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ORIGINAL PAPER

Key aroma volatile compounds of gulupa (*Passiflora edulis* Sims fo *edulis*) fruit

Natalia Conde-Martínez · Aleyda Jiménez · Martin Steinhaus · Peter Schieberle · Diana Sinuco · Coralia Osorio

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Abstract By application of the aroma extract dilution analysis of gulupa (*Passiflora edulis* Sims fo *edulis*), fruit pulp extract obtained by solvent-assisted flavour extraction, and also comparison of chromatographic, spectroscopic (mass spectrum), and odour properties with standards, β-ionone, γ-nonalactone, ethyl butanoate, and ethyl cinnamate were identified as volatiles exhibiting the highest flavour dilution (FD) factor. Among the nineteen odour-active compounds of gulupa, only those showing the highest FD factors were quantified by stable isotope dilution assay. After calculation of odour activity values (OAVs; ratio of concentration to odour threshold in water), ethyl butanoate, ethyl hexanoate, and β-ionone were identified as key aroma compounds in gulupa, responsible for the fruity and floral odour notes.

Keywords *Passiflora edulis* Sims fo *edulis* · Passifloraceae · Volatile compounds · Gulupa · AEDA · Stable isotope dilution assays

Abbreviations

AEDA	Aroma extract dilution analysis
FD	Flavour dilution factor
FID	Flame ionization detector
HS-SPME	Headspace-solid phase microextraction
GC–MS	Gas chromatography coupled to mass spectrometry
GC-O	Gas chromatography-olfactometry
OAV	Odour activity value
RI	Retention index
SAFE	Solvent-assisted flavour evaporation
SIDA	Stable isotope dilution assay

N. Conde-Martínez · A. Jiménez · D. Sinuco · C. Osorio (✉)
Departamento de Química, Universidad Nacional de Colombia, 14490 Bogotá, Colombia
e-mail: cosorior@unal.edu.co

M. Steinhaus · P. Schieberle
Deutsche Forschungsanstalt für Lebensmittelchemie, Lise Meitner Str. 34, 85354 Freising, Germany

Introduction

The cultivation of tropical fruits is one of the major agricultural activities in Colombia, and their exportation has gained economic importance during the last decade, particularly, fruits of the genus Passiflora. The pleasant organoleptic properties of gulupa (*Passiflora edulis* Sims fo *edulis*) explain its increasing popularity in Germany, the Netherlands, and France, with sales of close to 9,000.000 USD in 2011. The trade of this fruit has led the strategy of Colombian government to position exotic fruits in new markets.

It is worth to mention that world production and trade of fresh tropical fruit are expected to expand over the next years. Developing countries account for about 98 % of total production, while developed countries account for 80 % of world import trade [1]. This fact lies on there is a growing demand of consumers for healthy food like tropical fruits, which is more notably in the northern and western member states of European Union.

Taking into account this background information, the characterization of physicochemical properties of gulupa fruit (pH, °Brix, texture, titratable acidity) and peel colour changes in three maturity stages were recently done [2]. Additionally, the volatile composition during the three stages was followed by HS-SPME and GC–MS analyses, finding an increase in the amount of volatile during fruit

ripening, with aliphatic esters as major constituents [3]. The volatile compounds of purple passion fruit (*Passiflora edulis* Sims), the Passiflora species closest to gulupa, have been studied since 1972. The volatile compounds obtained by SDE (Simultaneous Distillation–Extraction) were acid–base fractionated, finding ethyl butyrate and methyl hexanoate as major constituents of neutral fraction [4]. Later, Chen et al. [5] analysed the volatile components of purple passion fruit by headspace over Tenax. As a result, sixty volatiles were identified by GC–MS, with ethyl acetate, ethyl butyrate, hexyl hexanoate, and ethyl hexanoate being the major volatile components. Similar results were obtained using other methods to isolate volatile compounds, such as flash vacuum expansion and liquid–liquid extraction [6]. Additionally, Strohalm et al. [7] determined the enantiomeric composition of acetates, butanoates, hexanoates, and octanoates of the secondary alcohols in purple and yellow passion fruits. These compounds were isolated by SDE, and the enantiodifferentiation was performed by multidimensional gas chromatography over cyclodextrin chiral stationary phase. The enantiomeric excess of 2-alkyl esters series in purple passion fruit was near to 99 % for (*R*)-enantiomers. The chiral analysis of free alcohols revealed that 2-heptanol and 2-pentanol exhibited opposite configuration in both varieties, being the (*R*)-enantiomer predominant in purple variety. Additionally, the study of glycosidically bound volatiles showed that C_{13}-norisoprenoids are predominant in purple passion fruit [8].

