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Thin-layer high-vacuum distillation to isolate volatile flavour compounds of cocoa powder

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Abstract The volatile fraction of commercial cocoa powders was isolated using thin-layer high-vacuum distillation (TLHVD) of Soxhlet extracts. Calculated and experimental recovery of the internal standard *n*-undecanoic acid methylester did agree, and a good reproducibility was found for the procedure. Around 70 volatile compounds were identified and semi-quantified using internal standard-based gas chromatography (GC), coupled GC–mass spectrometry (GC–MS) and GC–olfactometry (GC–O). Dehydromevalonic acid lactone, (*R*)-(–)-pantolactone and two diastereomer solerols were identified for the first time after purification by micropreparative GC and re-analysis using GC–MS and chiral GC. Strong sensory contributions also came from acetic acid, 2/3-methyl butanoic acid, phenylacetaldehyde, furaneol, dihydroxymaltol, vanillin and phenylacetic acid.

Keywords Cocoa powder · Thin-layer high-vacuum distillation · Volatile flavour · Dehydromevalonic acid lactone · (*R*)-(–)-Pantolactone · Solerol

Introduction

Raw cocoa beans, the fermented, dried and roasted seeds of *Theobroma cacao*, present a rich source of bioactive principles, such as methylxanthines, polyphenols, sal-solinol and biogenic amines. The detection of arachidonoylethanolamine (anandamide), a brain constituent that binds to the cannabinoid receptor [1], in chocolate and cocoa powder has received particular attention [2]. The average consumer is attracted to cocoa products by their pleasant aroma. To convert the cleaned and milled beans (cacao mass) into cocoa powder, the initial fat content of

54% (w/w) is reduced by warm pressing. Lightly defatted cocoa powders contain about 20–22% residual cocoa butter.

Early investigations on cocoa flavour [3] started with 2 tons of beans and yielded 24 g of aroma extract. Numerous investigations since then have aimed at disclosing the complex aroma [4–9]. Plant metabolism, microbial and each of the processing steps, particularly roasting, contribute to the finally obtained spectrum of more than 520 volatiles identified [5, 10, 11]. The key odorants were evaluated using aroma extract dilution analysis [12].

In view of this wealth of chemical data, progress can only come from more refined methods of aroma isolation and analysis. The primary principles to isolate aroma constituents are either based on volatility (variants of distillation) or partition (variants of extraction) or combinations of both [13]. However, the partial pressure of aroma compounds in foods rich in lipids is too low for headspace or conventional distillation [14], and solvent extraction does not separate lipids from non-polar aroma compounds. Thin-layer high-vacuum distillation (TLHVD) of mixtures of food lipids and extraction solvent has been described and validated recently, and the superior recovery compared to other methods was demonstrated [15]. Spiking a lipid phase with a model mixture, a significantly improved recovery especially for high-boiling aroma compounds was achieved. Even low-boilers, such as cyclopentanone (bp 131 °C) were well recovered (87% yield) [15]. The present study applied TLHVD to several customary cocoa powders to evaluate the performance and limitations of the technique for the analysis of aroma compounds from lipid phases of real foods.

Materials and methods

Foods

Cocoa powders “Bensdorp”, lightly defatted, from Unilever Bestfoods, Hamburg, Germany; “Krüger”, lightly defatted, from Krüger, Bergisch Gladbach, Germany; “Sarotti”,

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lightly defatted, from Sarotti Berlin, Germany; Sherry, Medium Dry, Jerez de la Frontera, Espana.

Chemicals

Pantolactone (>99%) and *R*-(-)-pantolactone (>99%) were purchased from Fluka, Germany; *n*-undecanoic and *n*-pentadecanoic acid methyl ester (>99%) were from Sigma, Germany and γ -butyrolactone (>99%) was from Merck, Germany.

