DIHYDROMALTOL (2,3-DIHYDRO-5-HYDROXY-6-METHYL-4*H*-PYRAN-4-ONE): IDENTIFICATION AS A POTENT AROMA COMPOUND IN RYAZHENKA KEFIR AND SENSORY EVALUATION

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Abstract

Dihydromaltol (DHM; 2,3-dihydro-5-hydroxy-6-methyl-4H-pyran-4-one) was identified as a novel potent aroma compound in a dairy product, Ryazhenka kefir, a Russian-style kefir made from cooked milk. Flavour impact of the structurally related caramelised smelling compounds, DHM, 2,5-dimethyl-4-hydroxy-3[2*H*]-furanone (DMHF; Furaneol®), 5-ethyl-4-hydroxy-2-methyl-3[2*H*]-furanone (EHMF; Homofuronol®) and maltol, was evaluated in various dairy samples by applying the odour activity value concept (OAV = concentration / odour threshold) using flavour thresholds. Besides DHM, also its novel homologue, dihydro-ethylmaltol (DHEM; 6-ethyl-2,3-dihydro-5-hydroxy-4*H*-pyran-4-one), was synthesised. DHEM has a caramelised odour but not been not found in nature, in contrast to its furanoid isomer, EHMF. Based on flavour thresholds in water, DHM (50-250 µg/kg) by itself was found to be approximately half as potent as DMHF but about 40-times more potent than maltol, and DHEM (10-100 µg/kg water) was found to be more potent than DHM.

Introduction

Ryazhenka kefir, a commercial, Russian-style kefir made from cultured cooked milk, has a rich, brown, creamy, cooked milk-like flavour. Because consumers desire “rich”, “creamy” flavour in dairy products, the objective of this study was to identify potent aroma compounds in Ryazhenka, and to estimate the flavour impact of selected, caramelised smelling cyclo-enolones in various liquid dairy products.

Experimental

Materials. “Ryazhenka Kefir - Cooked Cultured Milk” (3.8 % fat, live culture; Lifeway Foods Inc., IL, USA); Original Kefir (3.3 % fat; Lifeway); Evaporated Whole Milk (Carnation; Nestle Inc., USA); UHT whole milk (Gosners, UT, USA). UHT whole milk or Evaporated Whole Milk was inoculated with kefir (1 %) and incubated at 30 °C for 18 h. Cultured samples were heated (72 °C, 30 min) to inactivate cultures prior to analysis. Synthesised (Chemrise Inc.; Moscow, Russia) were 2,5-[$^{13}C_2$]-dimethyl-4-hydroxy-3[2H]-furanone ([$^{13}C_2$]-DMHF) (1), 5-[$^{2}H_3$]-ethyl-4-hydroxy-2-methyl-3[2H]-furanone ([$^{2}H_3$]-EHMF) (2), dihydromaltol (DHM; 2,3-dihydro-5-hydroxy-6-methyl-4H-pyran-4-one) and dihydro-ethylmaltol (DHEM; 6-ethyl-2,3-dihydro-5-hydroxy-4H-pyran-4-one) according to (3), starting with maltol or ethyl maltol, respectively. Chemical purity (>95 %) of DHM and DHEM was verified by ^{1}H-NMR and olfactory purity by GC/O. Flavour compound sources: DMHF (Furaneol®; Firmenich), EHMF (Homofuronol®; Givaudan), maltol (Phoenix Chemicals) and ethyl maltol (Citrus & Allied).

Isolation of volatiles. Similar to (4), dairy samples (25 g) were mixed with anhydrous sodium sulphate (~170 g). The resulting powder was placed in a glass vessel (3.5 x ∅11 cm) for purge-and-trap (P&T) sampling onto Tenax-TA® (180 mg), using nitrogen as purge gas channelled through the powder (60 °C, 70 ml/min, 90 min). For quantitation of cyclo-enolones, the liquid samples were spiked with the internal standards, [$^{13}C_2$]-DMHF and [$^{2}H_3$]-EHMF in ethanol, and equilibrated (5 °C) overnight before sample preparation for P&T.

GC/Olfactometry-Mass Spectrometry (GC/O-MS). After P&T, the volatiles of the dairy samples were thermo-desorbed (270 °C, 5 min) from the trap via a TDS-2 system (Gerstel; Germany) into a Cool Injection System (CIS4, Gerstel) for simultaneous GC/O-MS analysis (GC6890, Agilent Technologies, USA; ODP2, Gerstel; MSD5973, Agilent) using FFAP or DB5 capillaries (Agilent). Column temperature programming was similar to (3).