So far, no systematic study aiming at the identification of the key aroma compounds in gulupa has been performed before. In the case of fruits, aroma is one of the most appreciated characteristics that confirm the initial impression of consumers and can be used to either define maturity stage or distinguish different fruit types or varieties [9]. Thus, as part of our current studies on flavour of tropical fruits [10–12], the aim of the present work was to identify and quantify the key aroma volatile compounds in gulupa (*Passiflora edulis* Sims fo *edulis*) fruit pulp using the molecular sensory approach [13].

Materials and methods

Plant material

The fruits (exportation quality) from different cultivars of Cota (Cundinamarca, Colombia) were supplied by OCATI S.A. A voucher specimen (COL 527652) was deposited at the Instituto de Ciencias Naturales, Universidad Nacional de Colombia. Ripe fruits were selected according to their pH (2.8), °Brix (17.4), and fruit peeling colour (100 % purple) [2].

Chemicals

Pure reference standards of ethyl 2-methyl propanoate, 2,3-butanedione, ethyl butanoate, ethyl 3-methylbutanoate, (*Z*)-3-hexenal, ethyl hexanoate, 1-octen-3-one, methional, 3-methyl butanoic acid, 3-sulphanylhexyl acetate, linalool, butanoic acid, geraniol, β-ionone, δ-octalactone, γ-nonalactone, ethyl cinnamate, γ-decalactone, and vanillin were purchased from Aldrich (Sigma-Aldrich Chemie, Taufkirchen, Germany).

Sample preparation

Fruit pulp (100 g) was homogenized, and after 5 min, dichloromethane (300 mL) was added to the pulp, and the mixture was cooled in an ice bath. With continuous stirring and cooling, sodium sulphate (300 g) was added in small portions. The mixture was filtered through defatted cotton wool, and the sodium sulphate/gulupa powder obtained was washed with another portion of dichloromethane (200 mL). The combined organic phases, exhibiting the characteristic aroma of gulupa fruits, were submitted to solvent-assisted flavour extraction (SAFE) distillation [14]. The organic phase was dried over anhydrous sodium sulphate and concentrated to 1 mL distilling off the solvent by means of a Vigreux column (50 × 1 cm).

Gas chromatography–FID and gas chromatography-olfactometry (GC-O)

These analyses were performed using a Thermo Fischer Scientific gas chromatograph (Milano, Italy) equipped with an FID and using helium as the carrier gas. Samples were applied by the cold-on-column injection technique. Two capillary columns, DB-FFAP and DB-5 (each 30 m × 0.32 mm i.d., 0.25 µm film thickness, 70 kPa head pressure) (J&W Scientific, Chromatographie-Handel Müeller, Fridolfing, Germany), were used. The column oven was programmed from 40 (after 2 min) to 190 °C at 6 °C/min, and the final temperature, 240 °C for FFAP and 300 for DB-5, was reached at 12 °C/min and held for 10 min.

For GC-O analyses, FFAP column showed better resolution so the end of the capillary was connected to a deactivated Y-shaped glass splitter (Chromatographie Handel Müeller, Fridolfing, Germany) dividing the effluent into two equal parts, one for FID (240 °C) and the other for heated sniffing port (200 °C) using deactivated fused silica capillaries of the same length [10].

