On-Line Gas Chromatography Combustion/Pyrolysis Isotope Ratio Mass Spectrometry (HRGC-C/P-IRMS) of Pineapple (*Ananas comosus* L. Merr.) Volatiles

Christina Preston, Elke Richling, Sandra Elss, Markus Appel, Frank Heckel, Ariane Hartlieb, and Peter Schreier*

Lehrstuhl für Lebensmittelchemie, Universität Würzburg, Am Hubland, 97074 Würzburg, Germany

By use of extracts prepared by liquid−liquid separation of the volatiles from self-prepared juices of pineapple fruits (*Ananas comosus*) ($n = 14$) as well as commercial pineapple recovery aromas/ water phases ($n = 3$), 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 $\delta^{13}C_{VPDB}$ and δ^2H_{VSMOW} values of selected pineapple flavor constituents. In addition to methyl 2-methylbutanoate **1**, ethyl 2-methylbutanoate **2**, methyl hexanoate **3**, ethyl hexanoate **4**, and 2,5-dimethyl-4-methoxy-3[2H]-furanone **5**, each originating from the fruit, the $\delta^{13}C_{VPDB}$ and δ^2H_{VSMOW} data of commercial synthetic **1**−**5** and "natural" (biotechnologically derived) **1**−**4** were determined. With $\delta^{13}C_{VPDB}$ data of pineapple volatiles **1**−**4** varying from −12.8 to −24.4‰, the range expected for CAM metabolism was observed. Compound **5** showed higher depletion from −20.9 to −28.6‰. A similar situation was given for the δ^2H_{VSMOW} values of **3**−**5** from pineapple ranging from −118 to −191‰, whereas **1** and **2** showed higher depleted values from −184 to −263‰. In nearly all cases, analytical differentiation of **1**−**5** from pineapple and natural as well as synthetic origin was possible. In general, natural and synthetic **1**−**5** exhibited $\delta^{13}C_{VPDB}$ data ranging from −11.8 to −32.2‰ and −22.7 to −35.9‰, respectively. Their δ^2H_{VSMOW} data were in the range from −242 to −323‰ and −49 to −163‰, respectively.

INTRODUCTION

The pineapple plant (*Ananas comosus,* Bromeliaceae) originates from Brazil, and approximately 100 pineapple fruit varieties are known worldwide (*1*). Thailand is the world's largest producer of fresh and also canned pineapples. Thai pineapples account for nearly 18% of the global market, followed by the Philippines (14%) and Brazil (13%) (*2*). The pineapple fruit aroma has already been studied extensively, leading to the identifcation of the classical "pineapple furanone" 2,5-dimethyl-4-hydroxy-3[2H]-furanone (furaneol) (*3*, *4*), esters such as methyl and ethyl 2-methylbutanaote, methyl hexanoate, methyl and ethyl 3-(methylthio)propanoate, as well as several hydroxy and acetoxy esters and γ−lactones as "key constituents" (*5*−*11*).

Little information is available about isotope data of pineapple; it is limited to $^{13}C/^{12}C$ and $^2H/^1H$ ratios of sugars and leaf extracts (*12*−*14*). The $\delta^{13}C$ of organic material is largely determined by the carbon isotope discrimination of ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) (C3 plants) and phosphoenolpyruvate carboxylase (PEPC) (C4 plants), and $\delta^{13}C$ of organic material can be interpreted to a good approximation in terms of the carbon isotope discrimination of these enzymes (*15*). Pineapple performs the crassulacean acid metabolism (CAM), usually associated with succulent plants in arid habitats (i.e., the CO_2 concentrating mechanism operates by sequentially reducing CO_2 into carbohydrates at two different periods). The initial reduction of CO_2 into a four-carbon acid is done at night− when CAM plant stomata are open−by PEPC. Then, during the day, when CAM plant stomata are closed, the four-carbon sugar is decarboxylated, increasing the plants intercellular CO_2 concentration, and the resulting CO_2 is subsequently reduced back into carbohydrates, but this time by Rubisco. As a consequence, $^{13}C/^{12}C$, and in part, $^2H/^1H$ ratios of organic material differ between CAM and C3/C4 plants.

