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Dietary Linalool is Transferred into the Milk of Nursing Mothers

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Scope: Breast milk is repeatedly postulated to shape the first aroma and taste impressions of infants and thus impact their flavor learning. The objective of this study is to assess the transition of aroma compounds from a customary curry dish into milk.

Methods and Results: The article prepares a standardized curry dish and administers the dish to nursing mothers (n = 18) in an intervention study. The participants donate one milk sample before and three samples after the intervention. Due to their olfactory or quantitative relevance in the curry dish, 1,8-cineole, linalool, cuminaldehyde, cinnamaldehyde, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, sotolone, eugenol, vanillin, and γ-nonalactone are defined as target compounds, and their transition into milk is quantified by gas chromatography-mass spectrometry. A significant transition into the milk is observed for linalool, and its olfactory relevance in this respect is supported by calculated odor activity values. In contrast, no relevant levels are detected for the other eight target compounds.

Conclusion: Ingestion of a customary curry dish can lead to an alteration of the milk aroma, which might be perceived by the infant during breastfeeding. The current study also demonstrates that the extent of aroma transfer differs between both substances and individuals.

1. Introduction

Breast milk (hereafter: milk) is the first and in many cases the sole nourishment a human gets in contact with during the first months of life. A recent survey states that 86% of mothers with children younger than four years are or have been nursing their babies, and that 57% did so for more than 6 months.[1] The composition of milk is highly regulated by physiological processes to provide the infant with all required nutrients. At the same time, specific characterizing constituents define a highly variable yet individuum-specific and recognizable chemical signature of a mother's milk that is, for example reflected in the specific oligosaccharide profiles or immune components, amongst others.[2] This individual specificity also applies to milk odor, as demonstrated by Marlier & Schaal,[3] who showed that 4-day-old infants differentiate between the odor of their own mother's milk and the odor of another mother's milk. Milk odor can, nonetheless, be influenced by external factors such as the maternal diet. For certain odor-active compounds such as terpenes,[4] sulfurous compounds,[5] and ethanol,[6] a transfer of the original substance and/or its metabolites from the diet into the milk has been demonstrated and has been related to a concurrent alteration of milk odor (see[7] for recent reviews). The fact that these changes influence the nursling's behavior, as reported in the case of ethanol[6] and other odor-active compounds,[8] corroborates the notion that breastfed infants perceive diet-induced changes of milk odor. Therefore, the diet of the mother during the breastfeeding period is often stated to be an early influencing factor regarding the later acceptance of food in infancy, child- or even adulthood.[9]

According to a recent survey, 89% of the mothers alter their dietary habits upon breastfeeding.[1] Despite their daily relevance for many mother-infant-dyads, diet-induced flavor changes of milk are, however, still an under-researched area when it comes to the transfer of dietary flavor compounds and their metabolites from customary dishes usually ingested by the mother. Moreover, whereas several studies have investigated the transfer of aroma-active compounds into milk, much less is known about the potential transfer of taste-active compounds, evidence being limited to caffeine,[10] other bitter tasting alkaloids,[11] and a moderately

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positive correlation between the bitterness of the maternal diet and the bitterness of fore milk.[12] Expanding the scientific interest to comprise a combination of aroma- to taste-active substances, and from laboratory to more real-life conditions is therefore an essential step to advance our knowledge in this area of research. Accordingly, an intervention study was designed to characterize the transfer of aroma and taste compounds from a customary meal into milk. The results are presented in two publications, the current work focusing on volatile odor-active compounds, and N'Diaye et al. (this issue) reporting on the results obtained for taste-active components and non-volatile metabolites.

To study the transfer of both aroma- and taste-active compounds into milk in a realistic consumption scenario, a curry dish was selected for the intervention study, bearing a complex aroma and different taste impressions. Curry dishes come in numerous variants and are frequently consumed all over the world. The characterizing flavor-active ingredient of curry dishes is the curry spice, a mixture of spices that varies in its composition but generally includes coriander, fenugreek, cumin, pepper, turmeric, chili, cinnamon, cardamom, cloves, curry leaves, ginger, amchoor (a powder made of mangoes), asafoetida, mustard, star anise and fennel as basic ingredients. The complex composition of the curry spice aroma was used as representative example in the present study to investigate a real-life combinatory aroma substance mixture with components differing in their structural and sensory properties. Based on a characterization of the overall aroma and the most potent aroma compounds of the curry spice mixture and the standardized curry dish, representative target substances were selected to investigate the temporal and quantitative course of aroma transfer into milk.

2. Experimental Section

2.1. Chemicals

Dichloromethane HiPerSolv (DCM) and sodium sulfate (Na_2SO_4) were provided by VWR International GmbH (Darmstadt, Germany). DCM was freshly distilled prior to use. The analytical standards 1,8-cineole, linalool, cuminaldehyde, cinnamaldehyde, sotolone, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, eugenol, vanillin and γ-nonalactone were purchased from Sigma Aldrich (Steinheim, Germany). The isotopically labeled standards $^2H_3$-1,8-cineole, $^2H_{4\text{-}5}$-linalool, $^2H_8$-cuminaldehyde, $^2H_6$-cinnamaldehyde, $^{13}C_2$-sotolone, $^{13}C_2$-4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, $^2H_3$-eugenol and $^2H_4$-γ-nonalactone were from aromaLAB GmbH (Martinsried, Germany) and $^{13}C_6$-vanillin from Sigma Aldrich.

2.2. Ingredients of the Curry Dish

All ingredients were bought at local supermarkets in Erlangen, Germany: coriander seeds (H. D. DeSilva & Sons (Pvt) Ltd.; Colombo, Sri Lanka), curry leaves (Uttam Fresh & Clean; Groß-Gerau, Germany), cumin seeds, fenugreek, black pepper whole, cloves (all from Bajwa Asian Foods GmbH & Co. KG; Groß-Gerau, Germany), turmeric powder, green cardamom, cinnamon sticks, whole chilies extra hot (all from TRS Asia's Finest Foods; Southhall, England), fresh ginger, salt (Sonnensalz, Bernburg, Germany), sunflower oil (Zentrale Handelsgesellschaft – ZHG – mbH, Offenburg, Germany), coconut milk (Dunekacke & Wilms Nachf. GmbH & Co. KG, Hamburg, Germany), and rice (organic, long grain rice, parboiled from dm-drogeriemarkt GmbH & Co. KG, Karlsruhe, Germany).

2.3. Preparation of the Curry Spice Mixture

The curry spice mixture consisted of 480 g of coriander seeds, 40 g of fenugreek, 240 g of cumin seeds, 40 g of peppercorns, 80 g of turmeric powder, 48 g of dried red chilies, 32 g of cinnamon sticks, 16 g of cardamom, 8 g of cloves and 16 g of curry leaves. Coriander seeds, fenugreek, peppercorns, dried red chilies, cinnamon sticks, cardamom and cloves were added to a preheated pan on medium flame and dry roasted for 4 min. Subsequently, cumin seeds were added and after 3 min curry leaves were added. After 3 more min turmeric powder was added, the flame was switched off and all ingredients were cooled down in the pan. The roasted spices were then ground to a homogeneous powder and stored as 10 g aliquots at -20 °C in evacuated, coated aluminum bags (aluminum composite film: PET-layer ≈12 µm, aluminum-layer ≈9 µm, PE-layer ≈20 µm, Vipak Sp. z o. o., Jarocin, Poland).

2.4. Preparation of the Curry Dish and Dosage Information

The curry dish consisted of cooked rice and a curry sauce. The sauce was cooked by using the Thermomix TM4 (Vorwerk Deutschland Stiftung & Co. KG, Wuppertal, Germany). It consisted of 2.5 g of curry spice mixture, 1 g of salt, 6 g of fresh ginger, 10 g of sunflower oil, 80 mL of coconut milk and 20 mL of water. The preparation of the curry sauce started with cutting fresh ginger into small cubes (side length 1–2 mm) and frying them together with the sunflower oil at 100 °C for 3 min. Subsequently, water, coconut milk, salt and curry spice mixture were added, and all ingredients were cooked for 5 min at 100 °C. The sauce was then stored until usage in PET trays covered with aluminum foil at -20 °C. On the sampling day, 80 g of dry rice were cooked in 160 mL of tap water for 15 min and mixed with the curry sauce, which had been thawed in a microwave for 60 s. Finally, the curry dish was heated up for another 60 s at 600 Watt in a microwave, and served to the participant. The average weight of the curry dish was 246 ± 3 g.

