Tracing odour- and taste-active compounds in human milk

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Abstract

Scope

The alteration of the flavour of human milk by the maternal diet and thereby the possibility of sensory programming of the infant has been reported in several studies. The objective of this study was to exemplarily illustrate and compare the simultaneous transition of odour- and taste-active compounds from a customary curry dish into milk.

Methods and Results

A standardised curry dish was prepared and its flavour-active compounds were characterised. This dish was used in an intervention study with lactating mothers who donated four milk samples, one before and three after the intervention. These samples were used to quantify the transition of flavour-active compounds from the curry dish into milk via GC-MS and LC-MS. The concentration courses of linalool, 1,8-cineole and piperine are illustrated here for the milk samples of three exemplary test persons. These courses demonstrate a transition after the intervention as well as interindividual variations regarding transition time and amounts.

Conclusion

Flavour-active substances can migrate from the maternal diet into human milk. The transferred compounds do not necessarily reach concentrations perceivable by adults. Though, even at below threshold concentrations, the transferred compounds can interact with the nurslings' receptors and induce physiological changes in the chemosensory system.

Keywords: curry, metabonomics, piperine, linalool, 1,8-cineole

Note: Our use of the term "flavour" relates to compounds, which contribute to the overall oral impression and thus includes aroma-, taste- and trigeminus-moderated sensations.

Introduction

The fact that the interplay of smell, taste and trigeminally moderated oral impressions has more effect on our perception of food than the sum of its parts is the subject of current research in the field of sensory science [1-4]. For instance, a citrus flavouring is perceived as more intense when it is presented in a sugar solution than without the tastant [1]. The concept of multisensory integration encompasses all those sensory impressions whose combination is matched and harmonised as a pattern in the human brain [5]. Multisensory experiences and expectations develop in the course of life. During breastfeeding, infants already experience first flavour impressions, which can provide the basis for flavour learning [6-8]. The flavour of breast milk can be influenced by maternal nutrition [8-11], and for many odorants, the transition of dietary aroma into milk has been demonstrated on a molecular level [10, 12, 13]. For tastants and trigeminally active compounds, however, there are only a few indications of a transfer into breast milk. Up to now, only a transfer of alkaloids such as caffeine [14] has been demonstrated, and a general connection between a diet rich in bitter substances and bitterness of breast milk has been observed [15]. However, an investigation of the interplay of odorants and tastants from an everyday multi-component dish has not yet taken place. This is where our work comes in, in which we set ourselves the goal of characterising the transition of flavour compounds in parallel from a standardised curry dish into breast milk. For this purpose, breastfeeding mothers gave milk samples before and after curry consumption as part of an intervention study to determine the temporal course of the possible transition of flavour-active substances.

Experimental

Curry dish

The basis of our intervention study was the usage of a standardised curry dish. The curry spice powder used for this dish comprised coriander seeds, cumin seeds, turmeric powder, dried red chilies, fenugreek, black

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peppercorns, cinnamon sticks, green cardamom, curry leaves and cloves. For the preparation of the dish, the curry powder was mixed with coconut milk, water, salt and fresh ginger to prepare a curry sauce which was then served with rice. The detailed recipe is available from Debong et al. and N'Diaye et al. (2021, submitted).

Intervention study

For the intervention study, nursing mothers were asked to donate milk samples following a specific procedure. This procedure, illustrated in Figure 1, started with a two-day washout phase during which the mothers refrained from the intake of certain food and kept a nutrition diary. On the study day, the mothers first donated a blank milk sample, then consumed the curry dish and subsequently donated further milk samples (for the detailed procedure see Debong et al. and N'Diaye et al., 2021, submitted). The samples were then aliquoted for the determination of odorants as well as tastants. The study was performed in agreement with the Declaration of Helsinki and approved by the Ethical Committee of the Friedrich-Alexander-Universität Erlangen-Nürnberg (registration number 24_16 B).