Aroma extract dilution analysis (AEDA)

The extract obtained by SAFE was stepwise diluted to obtain dilutions of 2^n [15], and each solution was analysed

by GC-O using a capillary FFAP column under the above-described conditions. The odour activity of each compound, expressed as flavour dilution (FD) factor, was determined as the greatest dilution at which that compound was still detected by comparing all of the runs [16]. The FD factors obtained by three panellists were averaged.

Fractionation of volatiles by column chromatography

To diminish the complexity of volatile extract, the distillate obtained from 500 g of fruit pulp by SAFE was concentrated until 100 mL and extracted three times with an aqueous sodium carbonate solution (0.5 mol/L) to remove the acidic volatiles. The organic phase, containing the neutral and basic volatiles (NBF), was dried over anhydrous sodium sulphate and concentrated to 4 mL using a Vigreux column (50 × 1 cm) to be analysed by GC-O. The combined aqueous phases were acidified (pH 2) with hydrochloric acid (16 %) and extracted with dichloromethane (total volume: 200 mL) to yield the fraction of acidic volatiles (AF), which was also concentrated to 1 mL for GC-O analyses.

A portion of fraction NBF was applied at 12 °C onto a water-cooled glass column (25 × 1 cm i.d.) filled with slurry of purified silica gel (8 g) in pentane. Elution was performed with pentane (50 mL) followed by pentane/diethyl ether (9:1; v/v; 50 mL), pentane/diethyl ether (7:3; v/v; 50 mL), and finally diethyl ether (50 mL). The eluate was collected in 10 mL fractions (F1-F20). After concentration, the odorants detected during AEDA were localized in the fractions by GC-O, and mass spectra were recorded by GC–MS using capillaries DB-5 and FFAP, respectively.

Selective enrichment of thiols

Fraction NBF obtained from 100 g of fruit pulp was concentrated to 5 mL, and the thiols were isolated by affinity chromatography on mercurated agarose gel [17].

Gas chromatography–mass spectrometry (GC–MS)

Mass spectra were recorded on a gas chromatograph Trace Ultra (Thermo Scientific, Dreieich, Germany) coupled to a mass spectrometer Saturn 2200 (Varian, Darmstadt, Germany). Mass spectra in the electron ionization mode (MS/EI) were acquired at 70 eV (ionization energy). A FFAP column was used under the same temperature conditions as mentioned above for GC analysis. MS data were recorded in a mass range 40–350 μ.

Identification

Linear retention indices were calculated according to the Kovats method using a mixture of normal paraffin C_6-C_{28} as external references. The identification of volatile compounds was completed by comparison of their retention indices, mass spectra, and odour notes with those exhibited by solutions of standards if they are available (50 μg/mL).

Quantitation of aroma compounds by stable isotope dilution assays (SIDA)

The fruit pulp was mixed for 5 min by stirring. Then, the labelled standards (0.3–4 μg) dissolved in dichloromethane (50–100 mL) were added to portions of the mixed pulp (1–100 g) and further homogenized. With continuous mixing and cooling in an ice bath, anhydrous sodium sulphate was added (3–300 g). Then, the mixture was filtered through defatted cotton wool, and the sodium sulphate/gulupa powder obtained was washed with another portion of dichloromethane (50–100 mL). The dichloromethane extract was submitted to a SAFE distillation at 40 °C, and the distillates were dried over anhydrous sulphate and concentrated to 0.2 mL by means of a Vigreux column and a microdistillation apparatus.