In this paper, we report for the first time $\delta^{13}C_{VPDB}$ and δ^2H_{VSMOW} data of several pineapple fruit volatiles.

MATERIALS AND METHODS

Samples. Fresh pineapple fruits ($n = 13$) from seven regions (Costa Rica, Ghana, Honduras, Ivory Coast, La Réunion, South Africa, Thailand) were purchased from fruit distributors and provided by the

* To whom correspondence should be addressed. Fax: +49-931-8885484. E-mail: schreier@pzlc.uni-wuerzburg.de.

industry. Pineapple recovery aromas/water phases ($n = 3$) were kindly provided by the Schutzgemeinschaft der Fruchtsaftindustrie e.V. (SGF), Nieder-Olm, Germany, and flavor houses. Synthetic and biotechnologically derived (natural) samples of methyl 2-methylbutanoate (**1**), ethyl 2-methylbutanoate (**2**), methyl hexanoate (**3**), ethyl hexanoate (**4**), 2,5-dimethyl-4-methoxy-3-[2H]-furanone (**5**), and 2,5-dimethyl-4-hydroxy-3[2H]-furanone (furaneol) were purchased from Fluka (Deisenhofen, Germany), Roth (Karlsruhe, Germany), Sigma-Aldrich (Steinheim, Germany), Acros (Geel, Belgium), and Avocado (Karlsruhe, Germany). A part of the natural samples (**1**−**4**) was kindly provided by SAM GmbH, Mannheim. All other chemicals were purchased from Sigma-Aldrich (Steinheim, Germany). Solvents were redistilled before use.

Sample Preparation. Synthetic and natural reference samples **1**−**5** and furaneol were dissolved (1 mg/mL) in diethyl ether and the solutions directly analyzed by HRGC-MS and HRGC-C/P-IRMS.

After peeling, crushing, homogenizing, and centrifuging (3000g), the pineapple juice (average yield 70%) was subjected to liquid−liquid extraction (LLE) for 48 h using pentane/dichloromethane mixture (2:1, v/v). Recovery aromas/water phases were directly subjected to LLE.

Model experiments comprised LLE (pentane/dichloromethane, 2:1, v/v) of solutions of **1**−**5** (each 50−100 mg in 800 mL of water, respectively).

All of the extracts were dried over anhydrous sodium sulfate, filtered, and carefully concentrated to approximately 1 mL using a Vigreux column (45 °C).

Gas Chromatography-Mass Spectrometry (HRGC-MS). 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 flavor compounds were separated on a J&W DB-Wax fused silica capillary column (30 m × 0.25 mm, df= 0.25 μm). The temperature program was as follows: 3 min isothermal at 50 °C, then raised at 4 °C/min. Identification was performed by comparison of linear retention indices and mass spectral data of sample constituents with that of authentic reference compounds.

Gas Chromatography-Isotope Ratio Mass Spectrometry (HRGC-IRMS). 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). The following conditions were employed: A 1-μL "splitless" injection (250 °C). Temperature programs: 5 min isothermal at 40 °C, then raised to 200 °C at 2 °C/min (for **1** and **2**); 50−220 °C at 5 °C/min (for **3**−**5**); helium flow, 2 mL/min.

Interfaces. ^{13}C/^{12}C: Combustion by oxidative reactor (Al$_2$O$_3$, 0.5 mm i.d., 1.5 mm o.d., 320 mm) with Cu, Ni, and Pt (each 240 mm × 0.125 mm) to CO$_2$ at 960 °C; water separation by Nafion membrane.

*Pyrolysis.* ^{2}H/^{1}H: The effluent from the GC passes through a ceramic tube (Al$_2$O$_3$; 0.5 mm i.d., 320 mm) for pyrolysis to H$_2$ at 1440 °C.

