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On-line gas chromatography combustion/pyrolysis isotope ratio mass spectrometry (HRGC-C/P-IRMS) of major volatiles from pear fruit (*Pyrus communis*) and pear products

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Dedicated to Prof. Dr. H. Werner's 70th birthday

Abstract

Using extracts obtained by simultaneous distillation extraction (SDE) as well as liquid liquid extraction (LLE) of self-prepared juices from pear fruits ($n = 20$) and from commercial pear products (juices, $n = 11$; brandies, $n = 16$; baby food, $n = 8$), on-line capillary gas chromatography–isotope ratio mass spectrometry was employed in the combustion (C) and the pyrolysis (P) modes (HRGC-C/P-IRMS) to determine the $\delta^{13}C_{\text{V-PDB}}$ and $\delta^2H_{\text{V-SMOW}}$ values of major pear flavour constituents. In addition to butyl acetate **1**, 1-butanol **2**, hexyl acetate **3**, 1-hexanol **4**, as well as the 'pear esters' methyl *E,Z*-2,4-decadienoate **5**, ethyl *E,Z*-2,4-decadienaote **6**, and ethyl *E,E*-2,4-decadienoate **7**, each originating from the fruit, the $\delta^{13}C_{\text{VPDB}}$ and $\delta^2H_{\text{V-SMOW}}$ data of commercial synthetic and "natural" (biotechnologically derived) **1**–**7** were determined.
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1. Introduction

Early studies reported the identification of the volatiles responsible for the Bartlett pear aroma by gas chromatography in essences collected without loss of aroma characteristics (Heinz & Jennings, 1966; Jennings & Sevenants, 1964). Hexyl acetate and methyl *E,Z*-2,4-decadienoate, as major components, were identified by infrared spectroscopy. The former was described as a contributory flavour compound, and the latter a character impact compound. In addition, ethyl *E,Z*-2,4-decadienoate was identified as a typical Bartlett pear flavour constituent. Subsequent studies of several cultivars, employing gas chromatography, HPLC and sensory evaluation, have shown that high concentrations of 2,4-decadienoates are characteristic, not only for Bartlett pear, but also for other pear cultivars. Apart from these flavour constituents, many others have been identified over the years (Paillard, 1990). The data have been collected in the known list of volatile compounds of food (VCF & Volatile Compounds in Food Database, 1996–1999).

In spite of the industrial interest, information about the authentication of pear is rather scarce. The mentioned 2,4-decadienoates and, at least for several cultivars, octane-1,3-diol and 5(*Z*)-octane-1,3-diol are characteristic pear constituents (Beuerle & Schwab, 1997); however, the former can easily be synthesized

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and the latter is also present in some apple cultivars. Thus, simple detection of 'key compounds' is not sufficient for authentication purposes. Stable isotope analysis is the method of choice (Rossmann & Schmidt, 2000; Schmidt, Rossmann, & Werner, 1998) but, as far as we know, no information is yet available about isotope data of pear. In this paper, we report, for the first time, $\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$ data of volatiles from pear fruits and products made from them.

2. Materials and methods

2.1. Samples

Pear fruits ($n = 20$) from 7 regions (Argentina, Belgium, China, Germany, Italy, South Africa, Spain) were obtained by fruit distributors. Commercial pear products, i.e. juices ($n = 11$), brandies ($n = 16$), and baby foods ($n = 8$) were purchased from local supermarkets. Synthetic and biotechnologically derived ("natural") samples of butyl acetate **1**, 1-butanol **2**, hexyl acetate **3**, 1-hexanol **4**, methyl *E,Z*-2,4-decadienoate **5**, ethyl *E,Z*-2,4-decadienaote **6**, and ethyl *E,E*-2,4-decadienoate **7** were purchased from Acros (Geel, Belgium), Avocado (Karlsruhe, Germany), Fluka (Deisenhofen, Germany), Sigma–Aldrich (Steinheim, Germany), Silesia (Neuss, Germany), Quest (Naarden, Netherlands), Degussa (Düsseldorf, Germany), Merck (Darmstadt, Germany), ICN (Eschwege, Germany) and Lancaster (Frankfurt a. M., Germany). Some of the "natural" samples were kindly provided by the flavour industry.

