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ARTICLE

Identification of Dihydromaltol (2,3-Dihydro-5-hydroxy-6-methyl-4*H*-pyran-4-one) in Ryazhenka Kefir and Comparative Sensory Impact Assessment of Related Cycloenolones

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Dihydromaltol (DHM; 2,3-dihydro-5-hydroxy-6-methyl-4*H*-pyran-4-one) was identified as a novel potent aroma compound in a dairy product, Ryazhenka kefir, using GC−olfactometry−MS. The flavor impact of the structurally related caramelized-smelling compounds DHM, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF), 5-ethyl-4-hydroxy-2-methyl-3(2*H*)-furanone (EHMF) and maltol was assessed in various dairy samples by applying the odor activity value concept (OAV = concentration/odor threshold) using flavor (retronasal odor) thresholds instead of odor thresholds. Commercial Ryazhenka kefir, original kefir, and kefir-culture inoculated heated milk, as well as UHT milk, evaporated milk, heated cream, and fresh pasteurized cream, were analyzed. In all dairy samples containing DHM, DMHF appeared to dominate over DHM in its flavor impact. Although DHM, the pyranoid isomer of DMHF, has been found in nature, dihydroethylmaltol (DHEM; 6-ethyl-2,3-dihydro-5-hydroxy-4*H*-pyran-4-one), the pyranoid isomer of EHMF (the seven carbon DMHF homologue), has not been found in nature. Therefore, DHM and its novel homologue, DHEM, were synthesized to determine their flavor thresholds and to investigate structure−odor−relationships among cycloenolones. DHEM has a strong caramelized odor. On the basis of flavor thresholds in water, DHM (50−250 μg/kg) by itself was found to be less than half as potent as DMHF but about 40 times more potent than maltol. DHEM (2.5−5 μg/kg of water) by itself was found to be more potent than DHM and close to the odor intensity of EHMF. The novel data provided on DHM and DHEM support understanding of the relationship between chemical structure and flavor intensity within the important aroma compound class, of cycloenolones.

KEYWORDS: Dihydromaltol; kefir; olfactometry; dihydroethylmaltol; flavor; dairy

INTRODUCTION

Creaminess is a well-known key driver of consumers' preference for dairy foods. Therefore, systematic sensory-guided research has been conducted to define the chemical nature of creaminess-related flavor compounds in dairy products (*1*). Thermal treatment of full-fat cream was found to strongly enhance its overall aroma intensity and, in particular, creamy, buttery, popcorn-like, and sulfury notes. Heat-induced Maillard reaction compounds, such as maltol and certain furanones (e.g., 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF)), have been claimed to contribute to the flavor of sterilized milk that was heated at 145 °C for 30 s, followed by 115 °C for 20 min in bottles (*2*). These compounds are cycloenolones with caramel odor (see chemical structures in **Figure 1**). Using aroma extract dilution analysis (AEDA), a sensory-guided analytical screening of odor-active compounds in foods, the cycloenolones with caramel odor, DMHF and 4,5-dimethyl-3-hydroxy-2(5*H*)-furanone (sotolon), have been found among potent odorants in UHT milk (*3*). In contrast to these findings, no cycloenolones have been reported in AEDA results of high-heat-treated cream that was heated at 95 °C for 6 s (*1*). Also, no cycloenolones have been reported in the results of sensory-guided aroma analysis of commercial sweetened condensed milk (*4*) and UHT milk (*5*). The contrasting data suggest the need for a more clarifying investigation into the flavor impact of cycloenolones in creamy, heated liquid dairy products.

For the present study, flavor chemists informally tasted various commercial heated, liquid dairy products to screen them for pronounced rich, creamy flavor notes (data not shown). Ryazhenka kefir was found to have a particularly rich, brown, creamy, cooked milk-like flavor. Ryazhenka is a commercial, Russian-style kefir made from cultured cooked milk. No GC−olfactometry (GC-O) guided aroma analysis of kefir was found in the public literature.