GC-MS quantitation. As in GC/O-MS, the volatiles were thermo-desorbed from traps via a TDS-3 into a CIS4 for quantitative analysis on a HP-FFAP capillary (30 m x 0.25 mm ID x 0.25 um film; Agilent) coupled with an MSD5975 (Agilent) operated in EI-SCAN mode. For quantitation of dihydromaltol and maltol via [$^{13}C_2$]-DMHF, and DMHF and EHMF via their corresponding isotope standards, the peak area of the extracted molecular ion trace of each compound was normalized based on its percentage versus its TIC peak area in a standard mixture that was analysed under the same conditions. No recovery factors were applied in quantitative calculation.

Results and Discussion

Using P&T-TDS-GCO/MS, three GC-sniffers found the caramelised smelling 2,3-dihydromaltol (DHM; Kováts RI $_{FFAP}$: 1876; RI $_{DB-5}$: 1092-1098) among the ten most intense odorants in Ryazhenka kefir, besides hexanoic acid, trans-4,5-epoxy-(E)-2-decenal, DMHF, butyric acid, (E)-2-nonenal, 2-acetyl-1-pyrroline, methional, dimethyltrisulphide and 1-octen-3-one (data not shown). DHM was found for the first time as a potent odorant in a dairy product, and its identity confirmed by same analysis of synthesised DHM. DHM and DMHF coelute on a DB-5 capillary and have very similar odour and mass spectrum, hence the use of FFAP for detection of DHM being crucial.

In order to evaluate and compare the flavour impact of selected, caramelised smelling cyclo-enolones (DHM, DMHF, EHMF, maltol) in various dairy samples, the odour activity value concept (OAV = concentration / odour threshold) (5) was applied, using flavour/taste thresholds in water. The flavour thresholds for DHM were determined as 50-250 µg/kg in water and skim milk (Table 1), thus DHM by itself being about half as potent as DMHF (30 µg/kg water) (6) but much more (~ 40x) potent than maltol (7,100-13,000 µg/kg water) (6, 7).

Dihydro-ethylmaltol (DHEM; RI $_{FFAP}$: 1901; RI $_{DB-5\ (J\&W)}$: 1166) was synthesised as a novel homologue of DHM. DHEM also has caramelised odour but has not been found in nature, in contrast to its furanoid isomer, EHMF. DHEM (MS-EI data): *m/z* 43 (10 %), 57 (100 %), 58 (36 %), 69 (4 %), 86 (17 %), 99 (3 %), 114 (2 %), 127 (2 %), 141 (5 %), 142 (83 %, MW), 143 (7 %). DHEM (^{1}H-NMR ($CDCl_3$) data): d 1.69 ppm, 3H, tr.; d 2.44 ppm, 2H, q.; d 2.62 ppm, 2H, tr.; d 4.33 ppm, 2H, tr.. The flavour threshold of DHEM (10-100 µg/kg water) was determined and is lower than that of DHM, similar to the difference between DMHF and EHMF (5 µg/kg water) (8). Other derivatives of DHM also have caramel aroma, except for the non-planar 2,2-dimethyl-DHM (9), confirming the structure-odour hypothesis of (10).

Table 1. *Sensory threshold values (µg/kg) of dihydromaltol (DHM) and dihydro-ethylmaltol (DHEM) at 25 °C.*

Threshold (µg/kg) for:	Dihydromaltol [a]	Dihydro-ethylmaltol [b]
Flavour in water	50 (3/6) [c] - **250** (6/6)	**10** (4/5) - 100 (4/5)
fFavour in skim milk	50 (3/7) - **250** (6/7)	n.d.
Flavour in 2 % milk	**≤ 50** (7/7) (cooked milk like)	100 (2/3)
Odour in water	250 (3/6) - **1000** (4/6)	n.d.
Detection in water	**25** (3/6) (milky mouthfeel; no aroma or flavour; 6/6)	n.d.

