European Food Research and Technology (2022) 248:1513–1523
https://doi.org/10.1007/s00217-022-03981-5

ORIGINAL PAPER

Molecular characterisation of an atypical coconut-like odour in cocoa

Caterina Porcelli[1] · **Martin Steinhaus**[1]

Received: 3 December 2021 / Revised: 31 January 2022 / Accepted: 8 February 2022 / Published online: 12 March 2022

Abstract

Parallel application of an aroma extract dilution analysis (AEDA) to the volatiles isolated from a sample of fermented cocoa with an atypically pronounced coconut note and to the volatiles isolated from a reference cocoa sample revealed coconut-like smelling compounds *δ*-octalactone, *δ*-2-octenolactone, *γ*-nonalactone, *γ*-decalactone, *δ*-decalactone, and *δ*-2-decenolactone as potential causative odorants. Quantitation of these six compounds and calculation of odour activity values as ratios of the concentrations to the odour threshold values suggested *δ*-2-decenolactone as the crucial compound. Chiral analysis showed the presence of pure (*R*)-*δ*-2-decenolactone, commonly referred to as massoia lactone. Its key role for the coconut note was finally demonstrated in a spiking experiment: the addition of (*R*)-*δ*-2-decenolactone to the reference cocoa in an amount corresponding to the concentration difference between the two samples was able to provoke a coconut note in an intensity comparable to the one in the atypically smelling cocoa. To avoid an undesired coconut note caused by (*R*)-*δ*-2-decenolactone in the final products, the chocolate industry may consider its odour threshold value, that is 100 µg/kg, as a potential limit for the acceptance of fermented cocoa in the incoming goods inspection.

Keywords Cocoa (*Theobroma cacao* L.) · Coconut aroma · Aroma extract dilution analysis (AEDA) · (*R*)-*δ*-2-Decenolactone · Massoia lactone · (6*R*)-5,6-Dihydro-6-pentyl-2*H*-pyran-2-one

Abbreviations

AEDA	Aroma extract dilution analysis
AV	Acidic volatiles
FD	Flavour dilution
GC–O	Gas chromatography–olfactometry
HDMF	4-Hydroxy-2,5-dimethylfuran-3(2*H*)-one
NBV	Neutral and basic volatiles
OAV	Odour activity value
OTV	Odour threshold value
RI	Retention index
SAFE	Solvent-assisted flavour evaporation

Nomenclature

2-Acetyl-1-pyrroline	1-(3,4-Dihydro-2*H*-pyrrol-5-yl) ethan-1-one
Ethyl cinnamate	Ethyl (2*E*)-3-phenylprop-2-enoate
Geranial	(2*E*)-3,7-Dimethylocta-2,6-dien-1-al
Geraniol	(2*E*)-3,7-Dimethylocta-2,6-dien-1-ol
Linalool	3,7-Dimethylocta-1,6-dien-3-ol
Maltol	3-Hydroxy-2-methyl-4*H*-pyran-4-one
Sotolon	3-Hydroxy-4,5-dimethylfuran-2(5*H*)-one
Vanillin	4-Hydroxy-3-methoxybenzaldehyde

Introduction

Cocoa is the key raw material in chocolate manufacturing and its quality is crucial for the pleasant aroma of chocolate products. Cocoa is derived from the seeds of the cocoa tree (*Theobroma cacao* L.) which is grown throughout the tropics, particularly in Ivory Coast, Ghana, Nigeria, Cambodia, Indonesia, Ecuador, Peru, and Brazil. After harvest, the cocoa fruits, also known as the cocoa pods, are opened and the seeds, 30–50 per pod, are collected together with the surrounding white mucilaginous pulp. In a fermentation step of 2–10 days, the pulp is removed and the seeds, now referred to as the cocoa beans [1], are dried. Annually, more

✉ Martin Steinhaus
martin.steinhaus@tum.de

[1] Leibniz Institute for Food Systems Biology at the Technical University of Munich (Leibniz-LSB@TUM), Lise-Meitner-Straße 34, 85354 Freising, Germany

than 4 million metric tons of fermented and dried cocoa are produced [2]. The cocoa is then shipped internationally to confectionery companies. Chocolate production starts with roasting of the cocoa. The roasted material is ground into cocoa liquor to which further ingredients such as sugar, cocoa butter, and milk powder are added before chocolate is finally obtained by conching, tempering, and moulding.

At the incoming goods inspection in the chocolate industry, the fermented cocoa undergoes a critical sensory evaluation. Ideally, the cocoa shows a rich aroma with sour, malty, floral, and fruity notes [3]. During roasting, further pleasant odour notes develop and the overall aroma intensifies [4, 5]. Occasionally, however, the fermented cocoa beans are tainted with off-flavours among which smoky and mouldy-musty notes are most prevalent [6, 7]. These off-notes can persist during further processing and thus decrease the marketability of the final confectionery products. Whereas the compounds responsible for the pleasant cocoa aroma and their development during processing have been studied in detail [8–11], little has been known on the molecular background of cocoa off-flavours before we started to work on the subject recently. We screened fermented cocoa samples with smoky off-notes as well as samples with mouldy-musty off-notes for potential off-flavour compounds by gas chromatography–olfactometry (GC–O) in combination with aroma extract dilution analyses (AEDA) and substantiated the results by quantitation and calculation of odour activity values (OAVs) [12, 13]. Moreover, we studied the behaviour of the off-flavour compounds during chocolate manufacturing [14]. Finally, we suggested maximum tolerable concentrations for the individual compounds in fermented cocoa which are applicable at the incoming goods inspection level in the chocolate industry and allow for a more objective decision-making on acceptance or rejection of cocoa batches than sensory testing can provide.

Although smoky und mouldy-musty off-flavours occur most frequently in fermented cocoa, another atypical odour has recently become a problem for the quality control departments in the chocolate industry, namely an odour described as coconut-like. The coconut note is clearly not as aversive as the smoky and mouldy-musty off-flavours and such cocoa might even be suitable to make specialty chocolate. However, a strong coconut-like note just occasionally and unpredictably occurring in a mainstream chocolate product is clearly undesired. Customers used to a specific product flavour tend to be very susceptible even to minor variations.

Unequivocal information on the molecular background of such an atypically pronounced coconut-like note in cocoa was not available in the scientific literature. Therefore, the aim of our investigation was to identify the crucial odour-active compound(s) in a sample of fermented cocoa with a pronounced coconut-like odour by applying GC–O and AEDA followed by quantitation and calculation of OAVs. A reference sample with a typical aroma profile was analysed in parallel.

Materials and methods

Cocoa and massoia bark oil samples

Four cocoa samples were provided by chocolate manufacturers. Sample C1 showed the most pronounced coconut note. Samples C2 and C3 also showed the atypical coconut note, but less pronounced. The fourth sample (REF) served as a reference with a typical aroma profile. Sample C1 consisted of cocoa nibs, samples C2 and C3 were cocoa liquors, and REF consisted of whole fermented cocoa seeds. All the samples were stored at 5 °C before analysis. Massoia bark oil was purchased from Maienfelser Naturkosmetik Manufaktur (Wüstenrot, Germany).

