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Determination of the $^{2}H/^{1}H$ and $^{15}N/^{14}N$ Ratios of Alkylpyrazines from Coffee Beans (*Coffea arabica* L. and *Coffea canephora* var. *robusta*) by Isotope Ratio Mass Spectrometry

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The $\delta^{15}N_{AIR}$ and $\delta^{2}H_{VSMOW}$ data for several alkylpyrazines formed during the roasting process of coffee are reported. Samples of commercially available roasted ($n = 9$) as well as self-roasted ($n = 8$) coffee beans (*Coffea arabica* L. and *Coffea canephora* var. *robusta*) of different origins were investigated. By use of extracts prepared by simultaneous distillation extraction (SDE) and subsequently fractionated by liquid chromatography on silica gel, on-line capillary gas chromatography−isotope ratio mass spectrometry was employed in the combustion (C) and pyrolysis (P) modes (HRGC-C/P-IRMS) to determine the $\delta^{15}N_{AIR}$ and $\delta^{2}H_{VSMOW}$ values, respectively. In addition to the constituents of coffee beans, data for commercial synthetic alkylpyrazines and substances declared to be "natural" were determined. The $\delta^{15}N_{AIR}$ data for coffee alkylpyrazines under study—2-ethyl-5-methylpyrazine (**1**) and 2-ethyl-6-methylpyrazine (**2**) (measured as sum **1/2**), 2-ethyl-3-methylpyrazine (**3**), 2-methylpyrazine (**4**), 2,5-dimethylpyrazine (**5**) and 2,6-dimethylpyrazine (**6**) (measured as sum **5/6**), and 2,3-dimethylpyrazine (**7**), as well as 2,3,5-trimethylpyrazine (**8**)—varied in the range from +8.3 to −10.2‰, thus revealing their biogeneration from amino acids ($\delta^{15}N_{AIR}$ ranging from +8‰ to −10‰). The $\delta^{2}H_{VSMOW}$ values were determined in the range from −5‰ to −127‰. Owing to the analytical differentiation observed between coffee alkylpyrazines and synthetic/"natural" samples of **3**, **4**, and **7**, authenticity assessment of coffee-flavored products seems to be promising, provided that extended data will be available in the future. In the literature, there were no IRMS data available for the alkylpyrazines (**1**−**8**) under study.

KEYWORDS: Alkylpyrazines; coffee; isotope ratio mass spectrometry; IRMS; HRGC-C/P-IRMS; $^{15}N/^{14}N$ ratio; $^{2}H/^{1}H$ ratio

INTRODUCTION

Over a period of two centuries, researchers have tried with varying degrees of success to identify the compounds that give roasted coffee its characteristic aroma and taste. At present, about 850 compounds have been identified in the flavor of roasted coffee and 300 in the odor of green coffee (*1*). A significant step in coffee flavor chemistry was taken in the 1920s when Reichstein and Staudinger (*2*) reported pyrazines for the first time. Many members of this family were found in the 1960s and it was then proved that pyrazines derive, during the roasting, from a Maillard reaction between amino acids and sugars. They are formed in remarkable amounts and constitute about 14% of the overall volatile content (*3*). The sensory importance of alkylpyrazines (or alkenylpyrazines) has been highlighted by Grosch and co-workers (*4*−*6*).

Because of their high impact on the roast coffee quality, these substances are the focus of authenticity and origin studies. In the past, isotope ratio mass spectrometry (IRMS) has been shown to be a powerful tool in authenticity and origin assessment of flavors (*7*, *8*). Whereas $^{13}C/^{12}C$ ratio determinations in the combustion (C) mode have already been performed by on-line coupling with capillary gas chromatography (HRGC-C-IRMS) (*7*, *8*), the introduction of HRGC-coupled $^{18}O/^{16}O$ and $^{2}H/^{1}H$ (*9*−*11*) ratio determinations in the pyrolysis mode (HRGC-P-IRMS) has opened the way for HRGC on-line multi-element approaches (*12*). Thus, recently, first investigations with selected flavor compounds, such as estragole and methyl eugenol (*13*), anethole (*14*), pineapple volatiles (*15*), cinnamaldehyde (*16*), and methyl cinnamate (*17*) were successfully performed.

In our present study, $^{2}H/^{1}H$ and $^{15}N/^{14}N$ ratio determinations of alkylpyrazines from roasted coffee beans were carried out

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by on-line HRGC-C/P-IRMS coupling, with the aim to create, as no information was available about IRMS data for alkylpyrazines to date, a first database and to check the possibilities to assess authenticity and origin.