It is well-known that consumers desire rich, creamy flavor in dairy products, and the objective of this study was to screen potent aroma compounds in Ryazhenka. Because Ryazhenka was heated, also the potential flavor impact of selected cycloenolones should be estimated in various liquid dairy products and compared. Dried dairy products are not considered for discussion in this study, because the formation of Maillard reaction

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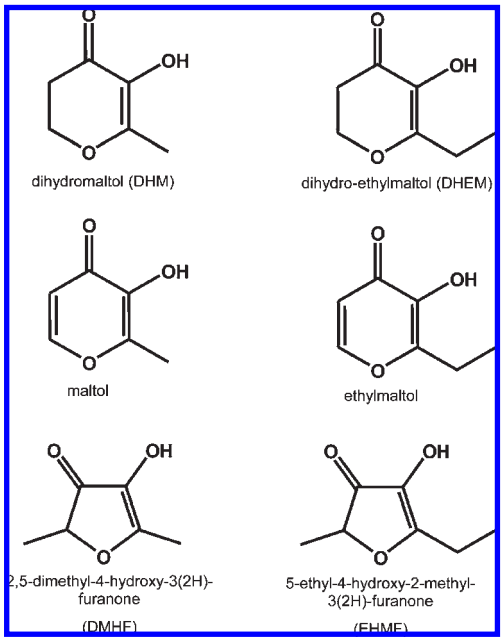

Figure 1. Chemical structures of dihydromaltol (DHM; 2,3-dihydro-5-hydroxy-6-methyl-4*H*-pyran-4-one), dihydroethylmaltol (DHEM; 6-ethyl-2,3-dihydro-5-hydroxy-4*H*-pyran-4-one), maltol (3-hydroxy-2-methyl-4*H*-pyran-4-one), ethylmaltol (2-ethyl-3-hydroxy-4*H*-pyran-4-one), 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF), and 5-ethyl-4-hydroxy-2-methyl-3(2*H*)-furanone (EHMF).

products, such as cycloenolones, is generally known to be enhanced by lowering the water content of a heated sample.

MATERIALS AND METHODS

Materials. Dairy products were sourced locally: Ryazhenka kefir, cooked cultured milk (3.8% fat, live culture; Lifeway Foods Inc., Morton Grove, IL), original kefir (3.3% fat; Lifeway), evaporated whole milk (Carnation; Nestle Inc., Solon, OH), and UHT whole milk (heated at "over 138 °C for several seconds"; Gossner Foods, Logan, UT). UHT whole milk or evaporated whole milk was inoculated with kefir (1%; w/w) and incubated at 30 °C for 18 h. Cultured samples were heated (72 °C, 30 min) to inactivate cultures prior to analysis. Fresh pasteurized cream (pooled from local farmers) was heated in aluminum jars at 80 °C for 8 h. For sensory analysis, fresh skim milk and 2% milk in glass bottles (Oberweis Dairy, Glenview, IL) were sourced locally. Chemicals were synthesized (Chemrise Inc., Moscow, Russia) according to the literature: 2,5-[$^{13}C_2$]-dimethyl-4-hydroxy-3(2*H*)-furanone ([$^{13}C_2$]-DMHF) (*6*) and 5-[$^{2}H_3$]-ethyl-4-hydroxy-2-methyl-3(2*H*)-furanone ([$^{2}H_3$]-EHMF) (*7*), as well as dihydromaltol (DHM; 2,3-dihydro-5-hydroxy-6-methyl-4*H*-pyran-4-one) and dihydroethylmaltol (DHEM; 6-ethyl-2,3-dihydro-5-hydroxy-4*H*-pyran-4-one) according to ref *8*, starting with maltol or ethylmaltol, respectively. The chemical purity of DHM (95%) and DHEM (94%) was determined by ^{1}H NMR (ratio of overall integration of proton signals from DHM or DHEM versus the overall integration of proton signals from impurities). The olfactory purity of DHM and DHEM was verified by GC-O.

Other chemicals were purchased from commercial sources: DMHF (Furaneol; Firmenich S.A., Switzerland), EHMF (homofuronol; Givaudan, Switzerland), maltol (Phoenix Chemicals, Calhoun, GA), ethylmaltol (Citrus & Allied Essences, Ltd., Elk Grove Village, IL), purified water (Purific; AquaCell Inc., Rancho Cucamonga, CA), and ethanol (95%, v/v, FCC grade; Aaper Inc., Shelbyville, KY) checked for olfactory purity by GC-O.

