Molecular characterisation of an atypical coconut-like odour in cocoa

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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 · (6R)-5,6-Dihydro-6-pentyl-2H-pyran-2-one

Abbreviations

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

Abbreviation Nomenclature

| Compound | Name |
|----------|------|
| δ-Octalactone | Geraniol |
| δ-2-Octenolactone | Geraniol (2E)-3,7-Dimethylocta-2,6-dien-1-ol |
| γ-Nonalactone | Linalool 3,7-Dimethylocta-1,6-dien-3-ol |
| γ-Decalactone | Maltol 3-Hydroxy-2-methyl-4H-pyran-4-one |
| δ-Decalactone | Sotolon 3-Hydroxy-4,5-dimethylfuran-2(5H)-one |
| δ-2-Decenolactone | 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
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]. Furthermore, 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 (2E)-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 synthesized according to literature procedures [15, 16].

The stable isotopically substituted odorants were prepared by approaches described in the literature: (3H2)34, [6-(2,3-2H2)propyloxan-2-one] [17]; (3H2)36 [5-(1,2-2H2)pentyloxolan-2-one], (3H2)42 [5-(1,2-2H2)hexyloxolan-2-one] [18]; and (3H2)44 [6-pentyl(3,3,4,4-2H4)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 purchased 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
In the second step, reference samples of the proposed odor-indices (RIs) and odour qualities were compared with previously obtained in fraction NBV were localized in the odour threshold values of compounds 34, 36, 42, 44 and 45 in low odour sunflower oil were determined orthogonally 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 (2E)-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 odors 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-methoxy pyrazine (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-phenylethanol-1-ol (32), metallic smelling trans-4,5-epoxy-(2E)-dec-2-enal (35), caramel-like smelling 4-hydroxy-2,5-dimethylfuran-3(2H)-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                             | Odour quality | RF<sup>a</sup> | FD factor<sup>b</sup> |
|----|-------------------------------------|---------------|----------------|-----------------------|
| 1  | Ethyl 2-methylpropanoate            | Fruity        | <1000          | 765                   |
| 2  | Butane-2,3-dione                    | Butter        | <1000          | <700                  |
| 3  | Ethyl butanoate                     | Fruity        | 1031           | 804                   |
| 4  | Ethyl 2-methylbutanoate             | Fruity        | 1060           | 849                   |
| 5  | Ethyl 3-methylbutanoate             | Fruity, blueberry | 1077           | 852                   |
| 6  | 3-Methylbutyl acetate               | Fruity, banana | 1130           | 878                   |
| 7  | 3-Hydroxybutan-2-one                | Butter        | 1262           | 800                   |
| 8  | 1-Octen-3-one                       | Mushroom      | 1298           | 979                   |
| 9  | 2-Acetyl-1-pyrroline                | Popcorn       | 1332           | 922                   |
| 10 | Dimethyl trisulfide                 | Cabbage       | 1370           | 967                   |
| 11 | 2,3,5-Trimethylpyrazine              | Earthy        | 1383           | 1002                  |
| 12 | Unknown                             | Rose, citrus  | 1411           | 4                     |
| 13 | Ethyl cyclohexanecarboxylate        | Fruity        | 1414           | 1131                  |
| 14 | 3-Isopropyl-2-methoxyprazine        | Bell pepper   | 1518           | 1184                  |
| 15 | Acetic acid                         | Vinegar, pungent | 1430           | 1095                  |
| 16 | 3-(Methylsulfinyl)propanal          | Cooked potato | 1454           | 905                   |
| 17 | 3-Isobutyl-2-methoxyprazine         | Bell pepper   | 1533           | 1160                  |
| 18 | (2E)-Non-2-enal                     | Green, fatty  | 1545           | 1102                  |
| 19 | Linalool                            | Citrus, bergamot | 1553           | 789                   |
| 20 | 2-Methylpropanoic acid              | Sweaty, cheese | 1618           | 821                   |
| 21 | Butanoic acid                       | Sweaty, cheese | 1695           | 1246                  |
| 22 | Phenylacetaldehyde                  | Honey         | 1643           | 1046                  |
| 23 | 2-f-Methylbutanoic acid             | Cheese        | 1655           | 874                   |
| 24 | 2-Acetylthiazole                    | Popcorn       | 1667           | 1038                  |
| 25 | Geranial                            | Floral, citrus | 1711           | 1269                  |
| 26 | (2E,4E)-Deca-2,4-dienal             | Fatty, deep-fried | 1780           | 1317                  |
| 27 | Ethyl phenylacetate                 | Honey         | 1795           | 1246                  |
| 28 | 2-Phenylethyl acetate               | Rose          | 1822           | 1256                  |
| 29 | Geraniol                            | Citrus, rose  | 1843           | 1256                  |
| 30 | 2-Methoxyphenol                     | Smoky         | 1858           | 1090                  |
| 31 | Ethyl 3-phenylpropanoate            | Cinnamon      | 1881           | 1418                  |
| 32 | 2-Phenylenethan-1-ol                | Honey, rose   | 1919           | 1116                  |
| 33 | Maltol                              | Caramel       | 1961           | 1110                  |
| 34 | δ-Octalactone                       | Coconut       | 1984           | 1250                  |
| 35 | trans-4,5-Epoxy-(2E)-dec-2-enal      | Metallic      | 2012           | 1382                  |
| 36 | γ-Nonalactone                       | Coconut       | 2029           | 1393                  |
| 37 | δ-2-Octenolactone                  | Coconut, creamy | 2047           | 1264                  |
| 38 | HDMFe                               | Caramel       | 2028           | 1071                  |
| 39 | Unknown                             | Burnt         | 2050           | 256                   |
| 40 | 4-Methylphenol                      | Faecal, horse stable | 2072           | 1078                  |
| 41 | Ethyl cinnamate                     | Sweet, cinnamon | 2106           | 1469                  |
| 42 | γ-Decalactone                       | Coconut, peach | 2140           | 1466                  |
| 43 | Sotolone                            | Soup seasoning | 2200           | 1107                  |
| 44 | δ-Decalactone                       | Coconut       | 2210           | 1494                  |
| 45 | δ-2-Decenoactone                    | Coconut       | 2255           | 1475                  |
| 46 | 3-Methyl-1H-indole                  | Faecal, mothball | 2513           | 1390                  |
| 47 | Pheny lacetic acid                  | Honey, beeswax | 2547           | 1261                  |
| 48 | Vanillin                            | Vanilla       | 2573           | 1408                  |