2.5. Participants

The milk samples were provided by 18 mothers (mean age: 32 ± 2 years), who all participated once and whose milk production was well above the needs of their infants (age range: 8–53 weeks). Only healthy (neither chronic nor acute diseases), non-smoking mothers with no allergies to the ingredients of the curry dish were included in the study.

All participants gave informed written consent prior to participating in the study. Withdrawal from the study was possible at any time without negative consequences. The study was designed in accordance with the Declaration of Helsinki and

the ethical committee of the Friedrich-Alexander-Universität Erlangen-Nürnberg approved the study protocol (registration number 24_16 B).

2.6. Procedure

All mothers were asked to refrain from the intake of ingredients of the curry dish, garlic and onions 2 days prior to the sampling day. On the sampling day, they were asked to eat one of two possible breakfast options (white bread with butter/margarine and non-flavored strawberry jam, or porridge) and drink water, and to use no perfume or nursing balm. The mothers kept a nutrition diary during the 2 days prior to, and on the sampling day. All mothers only consumed water and the curry dish during the sampling time. After their arrival at the study center (Chair of Aroma and Smell Research, Erlangen, Germany), the participants donated a baseline milk sample ("sample 1") of approx. 20 mL using a mechanical breast pump (Medela Harmony, Medela AG, Baar, Switzerland). The consumption of the curry dish was set at 12 o'clock and was followed by donation of further milk samples with an approx. volume of 20 mL (at 1 pm, 2 pm, 3 pm; corresponding to "sample 2", "sample 3", "sample 4"). If a sufficient amount was available, an aliquot (2 mL) of each milk sample was taken for the investigation of tastants and non-volatile metabolites (N'Diaye et al., this issue). All samples were stored in brown glasses at -80 °C for a maximum of 30 days before analysis. Orthonasal sensory evaluation of the milk samples (see next section) was conducted before freezing the samples.

2.7. Sensory Evaluation of the Curry Spice Mixture, Curry Dish, and Milk Samples

The orthonasal aroma profiles were established by trained panelists. The training consisted in weekly sessions dedicated to the description of odorants with an in-house developed flavor language, and weekly odor identification tests (>65% accuracy needed to participate in the sensory evaluations).

2.7.1. Curry Spice Mixture and Curry Dish

The samples (two teaspoons of the curry spice mixture and three teaspoons of the curry dish, respectively) were presented in glass beakers (WECK Mini-Sturzglas, 140 mL, 60 mm diameter aperture, J. Weck GmbH & Co. KG, Wehr-Öflingen, Germany) wrapped in aluminum foil to minimize a possible bias induced by the appearance of the samples. The aroma profiles were established within two sessions. In the first session, the aroma was first individually described by each panelist, and then discussed by the panel. The panel consisted of eight trained panelists (six female, two male, age range: 24–55 years) in the case of the curry dish and 10 trained panelists (seven female, three male, age range: 24–55 years) in the case of the curry spice mixture. All aroma attributes with more than 50% agreement became part of the aroma profile according to DIN EN ISO 13299:2016. In the second session, the intensities of these attributes in the samples were rated on a metric (continuous) unipolar scale from 0 (not perceivable) to 10 (very intensive).

2.7.2. Milk

The milk samples were evaluated with regard to pre-defined attributes according to previous work[13] and additional attributes defined by the panel according to the procedure above. The intensities of these attributes were rated by four panelists on a metric (continuous) unipolar scale from 0 (not perceivable) to 10 (very intensive). The reason for this rather low number of panelists was the fact that diet-induced aroma changes of milk can fade quickly,[14] and therefore sensory analyses need to be performed swiftly. The milk samples (15–20 mL) were presented in screw-off jars (100 mL, 40 mm diameter aperture, Glaswarenfabrik Karl Hecht GmbH & Co. KG, Sondheim vor der Rhön, Germany). Four sample sets were not sensorially evaluated due to a lack of trained panelists on the respective sampling days and subsequent freezing. The remaining 14 sample sets were used for the sensory evaluation.

2.8. Determination of Odor Thresholds

The odor thresholds of 1,8-cineole, linalool, cuminaldehyde, cinnamaldehyde, sotolone, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, γ-nonalactone, eugenol and vanillin were determined in distilled water and in cow milk (unhomogenized, pasteurized full fat (3.8%) cow milk, Gläserne Molkerei GmbH, Dechow, Germany). The thresholds were determined by 6–10 panelists (age range: 22–33 years, five male, five female), using a series of triangle tests. For each target compound, eight triangle tests were prepared by filling 30 mL of water or milk (for the two blanks) and 30 mL of the odorant solution (for the target; in water or milk) into three glass beakers (WECK Mini-Sturzglas, see Section 2.7). The panelists evaluated the samples in the three beakers by sniffing, and indicated which beaker contained the odorant (forced choice). The odorant concentration rose by a factor of three from one test to the next. The highest concentration level was 37 287 µg L^{-1} for cinnamaldehyde, 22 800 µg L^{-1} for eugenol, 20 200 µg L^{-1} for cuminaldehyde, 3672 µg L^{-1} for vanillin, 2280 µg L^{-1} for 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 595 µg L^{-1} for γ-nonalactone, 83 µg L^{-1} for 1,8-cineole, 26 µg L^{-1} for sotolone and 6 µg L^{-1} for linalool. Individual odor thresholds were determined by calculating the geometric mean of the highest undetected concentration and the lowest detected concentration after which all higher concentrations were detected as well. The average odor threshold for a substance was subsequently calculated as the geometric mean of the individual thresholds.

2.9. Isolation of Volatiles from the Milk Samples

DCM was added to the milk sample in a ratio of 1:1 (DCM:milk, v/v) and in the case of quantification, the respective isotopically labeled standards (Table S1, Supporting Information) were added followed by 30 min of stirring at room temperature. The emulsion was then subjected to solvent-assisted flavor evaporation[15] (SAFE) at 50 °C and a pressure of 10^{-4} mbar. The distillate was thawed, and the organic phase was separated from the aqueous phase and dried over anhydrous sodium sulfate. Finally, the organic phase was concentrated by Vigreux- and micro-distillation

at 50 °C to a volume of 100 µL. Three sample sets were used for the qualitative screening without the addition of isotopically labeled standards. Another two sample sets were used for pre-quantification experiments to elaborate the concentration ranges for targeted quantification. The remaining 13 sample sets were used for quantification.

In the case of the curry spice mixture 0.2 g and in the case of the curry dish 10 g were mixed with 100 mL of DCM, followed by the same procedure as described above to isolate the volatiles.

2.10. Identification of Odorants in the Curry Spice Mixture and the Curry Dish

The distillates obtained from the curry spice mixture and the curry dish were analyzed by gas chromatography-olfactometry (GC-O) and gas chromatography-mass spectrometry/olfactometry (GC-MS/O). An aroma extract dilution assay (AEDA) was performed by repeatedly diluting the distillates 1:3 v/v with DCM, and afterwards performing GC-O. The main odorants were determined as those which were still perceivable at the highest factors of dilution (flavor dilution factor, FD factor). The distillates and their dilutions were analyzed on two different capillary columns (DB-5 and DB-FFAP). A homologous series of alkanes from C5 to C34 was injected to determine retention indices (RIs) according to Kovats.[16] The RIs on the two columns, the perceived odor quality and the mass spectrum were compared to those of analytical standards to identify the odorants with the highest FD factors.