5,6-Dihydro-4-methyl-2H-pyran-2-one (dehydromevalonic acid lactone) was synthesised: To a portion of 100 mg of D,L-mevalonic acid lactone 20 mg of water-free oxalic acid was added. Using an oil bath the mixture was heated to 100 °C in a microdistillation apparatus for 20 min. A volume of 10 ml reaction product, dissolved in *n*-pentane/diethylether (azeotrope, 1:1.12), was deacidified with 10 ml of 0.1 sodium hydrogencarbonate and extracted three times with 10 ml of *n*-pentane/diethylether. The combined organic phases were dried with sodium sulfate overnight and concentrated to approximately 1 ml using a Vigreux column as described elsewhere [16]. The product yielded an EIMS at RI 1967 (DB WAX): 112 (M⁺, 50), 83 (10), 82 (100), 57 (7), 55 (14), 54 (33) 53 (15), 43 (10), 41 (10) 39 (37).

Mellein (culture medium of the basidiomycete *Marasmius alliaceus*) [17] and solerol (sherry) were freshly isolated from known sources: 500 ml of sherry or 300 ml of culture medium were mixed with 400 ml MeOH and homogenised using a Waring Blender. The homogenate was centrifuged at 5 °C and 3270 × *g*. The supernatant was made up to 1 l with saturated sodium chloride solution and extracted for 24 h with pentane/diethyl-ether in a continuous liquid–liquid extractor, dried over night and concentrated as described elsewhere [16]. The compounds were identified by GC-olfactometry and GC-MS according to their odour impression, RI (DB WAX) and EI mass spectra.

Cocoa powder extraction

Fifty grams of each cocoa powder was mixed with around 100 g sea-sand (pre-extracted with pentane/diethylether) and exhaustively extracted with pentane/diethylether using a Soxhlet extractor connected with a jacketed coil condenser cooled to 2 °C. The solvent containing lipids and aroma compounds was reduced to a volume of 30 ml using a Vigreux column.

Thin-layer high-vacuum distillation procedure

Samples were stored in a dropping funnel connected with a jacketed coil condenser connected to a 250 ml round-bottom flask with magnetic stirring. The round-bottom flask was placed in a thermostated water bath, and the coil of the condenser was thermostated with circulating

water, both at 60 °C (Julabo F10, Julabo Labortechnik, Seelbach, Germany). The temperature was chosen to maintain the liquid state of the cocoa butter. The apparatus was joined to a high-vacuum system (high-vacuum pump E2M-0-7, turbo molecular pump EXC 120 and active gauge controller, all BOC Edwards, Kirchheim, Germany) across four serial cryogenic traps. After a stable high vacuum below 5×10^{-4} Pa was achieved, the lipid sample containing aroma (30 ml, to which 100 μ g undecanoic acid methyl ester was added as an internal standard) was slowly introduced into the high-vacuum zone. The liquid lipid moved downwards as a thin film with an average flow velocity of about 0.5 ml min⁻¹. The lipid was collected under continuous stirring in the round-bottom flask and stirred for additional 30 min to conclude the distillation process (2 h total distillation time). Analytical blanks were carried out using stripped corn oil (Acros Organics, Geel, Belgium) instead of cocoa butter [15].

Analysis of volatiles

Aroma constituents were recovered from the traps using pentane/diethylether. The combined distillates were dried (Na₂SO₄) and concentrated to approximately 1.0 ml at atmospheric pressure and 40 °C bath temperature using a Vigreux column. Pentadecanoic methyl ester (100 μ g) was added as an external standard to normalise the injection volume.

Identification of cocoa volatiles

Fractionation of distillates: Twenty grams of silica gel (230–400 mesh, Merck, Darmstadt, Germany; activity adjusted to II–III) was poured into a glass column (15 mm × 200 mm) using *n*-pentane. One milliliter of each cocoa distillate was eluted using 150 ml of each *n*-pentane, *n*-pentane/diethylether, diethylether and methanol. The fractions were concentrated to 1 ml using either a Vigreux column (42 °C) or a rotary evaporator (methanol only) at 45 °C and 350 mbar.