In addition, coupling elemental analyzers (EA) (^{13}C/^{12}C, Euro Vector EA 3000, Milano, Italy; temperature, 1000 °C; ^{2}H/^{1}H, HT Sauerstoff-Analysator, HEKATech, 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 ^{2}H/^{1}H ratios. Stability check 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 ^{2}H/^{1}H ratios (for ^{13}C/^{12}C IAEA-CH-7 and for ^{2}H/^{1}H IAEA-CH-7, NBS 22 oil, and VSMOW).

The isotope ratios are expressed in per mil (‰) deviation relative to the VPDB and VSMOW international standards. For ^{13}C/^{12}C determinations, the mass spectrometer was calibrated against reference CO$_2$ gas (Messer Griesheim, Frankfurt, Germany) with a defined δ^{13}C$_{VPDB}$ = −24.9‰. Results are expressed in δ^{13}C$_{VPDB}$ values as

$$\delta^{13}C_{VPDB} \, [‰] = \left(\frac{R_{sample} - R_{VPDB}}{R_{VPDB}} \right) \cdot 1000$$

where R is the isotope ratio ^{13}C/^{12}C.
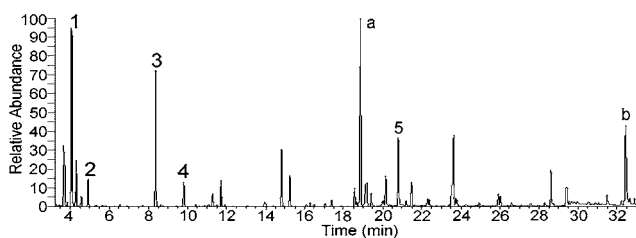
Figure 1. HRGC-MS separation of pineapple fruit (Victoria, La Réunion) volatiles. The peak numbers indicate the compounds under IRMS study. (**1**) methyl 2-methylbutzanoate, (**2**) ethyl 2-methylbutanoate, (**3**) methyl hexanoate, (**4**) ethyl hexanoate, (**5**) 2,5-dimethyl-4-methoxy-3[2H]-furanone, (a) methyl (3-methylthio)propanoate, (b) 2,5-dimethyl-4-hydroxy-3[2H]-furanone.

Table 1. δ^{2}H$_{VSMOW}$ Values (‰)^a of Synthetic Compounds **1-5** Recorded by P-IRMS via EA (A) and On-Line HRGC-P-IRMS (Single, B1; After Model Sample Preparation LLE, B2)

compound	δ^{2}H$_{VSMOW}$ [‰] ± sd		
	A	B1	B2
methyl 2-methylbutanoate (**1**)	−49 ± 3	−55 ± 4	−58 ± 1
ethyl 2-methylbutanoate (**2**)	−270 ± 1	−268 ± 3	−279 ± 1
methyl hexanoate (**3**)	−86 ± 2	−97 ± 3	−80 ± 2
ethyl hexanoate (**4**)	−115 ± 3	−105 ± 4	−105 ± 1
2,5-dimethyl-4-methoxy-3[2H]-furanone (**5**)	−103 ± 1	−116 ± 1	−103 ± 5

^{a} Mean values of six determinations and standard deviation (sd).

The isotope ratios for ^{2}H/^{1}H are expressed in per mil (‰) deviation relative to the Vienna standard mean ocean water (VSMOW) international standard. The mass spectrometer was calibrated against reference H$_2$ gas (Messer Griesheim, Frankfurt, Germany) with a defined δ^{2}H$_{VSMOW}$ = −270 ± 10‰.

Results are expressed in δ^{2}H$_{SMOW}$ units as

$$\delta^{2}H_{VSMOW}[‰] = \left(\frac{R_{sample} - R_{VSMOW}}{R_{VSMOW}} \right) \cdot 1000$$

where R = isotope ratio ^{2}H/^{1}H.
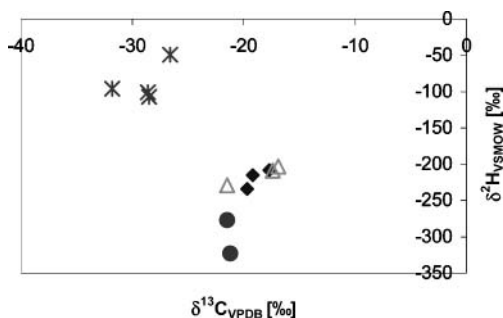
In general, 6-fold determinations were carried out and standard deviations calculated. The latter were ± 0.1 and ± 5‰ for δ^{13}C$_{VPDB}$ and δ^{2}H$_{VSMOW}$ determinations, respectively. Additional peak recognition was performed by reference compounds, and HRGC-MS registered under identical separation conditions as samples.