2.11. Quantification of the Target Odorants

The quantification of the target substances was carried out by a stable isotope dilution assay (SIDA). The masses selected for quantification are given in Table S1, Supporting Information. The substances were quantified via the area ratio of these quantifiers, measured via GC-MS in selected ion monitoring (SIM) mode (see Sections 2.12.2 and 2.12.3), and applying the respective calibration curves. The criteria for the calibration curves were an axis intercept of less than 0.2 as well as $R^2 \geq 0.99$.

The limit of detection (LOD) and the limit of quantification (LOQ) were determined according to DIN 32645. A calibration curve was set up in the range of the expected LOD in different concentrations in the respective blank matrix. The calculation of the LOD and the LOQ was carried out by using the formulas of the DIN 32645 presented in Formula 1.

$$x_{LOD} = s_{x0} \times t_{f,\alpha} \times \sqrt{\frac{1}{m} + \frac{1}{n} + \frac{x^2}{Q_x}}$$

$$x_{LOQ} = k \times s_{x0} \times t_{f,\alpha} \times \sqrt{\frac{1}{m} + \frac{1}{n} + \frac{[(k \times x_{LOD}) - x^2]}{Q_x}} \quad (1)$$

Formula 1. Formulas for the determination of the LOD and LOQ. k: 3 (relative result uncertainty) s_{x0}: standard deviation $t_{f,\alpha}$: t-value (f = degrees of freedom (n-2), α = significance niveau) m: number of calibration rows n: number of concentration niveaus Q_x: sum of squares of the residuals x: average of the calibration.

Cow milk (unhomogenized, pasteurized, 3.8% fat, Gläserne Molkerei GmbH) was used as a substitute for milk as the blank matrix. The unlabeled and the labeled analytical standards were added to this substitute in the range of the LOQ and the LOD, were then isolated according to the described work-up procedure, and measured analogously to the milk samples (see Sections 2.9, 2.12.2, and 2.12.3).

The equations used for quantification of the target compounds and their LOQs are listed in Table S2, Supporting Information.

2.12. Instrumental Analyses

Distillates obtained from the curry spice mixture and the curry dish was analyzed using GC-O (for AEVA; according to Section 2.12.1), GC-MS (for quantification; according to Sections 2.12.2 and 2.12.3) and 2-dimensional GC-MS (2dim GC-MS, for identification; according to Section 2.12.5). Distillates of the milk samples were analyzed using GC-O (for qualitative screening; according to Section 2.12.1) and GC-MS (for quantification; according to Sections 2.12.2 and 2.12.3) and 2dim GC-MS (for identification and qualitative screening of the milk samples, according to Section 2.12.4).

2.12.1. High Resolution Gas Chromatography – Flame Ionization Detection/Olfactometry (GC-FID/O)

The GC–O analyses were performed with a Trace Ultra gas chromatograph from Thermo-Fisher Scientific, Dreieich, Germany by using the following analytical columns: DB-FFAP (30 m × 0.32 mm, film thickness 0.25 µm, Agilent J&W Scientific, Santa Clara, CA, USA) and DB-5 (30 m × 0.32 mm, film thickness 0.25 µm, Agilent J&W Scientific, Santa Clara, CA, USA). After passing the column, the eluent was split and transferred to a flame ionization detector (FID) and to an olfactory detection port (ODP) in a volume ratio of 1:1. Manual injection of 2 µL of the distillate was performed into a cold-on-column injector at 40 °C directly onto the precolumn (uncoated, deactivated fused silica capillary, 2–5 m × 0.32 mm, Chromatografie Zubehör Trott, Kriftel, Germany). The temperature program started with a holding time of 2 min at 40 °C. Subsequently, the temperature was raised by 10 °C min^{-1} to the final temperature of 240 °C for DB-FFAP and 300 °C for DB-5 with a final hold time of 5 min. The carrier gas was helium at a flow rate of 2.5 mL min^{-1}. The temperatures of the FID and the ODP were set to 250 °C and 270 °C, respectively.

2.12.2. High Resolution Gas Chromatography – Mass Spectrometry (GC-MS)

The GC-MS analyses were performed with a GC 7890A and MSD 5975C from Agilent Technologies Deutschland GmbH, Waldbronn, Germany, which was equipped with a GERSTEL MPS2 autosampler and a GERSTEL CIS4 injection system (GERSTEL GmbH, Mülheim an der Ruhr, Germany). The analytical column used was a DB-FFAP (30 m × 0.25 mm, film thickness 0.25 µm; Agilent J&W Scientific). An uncoated, deactivated fused silica

capillary (2-5 m × 0.53 mm) was used as precolumn. Another uncoated fused silica capillary (0.3-0.6 m × 0.25 mm) was connected to the end of the analytical main column as a transfer-line into the MSD. The carrier gas was helium with a flow rate of 1.0 mL min^{-1}. The mass spectra were recorded in total ion current (TIC) mode (mass to charge ratio (m/z) range of 40–400) as well as in SIM mode with electron ionization (EI) at 70 eV. SIM conditions are provided in Table S1, Supporting Information. The temperature program of the oven and the injection volume were the same as given in Section 2.12.1.

2.12.3. High Resolution Gas Chromatography – Chemical Ionization Mass Spectrometry (GC-MS, CI)

The GC-MS (CI) analyses for the quantification of γ-nonalactone were performed with a Trace Ultra GC and ITQ 900, which was equipped with a Triplus autosampler from Thermo-Fisher Scientific. The analytical column used was a DB-FFAP (30 m × 0.25 mm, film thickness 0.25 µm; Agilent J&W Scientific). An uncoated, deactivated fused silica capillary (2-5 m × 0.53 mm) was used as precolumn. Another uncoated fused silica capillary (0.3-0.6 m × 0.25 mm) was connected to the end of the analytical main column as a transferline into the ITQ. The carrier gas was helium with a flow rate of 1.0 mL min^{-1}. The chemical ionization gas was methane with a flow of 2.2 mL min^{-1}. The mass spectra were recorded at 70 eV in TIC (m/z range of 40–400) and in SIM mode. For SIM the selected quantifiers were 157 m/z for the unlabeled and 161 m/z for the labeled γ-nonalactone. For the temperature program of the oven and the injection volume refer to Section 2.12.1.

2.12.4. Two-Dimensional Heart Cut—High Resolution Gas Chromatography—Mass Spectrometry/Olfactometry (GC-GC-MS/O) I

A GC system consisting of two 7890B GCs in combination with a 5977B MSD from Agilent Technologies Deutschland GmbH was applied. The first GC was equipped with a multi-column switching system MCS 2, and both GCs were connected by a cryo-trap system CTS 1 (both GERSTEL GmbH). The analytical columns were a DB-FFAP column (30 m × 0.32 mm, film thickness 0.25 µm; Agilent J&W Scientific) for the first oven and a DB-5 column (30 m × 0.25 mm, film thickness 0.25 µm; Agilent J&W Scientific) for the second oven. An uncoated, deactivated fused silica capillary was used as precolumn (3-5 m × 0.53 mm) as described above. The carrier gas was helium at a flow rate of 2.5 mL min^{-1}. In the first oven the effluent was split between an FID and an ODP (ODP3, GERSTEL GmbH), as well as a cryo-trap during the cut interval. After the specific cut interval, the trapped substances were transferred to the second analytical column, which was directly connected to the MSD. All split columns were made of uncoated, deactivated fused silica material. The FID and the ODP were held at 250 °C and 260 °C, respectively. Mass spectra were recorded at 70 eV in TIC mode (m/z range 40-400) as well as in SIM mode with EI. Application of the sample (2 µL) was performed at 40 °C using the cold-on-column technique. The oven was held at this temperature for 2 min; then, the temperature was raised by 10°C min^{-1} to 240 °C for the first oven and to 300 °C for the second oven. The final temperature was held for 5 min.

2.12.5. Two-Dimensional Heart Cut—High Resolution Gas Chromatography—Mass Spectrometry/Olfactometry (GC-GC-MS/O) II

A GC system consisting of two 7890A GCs in combination with a 220 Ion Trap from Agilent Technologies Deutschland GmbH was applied. All additional equipment and the settings of the system were equivalent to Section 2.12.4. At this instrument, the mass spectra were recorded at 70 eV in TIC mode with an m/z range of 35-250.