MATERIALS AND METHODS

Samples. Green coffee beans (*Coffea arabica* L. and *Coffea canephora var. robusta*) of different origins, that is, India, Brazil, Ethiopia, Malawi, Costa Rica, Hawaii, Indonesia, and Malabar ($n = 8$) were obtained from RONA Inc. (Kingston Court, U.K.) and Illycafé (Trieste, Italy). Roasted coffee beans from commercial German trademarks ($n = 9$) were purchased from a local supermarket.

Synthetic references and samples declared to be "natural" were 2-ethyl-5-methylpyrazine (**1**), 2-ethyl-6-methylpyrazine (**2**), 2-ethyl-3-methylpyrazine (**3**), 2-methylpyrazine (**4**), 2,5-dimethylpyrazine (**5**), 2,6-dimethylpyrazine (**6**), 2,3-dimethylpyrazine (**7**), and 2,3,5-trimethylpyrazine (**8**). They were obtained from Fluka (Deisenhofen, Germany), Treatt (Suffolk, U.K.), Sigma−Aldrich (Steinheim, Germany), Quest (Naarden, Netherlands), Bedoukian (Dunbury, USA), and Oxford (Duisburg, Germany). All other chemicals were purchased from Sigma−Aldrich (Steinheim, Germany). Solvents were redistilled before use.

Sample Preparation. Synthetic and "natural" references were dissolved (1 mg/mL) in diethyl ether and the solutions were analyzed by HRGC−MS and HRGC-C/P-IRMS and directly measured by EA−C/P-IRMS.

Roasting of raw coffee beans (in portions of 80 g) was performed with a model 40201 Precision Coffee Roaster (Hearthware Home Products, Inc.) with setting at position 5 (corresponding to 6 min at 210 °C). Commercially available and self-roasted coffee beans (each 350 g) were ground and, after addition of 2 L of distilled water, subjected to simultaneous distillation extraction (SDE) (2 h) with pentane−diethyl ether mixture (1:1 v/v). The extract obtained by SDE was dried over anhydrous sodium sulfate, filtered, and carefully concentrated to approximately 5 mL on a Vigreux column (45 °C). For the IRMS analysis, aroma extracts were purified by LC on silica gel (glass column, 2 × 30 cm, filled with silica gel 60 Merck, 0.2−0.5 mm) using a pentane−diethyl ether mixture (from 0 to 100% diethyl ether in 20% steps, each 150 mL; flow rate, 3 mL/min). The fraction eluted with 60% diethyl ether contained 2-ethyl-5-methylpyrazine (**1**), 2-ethyl-6-methylpyrazine (**2**), and 2-ethyl-3-methylpyrazine (**3**). 2-Methylpyrazine (**4**), 2,5-dimethylpyrazine (**5**), 2,6-dimethylpyrazine (**6**), 2,3-dimethylpyrazine (**7**), and 2,3,5-trimethylpyrazine (**8**) were recovered in the 80% diethyl ether fraction. After evaporation of the solvents, both fractions were applied to IRMS analysis. Because of insufficient separation of components **1** and **2** as well as **5** and **6** during HRGC-IRMS analysis, the isotope ratios were measured for the mixtures of **1**/**2** and **5**/**6**, respectively.

The influence of sample preparation on the $^2H/^1H$ and $^{15}N/^{14}N$ isotope ratios was checked for compounds **1/2**, **4**, and **5/6** by model SDE and subsequent silica gel column separation. No significant isotope discrimination effects were observed by the applied workup procedure.

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.). The flavor compounds were separated on a 30 m × 0.25 mm i.d., df = 0.25 μm DB-Wax fused silica capillary column (J&W, Agilent, Waldbronn, Germany). The temperature program was 3 min isothermal at 50 °C, then raised at 4 °C/min to 240 °C. Identification was performed by comparison of linear retention indices and mass spectrometric data for sample constituents with that of authentic reference compounds.

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 a 60 m × 0.32 mm i.d., df = 0.25 μm J&W DB-Wax fused silica capillary column. The following conditions were employed: 1 μL splitless injection (250 °C); temperature programs, from 50 to 220 °C at 5° C/min; helium flow, 2 mL/min.

Interfaces. $^{15}N/^{14}N$: combustion by oxidative reactor (Al_2O_3, 0.5 mm i.d., 1.5 mm o.d., 320 mm) with Cu, Ni, Pt (each 240 mm × 0.125 mm) at 960 °C and reduction reactor Al_2O_3, 0.5 mm i.d., 1.5 mm o.d., 320 mm, Cu, 600 °C) to N_2; water separation by Nafion membrane.

Pyrolysis. $^2H/^1H$: The effluent from the GC passed through a ceramic tube (Al_2O_3; 0.5 mm i.d., 320 mm) for pyrolysis to H_2 at 1440 °C.