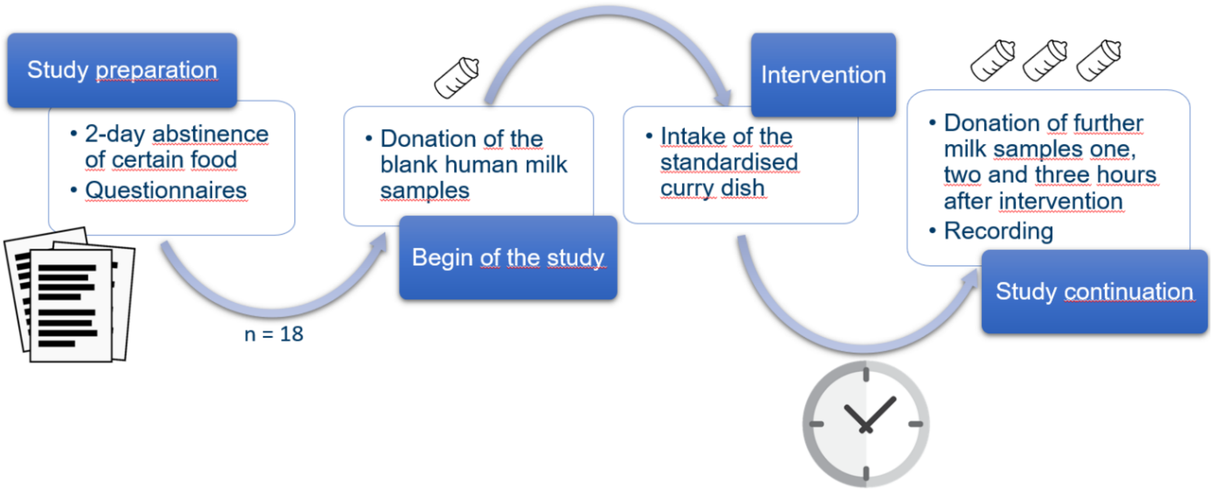

Figure 1: *Scheme of the intervention study procedure.*

Characterisation of the curry dish

To gain dosage information on the odour- and taste-active compounds we performed a targeted quantification of the compounds which were determined as the most relevant flavour-active compounds in the curry dish. These comprised, amongst others, the odorants 1,8-cineole and linalool, on which we will focus here. Those substances were quantified using a stable isotope dilution assay (SIDA) approach in combination with solvent assisted flavour evaporation (SAFE) and GC-MS with internal calibration. Detailed information is provided in Debong et al. (2021, submitted). The pungent oral sensation of the curry dish suggested pungent compounds as additional target analytes. These comprised, amongst others, piperine. Piperine was quantified by LC-MS/MS using an in-house synthesised d_{10}-piperine standard for an internal calibration. Detailed information is provided in N'Diaye et al. (2021, submitted).

Detection of odorants and tastants in milk and estimation of their sensory relevance

An instrumental-analytical approach was utilised to characterise the transfer of odorants and tastants from the curry dish into milk, analogously to the determination of the target compounds in the curry dish. In addition to that, odour and taste activity values (OAVs and TAVs) were calculated to evaluate the relevance of such flavour transition. Therefore, a trained panel determined the odour/taste thresholds of the target compounds in a cow-milk based substitution matrix (for details see Debong et al., N'Diaye et al., submitted). The OAVs and TAVs were then calculated as quotients of the flavour compound concentration in a sample and the odour/taste threshold of the respective compound.

Results and discussion

Flavour transition into milk

On average, the absolute dosages of the odorants linalool and 1,8-cineole were 224 µmol and 2.6 µmol per serving, respectively. The dosage amount of piperine was 32 µmol per curry dish. Exemplary transition courses of three milk sets are illustrated in Figure 2 and will be discussed in the following sections.

Considering the here illustrated examples, the concentrations of linalool in the milk samples donated before the intervention were about 1 nmol/L. Within one hour after the intervention the concentrations rose to values between 6 nmol/L (course A) and 22 nmol/L (course C). The concentrations of 1,8-cineole in the milk samples obtained before the intervention ranged between 2 nmol/L (course C) and 16 nmol/L (course A). Within one hour after the intervention the concentrations rose to values between 3 nmol/L (course C) and 22 nmol/L (course A). The piperine concentrations in the initial samples before the intervention ranged between 0.4 nmol/L (course A) and 102 nmol/L (course C). They rose to 26 nmol (courses A and B) and 135 nmol/L (course C) within one hour after the intervention.

Linalool, the compound with the highest dosage in the curry dish, had a transition rate of 2-4 x 10^{-4} % during the sampling period after intervention in the illustrated sample sets. 1,8-cineole had a transition rate of about 0.4-8 x 10^{-2} % and piperine of about 0.5-2 x 10^{-2} %. Due to these low transition rates, it can be expected that the substances underlie vast metabolization processes, which might have transformed their structure in phase I-reactions or conjugated them e.g. with glucuronic acid in phase II-reactions, and/or that they were eliminated via other pathways like urine, breath or faeces. The transition rates furthermore reveal substance-specific as well as interindividual differences. The transition amount can vary strongly, as can be seen by the comparison of course B to C. In course B the piperine maximum concentration is about five times lower and the 1,8-cineole maximum concentration about five times higher than in course C. Metabolic or other individual factors might explain such differences.

Additionally, the three exemplary transition courses reveal that interindividual differences can play a significant role with regard to temporal aspects of the transition of flavour-active substances. While the concentration courses of linalool in course A and C were similar – starting from a low initial level of linalool of around 1 nmol/L, rising to about 10 nmol/L within one hour after intervention and then continuously declining in the following samples – the linalool concentration in course B rose again in the last sample, obtained three hours after the intervention. For the transition courses of 1,8-cineole similar variations occurred. Basically, the 1,8-cineole concentration rose within one hour after intervention, but in course A the 1,8-cineole concentration increased again three hours after the intervention, similar to the linalool concentration in course B. For piperine such second maxima appeared in all of the three exemplary courses. The occurrence of these second transition maxima have recently been reported for ramson-derived metabolites in milk [13].

