparations are outlined in Figures 1 and 2, showing the results ob-

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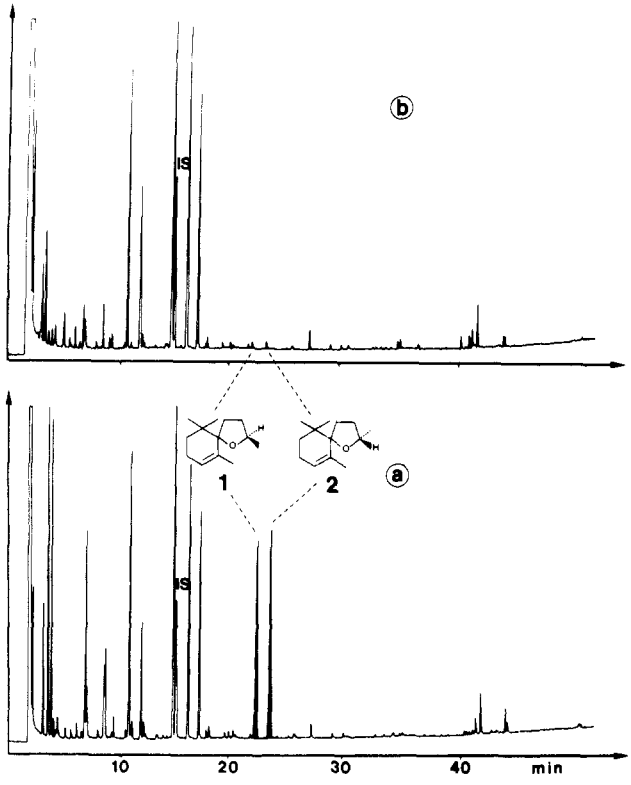

Figure 1. HRGC separation (J & W WCOT fused silica column, DB-Wax, 30-m length × 0.25-mm i.d.; df = 0.25 μm; on-column injection) of quince (*C. oblonga*, Mill.) volatiles after HVD/E sample preparation: a, pH of fruit juice (3.7); b, neutralization (pH 7.0) and enzymic inhibition.

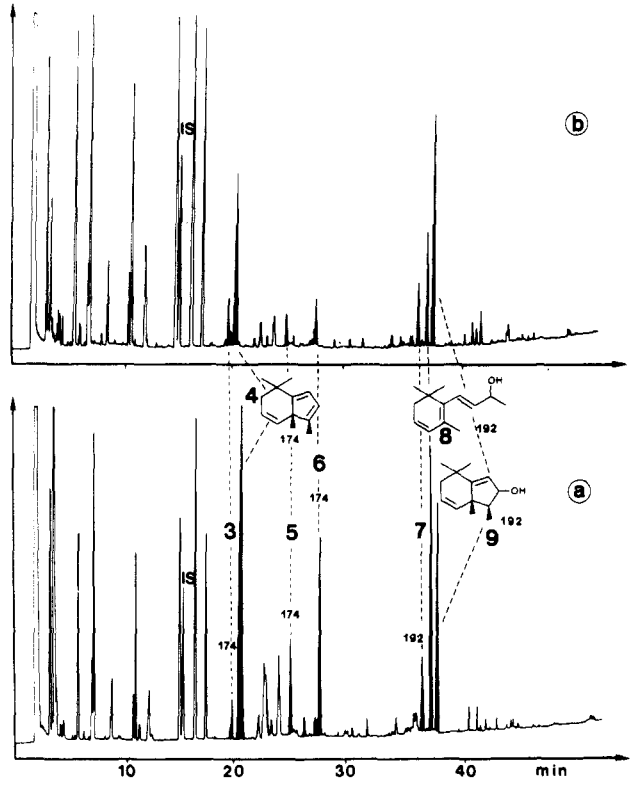

Figure 2. HRGC separation (J & W WCOT fused silica column, DB-Wax, 30-m length × 0.25-mm i.d.; df = 0.25 μm; on-column injection) of quince (*C. oblonga*, Mill.) volatiles after SDE sample preparation: a, pH of fruit juice (3.7); b, neutralization (pH 7.0) and enzymic inhibition.

tained after HVD/E and SDE each under a and b, respectively. In total, more than 150 volatiles were identified, which are not all represented here, since they have been mostly described in the previous work carried out on quince flavor (Schreyen et al., 1979; Tsuneya and Ishihara, 1983). In the following, the attention is focused on the influence of the sample preparation techniques used on the flavor compositions by means of several important flavor components numbered 1–9 in Figures 1 and 2.