In addition, coupling elemental analyzers (EA): for $^{15}N/^{14}N$ (Euro Vector EA 3000, Milano, Italy), temperature 1000 °C; for $^2H/^1H$ (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 $^{15}N/^{14}N$ and $^2H/^1H$ ratios. Stability check of the used reference gases was continuously performed by measuring International Atomic Energy Agency (IAEA, Vienna, Austria) standards.

The isotope ratios are expressed in per mil (‰) deviation relative to the Air as international standard. For $^{15}N/^{14}N$ determinations, the mass spectrometer was calibrated against reference N_2 gas (Messer Griesheim, Frankfurt, Germany) with a defined $\delta^{15}N = -10$ ‰ vs Air. For routine measurement, calibrated laboratory N_2 gas 5.3 ($\delta^{15}N_{AIR} = -5.5$ ‰ ± 0.2 ‰; calibrated with IAEA-N-1 and IAEA-N-2 standards) was used for daily measurement. Results are expressed as $\delta^{15}N_{AIR}$ values (per mil):

$$\delta^{15}N_{AIR} = \left(\frac{R_{sample} - R_{Air}}{R_{Air}}\right) \times 1000$$

where R is the isotope ratio $^{15}N/^{14}N$.

The isotope ratios for $^2H/^1H$ 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 $\delta^2H_{VSMOW} = -270$ ‰ ± 10 ‰.

Results are expressed in δ^2H_{VSMOW} units (per mil):

$$\delta^2H_{VSMOW} = \left(\frac{R_{sample} - R_{VSMOW}}{R_{VSMOW}}\right) \times 1000$$

where R = isotope ratio $^2H/^1H$.

In general, 6-fold determinations were carried out, and standard deviations were calculated. The latter were ±0.5 ‰ and ±5 ‰ for $\delta^{15}N_{AIR}$ and δ^2H_{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^2H_{VSMOW} values of the coffee bean volatiles, the system reliability was proven by measuring commercial references off-line via the equipped elemental analyzer (EA) (*18*). Data recorded by EA−C/P-IRMS were in good agreement with that determined by HRGC−C/P-IRMS analysis (data not shown). The areas of linearity for the δ^2H_{VSMOW} determinations were in the range 1−10 μg for compounds **1**, **2**, and **4**−**6** and 1−5 μg for pyrazine derivatives **3**, **7**, and **8** (each on column). The measurements of $\delta^{15}N_{AIR}$ values were carried out in the linearity range of 1−4 μg for the compounds under study (**1**−**8**). Finally, the influence of sample preparation on the $^2H/^1H$ and $^{15}N/^{14}N$ isotope ratios checked by model SDE and silica gel column separations was found to be within the range of standard deviation and thus negligible (data not shown).

RESULTS AND DISCUSSION

The $\delta^{15}N$ value of the total nitrogen in plant biomass is based on the primary inorganic nitrogen sources such as NO_3^-, NH_4^+, and N_2. Different origins therefore result potentially in $\delta^{15}N$ value discrepancies. Other determining factors are the discrimination effects during uptake and transformation. The primary form of organic nitrogen in plants is amide N in glutamine, which is converted into amino groups of amino acids. The relatively ^{15}N-depleted glutamate provides nitrogen for all other amino acids. However, each amino acid can exchange via

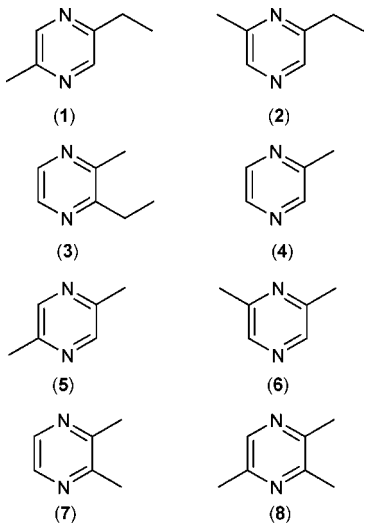

Figure 1. Structures of alkylpyrazines **1**–**8** under study: (**1**) 2-ethyl-5-methylpyrazine, (**2**) 2-ethyl-6-methylpyrazine, (**3**) 2-ethyl-3-methylpyrazine, (**4**) 2-methylpyrazine, (**5**) 2,5-dimethylpyrazine, (**6**) 2,6-dimethylpyrazine, (**7**) 2,3-dimethylpyrazine, and (**8**) 2,3,5-trimethylpyrazine.

transamination. Further isotope discriminations can occur in subsequent reactions such as oxidoreductions, amidations, and lyase and hydrolysis reactions. According to studies by Werner and Schmidt (*19*), the nitrogen in the amino acid fraction and in heteroaromatic compounds shows a relative ^{15}N enrichment above the bulk value by 1.7‰ or 4.9‰, respectively. Values of −10‰ to +8‰ are reported for different amino acids (*19*). The last-mentioned are the direct nitrogen suppliers for alkylpyrazines formed under roasting conditions and investigated in this study.