GC analyses: One microliter of the concentrated vacuum distillates was injected. Instrument was a Fisons GC-8060 (Fisons, Mainz-Kastel, Germany) equipped with a cool on-column injector and a flame ionisation detector (FID, 250 °C). A 30 m × 0.32 mm i.d. × 0.25 μ m DB-WAX (J&W Scientific) column and hydrogen as the carrier gas at 48 cm s⁻¹ were used. Oven temperature: 45 °C held for 5 min, to 150 °C at 5 °C min⁻¹, to 240 °C at 10 °C min⁻¹ held for 10 min.

GC-olfactometry (GC-O) was performed on a Sato Chrom GC equipped with a cool on-column injection port. The end of the analytical column was splitted (1:1); one part was led to the FID, the other one to a heated sniff port (both at 250 °C). A total of 10 experienced panellists analysed the samples and noted the odour descriptions imparted by compounds while eluting from the sniffing port. Analytical conditions were the same as described for GC-FID.

Chiral GC: Enantiomeric distribution of pantolactone isomers was measured using a double oven gas chromatograph (Sichromat 2–8, Siemens) equipped with a PTV (Programmable Thermal Vaporiser), hydrogen as the carrier gas (3 ml min⁻¹), a CW 20 M capillary column in one oven and a life T-switching device to cut onto a chiral β -cyclodextrin (Cyclosil-B, J&W Scientific) column (temperature program: CW 20 M column: 40 °C – 3 min, 3 °C min⁻¹, 220 °C – 5 min; chiral column: 100 °C – 50 min, 1 °C min⁻¹, 150 °C – 20 min, 1 °C min⁻¹, 220 °C). Identification of enantiomers was based on authentic standards.

Micropreparative GC: An autosampler introduced samples of 3 μ l into the CAS 2 injector of a 5890 series II gas chromatograph equipped with the MCS 2 (Gerstel, Mülheim, Germany). First column was a 5 m \times 0.53 mm i.d. \times 2 μ m CW 20 M (Leupold, Weihenstephan, Germany), second column was a 30 m \times 0.32 mm i.d. \times 0.4 μ m BC SE 54 (Leupold, Weihenstephan, Germany). Oven temperature was programmed 60 °C – 2 min, 5 °C min⁻¹ to 130 °C, 3 °C min⁻¹ to 240 °C – 2 min.

GC-MS: One microliter of each sample was injected on-column. Instruments used were a Fisons GC-8060 (Fisons, Mainz-Kastel, Germany) equipped with a cool on-column injector combined with a mass selective detector MD 800 (Fisons, Mainz-Kastel, Germany). Analytical conditions were: 30 m \times 0.32 mm i.d. \times 0.25 μ m DB-WAX (J&W Scientific) column, carrier gas helium at 3.1 ml min⁻¹, same temperature program as used for GC-FID analysis; detection in the electron impact mode, ionisation voltage 70 eV; continuous scanning from m/z 33 to 300, scan time 0.95 s, interscan delay 0.05 s.

Identification and semi-quantification: Mass spectral identification was achieved by comparing spectra with commercial mass spectral data bases WILEY, NIST and LIBTX. Retention indices were calculated according to the Kovats method, using n -alkanes as external references [15]. Experimental odour qualities and retention indices were compared with published data [17–22] and, if available, with authentic compounds. Approximate concentrations of volatile compounds were calculated according to the internal standard method using n -undecanoic acid methyl ester (100 μ g).

Analytical blanks of each isolation step (Soxhlet extraction, TLHVD, concentration) were carried out to detect all contaminants of the distillates (data not shown).