Isolation of Volatiles by Purge-and-Trap (P&T). Similar to the sample preparation in ref *9*, liquid dairy samples (25 g) were thoroughly mixed with an excess of prebaked sodium sulfate (anhydrous, granular powder, J. T. Baker, 3891-01; ca. 170 g) at room temperature. The resulting powder was placed in a custom-made, cylindrical glass vessel (3.5 × 11 cm i.d.) for sampling in a P&T device (model 1000/110; Dynatherm Analytical Instruments, Kelton, PA). Via a Teflon tube, nitrogen purge gas (grade 5.0) was channeled through the powder at 60 °C with a gas flow rate of 70 mL/min for 90 min. Purged volatiles were trapped onto Tenax TA (180 mg) in a glass tube (C03718; Gerstel Inc., Mülheim an der Ruhr, Germany) held at 62 °C. After sampling, the trap was dry-purged for another 10 min. Before sampling, the Tenax TA trap was conditioned for 1 h at 280 °C (P&T tube conditioner, model 9600; CDS Analytical, Oxford, PA).

Thermodesorption−GC−Olfactometry−Mass Spectrometry (TDS-GC-O-MS). After P&T, the volatile compounds of the dairy samples were thermodesorbed (270 °C, 5 min) from the trap via a TDS-2 system (Gerstel) into a Cool Injection System (CIS4, Gerstel) for simultaneous GC-O-MS analysis (GC6890, Agilent Technologies, USA; ODP2 sniffing port, Gerstel; MSD 5973, Agilent) using FFAP (HP-FFAP; 30 m, 0.25 mm i.d., 0.25 μm film; Agilent) or DB-5 (HP-5 ms; 30 m, 0.25 mm i.d., 0.25 μm film; Agilent) capillaries. The effluent of the GC capillary was split (1:1). One part of the effluent was evaluated by sniffing at the sniffing port (180 °C), and the retention time and character of odorous zones were recorded on paper. The other part of the effluent was analyzed by MS. Mass spectra of odorous zones were obtained by matching retention times of GC-O and MS recordings. The instruments were programmed as follows: TDS-2, the initial temperature of 40 °C was immediately ramped at a 60 °C/min heating rate to 270 °C and held for 5 min; CIS-4, the initial temperature of −80 °C was immediately ramped at a 12 °C/s heating rate to 250 °C and held for 10 min; GC (FFAP), the initial temperature of 40 °C was held for 1 min, ramped at a 8 °C/min heating rate to 220 °C, and held for 5 min; GC (DB-5), the initial temperature of −10 °C was held for 1 min, ramped at a 40 °C/min heating rate to 40 °C, then immediately ramped at a 5 °C/min heating rate to 200 °C, finally ramped at a 10 °C/min heating rate to 250 °C, and held for 5 min.

Quantitative Analysis. For quantitation of cycloenolones, the liquid dairy samples were spiked with known amounts (ca. 0.4 ppm) of internal standards, [$^{13}C_2$]-DMHF and [$^{2}H_3$]-EHMF, in ethanol (ca. 25 μL), stirred, and equilibrated (5 °C) overnight before sample preparation for P&T (see Isolation of Volatiles). As described in the section on TDS-GC-O-MS, the volatiles were thermodesorbed from Tenax traps via a TDS-3 (Gerstel) into a CIS4 (Gerstel) for separation on the reported FFAP capillary coupled with an MSD 5975 (Agilent) operated in EI-scan mode (*m*/*z* 30−300). Both dihydromaltol and maltol were quantified via [$^{13}C_2$]-DMHF. DMHF and EHMF were each quantified via their corresponding isotope standards. In quantitative calculation, the peak area of the extracted molecular mass ion trace of each compound was normalized on the basis of its percentage versus its TIC peak area in a standard mixture that was analyzed under the same conditions. No recovery factors were applied in quantitative calculation. Quantitative data were derived from one measurement per sample.

Sensory Thresholds. Neat DHM or DHEM was dissolved in ethanol (95%, v/v), all checked for olfactory purity by GC-O, and stock solutions of 1, 0.1, and 0.01% (w/w) were prepared. Purified water, skim milk, or 2% milk from glass bottles was spiked with the appropriate amount of DHM or DHEM stock solution and diluted with the matrix to yield sensory samples with defined DHM or DHEM concentrations (see footnotes in **Table 2**). The sensory samples contained <0.02% ethanol through spiking. The sensory panel consisted of up to seven flavor chemists (35−60 years of age; 2−3 males, 4 females) well-trained in tasting aroma chemicals. To familiarize the panelists with the flavor of the compounds to be examined, they tasted solutions of DHM and DHEM at 1 mg/kg in water. The following tests were performed in separate sessions. In a paired comparison test, spiked samples were each tasted against one sample of unspiked matrix at room temperature (ca. 25 °C). In sensory threshold determination, the panelists compared the unspiked matrix with the spiked matrix starting at the lowest spike concentration of a series. The panelists were asked to identify the spiked sample by a flavor (retronasal odor) or aroma (orthonasal odor) difference, respectively. For DHEM, the flavor threshold was first approximated in the described paired comparison test with DHEM spiked at 10, 100, 1000, and 12000 μg/kg in water. Then, the flavor threshold of DHEM in water was determined more precisely in a randomized triangle test against water. In each triangle test, the odd sample was spiked with DHEM at 2.5, 5, and 10 μg/kg DHEM, respectively, and the panelists were asked to identify the spiked sample. In all tests, the freshly prepared samples (ca. 15 g) were presented in