Among the high number of volatiles detected by HRGC−MS in the roasted coffee beans, only the major components as well as distinctly separated compounds were accessible to online HRGC−IRMS studies (**Figure 1**). The complex composition of the obtained aroma extracts required prior LC separation on silica gel. As a representative example, **Figure 2 A** shows the separation of the roasted coffee bean alkylpyrazines under study, that is, 2-ethyl-5-methylpyrazine (**1**), 2-ethyl-6-methylpyrazine (**2**), and 2-ethyl-3-methylpyrazine (**3**) identified in the 60% diethyl ether fraction after column separation. The alkylpyrazines 2-methylpyrazine (**4**), 2,5-dimethylpyrazine (**5**), 2,6-dimethylpyrazine (**6**), 2,3-dimethylpyrazine (**7**), and 2,3,5-trimethylpyrazine (**8**) were found in the 80% diethyl ether fraction (**Figure 2B**). The recorded $\delta^{15}N_{AIR}$ data varied in the range from +8.3‰ to −10.2‰, i.e., exactly in the range reported for amino acids (*19*). Obviously, no noticeable discrimination of nitrogen isotopes occurs during the roasting process. In the following, the individual values determined for each compound are discussed.

2-Ethyl-5-methylpyrazine, 1, and 2-Ethyl-6-methylpyrazine, 2. Because of their chromatographic coelution, **1** and **2** could not be measured separately by IRMS. The $\delta^{15}N_{AIR}$ and δ^2H_{VSMOW} data for both substances **1/2** are shown in **Figure 3**. The mixture of the synthetic samples **1** and **2** (3:2, as determined by HRGC−MS) revealed a $^{15}N/^{14}N$ ratio of +0.3‰ and a $^2H/^1H$ ratio of −63‰. No "natural" reference was available. For **1/2** from self-roasted Arabica and Robusta samples ($n = 6$), $\delta^{15}N_{AIR}$ and δ^2H_{VSMOW} values were recorded from +3.1‰ to −2.0‰ and from −64‰ to −98‰, respectively. As to nitrogen isotope values, a difference of about 3‰ was observed between Arabica and Robusta species. With the exception of one commercial sample ($\delta^{15}N_{AIR}$ value of +6.5‰ and δ^2H_{VSMOW} of −26‰), all other commercial coffees exhibited data in the same $\delta^{15}N_{AIR}$ range, such as self-roasted samples, but in a more depleted δ^2H_{VSMOW} area (from −101‰ to −127‰).