Chemicals were purchased from Sigma–Aldrich (Steinheim, Germany). Solvents were redistilled before use.

2.2. Sample preparation

Synthetic and "natural" reference samples **1–7** were dissolved (1 mg/ml) in diethyl ether and the solutions directly analyzed by HRGC–MS and HRGC-C/P-IRMS.

After crushing and homogenizing (3000 g), fresh pears were subjected to simultaneous distillation extraction (SDE) (2 h) using pentane–diethyl ether mixture (1 + 1, v/v). The same quantity was used for the SDE separation of volatiles from baby food samples (1000 g). Commercial juices, as well as brandies (700 ml), were diluted with distilled water and liquid–liquid extraction (LLE), using pentane dichromethane (2 + 1, v/v) at 40 °C, was performed for 24 h.

Model experiments comprised SDE and LLE of solutions of **1–7** (each 50 mg in 800 ml of water).

All of the extracts obtained by SDE were dried over anhydrous sodium sulphate, filtered, and carefully concentrated to approximately 1 ml using a Vigreux column (45 °C).

2.3. Gas chromatography–mass spectrometry

An HP Agilent 6890 Series gas chromatograph with split injection (220 °C; 1:20) was directly coupled to an HP Agilent 5973 Network mass spectrometer (Agilent Technologies Inc., CA). The flavour compounds were separated on a J&W DB-Wax fused silica capillary column (30 m × 0.25 mm i.d., df = 0.25 μm). The temperature programme was as follows: 3 min isothermal at 50 °C, then raised at 4 °C/min to 220 °C. Identification was performed by comparison of linear retention indices and mass spectral data of sample constituents with that of authentic reference compounds.

2.4. Gas chromatography–isotope ratio mass spectrometry

A Finnigan Delta plus XL isotope ratio mass spectrometer coupled by an open-split via a combustion/pyrolysis (C/P) interface to an HP 6890 gas chromatograph (GC) was used. The GC was equipped with an J & W DB-Wax fused silica capillary column (60 m × 0.32 mm i.d., df = 0.25 μm). A 1 μl "splitless" injection (250 °C) was employed. The temperature programme was 50–220 °C at 5 °C/min and helium flow of 2 ml/min was used.

Combustion was performed by oxidative reactor (Al_2O_3, 0.5 mm internal diameter, 1.5 mm outer diameter, 320 mm length) with Cu, Ni, and Pt (each 240 mm × 0.125 mm) to CO_2 at 960 °C; water separation was by Nafion membrane.

For pyrolysis to H_2 the effluent from the GC passed through a ceramic tube (Al_2O_3, 0.5 mm internal diameter, 320 mm length) at 1440 °C.

In addition, coupling of elemental analyzers (EA) ($^{13}C/^{12}C$, Euro Vector EA 3000, Milano, Italy; temperature, 1000 °C; $^2H/^1H$, HT Sauerstoff analysator, HEKA-Tech, Wegberg, Germany; temperature, 1460 °C) to the IRMS was realized for off-line control determination of reference samples.

Daily system stability checks were carried out by measuring reference samples with known $^{13}C/^{12}C$ and $^2H/^1H$ ratios. Stability checking of the used reference gases was continuously performed by measuring International Atomic Energy Agency (IAEA, Vienna, Austria) standards with defined $^{13}C/^{12}C$ and $^2H/^1H$ ratios (for $^{13}C/^{12}C$ IAEA-CH-7 and for $^2H/^1H$ IAEA-CH-7, NBS 22 oil, and V-SMOW).