2.13. Statistical Analyses

The effect of the consumption of a standardized curry dish on the aroma of the milk samples and on the concentrations of the target compounds was evaluated for each odor attribute and aroma compound, respectively, by a univariate analysis of variance (ANOVA), followed by Dunnett's post-hoc test. The average values of the intensity ratings of the panelists were used for each odor attribute and each sample. The data set of the samples before the consumption of the curry dish was used as control set to check for significant differences against the three intervention sets after the curry consumption.

3. Results

3.1. Sensory Evaluation of the Curry Spice Mixture and the Curry Dish

The aroma profiles of the curry spice mixture and the curry dish are presented in **Figure 1**. For the curry spice mixture, the attributes coriander-like/soapy, savory, citrus-like, clove-like, pepper-like, ginger-like, eucalyptus-like, turmeric-like, cooling, cinnamon-like and pungent were determined by the sensory panel. The coriander-like/soapy impression was evaluated as being the most intense with a mean value of 7 (±standard deviation $\sigma = 2$). A mean intensity of 5 was determined for the attributes savory, citrus-like, clove-like and pepper-like (5 ± 2 in each case). The attributes eucalyptus-like (4 ± 3), ginger-like (4 ± 2), turmeric-like (4 ± 2) and cooling (4 ± 3) were rated with a mean intensity of 4. The attributes with the lowest perceived intensities were cinnamon-like (3 ± 3) and pungent (3 ± 2).

For the curry dish, the attributes turmeric-like, ginger-like, savory, coriander-like/soapy, clove-like, cinnamon-like, flowery, cumin-like, pepper-like, coconut-like, eucalyptus-like, pungent, cooling, popcorn-like and caramel-like were determined by the sensory panel. The highest intensity with a mean of 5 was determined for the attribute turmeric-like (5 ± 2). The attributes ginger-like (4 ± 2), savory (4 ± 2) and coriander-like/soapy (4 ± 3) were evaluated with a mean value of 4. For the flowery (3 ± 2), clove-like (3 ± 2), cinnamon-like (3 ± 2) and cumin-like (3 ± 3) percepts a mean intensity of 3 was determined, followed by the

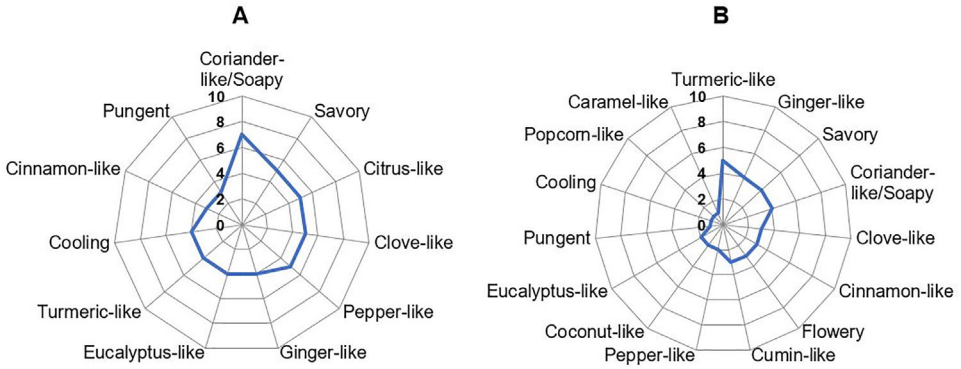


Figure 1. Aroma profiles of the curry spice mixture (A) and the curry dish (B): average odor intensity ratings of 10 (A) and eight (B) trained panelists, rated on a scale from 0 (not perceivable) to 10 (very intensive).

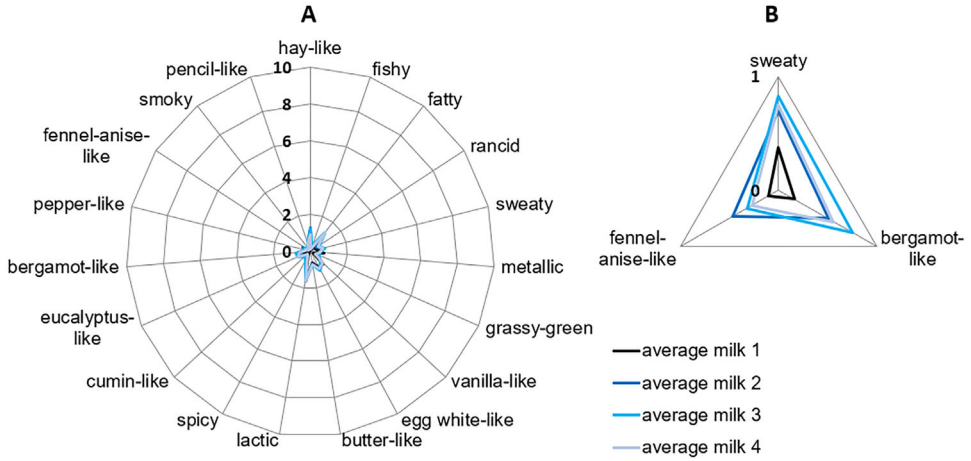


Figure 2. (A) Average intensities of aroma descriptors in milk samples (n = 14) obtained before (black line) and 1, 2, and 3 h after curry consumption (dark blue, blue, light blue lines). Intensities were rated by four trained panelists on a linear scale from 0 (not perceivable) to 10 (very intensive). (B) Enlarged figure for attributes with significant odor intensity changes: sweaty, fennel-anise-like and bergamot-like.

attributes eucalyptus-like (2 ± 3), coconut-like (2 ± 1) and pepper-like (2 ± 2). The odor attributes caramel-like, popcorn-like, cooling and pungent (1 ± 1 in each case) were perceived with the lowest odor intensities.

3.2. Sensory Evaluation of the Milk Samples

The results of the sensory evaluation of milk samples obtained before and after ingestion of the curry dish are presented in **Figure 2**. The intensities of the odor attributes hay-like, fishy, fatty, rancid, sweaty, metallic, grassy-green, vanilla-like, egg white-like, butter-like, lactic, spicy, cumin-like, eucalyptus-like, bergamot-like, pepper-like, fennel-anise-like, smoky and pencil-like were rated according to predefined descriptions by the sensory panel and previous studies on milk odor.[13b] Overall, the individual smell intensities were low. The results of the ANOVAs are shown in Table S3, Supporting Information. Dunnett's post-hoc tests revealed a significant ($p < 0.05$, see Table S3, Supporting Information) increase in intensity for the attributes bergamot-like (sample 2, 3 and 4 versus sample 1), fennel-anise-like (sample 2 versus sample 1) and sweaty (sample 3 versus sample 1) after curry consumption. No other significant differences became evident ($p > 0.05$).

3.3. Sensory-Analytical Characterization of the Curry Spice Mixture and the Curry Dish

The most important odorants and volatiles in the distillates of the curry spice mixture and the curry dish were determined by AEDA and GC-MS, and identified via GC-MS/O and GC-GC-MS/O. **Figure 3**A,B show the chromatograms of the distillates. Cuminaldehyde, zingiberene, linalool, eugenol, cinnamaldehyde, 1,8-cineole, *a*R-turmerone and 1,4-*p*-menthadien-7-al were quantitatively the most abundant volatile compounds.

Qualitatively, the AEDA revealed a total of 43 different odorants in the distillates of the curry spice mixture and the curry dish. The odorants with the highest FD factors in the curry spice mixture were linalool, cuminaldehyde, eugenol, and sotolone. In the curry dish, linalool, cuminaldehyde, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, eugenol, sotolone and vanillin reached the highest FD factors, as presented in **Table 1**.

