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Authenticity and Traceability of Vanilla Flavour by Analysis of Stable Isotopes

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How can you be sure that the vanilla flavour added to your food product is actually a natural vanilla flavour originating from Madagascar as indicated on the label? In order to protect consumers against mislabeling and local producers against counterfeits, it is necessary to develop methods that can verify authenticity.

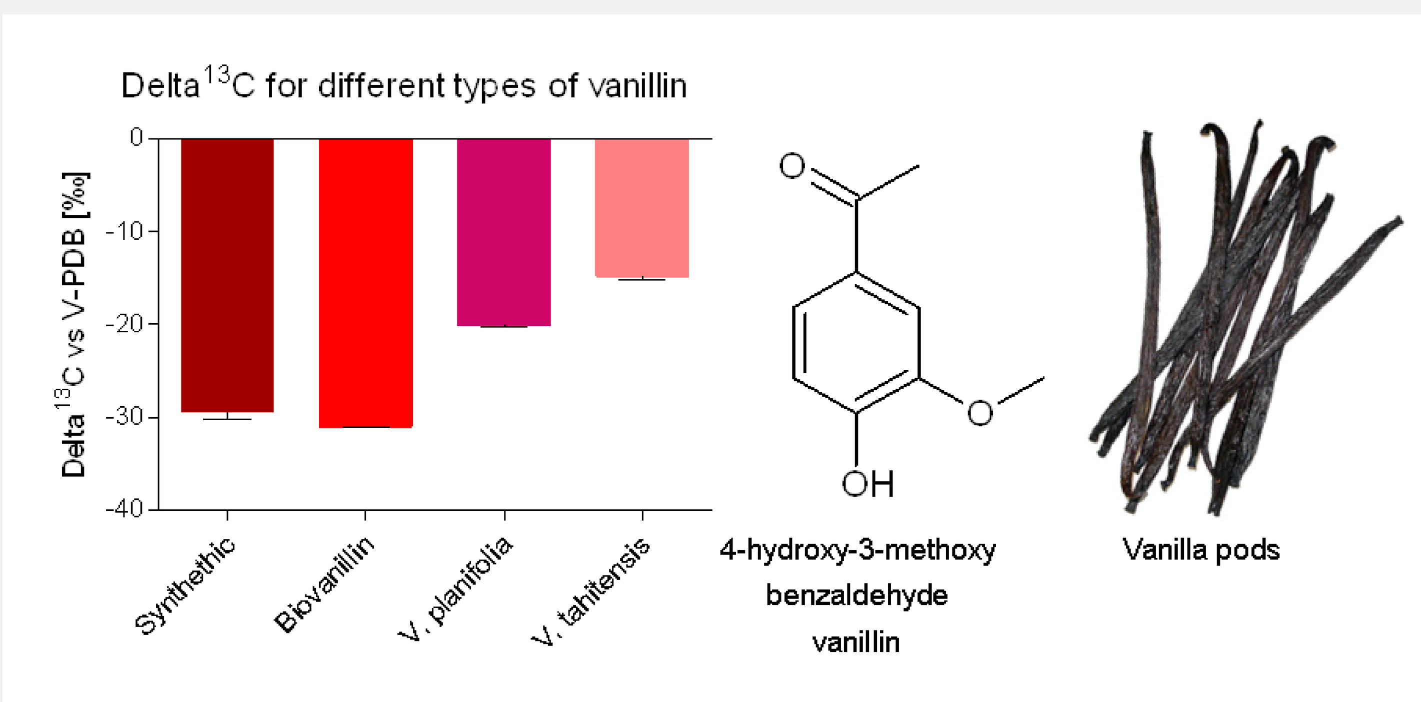
Introduction

The characteristic flavour from vanilla pods is mainly due to 4-hydroxy-3-methoxybenzaldehyde, also called vanillin. Vanillin is added to a wide range of food products to obtain vanilla flavour. In general there are three types of vanillin: **natural vanillin** extracted from the vanilla orchid, **synthetic vanillin,** and **biovanillin** made by fermentation of e.g. eugenol. Because the vanilla plant uses the Crassulacean Acid Metabolism (CAM) for CO_2 fixation, the $^{13}C/^{12}C$ ratio will differ from plants used as substrates for fermentations, as they uses the Calvin Cycle (C3) for carbon fixation. Synthetic vanillin is made from petrochemicals which often are more depleted in the heavy carbon isotopes compared to CAM plants. Therefore the differences in ratios of stable carbon isotopes of vanillin were, in this study, used to determine the method of production.