The isotope ratios were expressed per thousand (‰) deviation relative to the V-PDB and V-SMOW international standards. Results were expressed in δ values as follows (for $\delta^2H_{V\text{-}SMOW}$, corresponding formula is valid):

$$\delta^{13}C_{V-PDB}[\text{‰}] = \left(\frac{(^{13}C/^{12}C)_{sample}}{(^{13}C/^{12}C)_{V-PDB}} - 1 \right) * 1000.$$

For $^{13}C/^{12}C$ and $^2H/^1H$ measurements, calibration of the IRMS was done with certified CO_2 ($\delta^{13}C_{V-PDB}$ declared $= -24.90 \pm 0.2\%$) and H_2 (δ^2H_{V-SMOW} declared $= -270 \pm 10\%$) reference gases (Messer Griesheim, Frankfurt, Germany).

In general, sixfold determinations were carried out and standard deviations calculated. The latter were ± 0.1 and ± 5 for $\delta^{13}C_{V-PDB}$ and δ^2H_{V-SMOW} determinations, respectively. Additional peak recognition was performed by reference compounds, and HRGC–MS registered under separation conditions identical to samples.

As it is well-known that determinations in the P-IRMS mode depend on the structure and the quantity of samples to be analyzed (Bilke & Mosandl, 2002; Hör, Ruff, Weckerle, König, & Schreier, 2001), evaluation of the linear dynamic range for each target compound was required, in order to achieve reproducibility and linearity of data. The δ^2H_{V-SMOW} linear dynamic ranges of reference substances under study were determined via EA using different sample amounts. For the compounds **2–3**, as well as **5–7**, linearity ranges were found to be 0.5–4 µg, whereas linearities for **1** and **4** were in the range of 1–4.5 µg and 1.5–3.0 µg, respectively.

To determine the δ^2H_{V-SMOW} values of compounds **1–7**, the system reliability had further to be proven by measuring commercial references "off-line" via the equipped elemental analyser (EA) (Hör et al., 2001). Comparison of the data recorded by EA-P-IRMS revealed good agreement with that determined by HRGC-P-IRMS analysis (data not shown).

Finally, the influence of SDE or LLE sample preparation on the $^2H/^1H$ isotope ratio, checked by model SDE and LLE separations, was found to be within the range of standard deviation and thus negligible (data not shown).