The concentrates were analysed by means of two-dimensional GC–GC–MS. This system consisted of a Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland), a Trace Ultra GC (Thermo Scientific, Dreieich, Germany), a CP 3800 GC, and a Saturn 2200 mass spectrometer (Varian, Darmstadt, Germany). The Trace GC was equipped with a cold-on-column injector, an FFAP capillary (30 m × 0.32 mm i.d., 0.25 μm film thickness), a moving column stream switching system, and an FID (Thermo Scientific, Dreieich, Germany). The moving column stream switching system was connected to the CP 3800 via an uncoated but deactivated fused silica transfer line (0.32 mm i.d) in a heated (250 °C) hose (Horst, Lorsch, Germany). The GC hosted a cold trap (SGE, Griesheim, Germany) and a DB-1701 column (30 m × 0.25 mm i.d., 0.25 μm film thickness), which was connected to the mass spectrometer. Mass chromatograms were recorded in the chemical ionization (MS/CI) mode using methanol as the reactant. Analyte concentrations were calculated from the area counts of characteristic mass traces of analyte and standard (Table 1).

Isotopically labelled internal standards

The isotopically labelled internal standards, labelled with either deuterium (^{2}H) or carbon-13 (^{13}C), were obtained by synthesis according to the literature given in parenthesis: [$^2H_3$]-ethyl 2-methylpropanoate [18], [$^2H_3$]-ethyl butanoate and [$^2H_3$]-ethyl hexanoate [19], [$^2H_5$]-(*Z*)-3-hexenal [19], [$^2H_2$]-geraniol [20], [$^2H_3$]-β-ionone [21], [$^2H_2$]-δ-octalactone [21], [$^2H_2$]-γ-nonalactone [22], [$^2H_2$]-γ-decalactone [22], and [$^2H_5$]-ethyl cinnamate [23].

Table 1 Mass fragments and calibration factors used in the stable isotope dilution assays for the quantitation of aroma compounds in gulupa (*Passiflora edulis* Sims *fo edulis*)

No.	Compound	Isotopic labelling[a]	m/z[b]		Response factor[c]
			Analite	Internal standard	
1	Ethyl 2-methylpropanoate	$^2H_3$	117	120	1.03
2	Ethyl butanoate	$^2H_3$	117	120	0.96
3	(*Z*)-3-Hexenal	$^2H_5$	81	84:86	0.80
4	Ethyl hexanoate	$^2H_3$	145	148	1.01
5	Geraniol	$^2H_2$	137	139	0.89
6	β-Ionone	$^2H_3$	193	196	0.68
7	δ-octalactone	$^2H_2$	143	145	0.84
8	γ-Nonalactone	$^2H_2$	157	159	0.67
9	Ethyl cinnamate	$^2H_5$	177	182	0.71
10	γ-Decalactone	$^2H_2$	171	173	0.48

[a] Labelling in the isotopologues used as internal standards in the stable isotope dilution assays

[b] Mass traces obtained by GC–MS (CI) used for peak area evaluation of analyte and standard, respectively

[c] Response factor determined from reference mixtures of analyte and standard as described by Steinhaus, Fritsch, and Schieberle [27]

Odour thresholds

Odour thresholds were determined using a panel of 15–20 trained panellists recruited from the Deutsche Forschungsanstalt für Lebensmittelchemie and following the ASTM procedure for the determination of odour and taste thresholds by a forced-choice ascending concentration series method of limits in triangular tests [24].

Aroma profile determination

Samples of fresh gulupa (20 g) were placed into cylindrical ground neck glasses (7 cm × 3.5 cm) with lids and were orthonasally evaluated by a sensory panel (10 people). Descriptors used were determined in preliminary sensory experiments. Each descriptor used was defined on the basis of the odour of a reference compound dissolved in water at a concentration of 100 times above the respective threshold value. Reference odorants used in the experiments were ethyl butanoate (fruity), vanillin (sweet), β-ionone (flowery), (*Z*)-3-hexenal (grassy), and δ-octalactone (fatty). Panellists were asked to rate the intensities of each descriptor in the samples presented on a non-structural scale from 0 to 10, with 0 = not detectable, 5 = weak, 10 = strong. These results were plotted in a spider web diagram.

For aroma reconstitution, appropriate amounts (20–100 μL) of aqueous or ethanolic stock solutions of the odorants were mixed and made up to 1 L with water to yield the same concentrations as determined in gulupa fruit pulp. Final ethanol concentration was kept below 1 g/L, that is, below the odour threshold of ethanol. The overall aroma profile model mixture was determined in the same way as described above for the fresh fruit.