Chemicals

Reference odorants **1**–**6**, **8**, **11**–**21**, **23**–**33**, **36**–**48**, and (2*E*)-dec-2-enal were purchased from Merck (Darmstadt, Germany). Odorants **7** and **34** were obtained from Alfa Aesar (Karlsruhe, Germany). Odorants **10** and **22** were purchased from Acros Organics (Schwerte, Germany). Odorants **9** and **35** were synthetized according to literature procedures [15, 16].

The stable isotopically substituted odorants were prepared by approaches described in the literature: ($^2H_2$)-**34**, [6-(2,3-$^2H_2$)propyloxan-2-one] [17]; ($^2H_2$)-**36** [5-(1,2-$^2H_2$)pentyloxolan-2-one], ($^2H_2$)-**42** [5-(1,2-$^2H_2$)hexyloxolan-2-one] [18]; and ($^2H_{2\text{-}4}$)-**44** [6-pentyl(3,3,4,4-$^2H_4$)oxan-2-one] [19].

Dichloromethane, diethyl ether, and *n*-pentane were purchased from CLN (Freising, Germany). Before use, they were freshly distilled through a column (120 × 5 cm) packed with Raschig rings. Ethanol LiChrosolv® and hexane LiChrosolv® were obtained from Merck. Silica gel 60 (0.040–0.63 mm) was purchased from VWR (Darmstadt, Germany) and purified as detailed previously [20]. Low odour sunflower oil, brand Thomy, was from Nestlé (Neuss, Germany).

AEDA

Fermented cocoa seeds and cocoa liquor were flash frozen with liquid nitrogen and coarsely crushed using a laboratory mill Grindomix GM 200 (Retsch, Haan, Germany) at 3800 rpm (2 × 15 s). The material was ground into a fine powder by using a 6875 Freezer Mill (SPEX SamplePrep, Stanmore, UK). The powder (50 g) was stirred with dichloromethane (100 mL) at room temperature for 16 h. The mixture was filtered through a folded paper filter and the

residue was stirred with a second portion of dichloromethane (100 mL) for 1 h. After filtration, the combined extracts were dried over anhydrous sodium sulphate and nonvolatiles were removed by solvent-assisted flavour evaporation (SAFE) at 40 °C [21]. The distillate was shaken with an aqueous solution of sodium hydrogen carbonate (0.5 mol/L; 2 × 100 mL). The aqueous extracts were combined. The organic phase was washed with brine (50 mL), dried over anhydrous sodium sulphate and concentrated (1 mL), first using a Vigreux column (50 × 1 cm) and subsequently a Bemelmans microdistillation device to afford the neutral and basic volatiles (NBV) fraction [22]. The aqueous phase was washed with dichloromethane (50 mL), acidified (pH 2) with hydrochloric acid (5 mol/L), and the acidic volatiles were re-extracted with dichloromethane (3 × 50 mL). The combined organic phases were washed with brine (50 mL), dried over anhydrous sodium sulphate, and concentrated (1 mL) to afford the acidic volatiles (AV) fraction.

The fractions NBV and AV were analysed using a GC–O/FID system (cf. Supplementary Information file) and an FFAP column. Three experienced assessors (2 females, 1 male, aged 27–50) carried out the analyses. GC–O runs were repeated until the individual results were reproducible. Afterwards, fractions NBV and AV were stepwise diluted 1:2 with dichloromethane to obtain dilutions of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024, 1:2048, 1:4096, and 1:8192 of the initial solution and the diluted samples were also subjected to GC–O analysis by the same three experienced assessors. Finally, each odorant was assigned a flavour dilution (FD) factor defined as the dilution factor of the highest diluted sample in which the odorant was detected during GC–O analysis by any of the three assessors [23, 24].

To clarify the structures of the odorants, at first the retention indices (RIs) and odour qualities were compared with data from the Leibniz-LSB@TUM odorant database [25]. In the second step, reference samples of the proposed odorants were analysed by GC–O in parallel to the cocoa volatile isolates by using two capillary columns of different polarity (FFAP and DB-5). Final structure confirmation was achieved by GC–MS. To avoid coelution problems, fraction NBV was further fractionated into five subfractions by liquid chromatography before GC–MS analysis. For this purpose, hexane (1 mL) was added to fraction NBV (1 mL) and the mixture was re-concentrated to a volume of 1 mL. The concentrate was applied onto a slurry of purified silica gel (8 g) in pentane within a water-cooled (12 °C) glass column (1 cm i.d.). Elution was carried out with pentane/diethyl ether mixtures of 100 + 0, 90 + 10, 70 + 30, 50 + 50, and 0 + 100 (v + v; 50 mL each). The eluate was collected in five portions of 50 mL and each portion was dried over anhydrous sodium sulphate, filtered, and concentrated to 1 mL. The odorants previously detected in fraction NBV were localized in the individual subfractions by GC–O. Then the fractions were analysed in parallel to reference compound solutions with a GC–HRMS system (cf. Supplementary Information file) run in EI or CI mode using an FFAP or a DB-5 column.

Odorant quantitation

Samples were powdered as detailed above. Dichloromethane (50 mL) was added to the powder (5 g) and stable isotopically substituted odorants (~ 5 ng–2 µg) in dichloromethane (10 µL–2 mL) were added as internal standards. After magnetic stirring at room temperature for 16 h, the mixture was filtered through a folded paper filter. The residue was stirred (1 h) with a second portion of dichloromethane (50 mL). After filtration, the combined extracts were dried over anhydrous sodium sulphate. Nonvolatiles were removed by SAFE at 40 °C. The distillate was dried over anhydrous sodium sulphate and concentrated (100 µL).

The concentrates were analysed using a heart-cut GC–GC–HRMS system (cf. Supplementary Information file). First, the retention times of the target compounds and the internal standards in the first and second dimensions were determined by analysis of reference mixtures. During analysis of the cocoa volatiles, a heart-cut (1–2 min) of the eluate of the first column containing the respective target compound and the internal standard was transferred to the second column. Transferred substances were refocused in a cold trap. Finally, cooling of the trap was turned off, and the second oven and the mass spectrometer were started. Peak areas corresponding to the analyte and internal standard were obtained from extracted ion chromatograms using characteristic quantifier ions. The concentration of each target compound in the cocoa samples was then calculated from the area counts of the analyte peak, the area counts of the standard peak, the amount of cocoa sample, and the amount of standard added, by employing a calibration line equation previously obtained from the analysis of analyte/standard mixtures in different concentration ratios followed by linear regression. Individual quantifier ions and calibration line equations are available in the Supplementary Information file, Table S1.

