CHARACTERIZATION OF OFF-ODOR DEVELOPMENT IN HUMAN MILK DURING STORAGE AT -18 °C

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Aims of the study

- Sensory evaluation of ortho- and retronasal flavor profiles of fresh human milk in comparison to milk stored at -18 °C
- Characterization of odor active substances in fresh and stored human milk by application of sensitive and selective sensory and analytical tools
- Better understanding of the molecular principles of flavor changes in relation to the chemical compositions and the odor profiles of human milk during storage

Introduction

Human milk has been reported to be storable at 4 °C for 3 to 8 days and at -18 °C for a maximum of 3 to 6 months. These recommendations are based on the stability of beneficial milk constituents, as well as on hygienic considerations [1]. However, sensory changes of human milk during storage have not been investigated until today, despite chemo-sensory aspects of human milk being of enormous importance for the neonate [2].
Generally, the characterization of potent odorants secreted into human milk at trace levels requires special analytical tools, which allow direct extraction and preconcentration of the analytes from small-scale samples, such as solvent extraction and high vacuum distillation at low temperatures [3]. Our aim was to characterize potential flavor changes in human milk after storage. Therefore, changes in ortho- and retronasal aroma profiles of fresh human milk samples in comparison to those stored at -18 °C were monitored by descriptive sensory evaluation. Furthermore, molecular identification of predominant odor substances was accomplished by senso-analytical techniques such as high resolution gas-chromatography-olfactometry (HRGC-O) /mass spectrometry (MS) and comparative aroma extract dilution analysis (cAEDA).

Experimental

Samples

Two samples of human milk (each 200 mL) were collected and each sample was divided into 20 mL aliquot portions. One aliquot of each sample was immediately evaluated by means of ortho- and retronasal aroma profile analysis (see below), while the remaining portions were stored in brown glass bottles with plastic lids (total volume 50 mL) at -18 °C for 2 months.

Sensory analyses

Sensory analyses were performed in a sensory panel room at 21±1 °C. Four samples (20 mL each of fresh or stored human milk samples) were presented in covered glass vessels (capacity 120 mL) to the sensory panel for comparative orthonasal evaluation. The order of the samples was randomized and no information about the purpose of the experiment or the exact composition of the samples was disclosed to the panelists.
Panelists were asked to describe the samples and then to score the intensities of these attributes as well as the overall odor intensities on a scale from 0 (no perception) to 3 (strong perception) in four different sessions.

Enrichment of odor active compounds and analysis

After addition of 12.5 mL of dichloromethane to the respective milk samples (25 mL), the solutions were equilibrated by stirring (30 min) and subsequently subjected to Solvent Assisted Flavor Evaporation (SAFE; [4]), solvent extraction of the distillate with dichloromethane, and concentration. Then, the distillates were analyzed by HRGC-O/MS. Identification of odorants was based on comparison with reference compounds (EI mass spectra), retention indices on two analytical capillaries of different polarities, odor qualities and odor intensities of compounds (Table 1).
For elucidation of the odor changes with storage cAEDA was carried out as in [5] by stepwise diluting (1+1) the original extract and performing GC-O analysis on each dilution step (FD factors [3]), both for fresh and stored samples.

Table 1. Selected potent odorants as detected by means of cAEDA of a representative fresh and stored (-18 °C, 2 months) human milk sample exhibiting the specific fishy off-odor. Intensities of listed compounds changed by at least 3 dilution steps.
[1)] Tentatively identified based on retention indices and odor characteristics in comparison to references. nd = not determined.

No.	Odorant	Odor Quality during GC-O	RI value on		FD factor	
			FFAP	DB5	Fresh milk	Stored milk
1	Oct-1-en-3-one	mushroom-like	1289	975	<1	16
2	(*Z*)-Octa-1,5-dien-3-one [1)]	geranium leaf-like	1357	981	<1	64
3	(*E*,*Z*)-Nona-2,4-dienal	fatty	1640	1193	<1	512
4	(*E*,*Z*)-Deca-2,4-dienal	fatty	1741	1291	<1	32
5	*tr*-4,5-Epoxy-(*E*)-dec-2-enal	metallic	1984	1374	32	512
6	Unknown	metallic	2039	nd	<1	64
7	(*E*,*Z*,*Z*)-Trideca-2,4,7-trienal [1)]	bloody, egg white-like	2085	1578	<1	128
8	Decanoic acid	fatty, rancid	2253	-	<1	64
9	Dodecanoic acid	fatty, rancid	2465	-	8	64

Results

Sensory evaluation of the aroma profiles of fresh and stored human milk showed that the metallic and fishy attributes after storage were perceived orthonasally significantly more intensively. In retronasal evaluation, however, the soapy, metallic, fishy, sweaty and rancid attributes were dominant. In addition, retronasal evaluation of the stored samples induced medium to intense nauseatic and emetic response in the respective panelist (data not shown).
When analyzing the fresh and stored human milk samples by means of HRGC-O/MS, a series of odor-active compounds were detected as additional or more intense compounds in the stored sample compared to the fresh sample. Among the detected compounds were 9 odorants that specifically exhibited significantly higher FD factors in the stored sample (cf. Table 1, chemical structures are shown in Fig. 2).

Discussion

The presented data show that human milk can undergo dramatic sensory changes during storage. Predominantly fishy-metallic odor notes are formed that are intensely perceived both in ortho- as well as retronasal evaluation. In adults, the altered flavor profiles clearly induced rejection, escalating to nausea upon tasting the stored milk.
Nevertheless, preliminary observations of our group have shown that babies do not react with any clear sign of refusal to the observed flavor changes, and that they feed on stored human milk without major differences in total intake. Further studies need to be performed to clarify whether babies tolerate the respective flavor as it has not (yet) been associated with any negative physiological response, or whether they are not able to perceive the respective flavor impressions. Nevertheless, the latter seems unlikely when looking at previously reported olfactory skills of human infants [2].

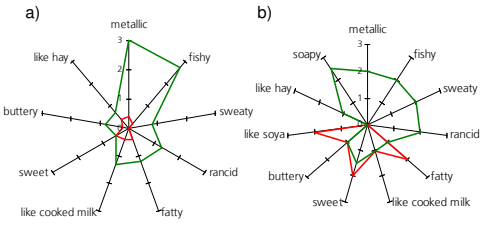

Fig. 1. Comparative Aroma Profile Analysis of a) ortho- and b) retronasal flavor profiles of fresh — and stored — (-18 °C, 2 months) human milk.

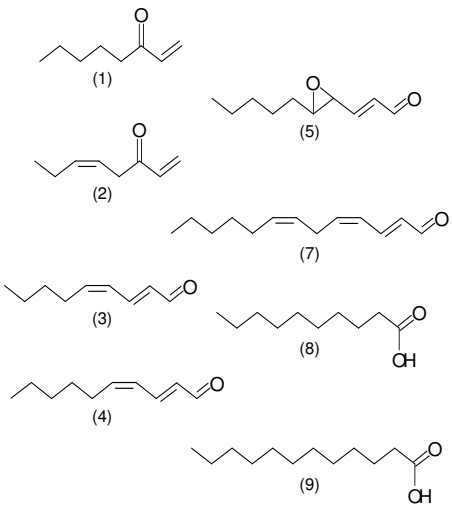

Fig. 2. Odor compounds found to be more intensive in stored human milk. Odorants 1-5 and 7 are typical oxidation products of (poly)unsaturated fatty acids (6).

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