3. Results and discussion

3.1. General

Among the more than 150 volatiles identified by HRGC–MS in pear fruit, only the major ones were accessible to on-line HRGC–IRMS studies. As a representative example, in Fig. 1 the HRGC–MS separation of main pear volatiles with the numbered target compounds, **1–7**, is shown. The amount of one of the "pear
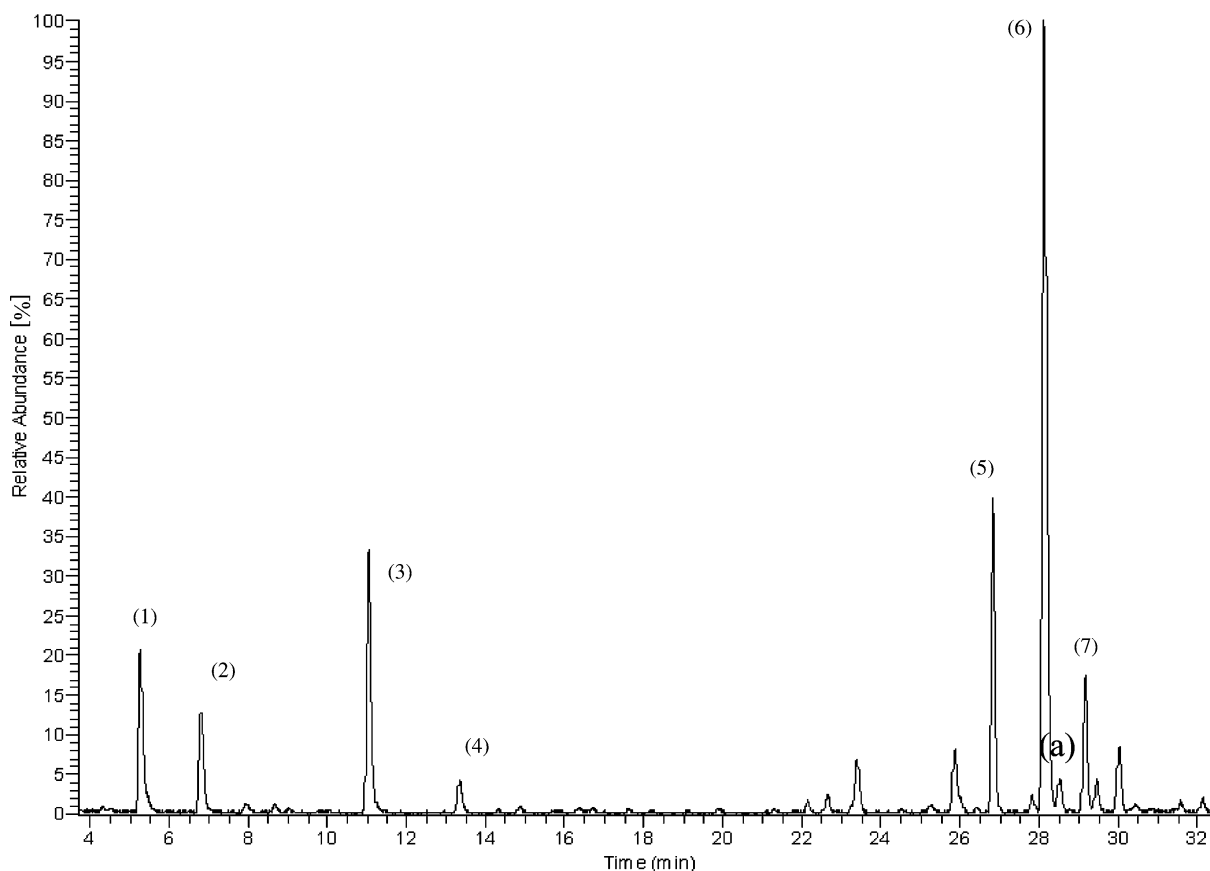

Fig. 1. HRGC–MS separation of pear fruit volatiles. The peak numbers indicate the compounds under IRMS study. (**1**) butyl acetate, (**2**) 1-butanol, (**3**) hexyl acetate, (**4**) 1-hexanol, (**5**) methyl *E,Z*-2,4-decadienoate, (**6**) ethyl *E,Z*-2,4-decadienoate, (**7**) ethyl *E,E*-2,4-decadienoate, (a) methyl *E,E*-2,4-decadienoate.

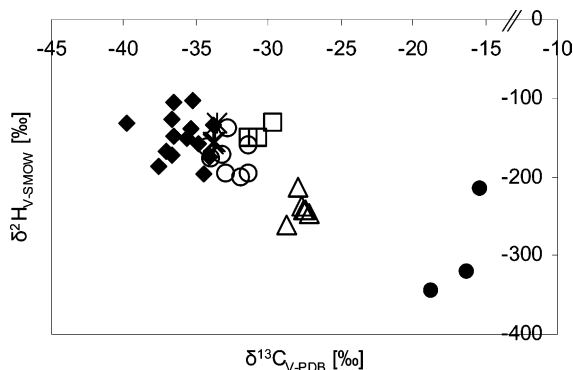Fig. 2. Correlation of $\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$ values (‰) of butyl acetate **1** from pear fruit (♦), pear juice (○), baby food (∗), brandy (△), as well as natural references (●) and synthetic references (□). Standard deviations: ±0.1‰ and ±5‰ for $\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$ determinations, respectively.

Fig. 3. Correlation of $\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$ values (‰) of 1-butanol **2** from pear fruit (♦), pear juice (○), baby food (∗), brandy (△), as well as natural references (●) and synthetic references (□). Standard deviations: ±0.1‰ and ±5‰ for $\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$ determinations, respectively.

esters", i.e. methyl *E,E*-2,4-decadienoate (a in Fig. 1), was too low to be analyzed by HRGC–IRMS.