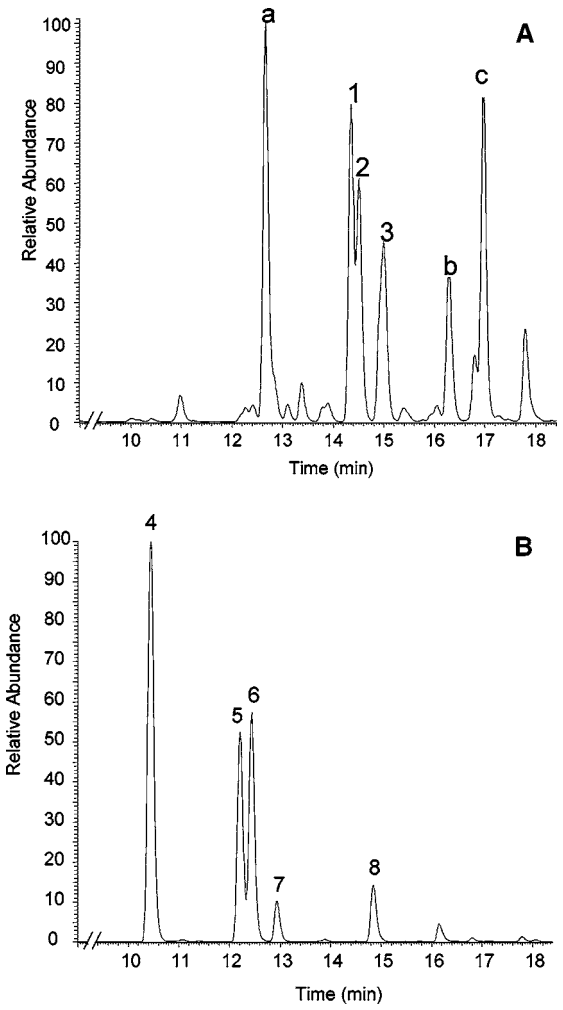

Figure 2. HRGC-MS chromatogram (sector) of the 60% (**A**) and 80% (**B**) diethyl ether fraction from the LC separation of roasted coffee bean (*C. arabica* L.) volatiles on silica gel. The peak numbers indicate the compounds under IRMS study in this fraction. Chromatogram **A**: (**1**) 2-ethyl-5-methylpyrazine, (**2**) 2-ethyl-6-methylpyrazine, (**3**) 2-ethyl-3-methylpyrazine, (a) 2-ethylpyrazine, (b) 3-ethyl-2,5-dimethylpyrazine, and (c) 1-(acetyloxy)-2-propanone. Chromatogram **B**: (**4**) 2-methylpyrazine, (**5**) 2,5-dimethylpyrazine, (**6**) 2,6-dimethylpyrazine, (**7**) 2,3-dimethylpyrazine, (**8**) 2,3,5-trimethylpyrazine.

2-Ethyl-3-methylpyrazine, 3. All references of alkylpyrazine **3**, including a reference declared to be natural, showed a very remarkable narrow range for $\delta^{15}N_{AIR}$ values between +0.7 and −0.5‰ (**Figure 4**). The δ^2H_{VSMOW} data were found between −95‰ and −179‰. These data are distinctly different from those observed for the coffee samples under study. They ranged from +3.4‰ to −5.5‰ for $\delta^{15}N_{AIR}$ and from −49‰ to −92‰ for δ^2H_{VSMOW}. As **3** is an organoleptically attractive coffee constituent, this clear-cut distinction could be of interest to assess its authenticity in coffee-flavored products.

2-Methylpyrazine, 4. The correlation of $\delta^{15}N_{AIR}$ and δ^2H_{VSMOW} data for **4** from various origins is outlined in **Figure 5**. The graph shows differences between synthetic samples ($\delta^{15}N_{AIR}$ from +0.3‰ to −1.2‰ and δ^2H_{VSMOW} from +15‰ to +40‰) and references declared to be natural ($\delta^{15}N_{AIR}$ from −0.9‰ to −2.4‰ and δ^2H_{VSMOW} from −102‰ to −117‰). Between these groups, data for pyrazine **4** from self-roasted

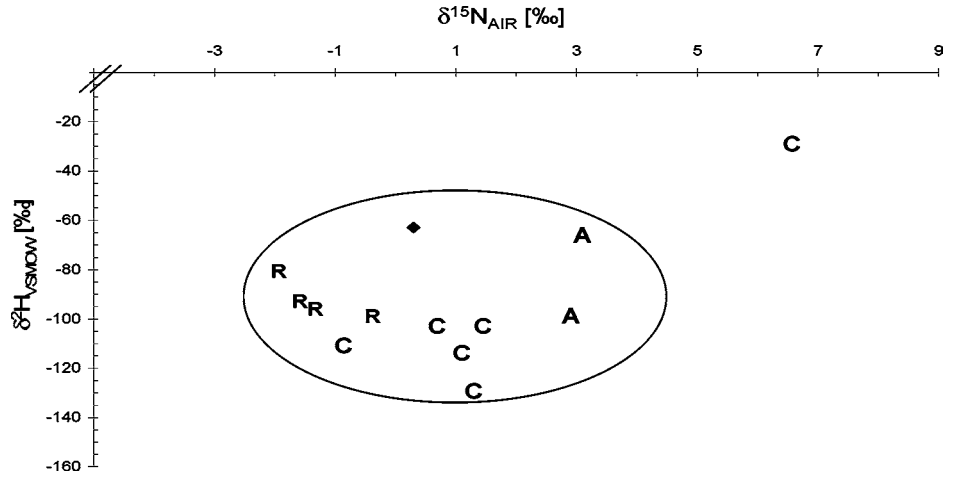

Figure 3. Correlation of $\delta^{15}N_{AIR}$ and $\delta^{2}H_{VSMOW}$ values (‰) of the 2-ethyl-5-methylpyrazine/2-ethyl-6-methylpyrazine mixture **1/2** from self-roasted Arabica coffee (A), self-roasted Robusta coffee (R), commercially available roast coffee (C), and synthetic reference (60% **1**, 40% **2**) (◆). Standard deviations were ±0.5‰ and ±5‰ for $\delta^{15}N_{AIR}$ and $\delta^{2}H_{VSMOW}$ determinations, respectively.