Results and discussion

Dichloromethane was selected as solvent for the extraction of aroma compounds; the odour evaluation of this extract showed greater similarity to the fruit flavour than those obtained with pentane or ethyl ether. Due to low temperature of extraction applied (40 °C) and low pressure used, SAFE method allows the characterization of all aroma-active components using GC-O analysis without artefact formation. Thus, after AEDA analyses 19 odour-active compounds were identified (Table 2) as responsible for fruity, floral, creamy, sweet, and green odour notes. Only 3-sulphanylhexyl acetate was present in the thiol-enriched extract.

Butanoic and 2/3-methyl butanoic acids were detected in acidic fraction. The ratio of 2-methylbutanoic acid to 3-methylbutanoic acid was established as 70:30 from their MS data [10]. Sixteen fractions were obtained by column chromatography of NBF fraction and analysed by GC-O and GC–MS. This procedure allows to unequivocally identify the odour-active volatiles of gulupa fruit pulp.

Volatile compounds with the highest FD values were β-ionone, γ-nonalactone, ethyl butanoate, and ethyl cinnamate. With exception of β-ionone, neither C_{13}-norisoprenoids nor chiral 2-alkyl esters were odour-active compounds in this fruit, in spite of the fact that these compounds are suitable to differentiate yellow and purple passion fruit varieties [7, 8]. By comparison of these results

Table 2 Aroma-active compounds detected in SAFE extract from gulupa fruit pulp

No.[a]	Compound[b]	Odour quality[c]	RI		FD	Fraction[d]
			FFAP	DB-5		
1	Ethyl 2-methyl propanoate	Fruity, sweet	967	758	64	NBF, F7
2	2,3-Butanedione	Creamy, buttery	980	589	8	NBF
3	Ethyl butanoate	Fruity	1027	803	4096	NBF, F7
4	Ethyl 3-methyl butanoate	Fruity, sweet	1064	853	8	NBF, F7
5	(*Z*)-3-Hexenal	Grassy	1147	793	16	NBF, F7
6	Ethyl hexanoate	Fruity	1227	998	64	NBF, F7
7	1-Octen-3-one	Mushrooms	1295	975	8	NBF, F7
8	Methional[e]	Cooked potato	1445	903	2	NBF, F11-F12
9	Linalool	Flowery, citrus	1538	1096	8	NBF, F11
10	Butanoic acid	Rancid	1620	811	1	AF
11	2/3-Methylbutanoic acid[f]	Rancid	1675	885	16	AF
12	3-Sulphanylhexyl acetate[e]	Sweaty	1711	1245	8	NBF, F7-F8
13	Geraniol	Flowery	1841	1236	256	NBF, F13
14	β-Ionone	Flowery	1928	1483	8192	NBF, F7-F11
15	δ-Octalactone	Coconut, caramel	1956	1283	64	NBF, F15
16	γ-Nonalactone	Coconut-like	2018	1360	8192	NBF, F15-F16
17	Ethyl cinnamate	Flowery	2128	1467	1024	NBF, F7-F8
18	γ-Decalactone	Coconut-like	2138	1468	64	NBF, F13-F15
19	Vanillin	Vanilla	2570	1414	128	AF

[a] Odorants were numbered according to their retention time on FFAP column

[b] Odorant were identified by comparing their retention indices on the FFAP and DB-5 column, their mass spectra, and their odour notes with respective data of reference compounds

[c] Odour note as perceived as the sniffing port during GC-O

[d] Fraction(s) in which the compound was detected by GC-O after fractionation into acidic volatiles (AF) and neutral and basic volatiles (NBF), as well as after further fractionation of fraction NBF by column chromatography; for details of column chromatographic fractionation, see "Materials and methods" section

[e] Tentative identification only based on retention index and odour note

[f] 2- and 3-methyl butanoic acid were not separated on the GC-columns used, mass spectral data showed a 70:30 mixture, respectively

with those obtained before [2], the difference between volatile compounds and "odour-active" volatiles is evident. Using HS-SPME several esters such as, ethyl octanoate, hexyl butanoate, and hexyl hexanoate were detected in different maturity stages of gulupa [2]; however, they did not show any influence in the human olfactory bulb. In spite of this fact, the emission of these volatiles through the ripening process is a response to different factors and allows the plant to interact with the environment [25], an important field to be studied.