Results and discussion

The best operating temperature for cocoa fat during the high-vacuum distillation was 60 °C: Crystallisation of cocoa fat commenced visibly below this temperature, and higher temperatures were avoided to maintain gentle conditions of operation. The net distillation time for a standard sample was around 2 h under these conditions. Three independent TLHVD experiments using each of the three cocoa powders spiked with the internal standard n -undecanoic acid methyl ester were carried out. The mean recovery was 59.2%, and the standard deviation was \pm 1.26. This

demonstrated the superior efficiency of recovery and high reproducibility of the method as compared with previous methods [15]. The measured recovery agreed well with previously published prediction derived from a set of model aroma compounds [15]: With a boiling point of 248 °C and a log P of 4.79 as the parameters describing volatility and partition of undecanoic acid methylester, a recovery of 62.6% was calculated [23]. Depending on the type of the lipid the partition coefficient will differ from the coefficient found with n -octanol; thus, a corrected log P value may result in an even better approximation of experimental and predicted data.

The concentrated distillates of the three cocoa samples contained more than 80 separated volatiles, of which around 40 imparted a sensory impression at the sniff port of the GC-O. Of all volatiles around 70 were identified by mass spectrum and retention index (Table 1). In good agreement with most of the earlier studies hydrocarbons, alcohols, aldehydes, fatty acids, ethers, esters, pyrazines, furanones and pyranones were found [4–8]. Another recent study used steam distillation followed by a Likens–Nickerson distillation step [9]. Presence of water and the two-fold heat stress resulted in a different spectrum of compounds clearly demonstrating the effects of the isolation procedure on the resulting composition of volatiles.

Due to the almost equal recoveries obtained for the three samples the quantities of the volatiles are comparable. The predominant peak in all samples was acetic acid, which was not well quantified because of the asymmetric shape of the peak eluted. The “Sarotti” cocoa powder possessed the highest overall volatile concentration (58.98 mg kg⁻¹ versus 50.25 mg kg⁻¹ for “Krüger” and 41.65 mg kg⁻¹ for “Bensdorp”). Its lead was most pronounced in the first half of the chromatogram, and many olfactory signals of lower boilers were recorded, which were missing in the “Krüger” and “Bensdorp” samples. After silica gel fractionation of the “Sarotti” distillate, aroma extract dilution analysis was applied to reduce the number of overlapping sensorial impressions. After a 1:100 dilution, 17 compounds were recognised at the sniff port of the GC-O. The most potent compounds, phenylacetaldehyde, furaneol, dihydroxymaltol and phenylacetic acid, were still detected at a dilution of the distillate of 1:1,000; acetic acid, 2/3-methyl butanoic acid and vanillin were still detected at a dilution of the distillate of 1:10,000 (Table 2). The isomer methyl butanoic acids were not separated under conditions. In earlier studies, the most important sensorial impressions were assigned to dimethyltrisulfide, 2/3-methyl butanal, acetic acid, 2/3-methyl butanoic acid, phenylacetaldehyde, furaneol and phenylacetic acid [4, 6, 8]. Although different methods of isolation were applied, the data agree well, considering that dimethyltrisulfide and the methyl butanals co-eluted with the solvent in the present study. The particular strength of TLHVD appears to rest in the isolation of relatively high-boiling aroma constituents. Compounds with a retention index $>$ 2,000 (CW 20M) were found in concentrations 20- to 40-fold of those reported previously.

As an immediate result, four higher-boiling compounds were identified in cocoa products for the first time:

Table 1 Comparison of composition of volatiles identified in three different cocoa powders