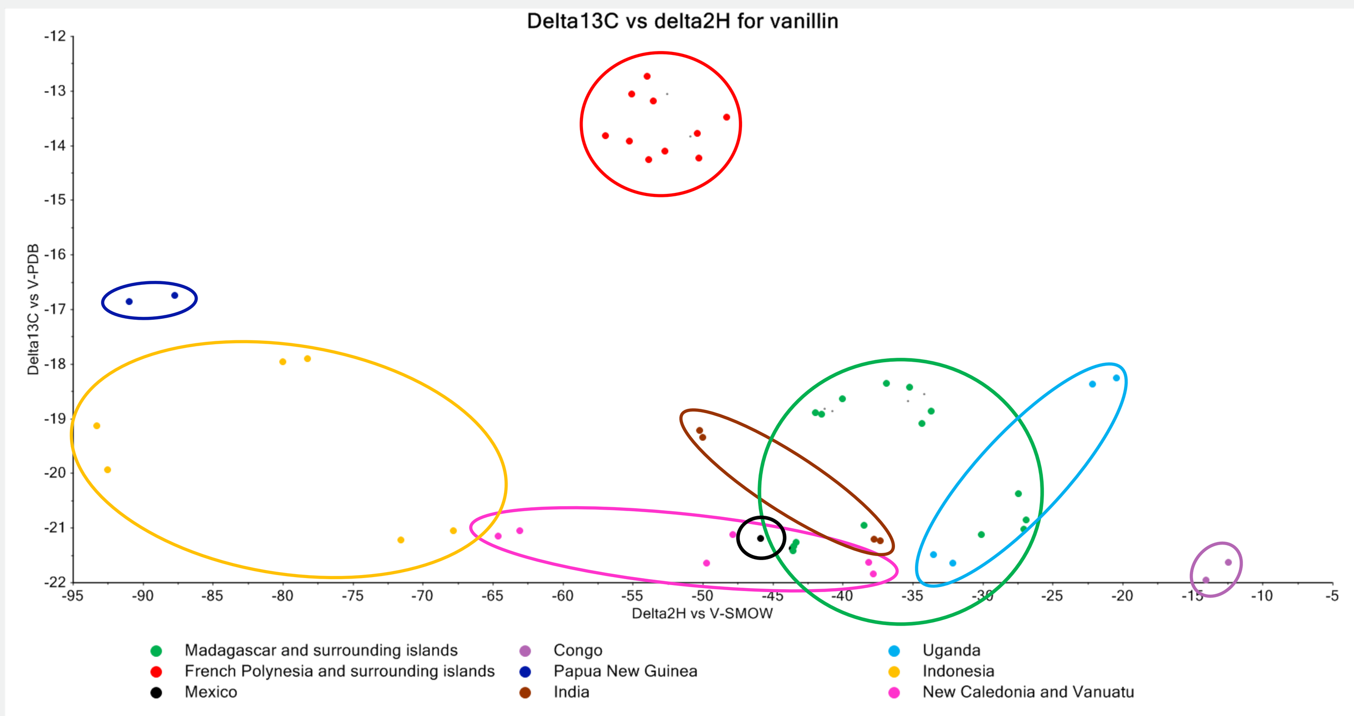
Stable isotope ratios of hydrogen was used to trace the geographical origin of a vanilla plant. The ratio of $^{2}H/^{1}H$ in precipitation vary due to longitudinal, altitudinal, and continental effects and this isotopic variation is incorporated into the plants metabolites including vanillin.