Odour threshold values

The odour threshold values of compounds **34**, **36**, **42**, **44** and **45** in low odour sunflower oil were determined orthonasally by a series of three alternative forced choice tests according to the standard practice of the American Society for Testing and Materials [26]. Test samples (10 g) consisted of sunflower oil which had been spiked with the odorant, blank samples consisted of pure sunflower oil. Between two consecutive three alternative forced choice tests, odorant concentrations increased threefold. Samples (10 g) were

presented in cylindrical plastic vessels (5.2 cm height, 3.5 cm i.d.) with lids. Tests were performed at a temperature of 22 ± 2 °C in a special room exclusively dedicated to sensory evaluations. The panel consisted of 15–20 trained assessors.

The odour threshold values of the individual δ-2-decenolactone enantiomers in air were determined by AEDA using the GC–O/FID system (cf. Supplementary Information file) with the chiral MEGA-DEX DAC-Beta column and the approach detailed in [27]. The internal standard was (2*E*)-dec-2-enal with an odour threshold value of 2.7 ng/L [28].

Isolation of massoia bark oil volatiles

Massoia bark oil (2 mL) was diluted with dichloromethane (50 mL) and the mixture was subjected to SAFE. The distillate was dried over anhydrous sodium sulphate, concentrated (1 mL) using a Vigreux column (50 × 1 cm), and stored at − 20 °C before analysis.

Quantitative olfactory profiles

Powdered reference cocoa (5 g) was spiked with δ-2-decenolactone (9 µg of the commercial mixture) in ethanol (150 µL). This sample was subjected to a quantitative olfactory profile analysis together with a sample of the powdered reference cocoa without addition of δ-2-decenolactone and a sample of powdered cocoa C1 with the pronounced coconut-like note. To the latter two samples, 150 µL of pure ethanol were added. Samples (5 g) were presented in cylindrical PTFE vessels (5.7 cm height, 3.5 cm i.d.) with lids. Tests were performed in the special room detailed above. A panel of 19 trained assessors evaluated the three samples orthonasally and assigned scores ranging from 0 to 3 with 0 = not detectable, 1 = weak, 2 = moderate, and 3 = strong to eight pre-defined odour descriptors previously collected by free-choice profiling. For each descriptor an odour reference was provided consisting of an aqueous odorant solution in a concentration ~ 100 times above the orthonasal odour threshold value. The eight descriptors and the corresponding reference odorants were "coconut-like" (γ-nonalactone), "vanilla-like" (vanillin), "honey" (phenylacetaldehyde), "banana-like" (3-methylbutyl acetate), "fruity" (ethyl 2-methylbutanoate), "vinegar-like" (acetic acid), "earthy" (2,3,5-trimethylpyrazine), and "malty" (3-methylbutanal). The scores of the individual assessors were averaged by calculating the arithmetic mean.

Results and discussion

Odorant screening

Parallel application of an AEDA to the volatiles isolated from a cocoa sample with an atypically pronounced coconut note (C1) and to the volatiles isolated from a reference cocoa (REF) revealed a total of 48 odorants with an FD factor of 4 or higher in at least one of the two samples, 46 of which were unequivocally identified (Table 1).

In both samples, the highest FD factors (2048–4096) were determined for fruity smelling compounds ethyl butanoate (**3**) and ethyl 2-methylbutanoate (**4**), bell pepper-like smelling 3-isopropyl-2-methoxypyrazine (**14**), cheesy smelling 2-/3-methylbutanoic acid (**23**), floral, citrusy smelling geranial (**25**), rosy smelling 2-phenylethyl acetate (**28**), cinnamon-like smelling ethyl 3-phenylpropanoate (**31**), honey-like, rosy smelling 2-phenylethan-1-ol (**32**), metallic smelling *trans*-4,5-epoxy-(2*E*)-dec-2-enal (**35**), caramel-like smelling 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (HDMF; Furaneol®) (**38**), and soup seasoning-like smelling sotolon (**43**). These compounds are all well-known cocoa odorants and have been reported in numerous studies before [3, 9, 29–31].

Six of the 48 odorants depicted in Table 1 showed a coconut odour. All six compounds were γ- or δ-lactones, namely δ-octalactone (**34**), γ-nonalactone (**36**), δ-2-octenolactone (**37**), γ-decalactone (**42**), δ-decalactone (**44**) and δ-2-decenolactone (**45**). Their structures are depicted in Fig. 1. Given the fact that their coconut-like odour quality exactly matched the atypical note detected in cocoa sample C1, the six lactones were identified as potential causative compounds and a substantial contribution of other compounds was considered unlikely. In general, γ-lactones and δ-lactones are important contributors to the aroma of different kinds of fruits such as apricots and peaches [32–35], wines and spirits [36], and milk products [19, 37]. Their biosyntheses start from fatty acids. After introduction of a hydroxy group, the chain length is reduced by β-oxidation. Under acidic conditions, the shortened hydroxy carboxylic acids finally undergo cyclization to form the lactones [38]. The six coconut-like smelling lactones identified in the current study have been found in cocoa before [9, 12, 13, 29]; however, none of them has been reported as causative for an atypical aroma note in fermented cocoa so far. Among these six lactones, δ-2-decenolactone (**45**) showed the highest FD factor in the cocoa sample C1 with the atypically pronounced coconut-like odour, namely 2048. In this sample, higher FD factors were only obtained for 2 of the 48 odorants, namely for 2-phenylethyl acetate and sotolon (both 4096). Moreover, with 256 the FD factor of δ-2-decenolactone in the reference sample was clearly lower. High FD factors in sample

Table 1 Odorants in the volatile isolates obtained from the cocoa with the atypically pronounced coconut odour (C1) and from the reference cocoa (REF)