3.2. Butyl acetate 1

The $\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$ values determined for **1** in pear fruits and pear products are graphically outlined in Fig. 2. Pear fruit ($n = 14$) showed $\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$ data ranging from −33.8‰ to −39.8‰, as well as −104‰ to −197‰, respectively. Baby food ($n = 4$) gave almost the same IRMS data as found for **1** from fruits. Pear juice ($n = 8$) exhibited $\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$ values ranging from −31.3‰ to −34.2‰ and −138‰ to −200‰, respectively. Brandies ($n = 6$) differed from the pear fruit values regarding both the $\delta^{13}C_{V\text{-}PDB}$ values (−27.2‰ to −28.7‰) and the $\delta^2H_{V\text{-}SMOW}$ values (−213‰ to −262‰). The $\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$ values of synthetic references ($n = 3$), ranging from −29.7‰ to −31.3‰ and from −131‰ to −152‰, respectively, did not clearly differ from the fruit data but did from the "natural" references ($n = 3$; −15.3‰ to −18.7‰ as well as −215‰ to −346‰ for $^{13}C/^{12}C$ and $^2H/^1H$, respectively). Obviously, the latter were produced from a C4 source. In the literature (Karl, Dietrich, & Mosandl, 1994), $\delta^{13}C_{V\text{-}PDB}$ values of −34.6‰ to −36.9‰ for different apple varieties were reported; thus they are in good agreement with the data determined for pear fruits.

3.3. 1-Butanol 2

The correlation of $\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$ data of **2** from different sources is displayed in Fig. 3. $\delta^2H_{V\text{-}SMOW}$ data for **2** from pear fruit ($n = 18$) ranged from −50‰ to −165‰ ; the $\delta^{13}C_{V\text{-}PDB}$ data varied from −37.1‰ to −41.8‰. The IRMS data recorded for **2** from the pear juices ($n = 6$) were in agreement with the
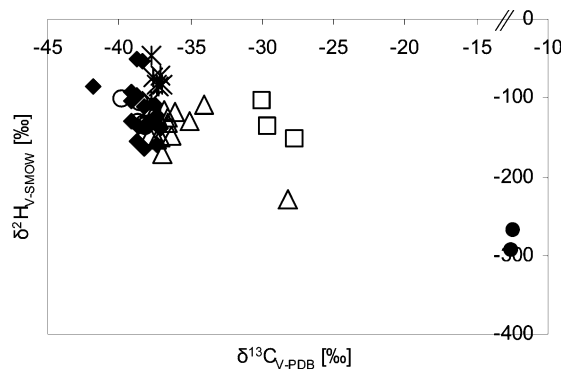
values recorded for pear fruit. Similar data were obtained for baby food ($n = 7$; $\delta^{13}C_{V\text{-}PDB}$ from −37.0‰ to −37.8‰ and $\delta^2H_{V\text{-}SMOW}$ from −46‰ to −103‰). Brandies showed $\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$ values ranging from −28.2‰ to −37.9‰ and from −109‰ to −230‰, respectively. The graph shows distinct differences between synthetic ($n = 3$; $\delta^{13}C_{V\text{-}PDB}$ from −27.7‰ to −30.0‰ and $\delta^2H_{V\text{-}SMOW}$ from −105‰ to −153‰) and "natural" references ($n = 2$; $\delta^{13}C_{V\text{-}PDB}$ from −12.4‰ to −12.6‰ and $\delta^2H_{V\text{-}SMOW}$ from −269‰ to −294‰). Again, the $\delta^{13}C_{V\text{-}PDB}$ values of the latter clearly indicate their origin from a C4 source. Previously, other groups have published $\delta^{13}C_{V\text{-}PDB}$ values from −13.3‰ to −28.3‰ and $\delta^2H_{V\text{-}SMOW}$ data from −155‰ to −290‰ for "natural" **2** ($n = 6$) as well as −27.8‰ and −138‰ for $^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$ for synthetic **2** ($n = 1$) (Krammer et al., 2001), as well as $\delta^{13}C_{V\text{-}PDB}$ values of −23.6‰, again for synthetic **2** ($n = 2$) (Mosandl, Hener, & Schmarr, 1990; Rautenschlein, Habfast, & Brand, 1991).

