Characterisation of the flavour of the old Austrian apple variety 'Ilzer Rose'

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Abstract

The old apple variety 'Ilzer Rose', coming from the region near the village Ilz (Austria), is an old variety that has been described since approximately 1900. The rather small, intense-red apples with white flesh have a very pleasant, intense fruity and rose-like flavour. The aim of this study was to characterize the flavour of the old apple variety 'Ilzer Rose' but also to identify differences in distribution of volatiles between the skin and the flesh of the apples. The use of comprehensive GC x GC-MS resulted in the detection of more than 600 volatile compounds and offers a completely new picture of the apple volatilome.

Introduction

Styria is Austrian's apple cultivation hot spot. About 80% of the annual yield (corresponding to about 130.000 tons) is harvested in this region. The majority of apples – mainly new apple varieties as Golden Delicious, Gala or Idared – are cultivated in plantations. However, about 25% of the apples are grown in so-called meadow orchards. The traditional meadow orchards have been part of a specific type of landscape for hundreds of years and have hosted an enormous number of old apple varieties since then. Even though these varieties have been cultivated in this region for many decades, their flavour properties have not been characterised so far. Most varieties lack a molecular characterisation of flavour compounds.

In general, the flavour of apples is composed by several hundred different volatile compounds such as alcohols, aldehydes, esters, etc. The composition of the apple volatiles depends on variety, climate, maturity/ripening level and storage conditions [1]. Primary flavour compounds are formed via the enzymatic and biological processes in the intact fruit during growth, maturation and ripening, whereas secondary flavour compounds develop as results of tissue disruption. Apple flavour compounds are produced by several biosynthetic pathways, such as the β-oxidation of fatty acids, which is the primary biosynthetic pathway for ester formation. After cell disruption, the lipoxygenase (LOX) pathway is active and is mainly responsible for the formation of straight chain C6 and C9 aldehydes whereas amino acid degradation reactions lead for example to methyl branched aldehydes and alcohols. It is generally assumed that terpene biosynthesis plays a minor role for apple flavour. However, terpenes are formed via the mevalonic (MVA) pathway or the 2-C-methyl-D-erythriol-4-phosphate (MEP) pathway. In general, compounds such as (*E*)-2-hexenal, hexanal, ethyl-2-methylbutanoate, ethyl butanoate and propyl butanoate are regarded to play a significant role for the apple flavour.

The formation of flavour compounds depends on the presence of precursor compounds and enzyme activities of the fruits, for processed fruits also on the conditions used during fruit processing. In this study we aimed to investigate primary flavour compounds in different parts of 'Ilzer Rose' apples. To reach this aim we applied 1-dimensional GC-MS as well as comprehensive GC x GC-MS for the identification of

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'Ilzer Rose' volatiles after enrichment by Headspace Solid Phase Microextraction (HS-SPME). The enormous capacity regarding separation as well as sensitivity of comprehensive GC x GC-MS allows deep insight into the flavour composition of this old apple variety. In addition, sensory methods were used to characterize the overall flavour properties.

Experimental

Apple samples

Apples were harvested in 2016 from traditionally grown trees from meadow orchards in Styria. Apple skin was carefully separated from the flesh. To inactivate apple enzymes as far as possible, apple flesh and skin were prepared separately according to Aprea et al [2] prior to GC analysis.

Gas chromatographic analysis

Aliquots of the homogenised samples (250 mg each for 1-dim GC-MS and 50 mg for comprehensive GC x GC-MS) were transferred into headspace vials, 2-octanol was used as internal standard (50 ng absolute). Four replicates of each sample were prepared and analysed. After enrichment of the volatiles by HS-SPME (30°C, 20 min, 50/30 µm DVB/CAR/PDMS fibre, 2 cm stable flex fibre) analyses were performed with 1-dimensional GC-MS (Agilent GC 7890, MS 5975c VL MSD, Santa Clara, CA, USA; HP5 30 m*0.25 mm*1 µm, EI (70eV)) and comprehensive GC x GC-MS (Shimadzu GC-2010 Plus coupled with Shimadzu GCMS-QP2010 Ultra, , Shimadzu Europa Gmbh; 1st dim.: ZB-5MS 30 m *0.25 mm*0.25 µm and 2nd dim.: BPX50 2.5 m *0.15 mm*0.15 µm, Zoex cryo modulator, 5s modulation frequency, Hot Jet 280°C, 350 msec pulse time; EI (70 eV)). Identification of the compounds was based on the comparison of the obtained mass spectra to those from MS libraries or authentic reference compounds as well as on retention indices (RI). Linear-temperature programmed RI were calculated using n-alkanes (C_5-C_{26}) and compared to data from authentic reference compounds and data from literature. For comprehensive GC x GC-MS retention indices were calculated for the 1st dimension.