No	Odorant[a]	Odour quality[b]	RI[c]		FD factor[d]	
			FFAP	DB-5	C1	REF
1	Ethyl 2-methylpropanoate	Fruity	< 1000	765	16	4
2	Butane-2,3-dione	Butter	< 1000	< 700	16	8
3	Ethyl butanoate	Fruity	1031	804	256	2048
4	Ethyl 2-methylbutanoate	Fruity	1060	849	512	2048
5	Ethyl 3-methylbutanoate	Fruity, blueberry	1077	852	32	128
6	3-Methylbutyl acetate	Fruity, banana	1130	878	64	64
7	3-Hydroxybutan-2-one	Butter	1262	800	64	32
8	1-Octen-3-one	Mushroom	1298	979	16	8
9	2-Acetyl-1-pyrroline[e]	Popcorn	1332	922	4	4
10	Dimethyl trisulfide	Cabbage	1370	967	256	8
11	2,3,5-Trimethylpyrazine	Earthy	1383	1002	64	4
12	Unknown	Rose, citrus	1411		4	< 1
13	Ethyl cyclohexanecarboxylate	Fruity	1414	1131	< 1	256
14	3-Isopropyl-2-methoxypyrazine	Bell pepper	1430	1095	2048	256
15	Acetic acid	Vinegar, pungent	1436	< 700	64	32
16	3-(Methylsulfanyl)propanal[e]	Cooked potato	1454	905	1024	512
17	3-Isobutyl-2-methoxypyrazine	Bell pepper	1518	1184	256	128
18	(2*E*)-Non-2-enal	Green, fatty	1532	1160	64	64
19	Linalool	Citrus, bergamot	1545	1102	32	64
20	2-Methylpropanoic acid	Sweaty, cheese	1553	789	64	64
21	Butanoic acid	Sweaty, cheese	1618	821	128	512
22	Phenylacetaldehyde	Honey	1643	1046	256	128
23	2-/3-Methylbutanoic acid	Cheese	1655	874	2048	2048
24	2-Acetylthiazole	Popcorn	1667	1038	256	16
25	Geranial	Floral, citrus	1711	1269	2048	2048
26	(2*E*,4*E*)-Deca-2,4-dienal	Fatty, deep-fried	1780	1317	16	8
27	Ethyl phenylacetate	Honey	1795	1246	32	256
28	2-Phenylethyl acetate	Rose	1822	1256	4096	512
29	Geraniol	Citrus, rose	1843	1256	256	256
30	2-Methoxyphenol	Smoky	1858	1090	1024	512
31	Ethyl 3-phenylpropanoate	Cinnamon	1881	1418	1024	2048
32	2-Phenylethan-1-ol	Honey, rose	1919	1116	2048	2048
33	Maltol	Caramel	1961	1110	512	64
34	*δ*-Octalactone	Coconut	1984	1250	512	512
35	*trans*-4,5-Epoxy-(2*E*)-dec-2-enal	Metallic	2012	1382	1024	2048
36	*γ*-Nonalactone	Coconut	2029	1393	1024	1024
37	*δ*-2-Octenolactone	Coconut, creamy	2047	1264	16	16
38	HDMF[e]	Caramel	2028	1071	2048	128
39	Unknown	Burnt	2050		256	256
40	4-Methylphenol	Faecal, horse stable	2072	1078	32	32
41	Ethyl cinnamate	Sweet, cinnamon	2106	1469	16	16
42	*γ*-Decalactone	Coconut, peach	2140	1466	1024	1024
43	Sotolon[e]	Soup seasoning	2200	1107	4096	1024
44	*δ*-Decalactone	Coconut	2210	1494	32	1
45	*δ*-2-Decenolactone	Coconut	2255	1475	2048	256
46	3-Methyl-1*H*-indole	Faecal, mothball	2513	1390	8	4
47	Phenylacetic acid	Honey, beeswax	2547	1261	32	16
48	Vanillin	Vanilla	2573	1408	512	256

[a]Each odorant was identified by comparing its retention indices on two GC capillaries of different polarity (FFAP, DB-5), its mass spectrum obtained by GC–MS, as well as its odour quality as perceived at the sniff-

Table 1 (continued)

ing port during GC–O with data obtained from authentic reference compounds analysed under the same conditions

[b]Odour quality as perceived during GC–O analysis at the sniffing port

[c]Retention index; calculated from the retention time of the odorant and the retention times of adjacent *n*-alkanes by linear interpolation

[d]Flavour dilution factor; dilution factor of the highest diluted sample prepared from the concentrated SAFE distillate in which the odorant was detected during GC–O by any of three assessors

[e]A clear mass spectrum could not be obtained in the cocoa volatile isolates; identification was based on the remaining criteria detailed in footnote a

C1 were additionally obtained for γ-nonalactone (1024), γ-decalactone (1024), and δ-octalactone (512). For these 3 compounds, however, the FD factors indicated no difference between sample C1 and the reference sample. Low FD factors of δ-2-octenolactone (**37**) and δ-decalactone (**44**) suggested only a minor role of these compounds for the atypical odour in C1.

In summary, the odorant screening resulted in six compounds that potentially contributed to the atypical odour of the coconut-like smelling cocoa C1, but their individual roles needed further clarification. Therefore, the next steps in our study were to quantitate the six lactones and compare the concentrations with the odour threshold values of the individual compounds by calculating odour activity values.

Quantitation and calculation of odour activity values

The concentrations of the six coconut-like smelling lactones were determined by GC–MS analysis of volatile isolates obtained by solvent extraction and SAFE. To compensate for losses during sample workup, stable isotopically substituted odorants (cf. Supplementary Information file, Table S1) were added prior to the workup as internal standards. For compounds **34**, **36**, **42**, and **44**, deuterated isotopologues of the target compounds were employed. Isotopologues were not available for compounds **37** and **45**. Instead, the deuterated isotopologues of the corresponding saturated lactones **34** and **44** were used as internal standards. The results obtained for samples C1 and REF previously used for the odorant screening are shown in Table 2 together with the data of two additional samples (C2, C3) having an atypical, though less pronounced coconut note than sample C1. The concentrations of compounds **34**, **36**, **37**, **42**, and **44** were hardly suitable to explain the sensory difference between the samples. Although, for example, compounds **36** and **44** showed clearly higher concentrations in the samples with the atypical note than in the reference sample, the highest concentrations were obtained in sample C2, and not in C1 which showed the most intense coconut note. In contrast, the concentration of δ-2-decenolactone (**45**) was well in line with the sensory rating, thus being highest in sample C1 and lowest in the reference sample. This would be in line with a major role of δ-2-decenolactone for the coconut odour.

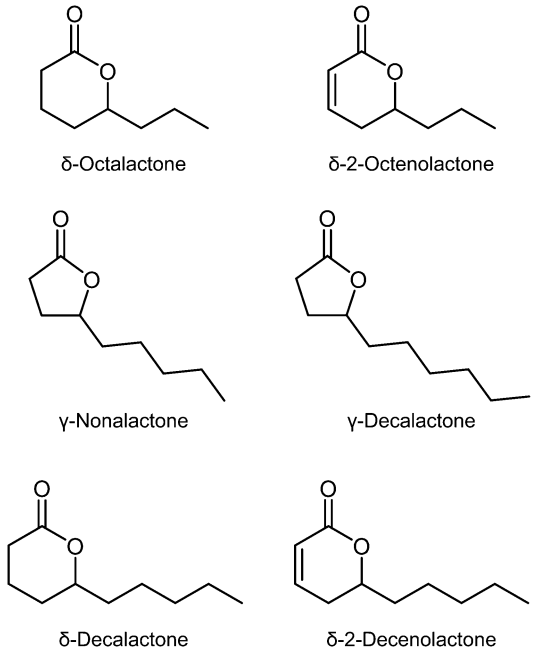


Fig. 1 Coconut-like smelling compounds identified in the cocoa samples

The picture became clearer, when the odour threshold values of the compounds were taken into account. For this

Table 2 Concentrations of lactones in three samples of fermented cocoa with an atypically pronounced coconut odour (C1, C2, C3) and in the reference cocoa sample (REF)