3.4. Hexyl acetate 3

The correlation of $\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$ data of **3** from various origins is outlined in Fig. 4. The graph shows distinct differences between pear fruit ($n = 16$; $\delta^{13}C_{V\text{-}PDB}$ from −29.7‰ to −38.3‰ and $\delta^2H_{V\text{-}SMOW}$ from −48‰ to −157‰) and "natural" references ($n = 7$; $\delta^{13}C_{V\text{-}PDB}$ from −28.0‰ to −35.9‰ and $\delta^2H_{V\text{-}SMOW}$ from −208‰ to −281‰). The synthetic references ($n = 2$) exhibited $\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$ values of −26.2‰ to −26.7‰ and −83‰ to −93‰, respectively. The pear juice showed $\delta^{13}C_{V\text{-}PDB}$ (−28.3‰) and $\delta^2H_{V\text{-}SMOW}$ (−218‰) values corresponding to the data of the "natural" references. No values could be measured for the brandies, owing to coelution of **3** with other substances. In the literature, IRMS values for **3** from apple varieties of $\delta^{13}C_{V\text{-}PDB}$ from −33.4‰ to −36.8‰ ($n = 8$)
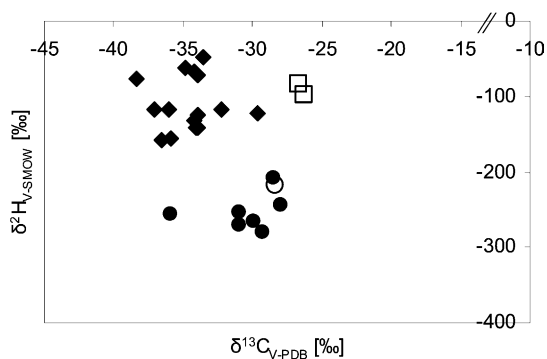
Fig. 4. Correlation of $\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$ values (‰) of hexyl acetate **3** from pear fruit (♦), pear juice (○), as well as natural references (●) and synthetic references (□). Standard deviations: ±0.1‰ and ±5‰ for $\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$ determinations, respectively.

have been given (Karl et al., 1994). Others have reported $\delta^{13}C_{V\text{-}PDB}$ data for nature-identical **3** from −31‰ up to −36‰ and, for natural **3**, from −27‰ to −34‰ (Schmidt & Gleixner, 1998).

3.5. 1-Hexanol 4

In Fig. 5, the $^{13}C/^{12}C$ and $^2H/^1H$ ratios determined for **4** in various samples are graphically correlated. Pear fruit ($n = 3$) showed $\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$ data ranging from −36.9‰ to −42.7‰ and −134‰ to −193‰, respectively. For pear juice ($n = 7$; $\delta^{13}C_{V\text{-}PDB}$ from −38.0‰ to −40.6‰ and $\delta^2H_{V\text{-}SMOW}$ from −145‰ to −212‰), IRMS data similar to pear fruit were obtained. The $\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$ values of brandies ($n = 13$) ranged from −34.3‰ to −39.1‰ and from −80‰ to −230‰, respectively. For $\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$, distinct differences were recorded between synthetic ($n = 11$; $\delta^{13}C_{V\text{-}PDB}$ from −25.0‰ to −27.0‰ and $\delta^2H_{V\text{-}SMOW}$ from −63‰ to −97‰) and "natural" references ($n = 8$; $\delta^{13}C_{V\text{-}PDB}$ from −29.3‰

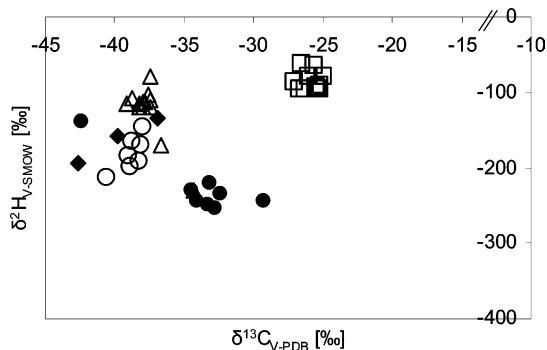Fig. 5. Correlation of $\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$ values (‰) of 1-hexanol **4** from pear fruit (♦), pear juice (○), brandy (△), as well as natural references (●) and synthetic references (□). Standard deviations: ±0.1‰ and ±5‰ for $\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$ determinations, respectively.