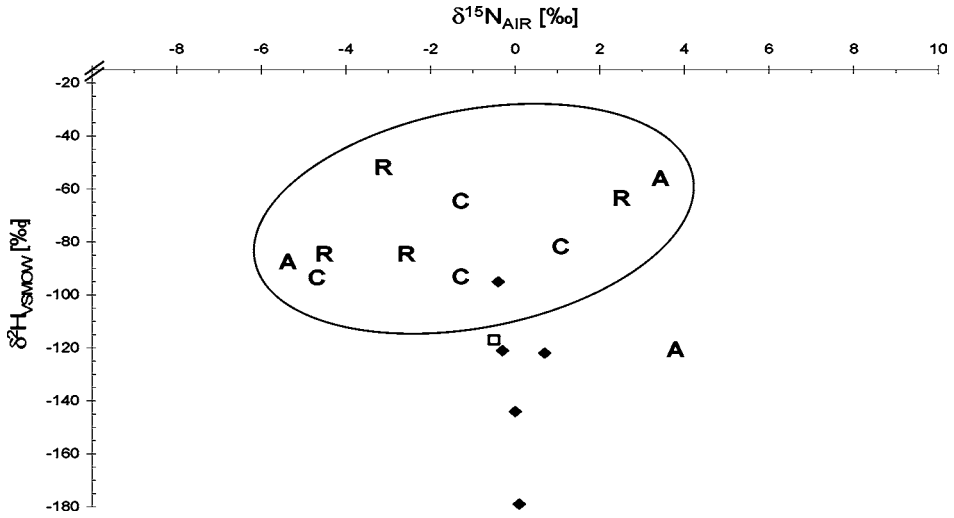

Figure 4. Correlation of $\delta^{15}N_{AIR}$ and $\delta^{2}H_{VSMOW}$ values (‰) of 2-ethyl-3-methylpyrazine **3** from self-roasted Arabica coffee (A), self-roasted Robusta coffee (R), commercially available roast coffee (C), synthetic references (◆), and references declared to be natural (□). Standard deviations were ±0.5‰ and ±5‰ for $\delta^{15}N_{AIR}$ and $\delta^{2}H_{VSMOW}$ determinations, respectively.

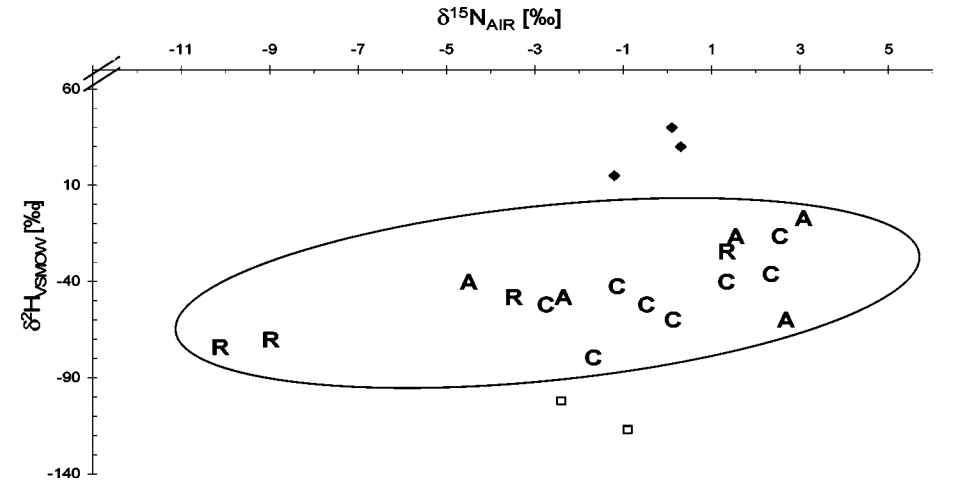

Figure 5. Correlation of $\delta^{15}N_{AIR}$ and $\delta^{2}H_{VSMOW}$ values (‰) of 2-methylpyrazine **4** from self-roasted Arabica coffee (A), self-roasted Robusta coffee (R), commercially available roast coffee (C), synthetic references (◆), and references declared to be natural (□). Standard deviations were ±0.5‰ and ±5‰ for $\delta^{15}N_{AIR}$ and $\delta^{2}H_{VSMOW}$ determinations, respectively.

Arabica and Robusta coffees ($n = 9$) are placed. They range from +3.0‰ to −10.2‰ for $\delta^{15}N_{AIR}$ and from −5‰ to −77‰ for $\delta^{2}H_{VSMOW}$. Two Robusta samples from India showed a stronger depletion in the $\delta^{15}N_{AIR}$ value compared to the rest of the investigated coffee samples. The $\delta^{15}N_{AIR}$ and $\delta^{2}H_{VSMOW}$ values of commercially roasted samples are concentrated in the