To determine the δ^{2}H$_{VSMOW}$ values of the pineapple volatiles **1**−**5**, the system reliability had to be proven by measuring commercial references "off-line" via the equipped elemental analyzer (EA) (*16*). Comparison of the data recorded by EA-C/P-IRMS revealed good agreement with that determined by HRGC-C/P-IRMS analysis (**Table 1**). The areas of linearity for the δ^{2}H$_{VSMOW}$ determination were from 0.5 to 4.5 μg and 2.0 to 4.5 μg (each on column) for compounds **1**−**4** and **5**, respectively.

The influence of sample preparation on the ^{2}H/^{1}H isotope ratio checked by model LLE separation was found to be negligible (**Table 1**).

RESULTS AND DISCUSSION

Among the more than 130 volatiles identified by HRGC-MS in pineapple fruit juices and recovery aromas/water phases, only a few major ones were accessible to on-line HRGC-IRMS studies. As a representative example, **Figure 1** shows the separation of pineapple juice volatiles with the numbered compounds **1**−**5** under study. Two of the major pineapple constituents, namely methyl (3-methylthio)propanoate and 2,5-dimethyl-4-hydroxy-3[2H]-furanone (furaneol) (a and b in

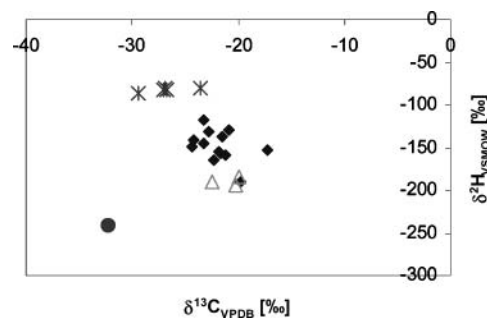Stable Isotope Analysis of Pineapple Volatiles

J. Agric. Food Chem., Vol. 51, No. 27, 2003 **8029**

Figure 2. Correlation of $\delta^{13}C_{VPDB}$ and δ^2H_{VSMOW} values (‰) of methyl 2-methylbutanoate **1** from pineapple fruits (◆), recovery aromas/water phases (△) as well as natural references (●), and synthetic references (∗). Standard deviations: ± 0.1 and ± 5‰ for $\delta^{13}C_{VPDB}$ and δ^2H_{VSMOW} determinations, respectively.

Figure 4. Correlation of $\delta^{13}C_{VPDB}$ and δ^2H_{VSMOW} values (‰) of methyl hexanoate (**3**) from pineapple fruits (◆), and recovery aromas/water phases (△), as well as natural reference (●), and synthetic references (∗). Standard deviations: ± 0.1 and ± 5‰ for $\delta^{13}C_{VPDB}$ and δ^2H_{VSMOW} determinations, respectively.
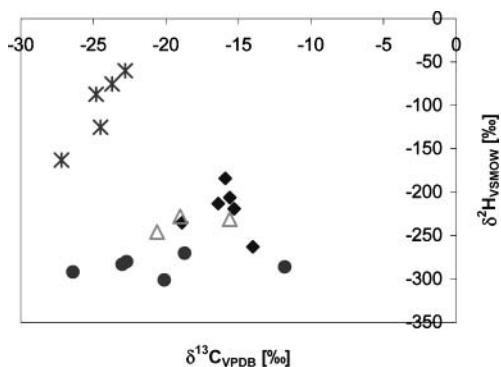
Figure 3. Correlation of $\delta^{13}C_{VPDB}$ and δ^2H_{VSMOW} values (‰) of ethyl 2-methylbutanoate **2** from pineapple fruits (◆), and recovery aromas/water phases (△), as well as natural references (●) and synthetic references (∗). Standard deviations: ± 0.1 and ± 5‰ for $\delta^{13}C_{VPDB}$ and δ^2H_{VSMOW} determinations, respectively.