No	Odorant	Concentration (µg/kg)[a]			
		C1	C2	C3	REF
34	δ-Octalactone	0.195	0.744	0.355	0.422
36	γ-Nonalactone	152	191	162	125
37	δ-2-Octenolactone	0.319	1.59	0.900	0.706
42	γ-Decalactone	51.3	413	52.3	109
44	δ-Decalactone	76.0	461	92.3	6.98
45	δ-2-Decenolactone	1580	570	210	86.1

[a]Mean of duplicates or triplicates; individual values and standard deviations are provided in the Supplementary Information file, Table S2

Table 3 Odour activity values (OAVs) of lactones in three samples of fermented cocoa with an atypically pronounced coconut odour (C1, C2, C3) and in the reference cocoa sample (REF)

No	Odorant	OTV_{oil}[a] (µg/kg)	OAV[b]			
			C1	C2	C3	REF
34	δ-Octalactone	1600[c]	0.00	0.00	0.00	0.00
36	γ-Nonalactone	1300[c]	0.12	0.15	0.12	0.10
37	δ-2-Octenolactone	4700[c,d]	0.00	0.00	0.00	0.00
42	γ-Decalactone	4800[c]	0.01	0.09	0.01	0.02
44	δ-Decalactone	4300[c]	0.02	0.11	0.02	0.00
45	δ-2-Decenolactone	120[e]	13	4.7	1.7	0.71

[a]Orthonasal odour threshold value in low odour sunflower oil

[b]Odour activity value; calculated as ratio of the concentration in the cocoa sample to the OTV

[c]OTV of a racemic mixture

[d]Data from literature [9]

[e]OTV of a commercial sample with unknown enantiomeric ratio

purpose, the concentrations (cf. Table 2) were divided by the respective odour threshold values previously determined with low odour sunflower oil as matrix to obtain the odour activity values of the compounds in the four samples. The odour activity values thus represented the factors by which the concentrations in the cocoa samples exceeded the respective odour threshold values. The results, however, included an approximation due to the chirality of the compounds. The odour threshold values of compounds **34**, **36**, **37**, **42**, and **44** were determined with racemic mixtures, whereas the enantiomeric distribution in the cocoa samples was unknown. Moreover, the odour threshold value of δ-2-decenolactone (**45**) was determined with a commercial mixture, the enantiomeric composition of which was also unknown. Nevertheless, the result was very clear, because the concentrations of compounds **34**, **36**, **37**, **42**, and **44** were by far below the respective odour threshold values of the racemates in all four samples corresponding to odour activity values between 0.00 and 0.15 (Table 3). In contrast, δ-2-decenolactone (**45**) showed approximated OAVs of 13 in sample C1, 4.7 in sample C2, and 1.7 in sample C3. In the reference sample, the concentration of δ-2-decenolactone was below the OTV resulting in an OAV of 0.71. The low OAVs of compounds **34**, **36**, and **42** were somewhat surprising, given their rather high FD factors in the AEDA (cf. Table 1). This exemplifies the importance of avoiding overinterpretation of AEDA data. Instead, AEDA results should always be substantiated by quantitation and calculation of OAVs [24]. In summary, the OAV calculations suggested that δ-2-decenolactone (**45**) was mainly responsible for the atypical odour of samples C1, C2, and C3.

δ-2-Decenolactone was first reported in 1937 by Abe who identified it as the primary odour-active compound in the bark of the massoia tree (*Cryptocaria massoia*), a tropical tree growing wild in the rain forests of New Guinea. Before cheaper synthetic flavourings became available, massoia bark oil was widely used as coconut flavouring [39, 40]. In massoia bark, only the (*R*)-isomer of δ-2-decenolactone is present [41, 42]. (*R*)-δ-2-decenolactone is therefore also often referred to as massoia lactone.

Chiral analysis of δ-2-decenolactone

To confirm the crucial role of δ-2-decenolactone for the atypical note in the coconut-like smelling cocoa sample C1, a spiking experiment was considered the method of choice: δ-2-decenolactone would be added to the reference sample to reach the same concentration as determined in C1 and the mixture would be sensorially evaluated. This experiment, however, needed to consider the enantiomeric distribution of the δ-2-decenolactone in the cocoa beans and in the spiking solution, because enantiomers often widely differ in their odour threshold values and sometimes even in their odour qualities. This has, for example, clearly been demonstrated for a homologous series of saturated γ- and δ-lactones [32, 36]. In most cases, the (*R*)-enantiomers showed a higher odour potency than the (*S*)-enantiomers. For δ-2-decenolactone, however, no such data was available. Therefore, we subjected the δ-2-decenolactone reference sample to enantioGC analysis using a chiral β-cyclodextrin-based GC column. The experiment revealed an enantiomeric ratio of 17/83. Analysis of a natural massoia bark oil allowed to assign the elution order as S before R. Thus, our commercially obtained reference compound consisted of 83% (*R*)-δ-2-decenolactone and 17% (*S*)-δ-2-decenolactone. GC–O showed that both enantiomers have a coconut-like odour; however, they clearly differed in their odour potency. Using the method of Ullrich and Grosch [27], the odour threshold values in air of both δ-2-decenolactone enantiomers were determined by AEDA. Results (Table 4) showed that also in δ-2-decenolactone, the (*R*)-enantiomer represented the more potent odorant. With 1.6 ng/L, its odour threshold value was ~ 30 times lower than that of the (*S*)-enantiomer.