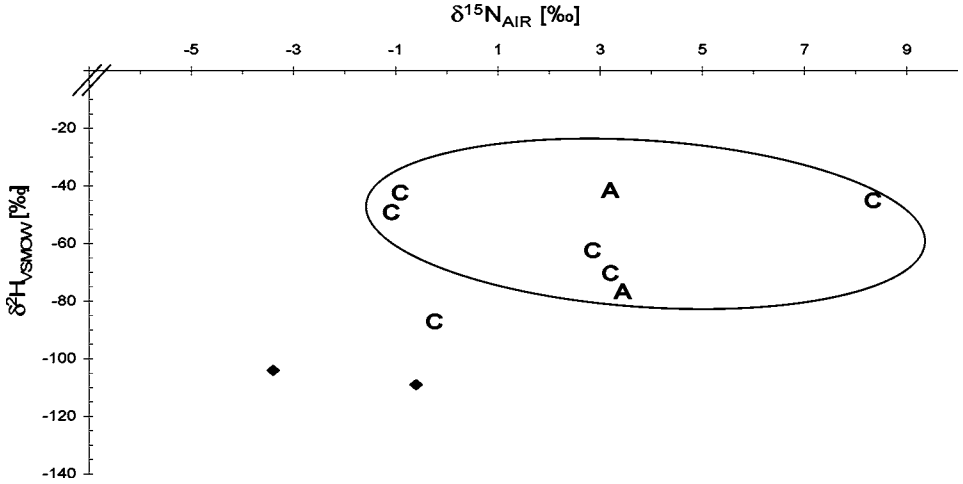

Figure 6. Correlation of $\delta^{15}N_{AIR}$ and $\delta^{2}H_{VSMOW}$ values (‰) of 2,5-dimethylpyrazine/2,6-dimethylpyrazine mixture **5/6** from self-roasted Arabica coffee (A), commercially available roast coffee (C), and synthetic reference mix (each 1:1) (◆). Standard deviations were ±0.5‰ and ±5‰ for $\delta^{15}N_{AIR}$ and $\delta^{2}H_{VSMOW}$ determinations, respectively.

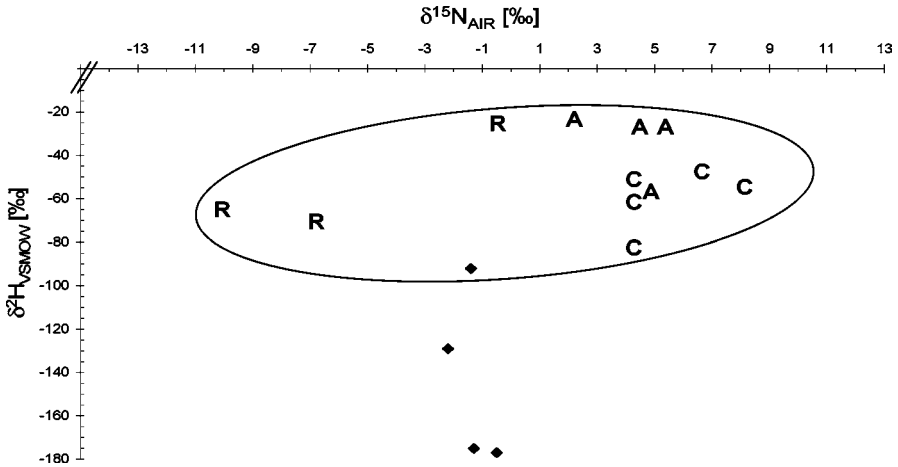

Figure 7. Correlation of $\delta^{15}N_{AIR}$ and $\delta^{2}H_{VSMOW}$ values (‰) of 2,3-dimethylpyrazine **7** from self-roasted Arabica coffee (A), self-roasted Robusta coffee (R), commercially available roast coffee (C), and synthetic references (◆). Standard deviations were ±0.5‰ and ±5‰ for $\delta^{15}N_{AIR}$ and $\delta^{2}H_{VSMOW}$ determinations, respectively.