From HVD/E of the natural quince fruit juice (pH 3.7) HRGC and HRGC–MS revealed the diastereoisomeric theaspiranes **1** and **2** among the major volatile constituents (each 1 mg/kg). After neutralization (pH 7.0) and enzymic inhibition of the fruit juice, these spiro ethers were only found in small amounts (each <30 μg/kg). These results show that **1** and **2** are obviously not original volatiles but were formed at natural pH (3.7) under HVD/E conditions from a nonvolatile and labile, still unknown precursor. The spiro ethers **1** and **2** have been already found in raspberries (Winter and Enggist, 1971), yellow passion fruit (Winter and Klöti, 1972), and black tea (Renold et al., 1974) as well as Osmanthus absolute (Kaiser et al., 1978), and they are used in the flavor industry to improve, e.g., tobacco, vanilla, and raspberry flavor (Skorianetz et al., 1975; Naegeli, 1977). In quince fruit oil, **1** and **2** have been previously found only in minor concentrations (Tsuneya and Ishihara, 1983).

According to the synthetic work carried out on spiro ethers by Schulte-Elte et al. (1978), 1-(2,6,6-trimethylcyclohex-1-enyl)butane-1,3-diol (**10**; Figure 3) can be considered as a natural precursor of **1** and **2**. As demonstrated by Schulte-Elte et al. (1978) **1** and **2** were formed as stable end products from **10** on strong acid treatment, whereas the dihydroedulanes **11** and **12** as well as the retro ionols **13** and **14** were obtained as unstable byproducts on mild acid treatment (Figure 3). However, in our study on quince, the potential precursor **10** could not be detected yet.

With SDE to isolate the quince juice volatiles, in experiment II-A again high amounts of theaspiranes **1** and **2** (each 0.6 mg/kg) were determined (Figure 2a) and lesser amounts (each 0.2 mg/kg) were found in the corresponding experiment II-B (Figure 2b). Additionally, as clearly shown in parts a and b in Figure 2, thermal treatment during SDE led to the formation of high quantities of a series of volatiles (3–9), which were not present under HVD/E conditions (Figure 1a,b). Among these compounds, **4**, **8** and **9** could be identified as 2,2,6,7-tetramethylbicyclo[4.3.0]-nona-4,7,9(1)-triene, 3,4-didehydro-β-ionol, and 2,2,6,7-tetramethylbicyclo[4.3.0]nona-4,9(1)-dien-8-ol, respectively, by HRGC and HRGC–MS. The compounds **3**, **5**, and **6** showed molecular weights each of 174, indicating isomeric structures of **4**, but exact structural elucidation was not successful yet. Analogously, compound **7** is obviously an isomer of **9**. In Table II, the MS and chromatographic data of quince volatiles 1–9 are summarized.

The volatiles **4**, **8**, and **9** newly formed under SDE conditions have been recently identified in steam-distilled quince fruit oil by Ishihara et al. (1986). From the results available, 3,4-didehydro-β-ionol (**8**) can be considered as a key component among the thermally formed volatiles shown in parts a and b of Figure 2. It is a thermal degradation product cleaved from a nonvolatile, still unknown precursor and, as a quite unstable compound, can be assumed, according to the findings of Ishihara et al. (1986), to be the precursor of bicyclic hydrocarbons **3**–**6**. Thus, these authors observed 80% conversion of **8** to **4** by refluxing it in acidic medium.

Finally, it has to be pointed out that the quantitative composition of quince flavor will be further changed, if instead of the juice quince pulp is used for SDE sample preparation. In this case, we found *trans*-α-farnesene as