Table 1. Potent Odorants Detected in a Russian-Style Kefir (Ryazhenka) by Purge and Trap (Tenax TA)−Thermodesorption−GC−Olfactometry[a]

no.	compound[b]	odor	sniffer 1[c]	sniffer 2[c]	sniffer 3[c]	RI (FFAP)	RI (lit.)	lit. ref[d]
1	hexanoic acid	sweaty, cheesy	4	2	0	1844	1841	*27*
2	dihydromaltol	caramel, sweet	4	2	3	1876	1879	*10*
3	*trans*-4,5-epoxy-(*E*)-2-decenal	metallic	4	2	2	2023	2025	*10*
4	2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF)	caramel, sweet	3	3	2	2049	2047	*10*
5	butyric acid	sweaty, cheesy	3	2	2	1617	1622	*27*
6	(*E*)-2-nonenal	cardboard, stale	3	1	2	1536	1537	*8*
7	2-acetyl-1-pyrroline	toasted, roasted	2	2	4	1351	1355	*8*
8	methional	cooked potato	2	2	4	1462	1473; 1457	*10*; *8*
9	dimethyl trisulfide	cooked cabbage, garlic	2	2	3	1382	1383	*8*
10	1-octen-3-one	mushroom	2	2	1	1309	1310	*10*
11	2-methylbutyric acid/2-methyl-3-(methyldithio)furan	acidic, cheesy + sweet, meaty	2	2	1	1673	1676/1670	*10/8*
12	(*E*,*Z*)-2,6-nonadienal	green, cucumber, floral	2	1.5	2	1589	1583	*20*
13	hexanal	green, grassy	2	1.5	2	1086	1080	*8*
14	heptanal	fatty, citrus, floral, herbaceous	2	1.5	2	1202	1200	*20*
15	nonanal	green, citrus, fatty	2	1.5	nd	1395	1383	*20*
16	maltol	caramel, sweet	2	1.5	nd	1990	2000	*8*
17	*p*-cresol	cowy, barny	2	1	2	2103	2100	*10*
18	octanal	green, citrus, fatty, aldehydic	2	1	2	1294	1280	*20*
19	(*E*)-2-heptenal	citrus, green, floral	2	1	1	1325	1305	*20*
20	unknown	green, grassy, herbaceous	2	1	nd	1388	ni	ni
21	isovaleric acid	sweaty, sweet, cheesy	2	1	nd	1668	1676	*10*
22	unknown	acidic, cheesy	2	1	nd	1737	ni	ni
23	(*Z*)-4-heptenal	putty	2	0.5	1	1251	1246	*8*
24	unknown	musty, metallic, putty	2	nd	3	1440	ni	ni
25	1-nonen-3-one	mushroom	2	nd	1	1406	1414	*10*
26	unknown	rubber, sweet, glue	2	nd	nd	1111	ni	ni
27	phenylacetaldehyde	honey, sweaty	2	nd	nd	1655	1651	*8*
28	unknown	mushroom	2	nd	nd	1330	ni	ni
29	2,3-butanedione (diacetyl)	sweet, solventy	1	1.5	2	1004	989	*10*

[a] Potency of odorants rated by intensity in GC-O (sniffers 1 and 2) or by a Chief Flavorist's opinion (sniffer 3) on being relevant for the typical flavor of Ryazhenka kefir. [b] Compound tentatively identified by its odor character and Kovats index (RI_{FFAP}) that were compared to corresponding data from the literature (nd, not detected; ni, not identified). [c] Odor rankings: sniffer 1, 1 (weak) to 4 (very strong); sniffer 2, 0.5 (very weak) to 3 (strong); sniffer 3, 0 (not important) to 4 (very important). [d] RI literature reference.

uncovered, odorless polypropylene cups (2 oz; Solo Cup Co., Highland Park, IL; item P200). Sensory evaluation was performed in an air-conditioned (21 °C), odor-free room under white fluorescent light.