[a] Matrix spiked with DHM at 25 (not for 2 % milk), 50, 250, 1000, 2000, 3000, 5000 and 10,000 µg/kg.
[b] Matrix spiked with DHEM at 10, 100, 1000 and 12,000 µg/kg.
[c] Ratio of panellists able to identify the spiked sample from the un-spiked matrix in comparison, starting with lowest spike concentration.
n.d. = not determined

Based on its highest OAVs (OAV: 18-56, Table 2) compared to the other enolones, DMHF is the dominant caramelised flavour impact compound in commercial Ryazhenka kefir made with cooked milk (*No. 1, 2*), in Ryazhenka grown on UHT-milk (*No. 4-6*) or on evaporated milk (*No. 8, 9*), and in evaporated milk (*No. 7*). However, DMHF has no or little flavour impact in UHT-milk (OAV: <1, *No. 3*), cream (OAV: <1, *No. 12*) or heated cream (80 °C for 8 h; OAV: 2, *No. 13*). DHM (OAV: 1-6) and EHMF (OAV: 1-18) have low to medium flavour impact in Ryazhenka samples (*No. 1, 2, 4-6, 8, 9*), but close to none in UHT-milk, evaporated milk, and cream (all OAV: <1). Only trace amounts of DHM (<10 µg/kg) were found in Original Kefir made commercially with pasteurized milk (*No. 10*) or grown on UHT-milk (*No. 11*). Maltol does not reach its flavour threshold in any of the measured liquid dairy samples, and therefore, has the lowest flavour impact among the analysed cyclo-enolones.

Based on GC/O data, DHM was reported as an important odorant in sweet bell pepper powder (11), was identified among the most odour active volatiles in barley malt (3), and was reported to contribute to the “toasty caramel” aroma of heated oak used in wine making (12). Among other volatile compounds, DHM was quantified in various wines (13) and its content monitored during aging of sweet fortified wines under aerobic and anaerobic conditions (14). Although the aroma impact of the measured wine volatiles was discussed using data similar to OAVs, sensory threshold data of DHM were not reported. In aqueous Maillard reaction models, DHM was identified from the reaction of D-glucose with L-phenylalanine under anaerobic boiling conditions (15). Using stable isotope labelled compounds in aqueous Maillard reaction models, a possible pathway has been suggested for the thermal generation of DHM from 2,3 dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (3-hydroxy-DHM), a Maillard reaction intermediate from hexoses (16).

Table 2. *Concentration and Odour Activity Values (OAV) of potent caramelised smelling aroma compounds in dairy samples [1].*

		Concentration (µg/kg)				OAV = conc. / flavour threshold [2]			
No.	Sample	Dihydro-maltol	DMHF	EHMF	Maltol	Dihydro-maltol	DMHF	EHMF	Maltol
1	RK1: "Ryazhenka Kefir"	746	1618	21	3312	3.0	54	4.2	0.3
2	RK2: "Ryazhenka Kefir"	574	781	<5	4576	2.3	26	<1	0.5
3	UHT-milk	<20	<20	<5	4831	<<1	<1	<1	0.5
4	RK3: UHT-milk fermented with RK1 inoculate	416	640	21	4995	1.7	21	4.2	0.5
5	RK4: UHT-milk fermented with RK1 inoculate (pH 4.1)	271	540	6	n.a.	1.1	18	1.2	n.a.
6	RK5: UHT-milk fermented with RK1 inoculate (pH 5.4)	217	574	8	n.a.	0.9	19	1.6	n.a.
7	Evaporated milk	25	526	<5	3671	<<1	18	<1	0.4
8	RK6: evaporated milk fermented with RK1 inoculate	1405	1404	56	7508	5.6	47	11	0.8
9	RK7: evaporated milk fermented with RK1 inoculate	1392	1677	88	5216	5.6	56	18	0.5
10	OK1: "Original Kefir"	8	n.a.	n.a.	n.a.	<<1	n.a.	n.a.	n.a.
11	OK2: UHT-milk fermented with OK1 inoculate	<10	79	<5	n.a.	<<1	2.6	<1	n.a.
12	Cream, fresh	<5	21	<5	12	<<1	0.7	<1	<<1
13	Cream, heated (80 °C for 8 h)	<5	55	<5	270	<<1	1.8	<1	<<1

[1] Aroma compounds quantified using internal standards, [$^{13}C_2$]-DMHF and [$^{2}H_3$]-EHMF (acronyms defined in text); no recovery factors applied.
[2] Flavour thresholds in water (see text): dihydromaltol (250 µg/kg; Table 1), Furaneol (30 µg/kg), EHMF (5 µg/kg); maltol (10,000 µg/kg).
n.a. = not analysed

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