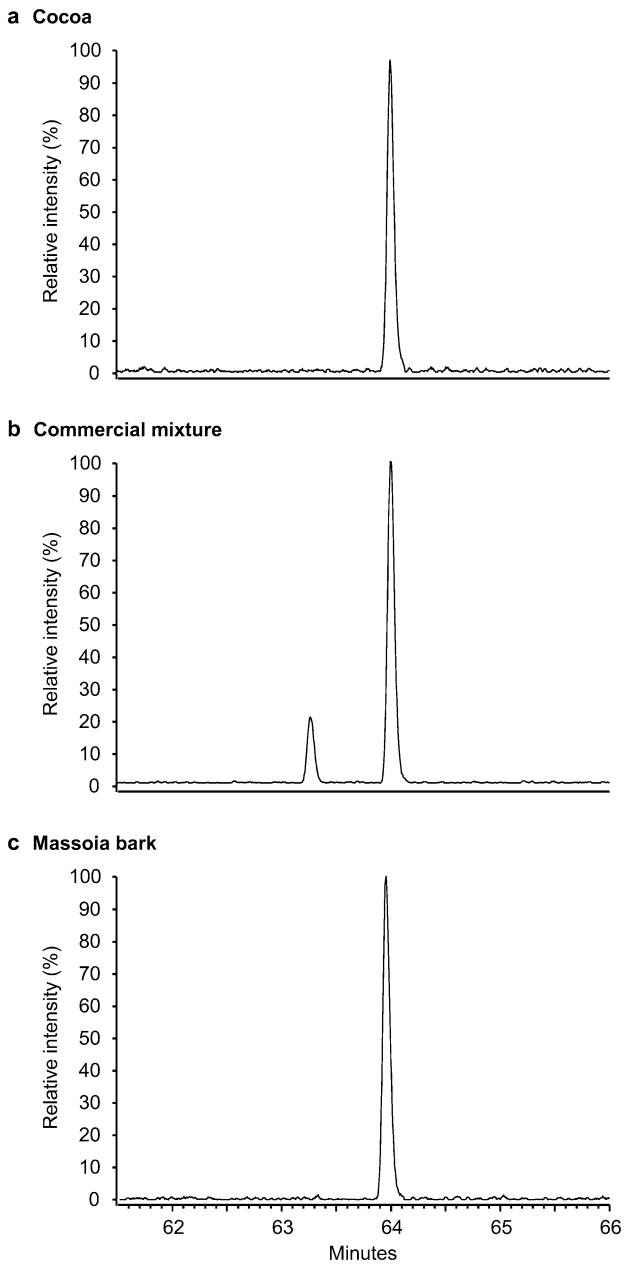


Fig. 2 Heart-cut GC–enantioGC–HRMS analysis of the volatile isolate obtained from the cocoa sample C1 with the atypically pronounced coconut note (**a**) in comparison to the commercial mixture of *δ*-2-decenolactone with 17% (*S*)- and 83% (*R*)-enantiomer (**b**) and the volatile isolate obtained from massoia bark oil (**c**)

As a next step, we determined the enantiomeric ratios of *δ*-2-decenolactone in the cocoa samples. For that purpose, the volatile isolates were subjected to two-dimensional gas chromatography with heart-cutting using the chiral column in the second dimension and a high resolution mass spectrometer as the detector. Results showed that the *δ*-2-decenolactone in cocoa was pure (*R*)-enantiomer. Figure 2 depicts the relevant chromatogram sections after injection

Table 4 Odour threshold values (OTVs) of (*R*)- and (*S*)-*δ*-2-decenolactone in air

No	Odorant	Odour quality	OTV_{air} (ng/L)
45a	(*R*)-*δ*-2-Decenolactone	Coconut	1.6
45b	(*S*)-*δ*-2-Decenolactone	Coconut	52

Table 5 Odour activity values (OAVs) of (*R*)-*δ*-2-decenolactone in three samples of fermented cocoa with an atypical coconut-like odour (C1, C2, C3) and in the reference cocoa sample without a coconut-like odour note (REF)

Odorant	OTV_{oil}[a] (µg/kg)	OAV[b]			
		C1	C2	C3	REF
(*R*)-*δ*-2-decenolactone	100	16	5.6	2.1	0.84

[a]Odour threshold value in oil; approximated from the odour threshold value in air (cf. Table 4) and the odour threshold value of the 83/17 (*R*)/(*S*)-mixture in oil as 0.83×120 µg/kg + $(1.6/51.5) \times 0.17 \times 120$ µg/kg

[b]Odour activity value; calculated as ratio of the concentration in the cocoa sample (cf. Table 2) to the OTV in oil

of the volatile isolate obtained from sample C1 with the atypically pronounced coconut note (Fig. 2a), the reference mixture with 83% (*R*)- and 17% (*S*)-enantiomer (Fig. 2b), and the volatile isolate obtained from massoia bark oil (Fig. 2c). In addition to massoia bark and fermented cocoa, enantiopure (*R*)-*δ*-2-decenolactone has also been reported in Merlot and Cabernet Sauvignon musts and wines, where it contributes to dried fruit aroma notes [40].

With the knowledge of the enantiomeric purity of *δ*-2-decenolactone in fermented cocoa and the knowledge on the difference in the odour potency of the two enantiomers, a better approximation of its relevance for the atypical coconut-like odour was possible. From the odour threshold values of the individual enantiomers in air (cf. Table 4) and the odour threshold value of the reference mixture with 83% (*R*)- and 17% (*S*)-enantiomer in oil (120 µg/kg; cf. Table 3), the odour threshold values of the individual enantiomers in oil were approximated. The calculations resulted in odour threshold values in oil of 3300 µg/kg for the (*S*)-*δ*-2-decenolactone and 100 µg/kg for the (*R*)-*δ*-2-decenolactone. Using the latter for the OAV calculations of (*R*)-*δ*-2-decenolactone in the cocoa samples resulted in values of 16 in C1, 5.6 in C2, and 2.1 in C3, whereas in the reference sample REF the OAV was below 1 (Table 5). In summary, these data supported the hypothesis that (*R*)-*δ*-2-decenolactone was the compound being responsible for the atypical coconut-like odour in the fermented cocoa samples.

Final evidence was eventually provided for sample C1 by a spiking experiment.

Odorant spiking

A sample of the reference cocoa REF was spiked with 1800 µg/kg of the commercially obtained δ-2-decenolactone which corresponded to 1490 µg/kg (*R*)-δ-2-decenolactone and thus the concentration difference between the reference sample REF and the sample with the atypically pronounced coconut note C1 (cf. Table 5). In this experiment, the odour contribution of the (*S*)-δ-2-decenolactone included in the commercially obtained δ-2-decenolactone was considered negligible; approximated from its percentage (17%) and its relative odour potency (1.6/51.5; cf. Table 4), this contribution was only 0.5%, whereas 99.5% of the odour could be attributed to the (*R*)-isomer.

The spiked sample was orthonasally compared to the reference cocoa without addition of (*R*)-δ-2-decenolactone and to the cocoa sample C1 with the atypical coconut-like odour note in a quantitative olfactory profile analysis. The result (Fig. 3) clearly showed that the spiking with the (*R*)-δ-2-decenolactone was able to provoke the atypically pronounced coconut odour. The rating of the coconut note in the spiked sample (Fig. 3a) was clearly higher than that in the reference cocoa without addition of (*R*)-δ-2-decenolactone (Fig. 3b) and in the same range as the rating in sample C1 (Fig. 3c).

Conclusion

The combination of a comparative odorant screening by AEDA, the targeted quantitation of potentially relevant compounds identified by their specific odour, and the calculation of odour activity values suggested δ-2-decenolactone as the compound causative for an atypically pronounced coconut odour in a sample of fermented cocoa. Chiral analysis indicated the presence of pure (*R*)-δ-2-decenolactone (Fig. 4). A spiking experiment finally confirmed the crucial role of (*R*)-δ-2-decenolactone for the coconut-like aroma note in this sample. In accordance with the concentrations determined in the three cocoa samples with an atypical coconut note and the reference cocoa without pronounced coconut note (cf. Table 5), the chocolate industry may consider 100 µg/kg, that is the odour threshold value of (*R*)-δ-2-decenolactone, as a provisional limit for the acceptance of fermented cocoa in the incoming goods inspection.