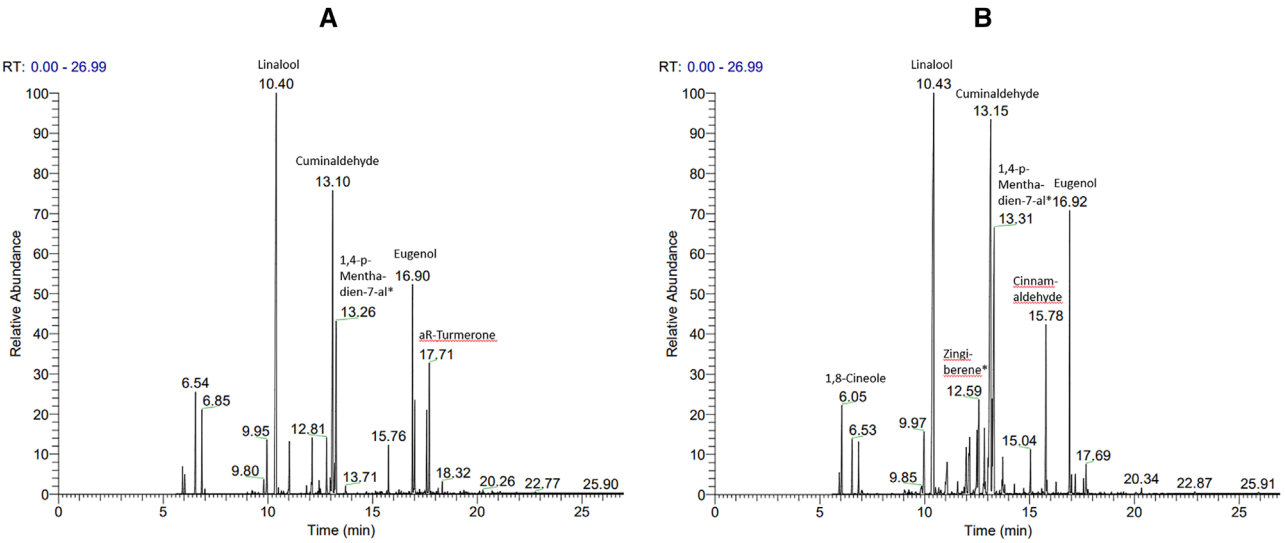


Figure 3. (A) Representative GC-MS chromatogram of a distillate obtained from the curry spice mixture (analytical column: DB-FFAP; quadrupole mass spectrometer; total ion current; mass range 40–400 *m/z*) and its most abundant volatiles (identified via comparison with reference compounds or (*) tentatively identified via comparison with NIST library). (B) Representative GC-MS chromatogram of a distillate obtained from the curry dish (analytical column: DB-FFAP; quadrupole mass spectrometer; total ion current; mass range 40–400 *m/z*) and its most abundant volatiles (identified via comparison with reference compounds or (*) tentatively identified via comparison with NIST library).

Table 1. The most important (FD factor ≥19 683) odorants identified in distillates of the curry spice mixture and the curry dish: compound names, odor qualities, retention indices (RI) on DB-5 and DB-FFAP columns, FD factors (column: FFAP) and identification criteria (RI compared to a standard on two different analytical columns, odor compared to a standard on two different analytical columns, MS compared with a standard).

Compound	Odor quality	RI DB-FFAP	RI DB-5	FD factor		Identification
				Curry spice mixture	Curry dish	
Linalool	Flowery, soapy	1534	1102	19 683	59 049	RI, Odor, MS
Cuminaldehyde	Cumin-like, fatty	1770	1246	19 683	19 683	RI, Odor, MS
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone	Caramel-like, strawberry-like	2022	1079	81	19 683	RI, Odor
Eugenol	Clove-like	2151	1360	19 683	59 049	RI, Odor, MS
Sotolone	Savory	2175	1102	19 683	19 683	RI, Odor
Vanillin	Vanilla-like	2539	1399	6561	59 049	RI, Odor, MS

Based on the GC-MS- and GC-O-analyses of the curry spice mixture and the curry dish, the following compounds were selected for quantification in milk: linalool, cuminaldehyde, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, eugenol, sotolone, vanillin (most potent aroma compounds), and additionally cinnamaldehyde as an abundant aroma-active volatile in the curry dish.

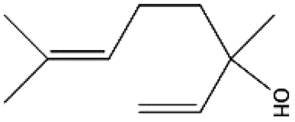
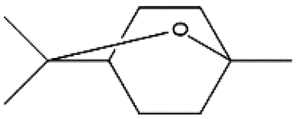
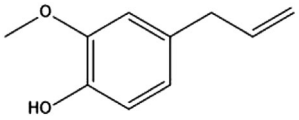
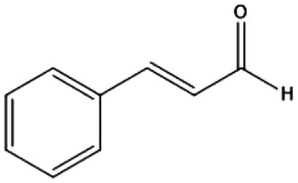
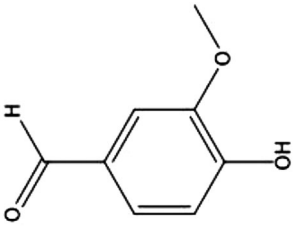
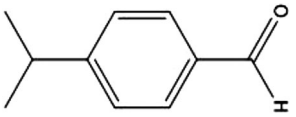
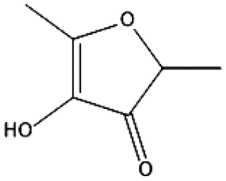
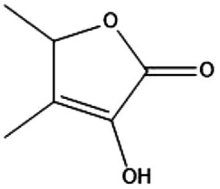
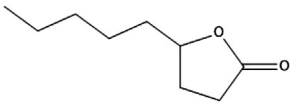
3.4. Odorant Screening of Milk Samples

Initially, the GC-MS data obtained from distillates of the milk samples of the intervention study were evaluated by means of a comparison of all chromatogram peaks with a signal to noise ratio ≥3 in order to discover potential additional target substances which are transferred into the milk. The only compounds with apparent differences in the samples were linalool and 1,8-cineole. Additionally, samples obtained from three participants were screened for odor-active compounds using GC-O, to unveil potential newly formed odor-potent constituents deriving from the curry dish. A coconut-like odor, namely γ-nonalactone, appeared to be a potential candidate due to its occurrence in one milk sample series. Based on the GC-MS and GC-O screening, it was decided to include both 1,8-cineole and γ-nonalactone in the following targeted analysis. The chemical structures of the nine target compounds, resulting from these two procedures as transmission candidates, together with physico-chemical information are provided in **Table 2**.

3.5. Quantification of the Target Compounds in the Curry Dish

The quantification of the target compounds in the curry dish revealed a dosage of 35 and 22 mg per serving for linalool and cuminaldehyde, respectively. Cinnamaldehyde and eugenol were quantified in the range of few milligrams whereas the dosage of vanillin, 1,8-cineole, γ-nonalactone and sotolone were in the range of 3–477 ng. The amount of 4-hydroxy-2,5-dimethyl-3(2*H*)-

Table 2. Chemical structures of the nine target odorants.

Substance	Structure	Chemical class	Molecular mass [u]	Log Pow
Linalool		Monoterpene, alcohol	154	3.0
1,8-Cineole		Monoterpene, ether	154	2.7
Eugenol		Phenylpropanoid, alcohol, ether	164	2.3
Cinnamaldehyde		Phenylpropanoid, aldehyde	132	1.9
Vanillin		Benzaldehyde derivative, aldehyde, ether, alcohol	152	1.2
Cuminaldehyde		Monoterpene, aldehyde	148	3.2
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone		Furanone, alcohol, ether, ketone	128	1.0
Sotolone		Lactone, alcohol	128	1.0
γ-Nonalactone		Lactone	156	2.1

Additional information on the respective chemical class, molecular mass and log Pow values (log n-octanol/water partition coefficient).