RESULTS AND DISCUSSION

Potent Odorants of Ryazhenka Kefir. Volatile compounds of Ryazhenka kefir were screened for potent odorants by purge and trap−thermodesorption−gas chromatography−olfactometry−MS (P&T-TDS-GC-O-MS). For isolation of volatile compounds from Ryazhenka by P&T, the kefir sample was mixed with an excess of sodium sulfate to enhance the yield of low-volatile polar compounds, such as cycloenolones. In GC-O of the volatiles thermodesorbed from the P&T trap, two experienced in the art GC sniffers arbitrarily evaluated the intensity of odorants in the GC effluent in separate experiments. A third GC sniffer, a Chief Flavorist, evaluated the odorants by their estimated relevance for the rich, brown, creamy, cooked milk-like flavor of Ryazhenka kefir. Using an FFAP capillary, all three GC sniffers found a caramelized-smelling compound in Ryazhenka kefir at a Kovats index (RI_{FFAP} 1876) unknown for dairy products (**Table 1**, no. 2). The unknown compound was detected among the 10 most intense or potentially relevant odorants in Ryazhenka. On the basis of RI values and odor quality, the other nine compounds were tentatively identified as hexanoic acid (**Table 1**, no. 1), *trans*-4,5-epoxy-(*E*)-2-decenal (no. 3), 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF; no. 4), butyric acid (no. 5), (*E*)-2-nonenal (no. 6), 2-acetyl-1-pyrroline (no. 7), methional (no. 8), dimethyl trisulfide (no. 9), and 1-octen-3-one (no. 10). Schlutt et al. (*1*) reported the compounds 1, 3, 5, 6, 7, 8, and 10 among the important odor-active compounds in high-heat-treated cream that had been screened for potent odorants by AEDA. Colahan-Sederstrom and Peterson (*3*) also applied AEDA and found odorants 1, 4, 5, 7, and 8 among the most aroma-active compounds in UHT milk heated at 141.1 °C for 6 s, whereas compounds 6 and 10 were found among the less potent odorants. The analytical method applied to Ryazhenka seems to be suitable for initial screening of potent odorants, because most intense smelling compounds detected in Ryazhenka by GC-O were also found as potent odorants in other heated dairy products with creamy, milky flavor. However, surprisingly, no lactones were detected among the most intense smelling odorants in Ryazhenka analysis. In contrast to these findings, lactones have been reported with highest flavor dilution (FD) factors in high-heat-treated cream (*1*) and relatively high FD factors in UHT milk (*3*), indicating their aroma contribution in these products.

Identification of DHM. In the screening of Ryazhenka odorants, the caramelized-smelling compound (RI_{FFAP} 1876) was tentatively identified as dihydromaltol (DHM; 2,3-dihydro-5-hydroxy-6-methyl-4*H*-pyran-4-one) by its RI and odor quality as described in ref *10*. Because, to the authors' knowledge, DHM was found for the first time as a potent odorant in a dairy product in this study, its identity needed to be confirmed by GC-O-MS of an authentic, synthesized standard compound. DHM was synthesized according to ref *8* and smelled caramelized. In GC-O-MS of Ryazhenka, the mass spectrum obtained from the well-separated peak at the caramelized-smelling zone matched that of the synthesized DHM at same retention index (RI_{FFAP} 1876), which confirmed the identity of DHM in Ryazhenka. It was noted that the mass spectrum of DHM (Figure 4a in ref *11*) strongly resembles that of DMHF (Figure 2 in ref *11*) analyzed under the same conditions but eluting much later in GC (RI_{FFAP} 2049). However, using a DB-5 capillary in GC-O-MS of Ryazhenka did not reveal DHM by sniffing or MS (data not shown), because

Table 2. Sensory Threshold Values of Dihydromaltol (DHM) and Dihydroethylmaltol (DHEM) at 25 °C

threshold for	DHM[a] (μg/kg)	DHEM[b] (μg/kg)
flavor in water	50 (3/6)−250 (6/6)[c]	2.5 (2/6)−5 (4/6)
flavor in skim milk	50 (3/7)−250 (6/7)	nd[d]
flavor in 2% milk	<50 (7/7)	nd
odor in water	250 (3/6)−1000 (4/6)	nd
detection in water	25 (3/6) (milky mouthfeel; no aroma or flavor; 6/6)	nd

