Determination of bioactive compounds in vanilla and its products

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Highlights

- Biological and biochemical profiles of 32 vanilla fruits and products gave relevant information on their array of active ingredients
- Development of a hyphenated HPTLC-UV/Vis/FLD-EDA-MS method for evaluation of the wide range of different qualities of vanilla
- Quantification of vanillin and also of the synthetic derivative ethyl vanillin
- Bioprofiles directly linked to the content of vanilla added to the food products and differed to those containing vanillin

Scope

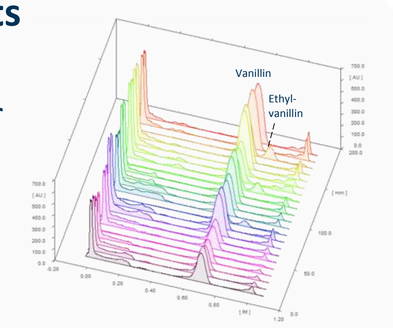
Vanilla is the most expensive spice (up to 600 $/kg) next to saffron. Out of 110 vanilla spieces (*Orchidaceae*) only three have been reported to be important in terms of commerce and cultivation: *V. planifolia, V. pompona and V. tahitensis.* As market prices of vanilla exorbitantly raised the last years, falsification of vanilla products might increase.

Vanillin is the main component responsable for the aroma of vanilla, whereas ethyl vanillin is a synthetic molecule used to imitate vanilla products for its lower costs and higher flavoring strength.

Though many studies, including HPTLC methods, were performed on its complex aroma, comprehensive bioprofiling was found to be exciting to evaluate the wide range of different qualities of vanilla, on the one hand obtained from the supermarket and on the other hand from producers of high-priced supreme qualities.

Quantification of vanillin in vanilla and products

- A study on extraction solvents showed that ethyl acetate - ethanol - water 1:1:1 was suited best for bioprofiling (*t*-butyl methyl ether for pudding-like products).
- The mobile phase *n*-hexane and ethyl acetate 1:1 enabled a reliable quantification of vanillin.



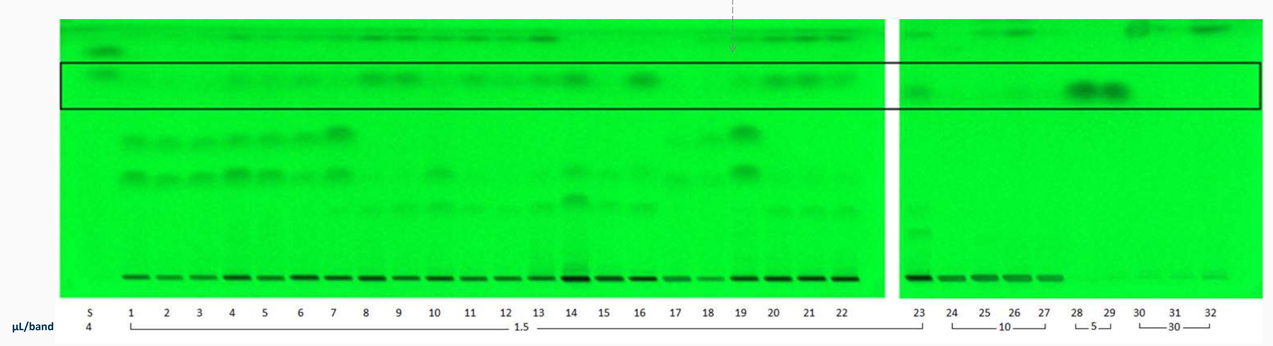
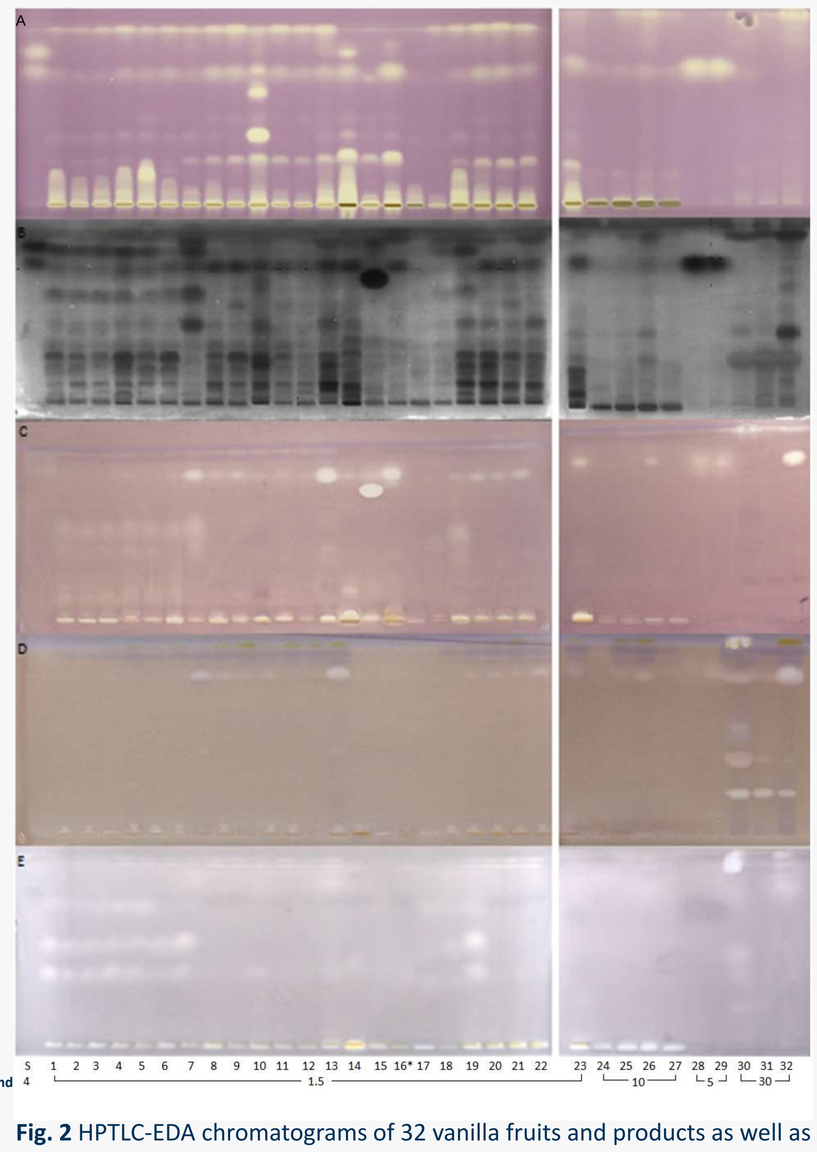