Sensory evaluation

For sensory evaluation, the fruits were cut into cylinders and treated with an antioxidant solution according to Corollaro et al. [3] to avoid (i) browning of the apple pieces and (ii) excessive formation of secondary flavour compounds. Sensory evaluation was performed by 14 well-trained panellists under standardised conditions using quantitative descriptive analysis (QDA®). All panellists had vast experience in evaluating fruits and had undergone apple-specific training prior to this study. Data acquisition was performed by the use of Compusense Sensory Software (Compusense Inc., Guelph, Canada).

Results and discussion

It was the aim of this study to characterize the flavour of the old apple variety 'Ilzer Rose', but also to investigate the distribution of the volatile compounds between the skin and the flesh of the apples.

Sensory evaluation was performed from standardised 'Ilzer Rose' apple pieces after inactivation of apple enzymes at the sample surface. Nine different odour/flavour attributes were chosen by the panel to describe the sensory characteristics of 'Ilzer Rose'.

Results from QDA® demonstrate the pronounced rose-like/floral and fruity properties of ‘Ilzer Rose’ apples (Figure 1).

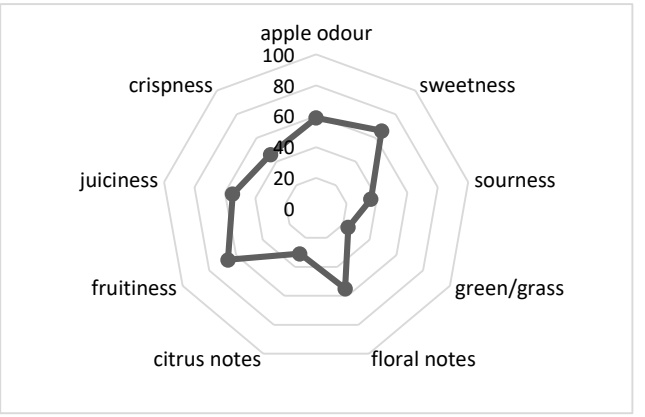

Figure 1: Results from QDA® of ‘Ilzer Rose’ after inactivation of fruit enzymes at the sample surface

A total of 82 volatile compounds was identified from the skin of the ‘Ilzer Rose’ by 1-dimensional GC-MS, in contrast to only 55 volatiles in the flesh alone**.** Significantly higher concentrations of most volatile compounds were found in the skin than in the flesh of the Ilzer Rose apples. Table 1 gives a comparison of the relative concentrations of selected volatiles in the skin and the flesh, respectively. Interestingly, not only the carotinoid cleavage product 6-methyl-5-hepten-2-one and the sesquiterpene α-farnesene – that had already been described in apple coating decades ago [5] – are significantly higher in concentration in the skin, but also esters like hexyl butanoate, hexyl 2-methyl butanoate and hexyl hexanoate (Table 1).

Table 1: Selected volatile compounds semi-quantified in the headspace of the apple skin and flesh samples by 1-dim GC-MS. Concentrations are expressed as relative concentrations to the internal standard 2-octanol

Compound	*RI (HP5)* $_{exp}$	*RI (HP5)* $_{lit}$	*Skin (**mg** kg^{-1})*	*Flesh (**µg** kg^{-1})*
6-Methyl-5-hepten-2-one	986	987[a]	1.6	n.d.
Hexyl acetate	1008	1014[b]	6.6	7
Hexyl butanoate	1188	1193[a]	3.5	3
Hexyl-2-methyl butanoate	1236	1236[c]	2.5	3
Hexylhexanoate	1384	1386[c]	5.0	n.d.
α-Farnesene	1516	1508[d]	24.6	25

[a] RI obtained from authentic reference compounds and collected in the SKAF Flavor database for Food Research Institute, Slovakia, © 2001−2002