[a] Threshold determined in a paired comparison test: matrix spiked with DHM at 25 (not for 2% milk), 50, 250, 1000, 2000, 3000, 5000, and 10,000 μg/kg. [b] Threshold determined in a triangle test: matrix spiked with DHEM at 2.5, 5, and 10 μg/kg. [c] Ratio of panelists able to identify the spiked sample from the unspiked matrix in comparison, starting with lowest spike concentration. [d] nd, not determined.

Table 3. Sensory Threshold Values of Caramelized Smelling Aroma Compounds in Water at Room Temperature

compound[a]	flavor (taste) threshold (μg/kg)	odor threshold (μg/kg)
dihydromaltol (DHM)	50−250	250−1000
2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF)	30 (*14*)[b]	60 (*28*)
5-ethyl-4-hydroxy-2-ethyl-3(2*H*)-furanone (EHMF)	5 (*16*)	20 (*29*)
maltol	7100−13000 (*14*, *15*)	35000 (*14*)
dihydroethylmaltol (DHEM)	2.5−5	nd[c]
ethylmaltol	44 (*30*)	10000 (estimated) (*15*)

[a] See chemical structures in **Figure 1**. [b] Reference for threshold value. [c] nd, not determined.

DHM and DMHF peaks overlapped on a DB-5 capillary (RI_{DB-5} 1092−1098) and have very similar odors and mass spectra. Engel et al. (*12*) reported nearly identical RI values for DHM (RI_{SE-54} 1079) and DMHF (RI_{SE-54} 1080) on a capillary similar to DB-5, confirming the findings of the present study. According to ref *10*, DHM and DMHF coelute also on an OV-1701 capillary ($RI_{OV-1701}$ 1240). Therefore, the use of the FFAP capillary is crucial for analytical separation, GC-O detection, and MS identification of DHM in the presence of DMHF.

Sensory and Structure−Odor Relationships of Related Enolones. Chemical structures of the discussed cycloenolones are depicted in **Figure 1**. To assess and compare the flavor impact of selected, caramelized-smelling cycloenolones (DHM, DMHF, EHMF, maltol) in various dairy samples, the odor activity value concept (OAV = concentration/odor threshold) (*13*) was applied, using (retronasal) flavor/taste thresholds in water. Because no flavor threshold for DHM was found in the public literature, sensory thresholds were determined in paired comparison tests against an unspiked matrix.

The flavor threshold for synthesized DHM was determined as 50−250 μg/kg in water and skim milk (**Table 2**) by at least 50% of the tasters. On the basis of flavor threshold values, DHM by itself is much more (ca. 40 times) potent than maltol (7100−13000 μg/kg of water) (*14*, *15*) but less than half as potent as its furanoid isomer, DMHF (30 μg/kg of water) (*14*). Similar relationships between DHM, maltol, and DMHF have been observed for their odor thresholds in air (*8*).

In 2% milk, all tasters perceived DHM at a concentration of 50 μg/kg, at which DHM started to impart cooked milk like character (**Table 2**). At already 25 μg/kg DHM in water, half the tasters perceived a milky mouthfeel from DHM, but none of the tasters perceived any aroma or flavor at that concentration. The orthonasal odor threshold for synthesized DHM was determined as 250−1000 μg/kg in water by at least 50% of the tasters (**Table 2**).

Besides DHM, its C7 homologue, dihydroethylmaltol (DHEM; 6-ethyl-2,3-dihydro-5-hydroxy-4*H*-pyran-4-one), was also synthesized similar to ref *8*, to determine its flavor threshold and to investigate structure−odor relationships among cycloenolones. To the authors' knowledge, DHEM has never been described in the public scientific literature before. The synthesized DHEM has a strong caramelized odor, and highly diluted, aqueous solutions have a sweet flavor. DHEM has not been found in Ryazhenka or in nature, in contrast to its furanoid isomer, EHMF.