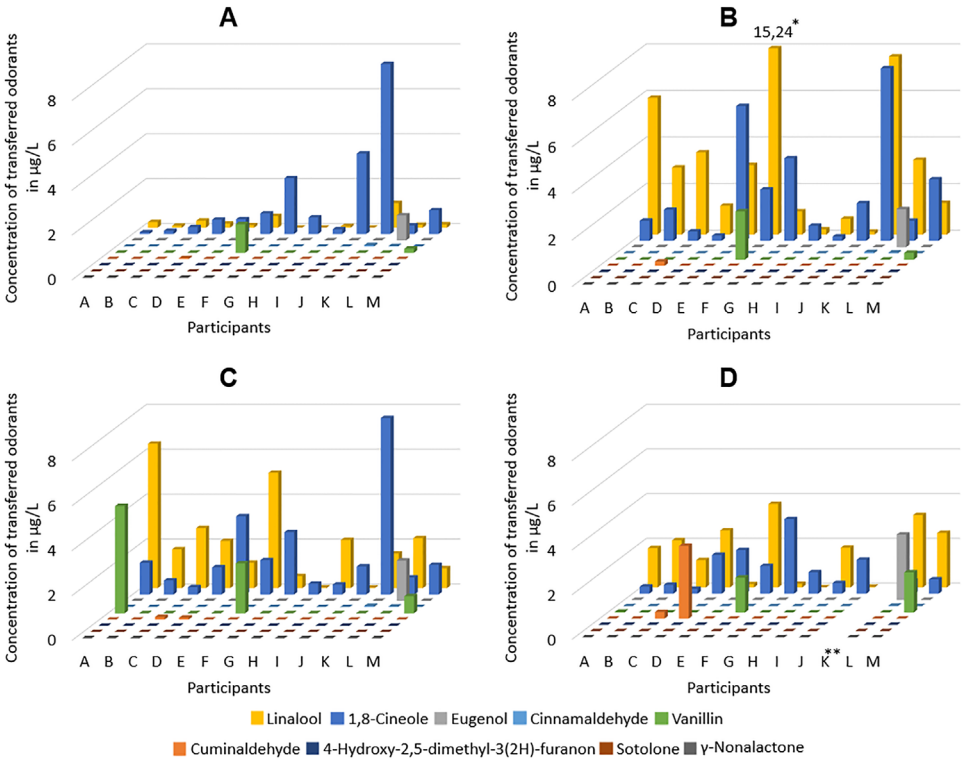


Figure 4. Overview of the concentration profiles of the nine target odorants in milk samples obtained from 13 participants before consumption of the curry dish (A), and 1 h (B), 2 h (C) and 3 h (D) after consumption of the curry dish. *Value exceeds the scale and is therefore displayed numerically. **No sample available.

Table 3. Average dosage [µg] and standard deviations (n = 3) of the target odorants in the curry dish.

Substance	Absolute dosage per curry dish [µg]	Standard deviation [%]
Linalool	34 615	8.6
1,8-Cineole	394	7.7
Eugenol	1055	4.5
Cinnamaldehyde	3575	4.8
Vanillin	477	8.7
Cuminaldehyde	21 522	5.0
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone	<1*	-
Sotolone	3	3.0
γ-Nonalactone	8	5.0

*Concentration was below the LOQ.

furanone in the curry dish was below its limit of detection. The average amounts of the target odorants per standardized curry meal are presented in **Table 3**.

3.6. Quantification of the Target Compounds in the Milk Samples

The concentrations of the target substances in the milk samples before and after the curry consumption are presented in **Figure 4** and listed in Table S4, Supporting Information. Despite the 48 h wash-out phase, some of the target compounds were already detected in the control samples donated before consumption of the curry dish. 1,8-Cineole was detected in each of the 13 control samples, with concentrations ranging between 0.07 and 7.57 µg L^{-1} (average: 1.44 µg L^{-1}). The concentrations of linalool ranged from below the LOD (n = 3; participants G, H and J) to 1.10 µg L^{-1} (average: 0.22 µg L^{-1}). Vanillin and cinnamaldehyde were detected in two samples each (F, M and K, L, respectively), with maximum concentrations of 1.27 and 0.06 µg L^{-1}. Cuminaldehyde and eugenol were detected above the LOQ in one control sample each (D and L), with a concentration of 0.04 and 1.11 µg L^{-1}, respectively. The concentrations of sotolone, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone and γ-nonalactone were below the LOD in all samples donated before consumption of the curry dish.

In the milk samples donated after the consumption of the curry dish, the concentrations of linalool and 1,8-cineol were without exception above the respective LOQs. The concentrations of linalool rose up to 15.24 µg L^{-1}, ranging between 0.12 and 15.24 µg L^{-1} (samples 2), 0.03–6.44 µg L^{-1} (samples 3), and 0.01–3.73 µg L^{-1} (samples 4). In the case of 1,8-cineole, concentrations increased up to 7.86 µg L^{-1}, with a range of 0.19–7.41 µg L^{-1} (samples 2), 0.33–7.86 µg L^{-1} (samples 3), and 0.22–3.33 µg L^{-1} (samples 4). In contrast, the other target compounds were not detected in each of the samples donated after consumption of the curry dish. Cuminaldehyde was above its LOD but below LOQ in a total of two samples and above its LOQ in a total of three samples (range: <LOQ to 3.25 µg L^{-1}). These samples had been donated by two mothers (C and D), including the mother for whom cuminaldehyde was also detected in sample 1

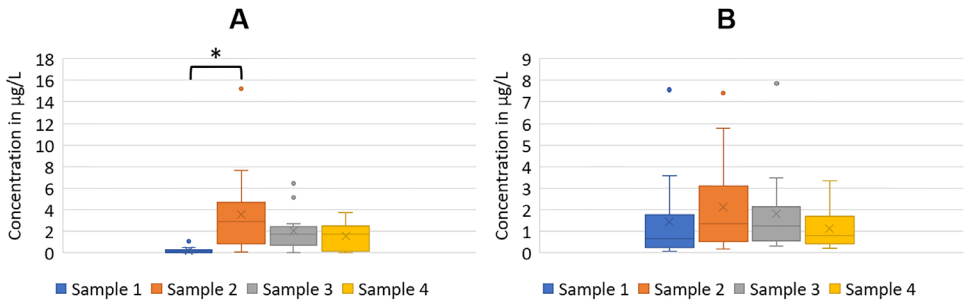


Figure 5. Concentration courses of linalool (A) and 1,8-cineole (B) in milk samples obtained before (sample 1, blue) and after (samples 2, 3, and 4) consumption of the curry dish, presented as boxplots indicating the median (middle line), the average (cross), the second and third quartile (box), the maxima and minima in the range of up to the 1.5-fold length of the interquartile distance (whiskers) as well as the overall maxima and minima (dots). *Significant with $p < 0.01$ (Dunnett's test).

(D). Vanillin was detected in a total of eight samples donated by three mothers (A, F, M). The concentration ranged between 0.03 and 4.78 µg L^{-1}. The control samples of two of these three mothers (F, M) contained vanillin in detectable amounts. Eugenol was above its LOQ in three samples (concentration range: 1.63 to 2.92 µg L^{-1}), donated from one mother (L). In the corresponding sample 1, eugenol had been detected as well. Cinnamaldehyde was above its LOD but below LOQ in a total of four samples and above its LOQ in one sample, donated from two mothers (K, L). In the control samples obtained from these mothers, the compound had been detected as well. Finally, the concentrations of sotolone, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone and γ-nonalactone were below the LOD in all samples obtained after consumption of the curry dish.

The ANOVAs revealed a significant impact of sampling time on the concentration of linalool only (**Figure 5**A; Table S5, Supporting Information). The linalool concentration in the control sample was significantly lower than the concentration in the milk sample obtained 1 h after ingestion of the curry dish ($p < 0.01$, Dunnett's test), and tended to be lower compared with the concentration in sample 3 (obtained 2 h after ingestion; $p = 0.07$, Dunnett's test). The differences in the concentrations of 1,8-cineole (Figure 5B) and the other target compounds were not significant ($p > 0.05$).