[b] RI obtained from www.flavornet.org

[c] RI obtained from http://webbook.nist.gov/

[d] RI obtained from literature [4]

Chromatograms obtained from comprehensive GC x GC-MS analysis clearly demonstrate the differences between flesh and skin (Figure 2). More than 600 volatile compounds were (tentatively) identified in ‘Ilzer Rose’ apples, many of them seen in the apples for the first time. These results are in accordance with recently published data on the volatilome of strawberries – nearly 600 volatiles were described from strawberries after analysis by comprehensive GC x GC-MS [6]. The identified compounds include

well-known apple volatiles like esters, alcohols, aldehydes and ketones, but also a large number of mono- and sesquiterpenes. The presence of high numbers of terpenes predominantly in the skin of 'Ilzer Rose' is of special interest as, so far, terpenes have not been regarded to be important contributors to apple flavour. However, they might be the reason for the expressed floral/rose-like notes that are known from 'Ilzer Rose' apples.

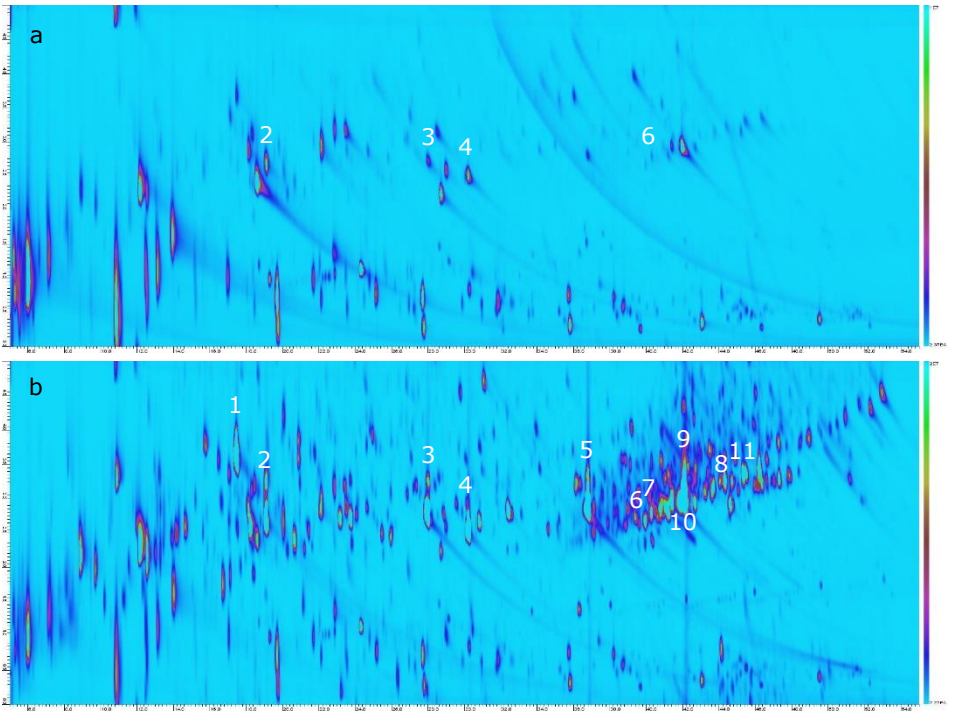

Figure 2: Chromatograms obtained from comprehensive GC x GC-MS; analysis of the (a) flesh and (b) skin of Ilzer Rose apple. Retention times in the first (x-axis) are given in minutes, retention times in the second dimension (y-axis) are given in seconds. (1) 6-methyl-5-hepten-2-one, (2) hexyl acetate, (3) hexyl butanoate, (4) hexyl-2-methylbutantoate, (5) hexyl hexanoate, (6) α-farnesene, (7) cis-β-farnesene^t, (8) cis-thujopsene^t, (9) β-longipinene^t, (10) β-vatirenene^t, (11) cis- α-santalol^t; ^{t}tentatively identified by probability-based matching of the obtained mass spectra with the mass spectra from the NIST library

The results obtained from this study demonstrate that the use of comprehensive GC x GC-MS offers a completely new insight into the apple volatilome. The preliminary results from this study serve as a basis for future investigations of volatiles in different parts of apples in general and of the floral, rose-like odour of 'Ilzer Rose' in particular.

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