In initial paired comparison tests with DHEM at 10, 100, 1000, and 12000 μg/kg in water, > 50% (4/5) of the tasters perceived DHEM at 10 μg/kg. In a subsequent, more precise triangle test, the flavor threshold of DHEM was determined as 2.5−5 μg/kg in water, with > 50% of the tasters identifying DHEM at 5 μg/kg (**Table 2**). Therefore, the flavor threshold of DHEM in water is lower than that of DHM (50−250 μg/kg), similar to the trend of flavor threshold difference between EHMF (5 μg/kg of water) (*16*) and DMHF (30 μg/kg of water). The higher flavor intensity of DHEM versus DHM is analogous to the report of ethylmaltol being 4−6 times more powerful than maltol as a flavor enhancer and sweetness synergist (*17*). The flavor threshold of ethylmaltol (44 μg/kg; **Table 3**) found in the literature seemed to be too low considering its estimated odor threshold of 10 mg/kg. However, in separate preliminary triangle tests with ethylmaltol at 10, 1.25, 0.68, 0.4, and 0.3 mg/kg in water as the odd sample, the sweet, brown, caramelized flavor of ethyl maltol was still perceived at 300 μg/kg by all tasters (5/5) in the present study. Therefore, a flavor threshold at the parts per billion range for ethylmaltol in water has been confirmed and the literature value of 44 μg/kg adopted.

Other derivatives of DHM (i.e., 2-methyl-DHM and 2,3-dimethyl-DHM) also have caramel aroma, except for 2,2-dimethyl-DHM (*18*), being aligned with the structure−odor hypothesis of planar cycloenolones having caramel aroma (*17*).

Comparing sensory thresholds of cycloenolones (**Table 3**) leads to the following conclusions. Substituting a methyl group by an ethyl group in a C-alkyl cycloenolone increases its flavor intensity (e.g., DHEM versus DHM, ethylmaltol versus maltol, EHMF versus DMHF), which has been reported earlier for odor in refs *17* and *19*. Introducing a dihydro function in the ring structure of a 2-alkyl-3-hydroxy-4-pyrone, while maintaining the enolone structure element, enhances the flavor intensity of the cycloenolone (e.g., DHM versus maltol, DHEM versus ethyl maltol). It seems that inhibiting the resonance stabilization of the enolone function with the "quasi-aromatic" heterocyclic structure intensifies the odor of the cycloenolone. Ohloff (*19*) reported similar findings about 3-methyl-2-hydroxy-2,4-cyclopentadien-1-one having a caramel and maple-like odor, but not the intensity of its dihydro derivative, cyclotene (3-methyl-2-hydroxy-2-cyclopenten-1-one). Similar enhancement of aroma intensity by inhibiting the resonance stabilization of the odiferous structural element with its neighboring heterocyclic structure is observed between 2-acetyl-2-thiazoline (odor threshold = 1 μg/kg of water) (*20*) and 2-acetylthiazole (10 μg/kg of water) (*20*).

Physical Data of Dihydroethylmaltol (DHEM). Synthesized DHEM is a white, crystalline substance at 21 °C, melting upon exposure to air. The Kovats indices of DHEM are RI_{FFAP} 1901 and RI_{DB-5} 1166. The spectral data of DHEM are MS/EI (MSD 5975; **Figure 2**), *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); and ^{1}H NMR (400 MHz Bruker, $CDCl_3$), δ 1.69 (3H, tr ($-CH_3$ on C8)), 2.44 (2H, q ($-CH_2$ on C7)), 2.62 (2H, tr ($-CH_2$ on C3)), 4.33 (2H, tr ($-CH_2$ on C2)).

Quantitation of Cycloenolones. To examine the yield increase of cycloenolones by applying sodium sulfate in sample preparation, volatile compounds of the same two Ryazhenka samples were

isolated by P&T directly from the liquid sample without addition of sodium sulfate, as well as after mixing the sample with sodium sulfate. In GC-MS, the extracted molecular mass peak areas of DHM, DMHF, EHMF, and the spiked isotope standards, [$^{13}C_2$]-DMHF and [$^{2}H_3$]-EHMF, were 11−23 times larger when Ryazhenka was mixed with sodium sulfate versus no sodium sulfate addition (data not shown). The peak areas were normalized to the weight of the liquid dairy sample.