For the odour-actives compounds which had the highest FD factors (Table 2), a stable isotopologue was synthesized and used as an internal standard in the quantitation [26]. The results of the quantitation experiments (Table 3) show the ranging of the concentration of each odorant. To estimate the aroma potency of the individual gulupa odorants, their concentrations were correlated with the respective odour thresholds using the odour activity value (OAV) concept [15]. Thus, ethyl butanoate, β-ionone, and ethyl hexanoate, responsible for the fruity and floral notes, showed the highest OAV exceeding their threshold by a factor of 362, 170, and 165. On the other hand, the OAVs of δ-octalactone and γ-nonalactone show that these compounds are not key aroma compounds in this fruit, in spite of the fact that γ-nonalactone has one of the highest FD.

In order to validate the identification of aroma-active compounds in gulupa, an aqueous solution containing the eleven odorants which exhibited an OAV greater than 1 (Table 3) was sensory compared with a gulupa fruit puree. The results of sensory panel revealed a close similarity between two samples (Fig. 1). All odour descriptors were qualified with a similar score in the fruit aroma profile as well as in the reconstitution experiment, with the exception of flowery odour note, which was described by the panellists to be more intense in the model mixture as compared to the original gulupa aroma. Unlike other Passifloraceae fruits, sulphury odour note was not relevant in gulupa.

Table 3 Concentrations, orthonasal odour threshold, and odour activity values of aroma-active volatile compounds in gulupa fruit pulp

No.	Compound	Quantitation data			
		No. of replicates	Conc ± SD (% CV) (μg/kg fresh fruit)	Threshold (μg/kg of water)	OAV
1	Ethyl 2-methylpropanoate	4	1.72 ± 0.14 (8 %)	0.089	19
2	Ethyl butanoate	4	275.0 ± 25.0 (9 %)	0.760	362
3	(*Z*)-3-Hexenal	4	0.74 ± 0.11 (15 %)	0.120	6
4	Ethyl hexanoate	2	198.31 ± 2.57 (1 %)	1.200	165
5	Geraniol	4	92.85 ± 3.92 (4 %)	1.100	84
6	β-Ionone	3	594.16 ± 88.3 (15 %)	3.500	170
7	δ-octalactone	4	108.7 ± 6.03 (6 %)	103	1
8	γ-Nonalactone	2	2.69 ± 0.25 (9 %)	9.700	0.3
9	Ethyl cinnamate	3	3.47 ± 0.66 (11 %)	16	0.2
10	γ-Decalactone	4	24.57 ± 1.72 (7 %)	1.100	22
11	Vanillin[b]	3	85.7 ± 5.4 (6 %)	53	2

[a] *OAV* concentration divided by threshold

[b] Quantitation was performed by external standard method

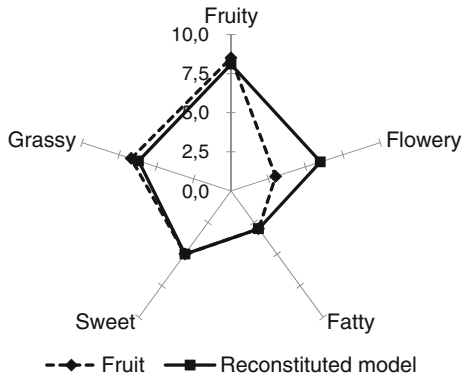

Fig. 1 Comparative aroma profiles of gulupa fruit and aroma reconstitute mixture

Thus, this is the first time that odour-active compounds from gulupa fruit pulp are studied. The above-mentioned results led us to conclude that for the case of this fruit; only a few esters play a role as odour-active compounds.