Figure 5. Correlation of $\delta^{13}C_{VPDB}$ and δ^2H_{VSMOW} values (‰) of ethyl hexanoate (**4**) from pineapple fruits (◆) and recovery aromas/water phases (△) as well as natural references (●) and synthetic references (∗). Standard deviations: ± 0.1 and ± 5‰ for $\delta^{13}C_{VPDB}$ and δ^2H_{VSMOW} determinations, respectively.
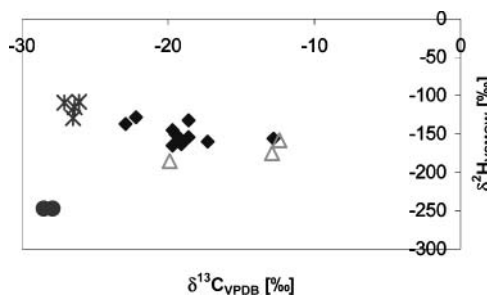
Figure 1, respectively) could not be analyzed by HRGC-IRMS for the following reasons: The thioester was not sufficiently separated from the subsequently eluting methyl 3-acetoxybutanoate, and furaneol analysis was basically hampered by its lack of linearity. Thus, the following information has to be limited to constituents **1**–**5**. Findings about the differences observed between the aroma profiles from pineapple fruit juices from different regions and various recovery aromas/water phases are discussed elsewhere (*17*).

Methyl 2-Methylbutanoate (1). In **Figure 2**, the $^{13}C/^{12}C$ and $^2H/^1H$ ratios determined for **1** in various samples are graphically correlated. Pineapple fruits ($n = 3$) showed $\delta^{13}C_{VPDB}$ and δ^2H_{VSMOW} data ranging from −17.7 to −19.7‰ as well as −208 to −234‰, respectively. Recovery aromas/water phases ($n = 3$) gave almost the same IRMS data as that found for **1** from fruits. The $\delta^{13}C_{VPDB}$ and δ^2H_{VSMOW} values of synthetic references, ranging from −26.6 to −31.8‰ and from −49 to −107‰, respectively, clearly differed from both the fruit data and that of natural methyl 2-methylbutanoate references ($n = 2$; −21.5 and −21.2 ‰ as well as −277 and −323‰ for $^{13}C/^{12}C$ and $^2H/^1H$, respectively). In the literature, no IRMS data were available for **1** to date.

Ethyl 2-Methylbutanoate (2). The correlation of $\delta^{13}C_{VPDB}$ and δ^2H_{VSMOW} data of **2** from various origin is outlined in **Figure 3**. The graph shows distinct differences between synthetic ($n = 5$; $\delta^{13}C_{VPDB}$ from −22.8 to −27.2‰ and δ^2H_{VSMOW} from −60 to −163‰) and natural samples ($n = 6$; $\delta^{13}C_{VPDB}$ from −11.8 to −26.4‰ and δ^2H_{VSMOW} from −270 to −301‰). The ester **2** from pineapple fruits ($n = 6$) exhibited

$\delta^{13}C_{VPDB}$ and δ^2H_{VSMOW} values ranging from −14.0 to −18.9‰ and −184 to −263‰, respectively. These data were in good agreement with that determined for **2** from the recovery aromas/water phases ($n = 3$) (**Figure 3**).

The scarce information available from literature about the IRMS data of **2** reveals δ^2H_{VSMOW} values ranging from −261 to −323‰ (origin not specified) (*18*). For **2** from apple fruit (a C3-plant), $\delta^{13}C_{VPDB}$ ratios of −30.9 to −35.0‰ have been reported (*19*).

