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Unravelling important odorants in horseradish (*Armoracia rusticana*)

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ABSTRACT

Horseradish (*Armoracia rusticana*) is a plant well known for its roots' spicy aroma. The present study investigates the main aroma constituents of horseradish roots in general by analysing the aroma profiles of six different horseradish varieties, with one variety grown in two different soils. Odorants were characterised by means of gas chromatography-olfactometry and identified via their mass spectra, retention indices on two columns with different polarity, and their characteristic odour. A series of new aroma compounds from different substance groups were identified that have hitherto not been described in horseradish. Moreover, several of these constituents were successfully shown to exhibit high odour potency, alongside a high potential to influence the overall aroma of horseradish roots, like (3*S*,3a*S*,7a*R*)-wine lactone and 3-isopropyl-2-methoxypyrazine.

1. Introduction

Armoracia rusticana (Gaertn., Mey. et Scherb.), commonly known as horseradish, is a hardy perennial plant belonging to the Brassicaceae family. It originates from the temperate eastern parts of Europe (De Candolle, 1885) but is cultivated nowadays in many regions of the world. Although perennial by nature it is mainly grown commercially as an annual crop (Shehata et al., 2009), and is valued for its fleshy root, providing an aromatic pungent smell when being cut. Horseradish is used as an ingredient for many traditional dishes all over the world (Agneta, Moellers, & Rivelli, 2013). Commonly the roots are grated, and mixed with salt and vinegar; the resulting condiment is enjoyed together with, e.g., roast beef, boiled beef or fish (Courter & Rhodes, 1969; Shehata et al., 2009). The pungent smell of horseradish is caused by isothiocyanates (ITCs) (Gilbert & Nursten, 1972; Masuda, Harada, Tanaka, Nakajima, & Tabeta, 1996), which are formed in the course of root cutting as degradation products of the horseradish-specific glucosinolates (GLSs). GLSs are plant secondary metabolites that consist of a β-d-thioglucose linked with a sulfonated oxime group with a variable side-chain (Ettlinger & Lundeen, 1956). These compounds are enzymatically degraded by myrosinase, a thioglucoside glucohydrolase, upon cell disruption; both partners, the enzyme and the glucosinolates are normally stored in different compartments of the cell but come into contact with each other when cells of the plant tissue are disrupted (Bones & Rossiter, 1996; Grob & Matile, 1979). Myrosinase splits off the glucose moiety of GLSs, resulting in the formation of an unstable aglucone, which further degrades to yield ITCs, thiocyanates, nitriles or epithionitriles (Hanschen, Lamy, Schreiner, & Rohn, 2014); the composition of these products depends on factors such as pH, structure of side-chain, the presence of metal ions and specific proteins that are likely to convert the unstable aglucone into different degradation products of the GLSs (cf. Fig. 1). Formation of these products is a means of the horseradish plant to fend off herbivores.

Numerous studies have investigated the GLS degradation products of horseradish. Kojima, Uchida, and Akahori (1973) performed a gas chromatography-mass spectrometry (GC–MS) analysis of a horseradish extract and identified eight different ITCs, namely isopropyl ITC, allyl ITC (AITC), 3-butenyl ITC, 4-pentenyl ITC, phenyl ITC, 3-methylthiopropyl ITC, benzyl ITC and 2-phenylethyl ITC (PEITC), whereas Grob and Matile (1980) tentatively identified 35 different GSL degradation products that were mainly composed of ITCs, but thiocyanates and nitriles were also found. An analysis of the whole volatile spectrum of horseradish was later provided by D'Auria, Mauriello, and Racioppi (2004), who characterised 18 substances in fresh, and 24 compounds in stored horseradish by

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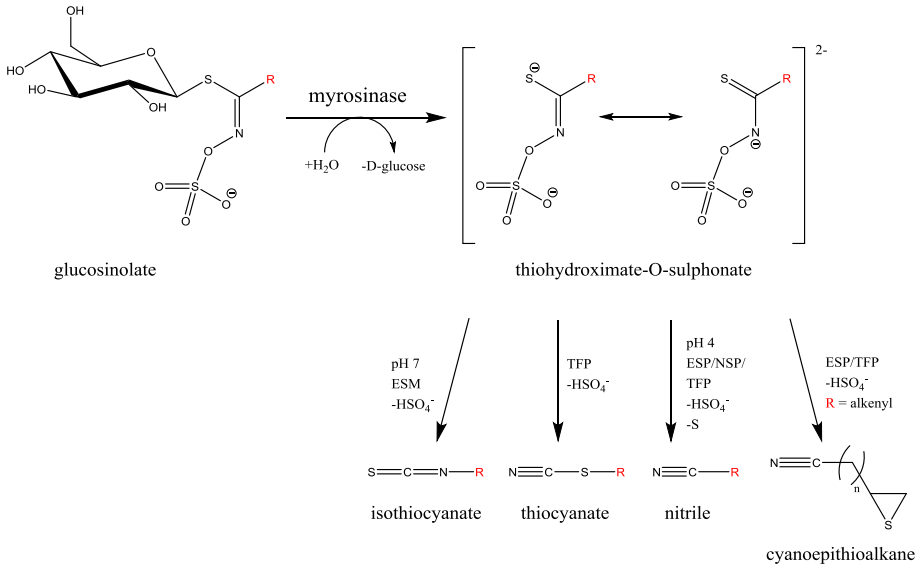