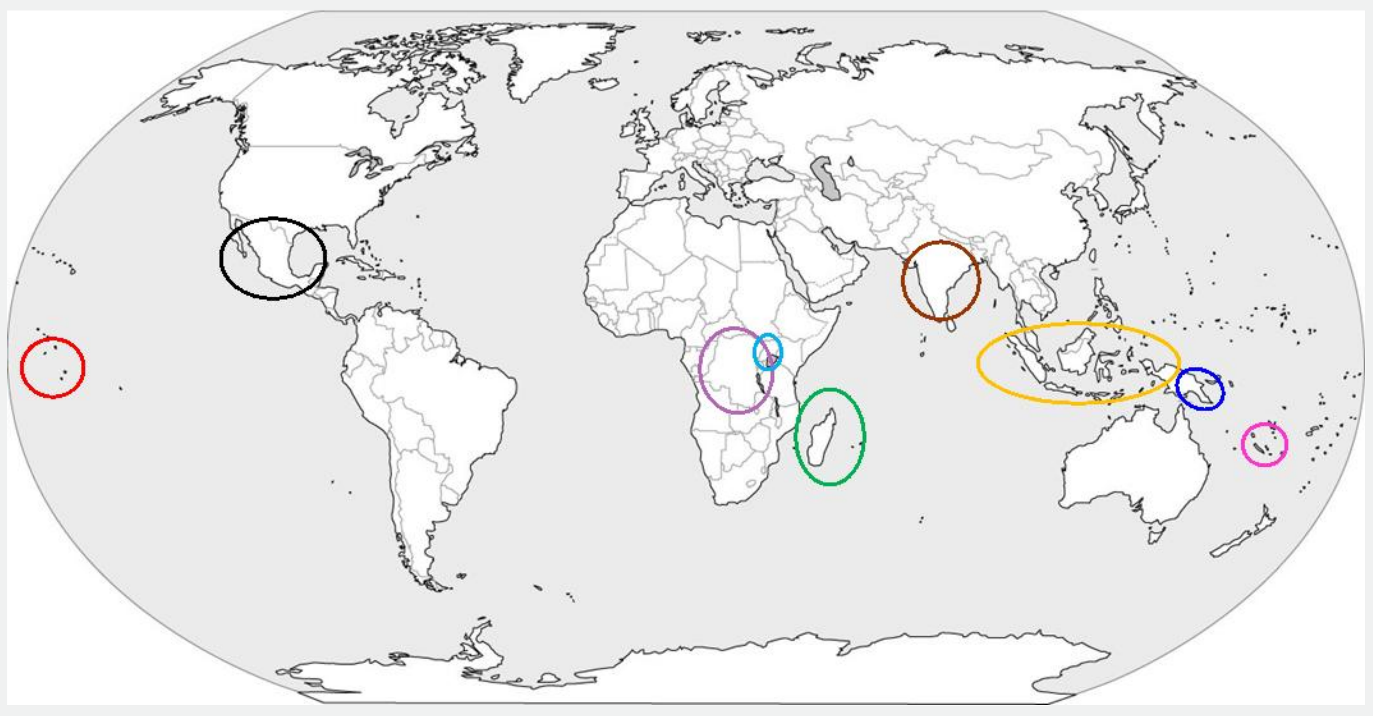
Results

$\delta^{13}C$ values of natural vanillin was significantly different ($P<0.001$) from the values of synthetic vanillin and biovanillin, while there was no difference between synthetic vanillin and biovanillin. Furthermore, a significant difference ($P<0.001$) of $\delta^{13}C$ for the two types of natural vanilla pods was found: *Vanilla planifolia* and *Vanilla tahitensis*.



Values of $\delta^{13}C$ for synthetic, bio and natural vanillin. Number of samples analyzed was 2 synthetic, 1 biovanillin, 28 *V. planifolia* and 10 *V. tahitensis*. Results are stated as δ values: $\delta^{13}C=((R_{sample}-R_{standard})/R_{standard})*1000$, where R is the ratio of the heavy to the light isotope.





Top: Ratios of stable isotopes of hydrogen and carbon for extracts of vanilla pods.
Bottom: Correlated origin of the vanilla pods analyzed.

Stable isotopes of carbon and especially hydrogen can provide information about the geographical origin of vanilla pods. There is a tendency towards a depletion of ^{2}H in areas with high precipitation.

Materials and methods

Vanillin was extracted from vanilla pods using $EtOH/H_2O$ (1:1) for 72h. After filtration the extract was further extracted with ethyl acetate/cyclo hexane (1:1).

GC-IRMS: Trace GC Ultra fitted with a DB-5 capillary column (Agilent Technologies, Böblingen, Germany) (30m x 0.250mm i.d., d_f 0.25 µm) coupled to Delta V Advantage Isotope Ratio Mass Spectrometer (Thermo Scientific, Bremen, Germany).

For measurements of $\delta^{13}C$ a **Combustion Reactor** (NiO tube with NiO/CuO/Pt) was operated at 1000 C. For determinations of $\delta^{2}H$ a **High Temperature Conversion Reactor** consisting of a ceramic tube with no catalyst was operated at 1420 C.

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