Fig. 1 HPTLC chromatograms at UV 254 nm of vanilla fruits (tracks 1-12), oleoresin (13), alcoholic extract (14-17), inner part (18), powders (19-23), aroma (24), vanilla sugars (25-27) in comparison to vanillin sugars (28-29), ice cream powder (30), puddings (31-32) and standard mixture of vanillin at hR_F 72 and ethylvanillin (S)

Bioprofiling of vanilla and its products

- Vanillin and ethyl vanillin showed antioxidant and antimicrobial properties
- Differences in the bioprofile of vanilla fruits were evident; bioprofiles were directly linked to the content of vanilla added to the food



DPPH•
Radical scavengers as light-yellow zones on purple background

A. fischeri
Gram-negative antimicrobials as dark zones on light-grey background (greyscale image of the bioluminescence)

B. subtilis
Gram-positive antimicrobials as white zones on a deep purple background

AChE
inhibitors as whitish zones on light red-brown background

Tyrosinase
inhibitors as light zones on gray background

Fig. 2 HPTLC-EDA chromatograms of 32 vanilla fruits and products as well as vanillin and ethyl vanillin standards (S) after different effect-directed assays

*B,D and E: 0.5 µL/band; C : 2 (no. 1-23), 1.5 (no. 15), 4 (no. 28-29) and 20 µL/band (no. 30-32)

HPTLC-ESI-HRMS of sample zones

Bioactive zones were eluted via an elution head-based interface into HRMS

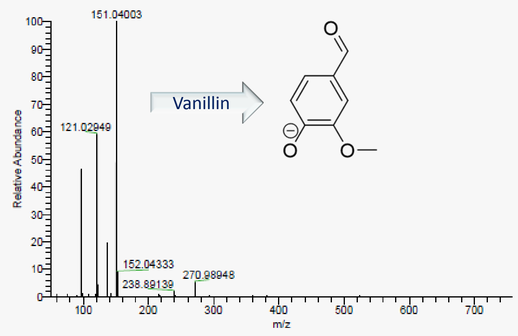


Fig. 3 HPTLC/MS of a zone in a vanilla product tentatively assigned as vanillin showing as basepeak the deprotonated molecule at *m/z* 151.04003 ± 0.3 ppm

Quantification of vanillin in 32 fruits and products

Vanillin content varied between 1 µg/g and 89 mg/g

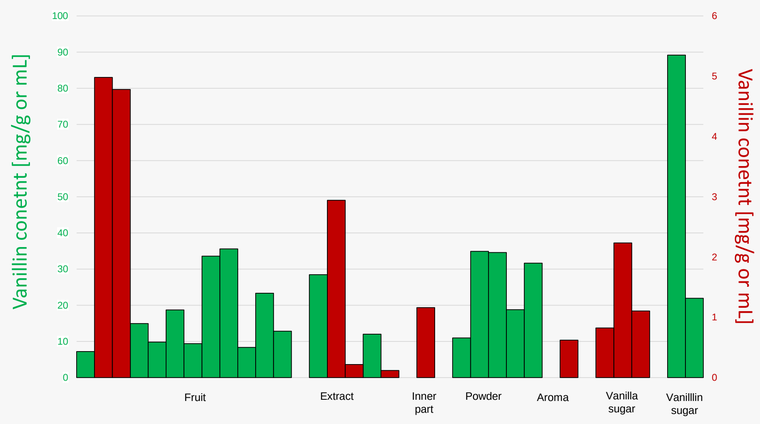


Fig. 4 Overview of the vanillin content determined in different vanilla samples; not depicted: ice cream powder (30) and pudding (31 and 32) due to their low vanillin content (<9 µg/g)

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