Volatile compound	RI ^a	RI ^b (Lit.)	Concentration (mg kg ⁻¹) ^c		
			Bensdorp	Krüger	Sarotti
<i>n</i> -Hexanal	n.d.	1080	0.07	<0.01	0.36
2-Methyl propanol	n.d.	1043	0.03	<0.01	0.07
3-Pentanol	n.d.	1100	<0.01	<0.01	0.02
1-Butanol	1134	1151	<0.01	<0.01	0.04
<i>n</i> -Heptanal	1170	1186	<0.01	<0.01	0.02
Methylpyrazine	1243	1251	<0.01	0.52	0.42
Acetoin	1263	1260	0.15	0.17	0.37
<i>n</i> -Octanal	1271	1278	<0.01	<0.01	0.05
2,5-Dimethylpyrazine	1281	1306	<0.01	0.65	0.66
2,6-Dimethylpyrazine	1298	1312	0.07	<0.01	0.21
Ethylpyrazine	1302	–	<0.01	0.08	0.13
2,3-Dimethylpyrazine	1306	1315	<0.01	0.09	0.18
2-Ethyl-6-methylpyrazine	1353	1351	0.11	0.21	0.15
2-Ethyl-5-methylpyrazine	1358	1364	<0.01	0.21	0.20
<i>n</i> -Nonanal	1373	1400	<0.01	0.21	0.37
2,4,6-Trimethylpyrazine	1378	1387	<0.01	0.23	0.59
Acetic acid	1418	1429	>10	>10	>10
3-Ethyl-2,5-dimethylpyrazine	1423	1400	<0.01	<0.01	0.25
Tetramethylpyrazine	1440	1458	<0.01	<0.01	<0.01
Benzaldehyde	1485	1502	<0.01	0.47	0.64
2,3-Butanediol	1528	1494	7.09	9.51	11.67
Propanoic acid	1538	1524	0.07	<0.01	0.36
2-Methyl propanoic acid	1566	1535	2.55	3.49	4.70
1,2-Propanediol	1583	1585	0.81	1.14	0.61
γ -Butyrolactone ^d	1592	1642	0.78	1.63	1.26
2-Methyl-3-propylpyrazine	1595	–	<0.01	<0.01	0.15
Menthol	1608	1612	<0.80	<0.01	<0.01
Phenylacetaldehyde	1614	1620	0.28	<0.01	0.66
Butanoic acid	1633	1627	1.06	0.67	0.60
2-Butyl tetrahydrofurane	1643	–	0.40	<0.01	0.57
2-Furanmethanol	1657	1631	0.41	0.60	0.59
2/3-Methyl butanoic acid	1671	1652	11.10	12.41	15.07
Pentanoic acid	1746	1720	0.48	0.49	0.33
Phenylethyl acetate	1794	1823	<0.01	0.36	<0.01
4-Methyl pentanoic acid	1809	1810	0.23	0.38	0.63
Dodecanoic acid methylethyl ester	1831	1840	0.81	1.27	2.52
Dodecanoic acid ethyl ester	1837	1825	<0.01	0.40	0.28
Hexanoic acid	1852	1837	1.28	1.37	0.87
Phenylethanol	1889	1890	0.60	1.33	0.88
3-Methyl butanamide	1903	–	0.21	<0.01	0.23
2-Acetylpyrrole	1950	1950	1.03	0.69	1.17
5,6-Dihydro-4-methyl-(2H)-pyran-2-one, dehydromevalonic acid lactone ^d	1967	–	0.31	0.34	0.20
2-Pyrrolidinone	1988	1966	0.43	0.41	0.31
Phenol	1993	1993	<0.01	0.05	0.06
2-Pyrrolaldehyde	1997	2023	0.79	1.01	0.94
Cinnamic aldehyde	2003	2040	0.13	<0.01	<0.01
Dihydro-3-hydroxy-4,4-dimethyl-2(3H)-furanone, pantolactone ^d	2006	–	0.32	0.41	0.31
4-Hydroxy-2,5-dimethyl-3(2H)-furanone, furaneol	2018	2040	<0.01	0.35	0.46
Octanoic acid	2063	2069	0.32	0.67	0.28
5-Methyl-2-pyrrolaldehyde	2079	–	0.24	0.34	0.47
Nonanoic acid	2110	2100	0.42	0.54	0.27