<sup>a</sup>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-
C1 were additionally obtained for \( \gamma \)-nonalactone (1024), \( \gamma \)-decalactone (1024), and \( \delta \)-octalactone (512). For these 3 compounds, however, the FD factors indicated no difference between sample C1 and the reference sample. Low FD factors of \( \delta \)-2-octenolactone (37) and \( \delta \)-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 \( \delta \)-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 \( \delta \)-2-decenolactone for the coconut odour.

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 | \( \delta \)-Octalactone | 0.195     | 0.744     | 0.355     | 0.422     |
| 36 | \( \gamma \)-Nonalactone | 152       | 191       | 162       | 125       |
| 37 | \( \delta \)-2-Octenolactone | 0.319     | 1.59      | 0.900     | 0.706     |
| 42 | \( \gamma \)-Decalactone   | 51.3      | 413       | 52.3      | 109       |
| 44 | \( \delta \)-Decalactone    | 76.0      | 461       | 92.3      | 6.98      |
| 45 | \( \delta \)-2-Decenolactone | 1580     | 570       | 210       | 86.1      |

aMean of duplicates or triplicates; individual values and standard deviations are provided in the Supplementary Information file, Table S2
In massoia bark, only the \((R)\)-isomer of \(\delta\)-2-decenolactone is present [41, 42]. \((R)\)-\(\delta\)-2-decenolactone is therefore also often referred to as massoia lactone.

### Chiral analysis of \(\delta\)-2-decenolactone

To confirm the crucial role of \(\delta\)-2-decenolactone for the atypical note in the coconut-like smelling cocoa sample C1, a spiking experiment was considered the method of choice: \(\delta\)-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 \(\delta\)-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 \(\gamma\)- and \(\delta\)-lactones [32, 36]. In most cases, the \((R)\)-enantiomers showed higher odour potency than the \((S)\)-enantiomers. For \(\delta\)-2-decenolactone, however, no such data was available.

Therefore, we subjected the \(\delta\)-2-decenolactone reference sample to enantioGC analysis using a chiral \(\beta\)-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\% \((R)\)-\(\delta\)-2-decenolactone and 17\% \((S)\)-\(\delta\)-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 \(\delta\)-2-decenolactone enantiomers were determined by AEDA. Results (Table 4) showed that also in \(\delta\)-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.
As a next step, we determined the enantiomeric ratios of \(\delta-2\)-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 \(\delta-2\)-decenolactone in cocoa was pure \((R)\)-enantiomer. Figure 2 depicts the relevant chromatogram sections after injection 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).

With the knowledge of the enantiomeric purity of \(\delta-2\)-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 \(83/17\) \((R)/(S)\)-mixture in oil as \(0.83 \times 120 \mu g/kg + (1.6/51.5) \times 0.17 \times 120 \mu g/kg\), the odour threshold values of the individual enantiomers in oil were approximated. The calculations resulted in odour threshold values in oil of \(3300 \mu g/kg\) for the \((S)\)-\(\delta-2\)-decenolactone and \(100 \mu g/kg\) for the \((R)\)-\(\delta-2\)-decenolactone. Using the latter for the OAV calculations of \((R)\)-\(\delta-2\)-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 \((R)\)-\(\delta-2\)-decenolactone was the compound being responsible for the atypical coconut-like odour in the fermented cocoa samples.

![Fig. 2](image-url)
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 odor 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 odor potency (1.6/51.5; cf. Table 4), this contribution was only 0.5%, whereas 99.5% of the odor 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 odor 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 odor, and the calculation of odor activity values suggested δ-2-decenolactone as the compound causative for an atypically pronounced coconut odor 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 odor 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

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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 \((R)\)-\(\delta\)-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 \((R)\)-\(\delta\)-2-decenolactone and other coconut-like smelling lactones in fermented cocoa. One possibility is their synthesis by microorganisms. For example, biosynthesis of \((R)\)-\(\delta\)-2-decenolactone was reported in \textit{Fusarium solani} [45] and \((R)\)-\(\delta\)-2-octenolactone was found in \textit{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.

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**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.

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