Further studies on the topic are required to achieve a more comprehensive understanding of the molecular basis of atypical coconut notes in cocoa. These should include a larger number of samples and clarify in particular whether in other cocoa samples further lactones may contribute to

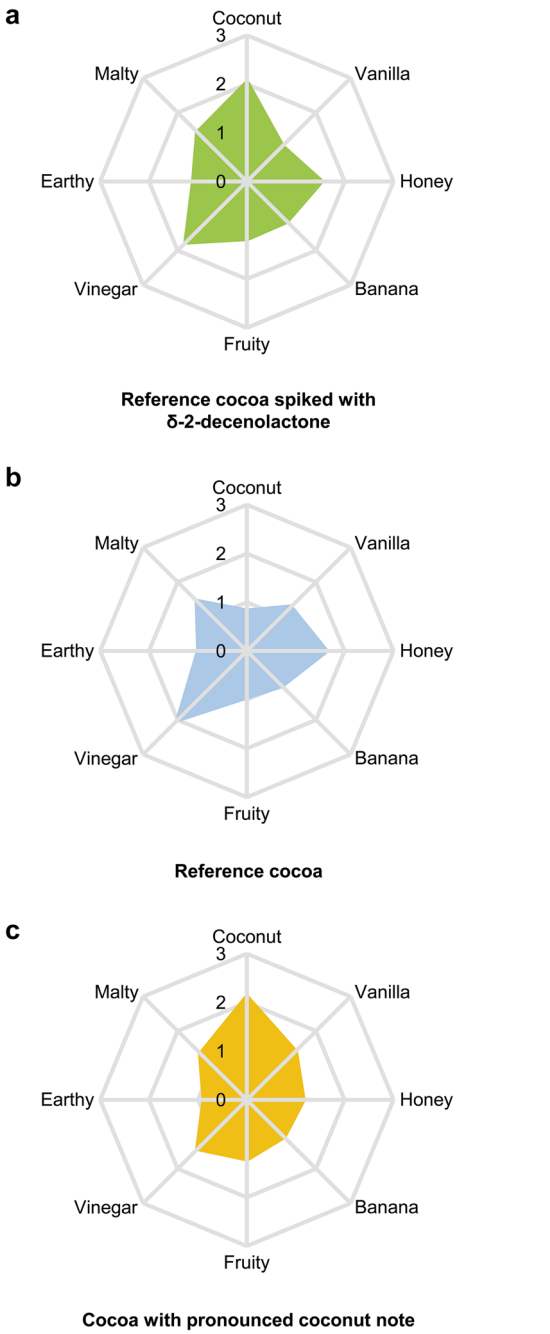
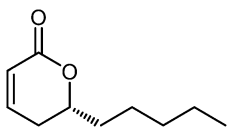


Fig. 3 Olfactory profiles of the reference cocoa sample spiked with δ-2-decenolactone (**a**), the reference sample without addition (**b**), and the sample C1 with the atypically pronounced coconut note (**c**). Assessors rated the intensity of each descriptor on a scale from 0 to 3 with 0 = not detectable, 1 = weak, 2 = moderate, and 3 = strong (details are available in the Supplementary Information file, Table S3)

Fig. 4 (*R*)-δ-2-Decenolactone



the atypical coconut-like odour. In the absence of high concentrations of (*R*)-*δ*-2-decenolactone, even subthreshold concentrations of other lactones might lead to a perceivable coconut note as recently hypothesized in a study on milk chocolate [43]. An aspect that could also be considered in future investigations is the influence of water presence on the concentrations of lactones in cocoa [44].

Another open question is the source of the (*R*)-*δ*-2-decenolactone and other coconut-like smelling lactones in fermented cocoa. One possibility is their synthesis by microorganisms. For example, biosynthesis of (*R*)-*δ*-2-decenolactone was reported in *Fusarium solani* [45] and (*R*)-*δ*-2-octenolactone was found in *Lasiodiplodia theobromae* [46]. The use of infested pods might be crucial for the development of atypically strong coconut notes in fermented cocoa. This could also explain their rather occasional occurrence.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00217-022-03981-5.

Acknowledgements The authors thank Julia Schweiger and Monika Riedmaier for the excellent technical assistance.

Funding Open Access funding enabled and organized by Projekt DEAL. This research was part of project no. 19455 N of the FEI supported via AiF within the programme for promoting the Industrial Collective Research (IGF) of the German Federal Ministry of Economic Affairs and Climate Action (BMWK), based on a resolution of the German Parliament.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

References

1. *Theobroma cacao* L. Plants of the world online http://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:320783-2 Accessed 14 Nov 2021
2. International Cocoa Organization. Production of cocoa beans. In: ICCO Quarterly Bulletin of Cocoa Statistics, Vol. XLV, No. 2, Cocoa year 2018/19. https://www.icco.org/quarterly-bulletin-of-cocoa-statistics-november-2019/ Accessed 14 Nov 2021
3. Aprotosoaie AC, Luca SV, Miron A (2016) Flavor chemistry of cocoa and cocoa products–an overview. Compr Rev Food Sci Food Saf 15:73–91
4. Frauendorfer F, Schieberle P (2008) Changes in key aroma compounds of Criollo cocoa beans during roasting. J Agric Food Chem 56:10244–10251
5. Jinap S, Rosli WIW, Russly AR, Nordin LM (1998) Effect of roasting time and temperature on volatile component profiles during nib roasting of cocoa beans (*Theobroma cacao*). J Sci Food Agric 77:441–448
6. Ney KH (1992) Cocoa off-flavors. In: Charalmbous G (ed) Off-flavors in foods and Beverages. Elsevier, Amsterdam
7. Perotti P, Cordero C, Bortolini C, Rubiolo P, Bicchi C, Liberto E (2020) Cocoa smoky off-flavor: chemical characterization and objective evaluation for quality control. Food Chem 309:125561
8. Ziegleder G (2009) Flavour development in cocoa and chocolate. In: Beckett ST (ed) Industrial chocolate manufacture and use. Blackwell Publishing, Oxford
9. Frauendorfer F, Schieberle P (2006) Identification of the key aroma compounds in cocoa powder based on molecular sensory correlations. J Agric Food Chem 54:5521–5529
10. Bainbridge JS, Davies SH (1912) The essential oil of cocoa. J Chem Soc Trans 101:2209–2221
11. Flamen I (1989) Coffee, cocoa, and tea. Food Rev Int 5:317–414
12. Füllemann D, Steinhaus M (2020) Characterization of odorants causing smoky off-flavors in cocoa. J Agric Food Chem 68:10833–10841
13. Porcelli C, Neiens SD, Steinhaus M (2021) Molecular background of a moldy-musty off-flavor in cocoa. J Agric Food Chem 69:4501–4508
14. Füllemann D, Neiens SD, Steinhaus M (2022) Impact of processing on important cocoa off-flavour compounds. Eur Food Res Technol. 248:197–205 https://doi.org/10.1007/s00217-021-03873-0
15. Schieberle P (1991) Primary odorants in popcorn. J Agric Food Chem 39:1141–1144
16. Schieberle P, Grosch W (1991) Potent odorants of the wheat bread crumb. Differences to the crust and effect of a longer dough fermentation. Z Lebensm-Unters Forsch 192:130–135
17. Christlbauer M, Schieberle P (2011) Evaluation of the key aroma compounds in beef and pork vegetable gravies a la chef by stable isotope dilution assays and aroma recombination experiments. J Agric Food Chem 59:13122–13130
18. Poisson L, Schieberle P (2008) Characterization of the key aroma compounds in an American Bourbon whisky by quantitative measurements, aroma recombination, and omission studies. J Agric Food Chem 56:5820–5826
19. Schütt J, Schieberle P (2017) Quantitation of nine lactones in dairy cream by stable isotope dilution assays based on novel syntheses of carbon-13-labeled *γ*-lactones and deuterium-labeled *δ*-lactones in combination with comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry. J Agric Food Chem 65:10534–10541
20. Steinhaus M (2015) Characterization of the major odor-active compounds in the leaves of the curry tree *Bergera koenigii* L. by aroma extract dilution analysis. J Agric Food Chem 63:4060–4067
21. Engel W, Bahr W, Schieberle P (1999) Solvent assisted flavour evaporation—a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. Eur Food Res Technol 209:237–241