Table 1 Continued

Volatile compound	RI ^a	RI ^b (Lit.)	Concentration (mg kg ⁻¹) ^c		
			Bensdorp	Krüger	Sarotti
5,6-Dihydro-6-pentyl-(2H)-pyran-2-one, massoi lactone	2185	2239	<0.01	0.21	0.20
Hexadecanoic acid methylester	2207	2203	<0.01	0.27	0.19
3,5-Dihydroxy-2,3-dihydro-6-methyl-4-pyran-4-one, dihydroxy maltol ^d	2240	–	<0.01	0.55	0.12
4-Methyl-5-(2-hydroxyethyl)thiazole	2275	2216	<0.01	0.05	0.19
5-(1-hydroxyethyl)-2(3H)-furanone, solerol isomer ^d	2287	–	0.32	0.36	0.18
5-(1-Hydroxyethyl)-2(3H)-furanone, solerol isomer ^d	2343	–	0.38	0.34	0.19
3-Hydroxypyridine	2370	–	0.07	0.07	0.07
2-Pyridone	2385	–	<0.01	<0.01	0.10
3,4-Dihydro-8-hydroxy-3-methyl-(1H)-2-benzopyran-1-one, mellein ^d (6)	2415	–	0.09	0.51	0.28
Benzoic acid	2432	2401	0.82	0.97	0.17
Vanillin	2532	2569	0.14	0.55	2.32
Phenylacetic acid	2553	2340	6.66	2.40	2.75
2-Phenyl acetamide	2660	2610	0.06	0.06	0.04
Caffeine	>3000	2900	0.53	1.21	1.00

^aRI: Retention index, experimental

^bRI: Retention index from published data

^cCalculated according to the internal standard

^dIdentity proven with authentic standard substances

Note: n.d. not determined

Table 2 Character impact compounds of cocoa powders (for numbering cf. Fig. 1)

Volatile compound	RI ^a	FD ^b	Odour quality ^c	Odour threshold (ng) ^d
Acetic acid	1401	1:10,000	Vinegar	
Propanoic acid	1538	1:100	Mouldy	0.24
2-Methyl propanoic acid	1568	1:100	Rancid	0.79
Phenylacetaldehyde	1614	1:1,000	Flowery	0.01
2/3-Methyl butanoic acid	1671	1:10,000	Sweaty	0.03
5,6-Dihydro-4-methyl-(2H)-pyran-2-one, dehydromevalonic acid lactone (1)	1978	1:100	Condensed milk, slightly sour	0.03
2-Pyrrolidinone	1988	1:100	Popcorn	0.05
Pyrrole-2-carboxaldehyd	1971	1:100	Nut	0.15
Dihydro-3-hydroxy-4,4-dimethyl-2(3H)-furanone, <i>R</i> -(–)-pantolactone (2)	2008	1:100	Caramel, coconut	0.05
4-Hydroxy-2,5-dimethyl-3(2H)-furanone, furaneol	2018	1:1,000	Caramel	0.08
5,6-Dihydro-6-pentyl-(2H)-pyran-2-one, massoi lactone (3)	2177	1:100	Coconut	0.04
3,5-Dihydroxy-2,3-dihydro-6-methyl-4-pyran-4-one, dihydroxy maltol (4)	2240	1:1,000	Caramel	0.002
4-Methyl-5-(2-hydroxyethyl)thiazol	2275	1:100	Peanut	0.03
5-(1-Hydroxyethyl)-2(3H)-furanone, solerol isomer (5)	2287	1:100	Red fruit, jam, green notes	0.03
5-(1-Hydroxyethyl)-2(3H)-furanone, solerol isomer (5)	2345	1:100	Red fruit, jam, green notes	0.03
Vanillin	2532	1:10,000	Vanilla	0.004
Phenyl-acetic acid	2553	1:1,000	Sweet, flowery	0.05