3.7. Odor Thresholds and Odor Activity Values

Overall, the odor thresholds determined in cow milk were higher than those determined in water. This effect was more or less pronounced, dependent on the respective compound. For instance, the average thresholds were in a comparable range in the case of vanillin but differed by a factor of 20 529 in the case of eugenol (**Table 4**). On the basis of the geometric mean value of the experimentally determined odor thresholds in cow milk and the concentrations of the target compounds in the milk samples, their odor activity values (OAV)[17] were calculated. For the respective highest concentrations that were determined per milk set, the average OAV for linalool was 6.8 ± 7.1 (range regarding individual subjects and their odorant concentration: 0.2 to 27.7) and for 1,8-cineole 0.7 ± 0.6 (range: 0.1 to 2.4). The OAVs considering the maximum concentration levels of vanillin, eugenol, cinnamaldehyde and cuminaldehyde in the milk samples were lower than 0.01. Likewise, the OAVs of sotolone, γ-nonalactone and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, calculated with their respective LODs, were lower than 0.01.

Table 4. Odor thresholds (µg L^{-1}; geometric mean ± geometric standard deviation) of the target substances in milk (unhomogenized, pasteurized cow milk with 3.8% fat) and in distilled water.

Compound	Number of panelists	Threshold in milk [µg L^{-1}]	Threshold in water [µg L^{-1}]
Linalool (I)	n = 8	0.6 ± 3.4	0.1 ± 3.2
1,8-Cineole (II)	n = 7	3.3 ± 15.6	0.5 ± 5.6
Sotolone (VIII)	n = 7	13.1 ± 2.5	0.2 ± 6.6
γ-Nonalactone (IX)	n = 8	172.9 ± 1.7	12.3 ± 2.5
Vanillin (V)	n = 8	615.9 ± 2.8	454.6 ± 10.6
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (VII)	n = 6	760.0 ± 4.0	20.1 ± 2.8
Eugenol (III)	n = 6	3042.4 ± 6.5	0.2 ± 6.8
Cinnamaldehyde (IV)	n = 10	6429.2 ± 3.2	174.7 ± 3.4
Cuminaldehyde (VI)	n = 7	9968.5 ± 2.0	70.9 ± 3.4

4. Discussion

In the current study, the sensorially most potent and the quantitatively most abundant odorants of a standardized curry dish were determined and quantified in milk samples donated before and after ingestion of the dish. The target compounds comprised 1,8-cineole, linalool, cuminaldehyde, cinnamaldehyde, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, sotolone, eugenol, vanillin and γ-nonalactone. We aimed to determine the time-dependent concentration changes of these aroma compounds and the concurrent changes in the aroma profile of the milk samples, by taking a combined sensory-analytical approach.

To evaluate whether the intake of a curry dish might impact the aroma of milk, two different approaches were taken. The most evident one was the sensory evaluation of the milk samples before and after the intervention by a trained panel. The chosen attributes for the intensity ratings comprised typical odors related to the ingredients of the curry dish and human milk.[13] Besides that, the panel determined a pencil-like odor. This odor attribute may derive from thymoquinone which has a typical

pencil-like odor and occurs in various herbs.[18] The sensory evaluation of the milk revealed significant changes for the attributes bergamot-like, sweaty and fennel-anise-like 1 to 2 h after the intervention. However, the average intensity ratings were <1 in the intervention samples, which would correlate with a very low intensity and would suggest that the differences were barely relevant. Nonetheless, the odor intensity ratings were higher for certain milk samples, as well as for individual panelists. For example, the bergamot-like aroma and the eucalyptus-like aroma were rated with an intensity of ≥4 in six and four out of the 14 sample sets, respectively. Accordingly, the magnitude of the aroma change differed between the individual test persons and their milk samples. In sum, these results suggest that the intake of a curry dish can indeed lead to perceivable aroma changes in milk. Apart from that, one needs to keep in mind that retronasal aroma perception may be even more pronounced as commonly observed for lipophilic foods such as milk. This is in line with the fact that retronasal odor thresholds are often lower than their corresponding orthonasal values, as for 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (retronasal odor threshold of 4 μg mL^{-1} and orthonasal odor threshold of 25 μg mL^{-1} in vegetable oil), vanillin (retronasal odor threshold 20 μg mL^{-1} in water and orthonasal odor threshold of 210 μg mL^{-1} in water) or linalool (retronasal odor threshold of 1.5 μg mL^{-1} and orthonasal odor threshold of 6 μg mL^{-1} in water).[19] It needs to be stated that the panellists were aware of the identity of the samples in the current study, which might have unconsciously influenced their ratings. This was due to the fact that sensory evaluations needed to be carried out as quickly as possible in conjunction with the sampling of the milk, to avoid any sensory changes due to storage effects. Nevertheless, in future studies, the sensory evaluations should be performed in a blind manner whenever possible.

The second approach and step of this study was to determine OAVs of the target substances as the ratio of their concentrations in the milk samples and their odor thresholds which were experimentally determined in a cow milk matrix. Generally, it is postulated that odorants with an OAV ≥1 contribute to the overall aroma of a food. This concept serves as a rough approximation of the real impact of an odorant to the overall aroma as synergistic effects are not taken into account. Moreover, human perception of odor intensity is not correlated in a linear relationship with odorant concentration as suggested by the OAV concept but rather follows non-linear psychophysical power functions so that OAVs do not directly express the real aroma impact but just serve as a guiding tool.[20] However, the procedure still allows for a matrix-adapted estimation of the aroma impact of an odorant. In this study, a cow milk matrix was chosen for this purpose as odor thresholds strongly depend on the lipophilicity of the matrix.[21] For comparison, odor thresholds were also determined in water. In accordance with Spitzer,[21] the odor thresholds determined in milk were higher compared to those determined in water, with 1.4- to 20 529-fold increase. This is likely due to the lipophilicity of the odorants, which interact with the substances in the fat phase and are hence, to some extent, retained in an oil-in-water emulsion such as milk. For linalool, this has been demonstrated by Miettinen et al.,[22] who showed in an emulsion model that besides the fat content, the droplet size also impacts aroma release. Other matrix components, e.g., proteins, may also affect aroma release. The retention of odorants in the matrix due to an increased fat content is contrary to the salting-out effect by which lipophilic odorants separate from the matrix due to a higher polarity caused by higher salt concentrations.[23] For comparatively polar odorants like vanillin (log Pow of 1.2) the retention can be expected to be substantially smaller than for more lipophilic odorants like eugenol (log Pow of 2.3). This effect is not linear, but it explains a basic tendency of the odor thresholds in milk and therefore of the calculated OAVs. The calculated OAVs are based upon thresholds determined with the static fat content of the cow milk matrix. The individual and varying fat content of milk samples, however, e.g., related to the lactation period or the individual breastfeeding session, could lead to some variation in the infants' aroma perception even with the same concentrations of aroma compounds. Furthermore, the perceptional variations for the infant are probably even more complex regarding dilution effects in the oral cavity by saliva, inhomogeneous mixing as well as swallowing processes.[24]

Based on the odor thresholds determined in the cow milk matrix, the average maximum OAV for linalool was 6.8 ± 7.1, whereas in case of 1,8-cineole, the average maximum OAV was 0.7 ± 0.6. This would suggest that a diet-induced change of the milk aroma due to 1,8-cineole in the detected concentrations would be less pronounced but would be still likely for some of these samples. The OAVs of the remaining seven target odorants were lower than 0.01. Therefore, the odors of linalool (bergamot-like) and 1,8-cineole (eucalyptus-like) can be supposed to be perceivable in milk samples according to the OAV concept, whereas the other odorants would be considered negligible for the overall aroma of the milk. Partially, these results are in accordance with the aroma profiles of the milk samples, which indeed revealed a significant change for the attribute bergamot-like. The odor thresholds were, however, determined with an adult panel, and the samples were not tasted retronasally due to work safety considerations. Earlier studies revealed that newborns are capable of differentiating between another and their own mother's milk,[3] that they are highly sensitive to olfactory stimuli,[25] and therefore might be better in detecting sensory differences in milk than adults. Accordingly, the retronasal tasting of milk by the nurslings could result in the perception of even low concentrated odorants. As a consequence, the observed odorant transfer might affect the aroma of milk for nurslings even if the determined OAVs are lower than one.