In conclusion, the systematic approach used in this work allowed focussing the quantitation efforts only in the aroma-relevant volatile compounds. These results are valuable to evaluate the fruit aroma of gulupa after both postharvest handling and package for transportation.

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Conflict of interest None.

Compliance with Ethics Requirements This article does not contain any studies with human or animal subjects.

References

1. FAO Corporate Document Repository (2013) http://www.fao.org/docrep/006/y5143e/y5143e1a.htm. Accessed Jan 2013
2. Jiménez AM, Sierra CA, Rodríguez-Pulido FJ, González-Miret M, Heredia FJ, Osorio C (2011) Food Res Intern 44:1912–1918
3. Jiménez A, Sierra CA, Osorio C (2010) In: Hofmann T, Meyerhof W, Schieberle P (eds) Advances and challenges in flavour chemistry and biology. Deutsche Forschungsanstalt für Lebensmittelchemie, Freising, Germany, pp 378–381
4. Parliment T (1972) J Agric Food Chem 20:1043–1045
5. Chen Ch, Kuo MCh, Hwang LS, Wu JWB, Wu ChM (1982) J Agric Food Chem 30:1211–1215
6. Brat P, Brillouet JM, Reynes M, Cogat PO, Ollé D (2000) J Agric Food Chem 48:6210–6214
7. Strohalm H, Dregus M, Wahl A, Engel KH (2007) J Agric Food Chem 55:10339–10344
8. Winterhalter P (1990) J Agric Food Chem 38:452–455
9. Ong BT, Nazimah SAH, Tan CP, Mirhosseini H, Osman A, Mat Hashim D, Rusul G (2008) J Food Comp Anal 21:416–422
10. Steinhaus M, Sinuco D, Polster J, Osorio C, Schieberle P (2008) J Agric Food Chem 56:4120–4127
11. Sinuco DC, Steinhaus M, Schieberle P, Osorio C (2010) Eur Food Res Tech 230:859–864
12. Sinuco DC, Steinhaus M, Osorio C, Schieberle P (2013) Eur Food Res Tech. doi:10.1007/s00217-012-1883-8
13. Schieberle P, Hofmann T (2011) In: Jelen H (ed) Food flavours—chemical, sensory and technological properties. CRC Press, Boca Raton, FL (USA), Taylor and Francis Group, pp 411–437
14. Engel W, Bahr W, Schieberle P (1999) Eur Food Res Technol 209:237–241
15. Schieberle P (1995) In: Gaonkar AG (ed) Characterization of food: emerging methods. The Netherlands, Elsevier, pp 403–431
16. Grosch W (1994) Flav Fragr J 9:147–158
17. Steinhaus M, Wilhelm W, Schieberle P (2007) Eur Food Res Tech 226:45–55
18. Guth H, Grosch W (1993) J Am Oil Chem Soc 70:513–518
19. Steinhaus M, Sinuco D, Polster J, Osorio C, Schieberle P (2009) J Agric Food Chem 57:2882–2888
20. Fischer A, Schieberle P (2009) Eur Food Res Tech 229:319–328
21. Greger V, Schieberle P (2007) J Agric Food Chem 55:5221–5228

22. Poisson L, Schieberle P (2008) J Agric Food Chem 56:5820–5826
23. Guth H (1997) J Agric Food Chem 45:3027–3032
24. American Society of Testing and Materials (ASTM), Standard E679-04 (2005) In: ASTM Book of Standards, American Society of Testing and Materials, West Conshohocken, PA, 15.08: 38–44
25. Tholl D, Boland W, Hansel A, Loreto F, Röse USR, Schnitzler JP (2006) Plant J 45:540–560
26. Schieberle P, Molyneux RJ (2012) J Agric Food Chem 60:2404–2408
27. Steinhaus M, Fritsch HT, Schieberle P (2003) J Agric Food Chem 51:7100–7105