# Characterisation of aroma-active compounds in horseradish (*Armoracia rusticana*)

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## Abstract

The aroma composition of freshly ground main roots of horseradish was investigated. Purified extracts of horseradish roots were analysed using the concept of aroma extract dilution analysis (AEDA) and gas chromatography-olfactometry, gas chromatography-mass spectrometry and two-dimensional heart-cut gas chromatography-mass spectrometry/olfactometry. Besides already reported compounds like allyl isothiocyanate and 2-phenylethyl isothiocyanate, a series of odorous substances belonging to different structural classes could be identified, some of them previously unknown or first-time reports in horseradish, and some with high odour potency and potential impact on the overall aroma of horseradish.

## Introduction

Horseradish (*Armoracia rusticana* Gaertn., Mey. et Scherb.) (Figure 1) is a hardy perennial plant belonging to the family of *Brassicaceae* [1] and is distantly related to well-known representatives of this family like cabbage, broccoli, mustard and rapeseed. Horseradish plants possess large leaves [2] and produce white flowers [1], though horseradish is mainly propagated asexually via sets that are obtained from the harvested secondary roots of the previous growing season [3]. It is cultivated in temperate climates in many parts all over the world, but is supposed to have originated from the eastern, temperate regions of Europe [4]. In Europe, the countries with the main growing areas are Austria, Germany, Hungary and Poland. The main reason for cultivation is its white and fleshy root, which is processed to condiments, mainly spicy pastes or sauces; moreover, horseradish is used in traditional phytomedicine, for example as treatment for bronchitis and coughs [5], and has been reported in relation to antimicrobial effects [6, 7].

**Figure 1:** Photograph of harvested horseradish plants

Responsible for the pungent note of the typical horseradish aroma are isothiocyanates (ITCs), which are enzymatically formed from glucosinolates upon cell disruption, when the root is cut or ground [1]. Thiocyanates, nitriles and epithionitriles

*B. Siegmund & E. Leitner (Eds): Flavour Sci.*, 2018, Verlag der Technischen Universität Graz
DOI: 10.3217/978-3-85125-593-5-9, 

are other possible glucosinolate degradation products that can, besides ITCs, undergo further reactions to various chemical substances [8, 9]. ITCs activate branches of the trigeminal nerve and generate a pain sensation. Numerous studies have dealt with the ITC composition and their content in horseradish roots [10-12], but only few have attempted to explore the substances responsible for the overall aroma impression of horseradish [12, 13]. Accordingly, we applied state-of-the-art methods that cover both sensory and analytical techniques to unravel the composition of horseradish aroma.

## Experimental

*Samples and sample preparation*

Seven horseradish main roots from different varieties were chosen for investigation (n = 7). They were grown on different acreages around the city of Baiersdorf, Germany from April till November 2014. Each main root was peeled and shredded with a Moulinex Moulinette. 1 g ground material was extracted with 30 ml dichloromethane for 30 min, the extract filtrated and dried over anhydrous $Na_2SO_4$. The extract was purified via solvent-assisted flavour evaporation (SAFE) and the aroma fraction thereby recovered. Afterwards the purified sample extracts were gently concentrated up to 100 µl through Vigreux distillation and micro-distillation.

*Sensory evaluation*

Aroma extract dilution analysis (AEDA) was applied to determine the relative contribution of the aroma-active compounds to the overall aroma of horseradish roots. Therefore, the 100 µl root extracts were diluted stepwise in a ratio of 1:2 (v/v). The original extracts (= FD 1) and their dilutions were consecutively analysed by means of gas chromatography-olfactometry, applying the cold on-column application technique, until no odour could be perceived at the sniffing port, and the flavour dilution (FD) factors of each odorous substance were determined. The sniffing analysis was primarily conducted by one trained panellist on two different capillary columns, DB-5 and DB-FFAP (J&W Scientific), and cross-checked by two other trained panellists.

*Mass spectrometric analysis*

The horseradish extracts were analysed via gas chromatography-mass spectrometry and two-dimensional heart-cut gas chromatography-mass spectrometry/olfactometry as has been described previously [14]. EI-mass spectra were created at 70 eV ionisation energy in full scan mode (m/z range 40-250/400).

## Results and discussion

A total of 39 odour-active substances was detected [14]. 30 of these substances could be identified, nine of them tentatively via matching retention indices (RI) and odour impressions with those of authentic reference standards.

21 aroma-active compounds from different structural groups could be detected in all seven samples (cf. Table 1). From the group of organic ITCs and nitriles allyl ITC, *sec*-butyl ITC, isobutyl ITC, 3-butenyl ITC, 4-pentenyl ITC, 2-(methylthio)ethyl ITC, 3-(methylthio)propyl ITC, benzyl ITC, 2-phenylethyl ITC and 1-cyano-2,3-epithiopropane (CETP) could be found. Those substances derive enzymatically from glucosinolates when the horseradish root is ground, and exhibit an overall pungent aroma sensation. As group they were detected in a very wide range of FD factors, starting from FD 1 for isobutyl ITC up to FD 2048 for 2-phenylethyl ITC. Acetic acid, *(Z)*-3-hexenal, 3-(methylthio)propanal, 2-phenylacetaldehyde, 1-octen-3-one, 1-nonen-3-one and 2-

phenylethanol, belonging to the structural group of carbonyl compounds, were detected with low or medium FD factors, for example FD 1 to 4 for acetic acid and FD 16 to 64 for *(Z)*-3-hexenal. As the ITCs, many carbonyl compounds are likely to be primarily formed upon root cutting from fatty acids undergoing lipid oxidation. The pyrazines 3-*sec*-butyl-2-methoxypyrazine and 3-isopropyl-2-methoxypyrazine showed ranges from FD 8 to 256 and FD 512 to 4096 respectively. Those pyrazines are most likely formed within the horseradish root through amidation of α-amino acids, condensation with α,β-dicarbonyls and subsequent methylation [15]. The same FD range as for 3-isopropyl-2-methoxypyrazine with likewise high FD factors was determined for the sweet, smoky, peach- and coconut-like smelling (3*S*,3a*S*,7a*R*)-wine lactone (FD 512-4096). 3-Methylindole with its faecal odour impression was detected with medium FD factors of 16 to 128.