Generally, the bergamot-like odor in the milk samples can be explained by the transfer of linalool into the milk, this study being, to the best of our knowledge, the first-time report of a transfer of this substance. Linalool concentrations were significantly higher in the milk samples after curry consumption than before. For 1,8-cineole, however, the transfer was not significant. Nonetheless, the quantification data showed a strong tendency for higher concentrations after curry consumption, as the median 1,8-cineole concentration doubled after curry consumption (0.66 μg L^{-1} before and 1.33 μg L^{-1} afterwards). A transfer of 1,8-cineole into milk has been previously reported by Kirsch et al.[26] The fact that no significant transfer of 1,8-cineole was observed in this study is obviously related to the comparatively low dosage of about 400 μg per curry dish (in contrast to 100 mg per Soledum capsule, administered by Kirsch et al.[26]). Otherwise, an inadvertent intake of mint pastilles,

toothpaste or other products scented with 1,8-cineole could have led to higher base levels of 1,8-cineole as these are products which participants might not include in their provided nutrition diary.

None of the other seven target compounds were significantly transferred into milk. This is particularly surprising with regard to cuminaldehyde, which is structurally related to linalool and 1,8-cineole and was the odorant with the second highest dosage concentration in the curry dish. Overall, the transfer profiles of the aroma compounds do not reflect their concentration ratios in the initial curry dish. For instance, 1,8-cineole was detectable in all intervention samples whereas vanillin, cinnamaldehyde, eugenol and cuminaldehyde, which were higher concentrated in the dish, were only detectable in one or two sample sets. In previous studies, which showed an odorant transmission into milk, dosages of 100 mg were customary.[26–27] The dosages of vanillin, eugenol and cinnamaldehyde of 0.5 to 3.6 mg per serving within this study were therefore comparatively low. Still, in case of a substantial transition, the dosage concentrations would have been high enough to be detected and, according to their odor thresholds in milk, to potentially alter the overall aroma. As we only detected small amounts of <0.75 to 42.8 ng of these substances in the milk samples, they probably underlie significant phase I and/or phase II metabolization processes, or are not substantially transferred to milk for other reasons which are not known yet. Due to their free hydroxy and aldehyde groups they might also be metabolized and conjugated faster than 1,8-cineole which would explain a smaller transition rate of the initial substance. For eugenol it has been stated that the formation of phase-II-metabolites followed by an excretion into urine is the most common pathway for humans.[28] Similarly high metabolization rates are known for vanillin in rats[29] and cinnamaldehyde in humans.[30] Only for γ-nonalactone, sotolone and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, which were more than 40-fold lower concentrated in the dish than 1,8-cineole, the lack of their detection in the samples can be explained by small dosage amounts.

Linalool was quantified in the highest amounts in the dish as well as in most of the milk samples collected after intervention, whereas cuminaldehyde, which is also a terpene and has a similar molecular weight and polarity (see Table 2), was barely detectable in the samples even though its amount (22 mg) in the dish was nearly as high as that of linalool (35 mg). Different routes and time courses of metabolization and excretion might explain this observation. Cuminaldehyde was shown to be metabolized to its corresponding carboxylic acid or its primary alcohol during phase I reactions in rabbits,[31] and might be predominantly excreted via urine. Another possibility is a delayed excretion of cuminaldehyde via milk several hours after the intervention. Indeed, for two mothers, the highest concentration levels of cuminaldehyde were reached at the last sampling point. Such a late transition of cuminaldehyde has previously been reported in sheep's milk[32] where its concentration was quantified above the LOQ 9 h after intervention. Such an effect could be due to specific routes of metabolization and potential depot effects within the body, for example the adipose tissue. Besides these aspects, different degrees of plasma protein binding could also contribute to the different extent of odorant transfer.[33]

The nine target compounds are representatives of several different chemical classes (see Table 2). Their molecular masses range from 128 to 164 u and their polarities defined by their respective log Pow range from 1.0 to 3.2. The functional groups, chemical class, polarity, molecular mass and the stereometrics do not correlate to the here observed transition profiles and can therefore not be the sole factors defining a transfer in milk. The unmodified transfer into milk is only one aspect of the fate of aroma compounds in the human body and is in competition with (bio)transformation and partitioning processes and excretion via breath, feces, urine, sweat or potentially other minor substrates like saliva, tears, hair, vomit etc. Moreover, in a real-life-scenario as used in the present study, the interaction of different dietary constituents in the gastro-intestinal tract and other sites of the body may affect the bioavailability of individual constituents on the one hand, and their (bio)transformation as well as excretion processes on the other hand. The complexity of these underlying mechanisms and their linkages in different individuals is, however, barely possible to be resolved with today's technologies.

In addition to the substance-specific differences in the transferred amounts, inter-individual differences became evident. Whereas a transfer of linalool and 1,8-cineole was recorded as being relevant for all participants, vanillin, cuminaldehyde and cinnamaldehyde could only be detected above their respective LOQ for two participants, and eugenol for one participant only. This implies that there might be an individual component to transmission of odorants into breastmilk. Apart from that, the relative quantification ratios differed between the test persons; e.g., for eight participants linalool was on average the most concentrated odorant whereas for the remaining five participants this was the case for 1,8-cineole. An explanation might be inter-individual differences in the metabolism of odorants due to genetic and enzymatic differences. Inter-individual polymorphisms in the human metabolism are well-known in pharmacogenomics,[34] whereas only few studies have targeted this phenomenon with respect to odorants. A recent study suggests, for example, that the individuum-specific composition of saliva and age can account for differences in metabolism; this has been reported in the context of odorant degradation as well as aroma release.[35] Further, the BMI, the hormonal status or the health status might impact such inter-individual variations.

4.1. Summary and Outlook

In this study, a significant time-dependent transfer of the odorant linalool into the milk of nursing mothers was observed after ingestion of a customary curry dish. Additional determinations of matrix-adapted odor thresholds and OAVs corroborated the olfactory relevance of this transfer, which therefore could contribute to an early flavor learning of the infant. For the other eight target components, no statistically significant transfer became evident. However, on the level of individual samples, the appearance of several substances was observable to some extent. In relation to cuminaldehyde, the results point to a potentially longer transition period as well as metabolic transformations, which however await to be confirmed by future studies. Furthermore,

the transfer profiles differed between individual participants. For example, a transition of eugenol, vanillin, cuminaldehyde and cinnamaldehyde was traceable only for certain individuals.

Future studies should, on the one hand, investigate metabolites of the aroma compounds in different human substrates, besides milk, more comprehensively. This is necessary to better understand the metabolic pathways and routes of excretion of these substances and the impact of nursing on that system. On the other hand, a sufficiently long sampling period should be applied in these studies to allow for the excretion of all parent compounds and their metabolites. There is also a need to unravel the reasons beyond inter-individual differences in the transition of aroma compounds. With the application of appropriate metadata and statistical methods, conclusions could be drawn as to how much influencing factors such as the mother's eating habits have an impact on the transition of aroma substances into milk.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

M.D. did quantitative and qualitative analysis, untargeted data acquisition, data analysis and interpretation, human intervention study and wrote the manuscript. D.S. realized the human intervention study, did qualitative analysis. D.O. did qualitative analysis of the curry spice mixture and dish. K.N. contributed to data interpretation and the manuscript. T.A. composed the curry spice mixture and recipe for the curry dish. R.L. conceptualized and supervised the project and contributed to data interpretation and the manuscript. T.H. conceptualized and supervised the project and contributed to data interpretation and the manuscript. A.B. conceptualized and supervised the project and contributed to data interpretation and the manuscript. H.L. realized the human intervention study, conceptualized and supervised the project and contributed to data interpretation and the manuscript.

Data Availability Statement

The data that support the findings of this study are available upon reasonable request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Keywords

aroma transition, breast milk, flavor learning, linalool, maternal diet

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