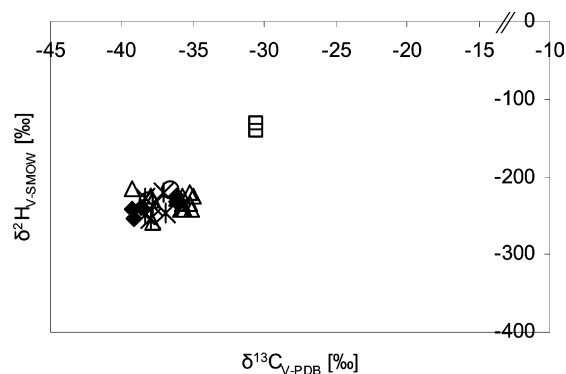
Fig. 6. Correlation of $\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$ values (‰) of methyl E,Z-2,4-decadienoate **5** from pear fruit (♦), pear juice (○), baby food (∗), brandy (△), as well as synthetic references (□). Standard deviations: ±0.1‰ and ±5‰ for $\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$ determinations, respectively.

to −42.4 ‰ and $\delta^2H_{V\text{-}SMOW}$ from −140‰ to −255‰). One of these references, declared as "natural", exhibited $\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$ values (−42.4‰ and −140‰) in agreement with the data from **4** originating from pear fruits. The authenticity of this sample was unknown. One of the brandy samples under study showed $\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$ data in the range of "natural" references.

3.6. Methyl E,Z-2,4-decadienoate 5

The correlation of $\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$ data of **5** from different sources is shown in Fig. 6. For **5** from pear fruit ($n = 6$), ranges from −36.1‰ to −39.3‰ and from −224‰ to −255‰ were recorded for $^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$, respectively. The values registered for both brandies ($n = 14$; −35.0 to −39.3‰ and −215‰ to −258‰ for $^{13}C/^{12}C$ and $^2H/^1H$, respectively) and baby food ($n = 6$; −36.9‰ to −38.3‰ and −221‰ to −254‰ for $^{13}C/^{12}C$ and $^2H/^1H$, respectively) were in agreement with the IRMS data from the pear fruit and that from pear juice ($n = 2$; $\delta^{13}C_{V\text{-}PDB}$ from −36.6‰ to −38.1‰ and $\delta^2H_{V\text{-}SMOW}$ from −219‰ to −233‰). Synthetic references ($n = 2$), exhibiting $\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$ values of −30.6‰ and −131‰ to −142‰, respectively, clearly differed from those of pear fruits and pear products.

3.7. Ethyl E,Z-2,4-decadienoate 6

$\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$ values of **6**, as determined in various sources, are correlated in Fig. 7. $\delta^2H_{V\text{-}SMOW}$ data for **6** from pear fruit ($n = 10$) ranged from −187‰ to −236‰; the $\delta^{13}C_{V\text{-}PDB}$ data varied from −34.3‰ to −38.4‰. The IRMS data recorded for **2** from baby food ($n = 7$) and the pear juice ($n = 1$) were in agreement with the values recorded for the pear fruits. Brandies ($n = 15$) exhibited $\delta^{13}C_{V\text{-}PDB}$ and $\delta^2H_{V\text{-}SMOW}$