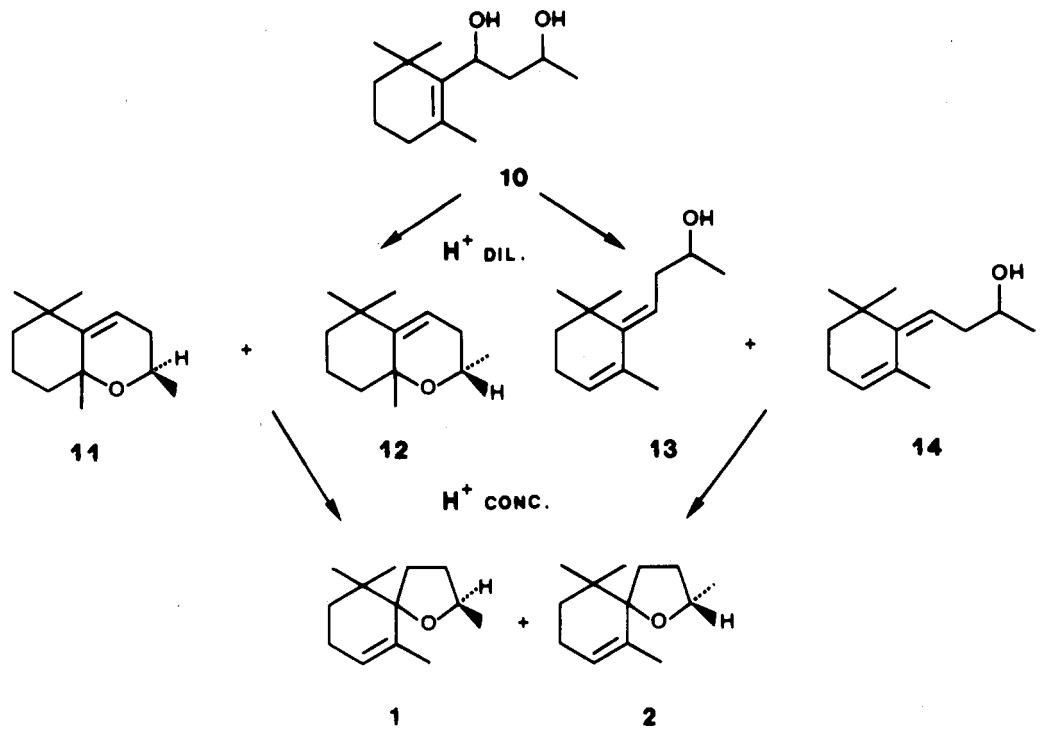

Figure 3. Chemical formation of theaspiranes 1 and 2 by strong-acid treatment of 1-(2,6,6-trimethylcyclohex-1-enyl)butane-1,3-diol (10). Dihydroedulanes 11 and 12 as well as retro ionols 13 and 14 are intermediates after weak-acid treatment of 10 (Schulte-Elte et al., 1978).

Table II. Mass Spectrometric (EI-MS, 70 eV) and Chromatographic Data (R_i; DB-Wax) of Quince Volatiles 1–9

no.[a]	compound	R_i	m/z (%)
1	theaspirane A	1514	138 (100), 82 (58), 96 (52), 43 (50), 41 (45), 55 (33), 109 (14), 123 (8)
2	theaspirane B	1550	138 (100), 82 (59), 96 (48), 41 (38), 43 (32), 55 (27), 109 (18), 123 (10)
3	isomeric to 4 (MW 174)	1436	159 (100), 131 (72), 91 (63), 117 (55), 174 (53), 118 (43), 105 (40), 77 (29)
4	2,2,6,7-tetramethylbicyclo-[4.3.0]nona-4,7,9(1)-triene	1461	159 (100), 174 (26), 131 (24), 144 (21), 129 (19), 105 (18), 91 (17), 41 (15)
5	isomeric to 4 (MW 174)	1594	159 (100), 131 (42), 117 (40), 91 (36), 174 (35), 105 (33), 129 (27), 144 (23)
6	isomeric to 4 (MW 174)	1675	159 (100), 144 (41), 174 (33), 129 (31), 128 (21), 119 (15), 91 (14), 105 (13)
7	isomeric to 9 (MW 192)	1982	43 (100), 159 (97), 41 (87), 177 (81), 136 (59), 121 (52), 119 (44), 192 (23)
8	3,4-didehydro-β-ionol	2013	119 (100), 43 (40), 192 (25), 41 (23), 91 (21), 121 (20), 159 (17), 105 (12)
9	2,2,6,7-tetramethylbicyclo-[4.3.0]nona-4,9(1)-dien-8-ol	2033	159 (100), 121 (74), 119 (58), 43 (55), 177 (51), 91 (39), 105 (38), 192 (14)

[a] Cf. Figures 1 and 2. R_i = linear retention index.

the main volatile component (12.5 mg/kg at pH 3.7 and 25 mg/kg at pH 7.0, respectively). This sesquiterpene hydrocarbon is a well-known volatile constituent of natural coatings of Rosaceae pome fruits such as apple (Huelin and Murray, 1966) and quince (Shimizu and Yoshihara, 1977; Tsuneya and Ishihara, 1983).

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