22. Bemelmans JMH (1979) Review of isolation and concentration techniques. In: Land GG, Nursten HE (eds) Progress in flavour research. Applied Science Publishers, London
23. Schieberle P, Grosch W (1987) Evaluation of the flavor of wheat and rye bread crusts by aroma extract dilution analysis. Z Lebensm-Unters Forsch 185:111–113
24. Steinhaus M (2020) Gas chromatography-olfactometry: principles, practical aspects and applications in food analysis. In: Tranchida PQ (ed) Advanced gas chromatography in food analysis. The Royal Society of Chemistry, Cambridge
25. Kreissl J, Mall V, Steinhaus P, Steinhaus M (2019) Leibniz-LSB@TUM odorant database, version 1.0. Leibniz Institute for Food Systems Biology at the Technical University of Munich, Freising. https://www.leibniz-lsb.de/en/databases/leibniz-lsbtum-odorant-database/odorantdb/ Accessed 14 Nov 2021
26. American Society of Testing and Materials (2019) Standard E679–19. Standard practice for determination of odor and taste thresholds by a forced-choice ascending concentration series method of limits. ASTM Book of Standards, West Conshohocken
27. Ullrich F, Grosch W (1987) Identification of the most intense volatile flavour compounds formed during autoxidation of linoleic acid. Z Lebensm-Unters Forsch 184:277–282
28. Teranishi R, Buttery RG, Guadagni DG (1974) Odor quality and chemical structure in fruit and vegetable flavors. Ann NY Acad Sci 237:209–216
29. Schnermann P, Schieberle P (1997) Evaluation of key odorants in milk chocolate and cocoa mass by aroma extract dilution analyses. J Agric Food Chem 45:867–872
30. Seyfried C, Granvogl M (2019) Characterization of the key aroma compounds in two commercial dark chocolates with high cocoa contents by means of the sensomics approach. J Agric Food Chem 67:5827–5837
31. Chetschik I, Pedan V, Chatelain K, Kneubühl M, Hühn T (2019) Characterization of the flavor properties of dark chocolates produced by a novel technological approach and comparison with traditionally produced dark chocolates. J Agric Food Chem 67:3991–4001
32. Guichard E, Kustermann A, Mosandl A (1990) Chiral flavor compounds from apricots: distribution of γ-lactone enantiomers and steoredifferentiation of dihydroactinidiolide using multi-dimensional gas chromotography. J Chromatogr A 498:396–401
33. Greger V, Schieberle P (2007) Characterization of the key aroma compounds in apricots (*Prunus armeniaca*) by application of the molecular sensory science concept. J Agric Food Chem 55:5221–5228
34. Maga JA, Katz I (1976) Lactones in foods. Crit Rev Food Sci Nutr 8:1–56
35. Mosandl A, Hener U, Hagenauer-Hener U, Kustermann A (1989) Direct enantiomer separation of chiral γ-lactones from food and beverages by multidimensional gas chromatography. J High Resolut Chromatogr 12:532–536
36. Cooke RC, Van Leeuwen KA, Capone DL, Gawel R, Elsey GM, Sefton MA (2009) Odor detection thresholds and enantiomeric distributions of several 4-alkyl substituted γ-lactones in Australian red wine. J Agric Food Chem 57:2462–2467
37. Dunkel A, Steinhaus M, Kotthoff M, Nowak B, Krautwurst D, Schieberle P, Hofmann T (2014) Nature's chemical signatures in human olfaction: a foodborne perspective for future biotechnology. Angew Chem, Int Ed 53:7125–7143
38. Silva R, Coelho E, Aguiar TQ, Domingues L (2021) Microbial biosynthesis of lactones: Gaps and opportunities towards sustainable production. Appl Sci 11:8500
39. Fricke K, Schieberle P (2020) Characterization of the key aroma compounds in a commercial milk chocolate by application of the sensomics approach. J Agric Food Chem 68:12086–12095
40. Abe S (1937) The main component of the essential oil of massooi. Part 1. J Chem Soc Japan 58:246–248
41. Pons A, Allamy L, Lavigne V, Dubourdieu D, Darriet P (2017) Study of the contribution of massoia lactone to the aroma of Merlot and Cabernet Sauvignon musts and wines. Food Chem 232:229–236
42. Arnaudo JF, Delbecque C, Perichet C, Bouillet S (2005) Production of natural flavor and fragrance ingredients in 2003: massoia, orris, and vetiver oils. In: Frey C, Rouseff R (eds) Natural flavors and fragrances. American Chemical Society, Washington DC
43. Mori K (1976) Absolute configuration of (−)-massoilactone as confirmed by a synthesis of its (*S*)-(+)-isomer. Agric Biol Chem 40:1617–1619
44. Ullrich L, Neiens S, Hühn T, Steinhaus M, Chetschik I (2021) Impact of water on odor-active compounds in fermented and dried cocoa beans and chocolates made thereof. J Agric Food Chem 69:8504–8510
45. Nago H, Matsumoto M, Nakai S (1993) 2-Deceno-δ-lactone-producing fungi, strains of Fusarium solani, isolated by using a medium containing decano-δ-lactone as the sole carbon source. Biosci Biotechnol Biochem 57:2107–2110
46. Matsumoto M, Nago H (1994) *(R)*-2-Octeno-δ-lactone and other volatiles produced by *Lasiodiplodia theobromae*. Biosci Biotechnol Biochem 58:1262–1266