**Table 1:** Aroma-active compounds detected in all horseradish samples (n = 7)

| Odorant [a] | *Odour quality* [b] | *RI value* [c] *on* | | *FD factor range* [d] |
|---|---|---|---|---|
| | | *DB-5* | *DB-FFAP* | |
| Acetic acid [f] | Vinegar-like | 636 | 1447 | 1-4 |
| *(Z)*-3-Hexenal [f] | Grassy, green | 806 | 1145 | 16-64 |
| Allyl ITC [f] | Pungent, mustard-like, horseradish-like, onion-like | 883 | 1353 | 512-1024 |
| 3-(Methylthio)propanal [e] | Cooked potato-like | 910 | 1450 | 4-16 |
| *sec*-Butyl ITC [f] | Pungent, green | 933 | 1263 | 4-32 |
| Isobutyl ITC [f] | Pungent, mustard-like | 954 | 1313 | 1-2 |
| 1-Octen-3-one [e] | Mushroom-like | 979 | 1303 | 4-16 |
| 3-Butenyl ITC [f] | Pungent | 982 | 1447 | 4 |
| 1-Cyano-2,3-epithiopropane [f,g] | Onion-like, pungent | 1000 | 1827 | <1-64 |
| 2-Phenylacetaldehyde [f] | Honey-like, sweet | 1044 | 1636 | 2-4 |
| 1-Nonen-3-one [e] | Mushroom-like, fatty | 1079 | 1397 | 1-8 |
| 4-Pentenyl ITC [f] | Pungent | 1082 | 1524 | 2-4 |
| 3-Isopropyl-2-methoxypyrazine [f] | Pea-like, green pepper-like | 1095 | 1423 | 512-4096 |
| 2-Phenylethanol [f] | Flowery, rose-like | 1119 | 1904 | 1-8 |
| 3-*sec*-Butyl-2-methoxypyrazine [f] | Green pepper-like | 1172 | 1493 | 8-256 |
| 2-(Methylthio)ethyl ITC [f] | Pungent | 1206 | 1892 | 1-4 |
| 3-(Methylthio)propyl ITC [f] | Mushroom-like | 1309 | 1967 | 8-128 |
| Benzyl ITC [f] | Pungent, watercress-like, green | 1363 | 2087 | 8-32 |
| 3-Methylindole (skatole) [f] | Faecal | 1391 | 2480 | 16-128 |
| (3*S*,3a*S*,7a*R*)-Wine lactone [e] | Sweet, peach-like, coconut-like, smoky | 1463 | 2214 | 512-4096 |
| 2-Phenylethyl ITC [f] | Horseradish-like, pungent, watercress-like, green | 1467 | 2205 | 512-2048 |

[a] The compounds were identified by comparing them with the reference odorant based on the given criteria (see below).
[b] Odour quality as perceived at the sniffing port.
[c] Retention indices according to Kovats (1958) [16].
[d] Flavour dilution (FD) factor on the capillary column DB-5.
[e] Identification criteria: RIs on capillaries named in table, odour quality and intensity at the sniffing port.
[f] Identification criteria: same as in ([e]) and MS-EI data.
[g] Detected via GC-MS in all samples, but only in four samples contents above the odour threshold.

Based on the FD factor values for each aroma compounds, the most potent odorants in freshly grated horseradish roots were 3-isopropyl-2-methoxypyrazine, (3*S*,3a*S*,7a*R*)-wine lactone, 2-phenylethyl ITC and allyl ITC. Accordingly, it is conceivable that the general aroma impression of horseradish is mainly defined by those four substances. Nevertheless, other substances, particularly those with medium FD factors like the faecal smelling 3-methylindole and the mushroom-like smelling 3-(methylthio)propyl ITC are also likely to contribute to the overall aroma of horseradish. ITCs with medium or low FD factors like benzyl ITC and isobutyl ITC may further enhance the overall pungency of the horseradish flavour, thereby acting as a group. Likewise, green, vegetable-like notes are likely to be contributed by the green pepper-like smelling 3-*sec*-butyl-2-methoxypyrazine and the grassy, green smelling *(Z)*-3-hexenal.

We further detected four substances with medium or low FD factors, respectively, that were found in six out of the seven samples. Those were the cheesy smelling butanoic acid (FD 2-4) and the pungent 3-methylbutyl ITC (FD 1-2), as well as two unknown odorous substances. One had an onion-, vinegar- and cabbage-like smell (FD 4-32), the other was perceived as earthy, mouldy and dusty (FD 1-16). We assume that the former substance could be a sulphur-containing molecule and the other an alkylated pyrazine, as they show sensory traits typical for these substance groups.

Summarising our findings, we detected substances that covered a wide FD range between the different samples, like CETP with a difference of seven dilution steps, whereas others were surprisingly consistent in the investigated varieties, like allyl ITC with a difference of one dilution step only between samples.

Accordingly, the results of this study make a significant contribution to the general knowledge of the chemical principles of horseradish aroma.

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