Methyl Hexanoate (3). The correlation of $\delta^{13}C_{VPDB}$ and δ^2H_{VSMOW} data of **3** from different sources is displayed in **Figure 4**. δ^2H_{VSMOW} data for **3** from pineapple fruit ($n = 14$) ranged from −118 to −191‰; the $\delta^{13}C_{VPDB}$ data varied from −17.2 to −24.4‰. The IRMS data recorded for **3** from the recovery aromas/water phases ($n = 3$) were in agreement with those of the values from pineapple fruits (**Figure 4**). In general, the $\delta^{13}C_{VPDB}$ and δ^2H_{VSMOW} values of synthetic reference samples ($n = 5$) − ranging from −23.6 to −29.4‰ and from −80 to −86‰, respectively, − clearly differed from that of a natural reference ($\delta^{13}C_{VPDB} = −32.2$‰ and $\delta^2H_{VSMOW} = −242$‰) and from the data recorded for **3** from the fruit.

Ethyl Hexanoate (4). The correlation of $\delta^{13}C_{VPDB}$ and δ^2H_{VSMOW} data of **4** from different sources is outlined in **Figure 5**. For **4** from pineapple fruits ($n = 11$) ranges from −12.8 to −22.9‰ and from −128 to −165‰ were recorded for $\delta^{13}C_{VPDB}$ and δ^2H_{VSMOW}, respectively. Similar data were obtained for the ester from recovery aromas/water phases ($n = 3$), whereas natural and synthetic samples of **4** ($n = 5$) differed distinctly in their IRMS data (**Figure 5**).

Comparing the δ^2H_{VSMOW} data recorded for the 2-methylbutanoates **1** and **2** with that of the hexanoates **3** and **4**, it will be obvious that in pineapple fruits the δ^2H_{VSMOW} values are
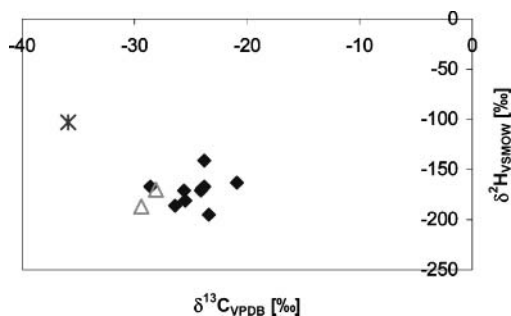
Figure 6. Correlation of $\delta^{13}C_{VPDB}$ and δ^2H_{VSMOW} values (‰) of 2,5-dimethyl-4-methoxy-3[2H]-furanone (**5**) from pineapple fruits (◆), recovery aromas/water phases (△), and synthetic references (∗). Standard deviations: ± 0.1 and ± 5‰ for $\delta^{13}C_{VPDB}$ and δ^2H_{VSMOW} determinations, respectively.

depleted in the branched esters. The hexanoates **3** and **4** showed δ^2H_{VSMOW} values ranging from −118 to −191‰, whereas those of the 2-methylbutanoates **1** and **2** were found to be between −184 and −263‰. Obviously, the influence of CAM metabolism is effective in the case of the fatty acid ester biogenesis of **3** and **4**, but not in the L-isoleucine-derived biosynthetic origin of 2-methylbutanoates (*20*). Owing to the present scarce information available on hydrogen isotopic variability of components from the secondary metabolism (*21*), causality for these findings has to remain open. A similar situation is given for the natural (not a scientific, but regulatory term) compounds under study, whose biotechnological production methods were not known.

2,5-Dimethyl-4-methoxy-3-[2H]-furanone (5). The $\delta^{13}C_{VPDB}$ and δ^2H_{VSMOW} data of **5** are graphically correlated in **Figure 6**. A synthetic sample showed $^{13}C/^{12}C$ and $^2H/^1H$ ratios of −35.9 and −103‰, respectively. No natural **5** was available. For **5** from pineapple fruits (*n* = 10), the $\delta^{13}C_{VPDB}$ (−20.9 to −28.6‰) and δ^2H_{VSMOW} data (from −141 to −195‰) exhibited the similar data as the recovery aromas/water phases (*n* = 2) ($\delta^{13}C_{VPDB}$ −28.1 and −29.4 ‰ and δ^2H_{VSMOW} −170 and −187 ‰, respectively). Previous information about **5** from pineapple revealed $\delta^{13}C_{VPDB}$ of −18.5‰ (*16*); δ^2H_{VSMOW} data were not available as yet.