^aRetention index

^bFlavour dilution factor

^cExperimental data as obtained by 10 panellists

^dAt the sniff port exit

Pantolactone, dehydromevalonic acid lactone and the two solerol isomers (Fig. 1). The identity of the impact compounds was confirmed using authentic standards. Most of the impact compounds of cocoa were *O*-heterocycles, among them numerous lactones. The occurrence of γ -butyrolactone in cocoa was described as early as in 1965 [24]. In the cocoa powders examined in this study, the compound showed the highest concentration of all

lactones. Its frequent presence in fermentation flavours has induced speculations on its biogenesis from a deaminated and decarboxylated glutamic acid precursor [25, 26]. Still it did not occur in the group of the most contributory aroma compounds because of its rather high odour detection threshold (Table 2). Dehydromevalonic acid lactone was first identified in smoked meat [27] and more recently in Gorgonzola cheese [28]. Its obvious origin from the

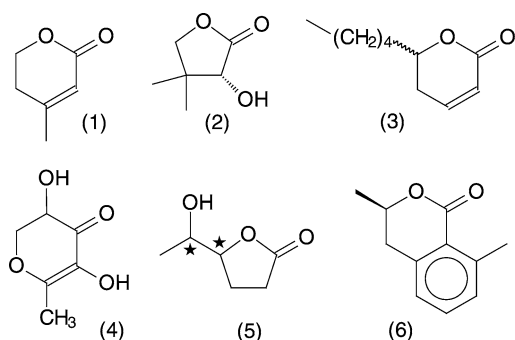


Fig. 1 Some of the most potent volatile flavour compounds of cocoa powder are *O*-heterocycles

ubiquitous terpene precursor mevalonic acid suggests that the compound may also be found in other acidic and heated food products, but has not been isolated in amounts sufficient for its detection by routine GC–MS analyses.

As indicated by its trivial name, pantolactone occurs widespread in food flavours [29–32]. The *R*-(–)-configuration of pantolactone from cocoa was derived from chiral GC by comparing the retention time of the compound from cocoa with the pure compound and a racemate. This configuration dominates in nature. Massoi lactone, a constituent of coffee, soy, pea and milk fat flavour, usually occurs also as the *R*-(–)-enantiomer [12, 33] in nature. As the compound is not commercially available, attempts have been made to detect the compound by submitting fresh butter to TLHVD. However, the distillates did not contain detectable concentrations of the compound.

Solerols are predominant constituents of various wine varieties [26, 34], but have also been found in soy sauce [35] and in fruits [36, 37]. The literature data on stereochemistry and odour properties of solerols are not fully consistent [26, 35]. Multidimensional GC analysis of Sherry aroma resulted in the identification of the *R,S* and *S,R*-enantiomers [38], while another group found the *R,S* and *R,R*-diastereomer pair [34]. All four possible stereoisomers were isolated from dried figs [37]. The solerols contained in the methanol fraction of the total cocoa powder distillate were isolated by micropreparative GC and then submitted to GC–O, GC–MS and chiral GC. The same procedure was applied to a Sherry extract obtained by liquid–liquid extraction. Samples from both origins showed the same chromatographic behaviour and mass spectral data, and the same pair of odorous peaks was detected by GC–O. However, as pure reference compounds were not available, the actual number and absolute stereochemistry of solerols in cocoa could not be conclusively determined.

Conclusion

One of the few remaining challenges of flavour research is the detection of high-boiling compounds in lipidic matrices. Compounds such as furaneol, raspberry ketone or vanillin possess impact character, although they are not very volatile. The above version of TLHVD was shown to

be capable of separating these and other, new high-boiling compounds from a real lipidic food matrix with satisfactory recovery [15]. Careful operation and maintenance of a stable high vacuum will result in a good reproducibility of results. However, the process is time-consuming and, without process automatization, only suitable for use on the laboratory scale. The conditions of operation need to be fine-tuned according to the physico-chemical properties of the bulk phase.

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