Fig. 1. General scheme of enzymatic glucosinolate degradation and formation of glucosinolate-breakdown products. ESP: epithiospecifier protein; TFP: thiocyanate-forming protein; ESM: epithiospecifier modifier protein; NSP: nitrile-specifier proteins. Adapted from Hanschen et al. (2014).

means of headspace solid-phase microextraction (HS-SPME) and GC–MS, based on comparison of the mass spectra with commercially available databases: amongst these were sulphides like methylthiomethane, ITCs, aldehydes like hexanal or 2-phenylacetaldehyde, nitriles, and terpenes like italicene. Nevertheless, none of the aforementioned studies aimed at elucidating the sensory impact of these individual substances on the overall aroma profile of horseradish.

A first attempt to unravel the substances that are responsible for the aroma of horseradish was accomplished by Gilbert and Nursten (1972). For this aim, the authors performed a gas chromatography-olfactometry (GC-O) analysis on the extract of English and Hungarian horseradish. They identified AITC, allyl thiocyanate, *sec*-butyl ITC, 4-pentenyl ITC and PEITC as aroma-active compounds, with AITC having been found as the most important, and PEITC as the second most important substance with regards to the overall horseradish aroma. Their rating of importance in terms of aroma contribution was based on odour intensity perceived during GC-O, and peak area as determined by means of gas chromatography with flame ionisation detection (GC-FID). The authors further stated that the English horseradish had a more pronounced vegetable-like background aroma than the Hungarian one. Nevertheless, they were not able to identify the underlying substances responsible for this sensory impression. In 1981, Ina, Sano, Nobukuni, and Kishima separated a horseradish extract into neutral, acidic and basic fractions, and investigated the separate fractions via GC–MS. Thereby, the authors were able to detect 10 different ITCs that they evaluated regarding their aroma by means of GC-O. Based on this evaluation they described the aroma impressions of AITC as strong mustard-like, of benzyl ITC and PEITC as radish-like, and of the remaining ITCs as weak mustard-like. GC-O analysis of horseradish extracts was also performed in another study, where 12 aroma-active substances, all GLS degradation products, could be identified; 12 sulfurous-smelling compounds were additionally detected that could, however, not be resolved with regard to their chemical structures (Tokarska & Karwowska, 1983). Later on, a horseradish extract obtained by steam distillation was also evaluated by means of GC-O leading to the detection of six ITCs and a nitrile, namely *sec*-butyl ITC, AITC, 3-butenyl ITC, 4-pentenyl ITC, 2-phenylethylcyanide, benzyl ITC and PEITC (Siegl, Hanke, & Schnitzler, 1995); however, this spectrum rather represented the profile of cooked horseradish, as stated by the authors, and no information about the method of identification was given.

Summarising the above-cited literature on horseradish aroma, it needs to be emphasised that the compiled data did not account for the contribution of single aroma compounds to the overall aroma of horseradish. To close this gap, Masuda et al. (1996) conducted an aroma extract dilution analysis (AEDA) on an extract of horseradish from New Zealand, to comparatively assess the relative aroma strength of specific ITCs in the horseradish sample. Using this approach, the authors confirmed AITC to have the highest and PEITC to have the second highest contribution to the overall horseradish aroma, followed by *sec*-butyl ITC, 3-butenyl ITC and 4-pentenyl ITC.

A comprehensive study exploring the potential contribution of all odour-active compounds responsible for the aroma of horseradish has not been accomplished to date. The question of whether there is more to the aroma of horseradish than just the characteristic pungent smell of ITCs remains unanswered. Accordingly, the aim of our study was to investigate the aroma of horseradish according to a coupled approach combining human-sensory and chemo-analytical strategies for targeted odorant analysis. Our goal was to elaborate which odorants are common in different horseradish varieties and, accordingly, constitute the characteristic aroma profile of horseradish. Moreover, we aimed at comparatively screening different varieties of horseradish for their main aroma constituents, and to elucidate their chemical structures, using methods that are state-of-the-art in modern aroma analysis, such as one- and two-dimensional gas-chromatography-olfactometry, as well as mass spectrometry.

2. Materials and methods

2.1. Horseradish samples

The horseradish varieties VFS × BA, BA × VFS, BA, NA, Kroener and Nyehemes were selected for investigation. These varieties are commercially available and have been cultivated through sets via vegetative reproduction for several decades. They were sourced from the acreage of Norbert Kroener, located around the city of Baiersdorf, Germany, in a radius of about 1.5 km. All varieties were planted in April 2014, and harvested in November of the same