As a general conclusion, it can be summarized that the δ^2H analysis via on-line HRGC-P-IRMS analysis is, despite its limited dynamic and linearity (*16, 22*), a helpful tool in the authenticity evaluation of food flavors. Similar to the combustion (C) mode, whose importance for the judgment of CAM constituents (*23*) was confirmed, the intrinsic limit is the purity of HRGC separated peaks, which can, in many cases, only be achieved by off-line or on-line preseparations (*24*). However, for a first, but decisive insight into the authenticity of major peaks of an aroma profile, as done here with pineapple, the δ^2H determination by HRGC-P-IRMS analysis has become an indispensable technique of modern flavor analysis. Of course, the quality of values will increase with the number of samples (e.g. by including important provenance regions such as Philippines, Brazil and Indonesia). The presented data form only the framework, which has to be filled up in the future.

LITERATURE CITED

(1) Bartholomew, D.; Pauli, R. E.; Rohrbach, K. *The Pineapple: Botany, Production, and Uses*; CABI Publ.: Cambridge, MA, 2003.

(2) Ti, T. C. The global pineapple economy FAO keynote address. *Acta Hort.* (ISHS) **2000**, *529*, 49−52.

(3) Rodin, J. O.; Himel, C. M.; Silverstein, R. M.; Leeper, R. W.; Gortner, W. A. Volatile flavor and aroma components of pineapple. 1. Isolation and tentative identification of 2,5-dimethyl-3[2H]-furanone. *J. Food Sci.* **1965**, *30*, 280−284.

(4) Willhalm, B.; Stoll, M.; Thomas, A. F. 2,5-Dimethyl-4-hydroxy-2,3-dihydrofuran-3-one. *Chem. Ind.* **1965**, *18,* 1629−1630.

(5) Näf-Müller, R.; Willhalm, B. Über den flüchtigen Teile der Ananas. *Helv. Chim. Acta* **1971**, *54*, 1880−1889.

(6) Takeoka, G.; Buttery, R. G.; Flath, R. A.; Teranishi, R.; Wheeler, E. L.; Wieczorek, R. L.; Guentert, M. Volatile constituents of pineapple (*Ananas comosus* [L.] Merr.) In *Flavor Chemistry. Trends and Developments*; Teranishi, R., Buttery, R. G., Shahidi, F., Eds.; ACS Symposium Series 388, American Chemical Society, Washington, D. C., 1989; pp 223−237.

(7) Engel, K.-H.; Heidlas, J.; Tressl, R. The flavor of tropical fruits (banana, melon, pineapple). In *Developments in Food Science. Food Flavours, Part C, The Flavour of Fruits*; Morton, I. D., MacLeod, A. J., Eds.; Elsevier: Amsterdam, 1990; pp 195−219.

(8) Takeoka, G. R.; Buttery, R. G.; Teranishi, R.; Flath, R. A.; Guentert, M. Identification of additional pineapple volatiles. *J. Agric. Food Chem.* **1991**, *39*, 1848−1851.

(9) Wu, P.; Kuo, M. C.; Hartman, T. G.; Rosen, R. T.; Ho, C. T. Free and glycosidically bound aroma compounds in pineapple (*Ananas comosus* L. Merr.). *J. Agric. Food Chem.* **1991**, *39*, 170−172.

(10) Umano, K.; Hagi, Y.; Nakahara, K.; Shoji, A.; Shibamoto, T. Volatile constituents of green and ripened pineapple (*Ananas comosus* [L.] Merr.). *J. Agric. Food Chem.* **1992**, *40*, 599−603.

(11) Teai, T.; Claude-Lafontaine, A.; Schippa, C.; Cozzolino, F. Volatile compounds in fresh pulp of pineapple (*Ananas comosus* [L.] Merr.) from French Polynesia**.** *J. Essential Oil Res.* **2001**, *13*, 314−318.