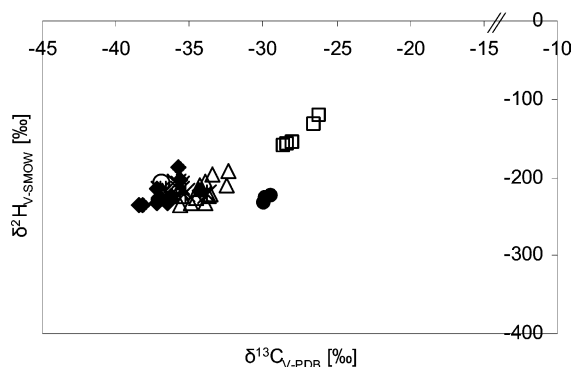Fig. 7. Correlation of $\delta^{13}C_{V-PDB}$ and δ^2H_{V-SMOW} values (‰) of ethyl E,Z-2,4-decadienoate **6** from pear fruit (♦), pear juice (○), baby food (∗), brandy (△), as well as natural references (●) and synthetic references (□). Standard deviations: ±0.1‰ and ±5‰ for $\delta^{13}C_{V-PDB}$ and δ^2H_{V-SMOW} determinations, respectively.

values ranging from −32.4‰ to −36.8‰ and −193‰ to −236‰, respectively. The graph shows distinct differences between pear fruit and synthetic references ($n = 5$; $\delta^{13}C_{V-PDB}$ from −26.2‰ to −28.6‰ and δ^2H_{V-SMOW} from −120‰ to −159‰). For "natural" references ($n = 3$), values for $\delta^{13}C_{V-PDB}$ ranging from −29.5‰ to −29.8‰ and for δ^2H_{V-SMOW} from −225‰ to −232‰ were measured.

3.8. Ethyl E,E-2,4-decadienoate 7

The correlation of $\delta^{13}C_{V-PDB}$ and δ^2H_{V-SMOW} data of **7** from different origins is outlined in Fig. 8. Pear fruit ($n = 2$) showed $\delta^{13}C_{V-PDB}$ and δ^2H_{V-SMOW} data ranging from −41.0‰ to −41.9‰ as well as −198‰ to −234‰, respectively. Baby food ($n = 2$) varied from −33.8‰ to −41.9‰ for $\delta^{13}C_{V-PDB}$ and from −203‰ to −232‰ for δ^2H_{V-SMOW}. The values for brandies ($n = 13$; −36.4‰ to −40.7‰ and −194‰ to −239‰ for $^{13}C/^{12}C$ and $^2H/^1H$, respectively) were in agreement with the IRMS data from the pear fruit and baby food.
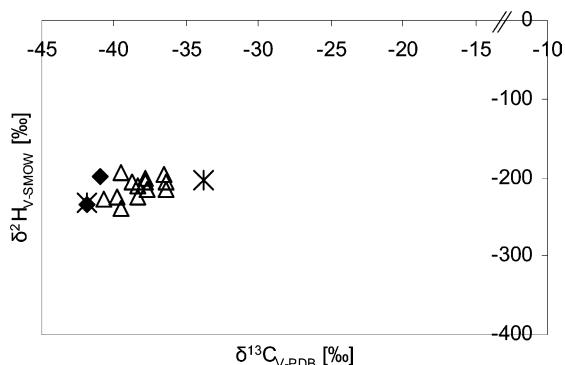

Fig. 8. Correlation of $\delta^{13}C_{V-PDB}$ and δ^2H_{V-SMOW} values (‰) of ethyl E,E-2,4-decadienoate **7** from pear fruit (♦), baby food (∗) and brandy (△). Standard deviations: ±0.1‰ and ±5‰ for $\delta^{13}C_{V-PDB}$ and δ^2H_{V-SMOW} determinations, respectively.

4. Conclusions

Summarizing, the HRGC–IRMS technique provided distinct authentication of **1–7** originating from synthetic to "ex-fruit" sources. Interestingly, the isotope data of brandies were found to be enriched. Such inverse isotope effects have already been observed many years ago in the course of liquid-vapour transformation of alcohols (Moussa, Naulet, Martin, & Martin, 1990). For some compounds under study, literature data were available; they were in good agreement with data measured for them in pear fruits and products thereof.

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