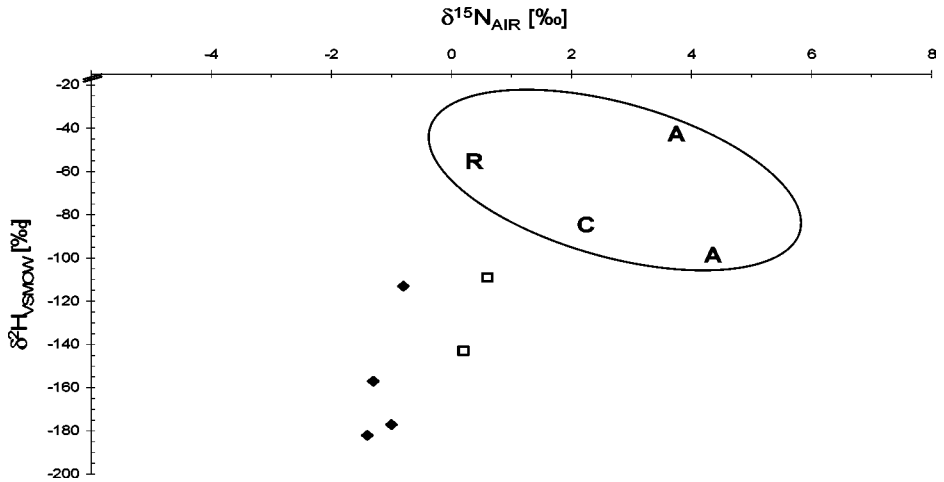

Figure 8. Correlation of $\delta^{15}N_{AIR}$ and $\delta^{2}H_{VSMOW}$ values (‰) of 2,3,5-trimethylpyrazine **8** from self-roasted Arabica coffee (A), self-roasted Robusta coffee (R), commercially available roast coffee (C), synthetic references (◆), and references declared to be natural (□). Standard deviations were ±0.5‰ and ±5‰ for $\delta^{15}N_{AIR}$ and $\delta^{2}H_{VSMOW}$ determinations, respectively.

region from +2.5‰ to +2.8‰ for $\delta^{15}N_{AIR}$ and from −13‰ to −77‰ for $\delta^{2}H_{VSMOW}$.

2,5-Dimethylpyrazine, 5, and 2,6-Dimethylpyrazine, 6. It was not possible to separate **5** and **6** by the HRGC−IRMS analysis. Thus, sum parameters (**5/6**) were measured. The correlation of $\delta^{15}N_{AIR}$ and $\delta^{2}H_{VSMOW}$ data for **5/6** is displayed in **Figure 6**. $\delta^{15}N_{AIR}$ data for **5/6** from self-roasted Arabica coffees ($n = 2$) as well as commercially available roasted coffees

($n = 6$) ranged from +8.3‰ to −1.1‰; the δ^2H_{SMOW} data varied from −40‰ to −85‰. The $\delta^{15}N_{AIR}$ and δ^2H_{VSMOW} values of the synthetic reference mix **5/6** (1:1, as determined by HRGC−MS) ($n = 2$) showed a depletion in both isotope ratios, ranging from −0.6‰ to −3.4‰ for $\delta^{15}N_{AIR}$ and from −104‰ to −109‰ for δ^2H_{VSMOW}.

2,3-Dimethylpyrazine, 7. The correlation of $\delta^{15}N_{AIR}$ and δ^2H_{VSMOW} data for **7** from different sources is outlined in **Figure 7**. The $\delta^{15}N_{AIR}$ values for **7** from self-roasted coffees ($n = 7$) varied from +5.2‰ to −10.2‰. The δ^2H_{VSMOW} results were determined in the range from −21‰ to −69‰. Again the two Robusta samples (from India and Indonesia) revealed stronger depleted values in comparison to all other samples. Commercially available coffees (n=5) showed values concentrated in the area from +8.0‰ to +4.1‰ for $\delta^{15}N_{AIR}$ and from −45‰ to −80‰ for δ^2H_{VSMOW}. Measurements of references resulted in a range from −92‰ to −177‰ for δ^2H_{VSMOW} and in a very narrow range for $\delta^{15}N_{AIR}$ between −0.5‰ and −2.2‰. By analogy with the data recorded for **3**, authenticity assessment seems to be promising, provided that extended data information will be available in the future.

2,3,5-Trimethylpyrazine, 8. Compound **8** was not detectable in sufficient amounts in each coffee sample for the HRGC−IRMS analysis. The correlation of $\delta^{15}N_{AIR}$ and δ^2H_{VSMOW} data for **8** is outlined in **Figure 8**. The $\delta^{15}N_{AIR}$ and δ^2H_{VSMOW} values from self-roasted coffees (two Arabica and one Robusta coffee) and one commercial sample constituted from +4.3‰ to +0.3‰ and from −42‰ to −98‰, respectively. Measurements of synthetic references resulted in a more depleted range from −0.8‰ to −1.4‰ for $\delta^{15}N_{AIR}$ and from −113‰ to −182‰ for δ^2H_{VSMOW}. Two references declared to be natural showed values of +0.6‰ and +0.2‰ for $\delta^{15}N_{AIR}$ as well as −109‰ and −143‰ for δ^2H_{VSMOW}.

In conclusion, for the first time stable isotope data for alkylpyrazines are provided. Neither the δ^2H_{VSMOW} nor the $\delta^{15}N_{AIR}$ values of these compounds were known before. The findings of our study demonstrate that an analytical differentiation between some synthetic alkylpyrazines—all ranging in the area of ±0‰ for $\delta^{15}N_{AIR}$—and coffee bean-derived alkylpyrazines is possible. Analytical differences between Robusta and Arabica were noticeable only in the case of one compound. Future investigations are required to extend these first insights into the authenticity assessment of selected coffee volatiles.

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