(12) Medina, E.; Lüttge, U.; Leal, F.; Ziegler, H. Carbon and hydrogen isotope ratios in Bromeliads growing under different light environments in natural conditions. *Bot. Acta* **1991**, *104*, 47−52.

(13) Pierce, S.; Winter, K.; Griffith, H. Carbon isotope ratio and the extent of daily CAM use by Bromeliaceae. *New Phytologist* **2002**, *156*, 75−83.

(14) Zhang, B. L.; Billault, I.; Li, X.; Maon, F.; Remaud, G.; Martin, M. L. Hydrogen isotope profile in the characterization of sugars. Influence of the metabolic pathway. *J. Agric. Food Chem.* **2002**, *50*, 1574−1580.

(15) Gillon, J. S.; Borland, A. M.; Harwood, K. G.; Roberts, A.; Broadmeadow, M. S. J.; Griffiths, H. Carbon isotope discrimination in terrestrial plants: carboxylations and decarboxylations. In *Stable Isotopes. Integration of Biological, Ecological, and Geochemical Processes*, Griffiths, H., Ed.; Bios. Scientific Publ.: Oxford, 1998; pp 111−131.

(16) Hör, K.; Ruff, C.; Weckerle, B.; König, T.; Schreier, P. Flavor authenticity studies by $^2H/^1H$ ratio determination using on-line gas chromatography pyrolysis isotope ratio mass spectometry. *J. Agric. Food Chem.* **2001**, *49*, 21−25.

Stable Isotope Analysis of Pineapple Volatiles

J. Agric. Food Chem., Vol. 51, No. 27, 2003 **8031**

(17) Elss, S.; Preston, C.; Hertzig, C.; Heckel, F.; Richling, E.; Schreier, P. Aroma profiles of pineapple fruit (Ananas comosus L. Merr.) and products made thereof. Lebensm. Wiss. Technol., in press.

(18) Schmidt, H.-L.; Rossmann, A.; Werner, R. A. Stable isotope ratio analysis in quality control of flavourings. In *Flavourings*, Ziegler, E., and Ziegler, H., Eds.; Wiley-VCH: Weinheim, 1998; pp. 539−594.

(19) Karl, V.; Dietrich, A.; Mosandl, A. Gas chromatography − isotope ratio mass spectrometry measurements of some carboxylic esters from different apple varieties. *Phytochem. Anal.* **1994**, *5*, 32−37.

(20) Rowan, D. D.; Lane, H. P.; Allen, J. M.; Fielder, S.; Hunt, M. B. Biosynthesis of 2-methylbutyl, 2-methyl-2-butenyl, and 2-methylbutanoate esters in red delicious and granny smith apples using deuterium-labeled substrates. *J. Agric. Food Chem.* **1996**, *44*, 3276−3285.

(21) Sessions, A. L.; Burgoyne, T. W.; Schimmelmann, A.; Hayes, J. M. Fractionation of hydrogen isotopes in lipid biosynthesis. *Org. Geochem.* **1999**, *30*, 1193−1200.

(22) Bilke, S.; Mosandl, A. Measurements by gas chromatography/pyrolysis/mass spectrometry: fundamental conditions in 2H/1H isotope ratio analysis. *Rapid Comm. Mass Spectrom.* **2002**, *16*, 468−472.

(23) González, J.; Remaud, G.; Jamin, E.; Naulet, N.; Martin, G. G. Specific natural isotope profile studied by isotope ratio mass spectrometry (SNIP−IRMS): $^{13}C/^{12}C$ ratios of fructose, glucose, and sucrose for improved detection of sugar addition to pineapple juices and concentrates. *J. Agric. Food Chem.* **1999**, *47*, 2316−2321.

(24) Juchelka, D.; Beck, T.; Hener, U.; Dettmar, F.; Mosandl, A. Multidimensional gas chromatography coupled online with isotope ratio mass spectrometry (MDGC−IRMS). Progress in the analytical authentication of genuine flavor components. *J. High Resol. Chromatogr.* **1998**, *21*, 145−151.