The three participants also differed in their basis levels of the investigated flavour-active compounds, despite the instruction of avoiding certain food during the wash-out phase. Especially for piperine and 1,8-cineole comparatively high basis levels were detected in some participants. Non-compliance might be one explanation, but also unintentional uptake of flavour compounds via products like tooth paste or chewing gums, which might contain 1,8-cineole. For the case of the high piperine basis level, the provided nutrition diary of the test person revealed that a dish seasoned with pepper was consumed on the evening before the intervention. This is especially interesting as it shows that the transition of flavour compounds apparently influences the composition of human milk not just in the first hours after intake but for a prolonged time. Such transitions could even cumulate, especially for those substances which occur in a diet very often. Pepper is one of the world's most consumed spices and contains piperine as well as linalool. The fact that pepper is often consumed all over the day during breakfast, lunch and supper could lead to a continuous and cumulated transition into milk.

On the basis of our results, the transition of flavour-active compounds is compared in terms of transition time and transition rate. The concentrations of all of the three target compounds rose within 1 h after the curry consumption, which demonstrates the possibility of a simultaneous transfer of both odour- and trigeminally-active substances into milk. The transition rates during the first three hours after intervention were 2-4 x 10^{-4} % for linalool, 0.4-8 x 10^{-2} % for 1,8-cineole and 0.5-2 x 10^{-2} % for piperine in the three exemplary milk sets. Therefore, it is suggested that 1,8-cineole and piperine have a similar and stronger tendency to migrate into human milk than linalool.

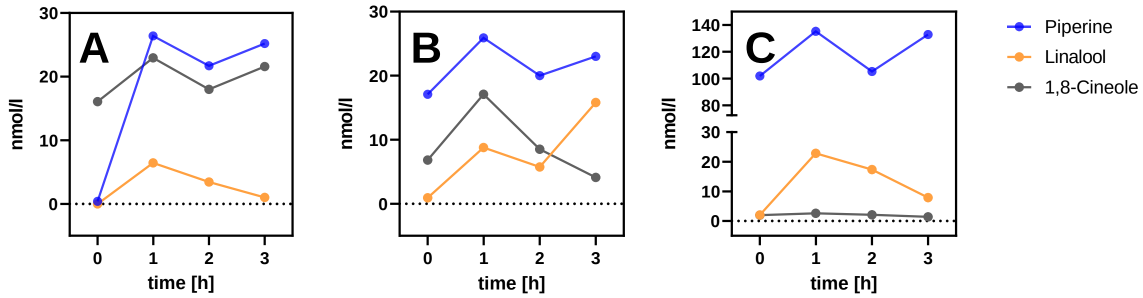

Figure 2: *Concentration course of piperine, linalool and 1,8-cineole before (0h) and after the intervention (1-3h) for three exemplary milk sets.*

Estimation of the sensory relevance of flavour transfer

The odour thresholds of linalool and 1,8-cineole were 4 nmol/L and 21 nmol/L, respectively. The threshold for pungency of piperine in milk matrix was 17500 nmol/L. Linalool reached OAVs > 1 in all three exemplary courses and even in seven of the nine illustrated samples after intervention. For 1,8-cineole, OAVs > 1 were calculated for the first and third sample after the intervention in course A. The piperine concentrations resulted in TAVs < 1 in all samples. Compounds with OAVs or TAVs > 1 are suggested to potentially alter the overall flavour of a milk sample, because their concentrations exceed their threshold levels. Accordingly, for linalool it can be argued that its ingestion with the curry dish leads to an impact on the overall aroma of the milk. For 1,8-cineole this might only be the case for few participants. Piperine can, based on its TAVs, not be suggested to have an impact on the overall flavour of the human milk.

The OAVs and TAVs were calculated based on average threshold levels of adult panellists. Odour perception of nurslings might be more sensitive [16] compared with adults. Moreover, retronasal odour perception is in general more intense [17-19]. Accordingly, the transition of 1,8-cineole, and possibly also of piperine, might lead to a sensory effect in the infant perception during nursing. Additionally, it is important to consider that early exposure could also impact later preferences towards, or tolerance of, the respective flavour substances when being below perception threshold. Finally, cross-modal summation, which is the concept that mixtures of e.g. odour- and taste-active substances can be perceived even if both stimuli are below their perception threshold, could lead to an actual perception of the given odour-taste mixture [20]. This effect has been shown in several current studies in which the intensity of taste impressions could be increased by flavourants [21-23] and as shown by Sinding et al. (2021), this could even lower the perception threshold of certain compounds [4].

Conclusion

A simultaneous transition of odour- and trigeminally-active substances from a curry dish into milk could be outlined in this work. This transition can be described as fast but marginal with an average maximum in milk samples donated one hour after the intervention and a rate of about 2 x 10^{-4} - 0.08 % of the initial dosage within the here considered sampling period. While the transfer did not seem to have an impact on the overall flavour of the milk in the case of piperine, for the investigated odorants that was at least partly the case. Additional evidence reported in Debong et al. (2021, submitted) and N'Diaye et al. (2021, submitted) suggests that not all flavour-active substances migrate from the curry dish into milk. As shown here, among those substances for which a transfer takes place not all might be perceivable. But some of them might be perceived or activate the nurslings' receptors and thus provide the basis for an early flavour learning and the creation of multisensory associations.

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