As previously mentioned, the odor activity value concept (OAV = concentration/odor threshold) was applied to assess and compare the potential flavor impact of selected, caramelized-smelling cycloenolones (DHM, DMHF, EHMF, maltol) in various dairy samples. Therefore, these compounds were quantified. All kefir samples containing DHM in the 200−1500 μg/kg range (**Table 4**; no. 1, 2, 4, 5, 6, 8, 9) contained also the similarly smelling DMHF at higher levels. EHMF occurred at detectable and varying levels (< 90 μg/kg) only in kefirs made from heated milks (no. 1, 4, 8, 9). Maltol was found at parts per million levels only in samples made with milk heated above 100 °C (no. 1−4, 7−9). Because the quantitative data alone do not indicate a potential flavor contribution of an odorant, the quantitative data were converted to OAVs (**Table 5**) for further discussion. In OAV calculation, (retronasal) flavor/taste thresholds in water were applied (**Table 3**).

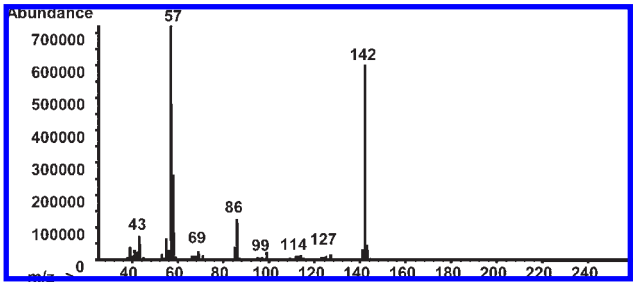

Figure 2. Mass spectrum (EI) of synthesized dihydroethylmaltol (DHEM; 6-ethyl-2,3-dihydro-5-hydroxy-4*H*-pyran-4-one).

Potential Flavor Impact of Selected Cycloenolones. According to the OAV concept, odorants contribute to the flavor of a sample only at concentrations above their odor/flavor threshold (OAV > 1). On the basis of its highest OAVs (OAV = 18−56, **Table 5**) compared to the other enolones, DMHF is the dominant caramelized flavor 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 flavor impact in UHT milk (OAV < 1, no. 3), which was heated at > 138 °C for several seconds, and in fresh pasteurized cream (OAV < 1, no. 12). However, DMHF has some flavor impact in cream that was heated at 80 °C for 8 h (OAV = 1.8, no. 13).

DHM (OAV = 1−6) and EHMF (OAV = 1−18) have low to medium flavor impact in Ryazhenka samples (no. 1, 2, 4−6, 8, 9), but close to none (all OAV < 1) in UHT milk (no. 3), evaporated milk (no. 7), and cream (no. 12 and 13). Only trace amounts of DHM (all OAV ≪ 1) were found in original kefir made commercially with pasteurized milk (no. 10) or grown on UHT milk (no. 11), indicating no flavor impact of DHM in those samples.

Table 4. Concentrations of Potent Caramelized-Smelling Aroma Compounds in Dairy Samples[a]

no.	sample	dihydromaltol (μg/kg)	DMHF[b] (μg/kg)	EHMF[b] (μg/kg)	maltol (μg/kg)
1	RK1: Ryazhenka kefir	746	1618	21	3312
2	RK2: Ryazhenka kefir	574	781	<5	4576
3	UHT milk	<20	<20	<5	4831
4	RK3: UHT milk fermented with RK1 inoculate	416	640	21	4995
5	RK4: UHT milk fermented with RK1 inoculate (pH 4.1)	271	540	6	na
6	RK5: UHT milk fermented with RK1 inoculate (pH 5.4)	217	574	8	na
7	evaporated milk	25	526	<5	3671
8	RK6: evaporated milk fermented with RK1 inoculate	1405	1404	56	7508
9	RK7: evaporated milk fermented with RK1 inoculate	1392	1677	88	5216
10	OK1: original kefir	8	na	na	na
11	OK2: UHT milk fermented with OK1 inoculate	<10	79	<5	na
12	cream, fresh pasteurized	<5	21	<5	12
13	cream, heated (80 °C for 8 h)	<5	55	<5	270

[a] Aroma compounds quantified using internal standards, [$^{13}C_2$]-DMHF and [$^{2}H_3$]-EHMF (acronyms defined below) with no recovery factors applied; na, not analyzed. [b] DMHF, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone; EHMF, 5-ethyl-4-hydroxy-2-methyl-3(2*H*)-furanone.

Table 5. Odor Activity Values (OAV = Concentration/Flavor Threshold[a]) of Potent Caramelized-Smelling Aroma Compounds in Dairy Samples

no.	sample	dihydromaltol	DMHF	EHMF	maltol
1	RK1: Ryazhenka kefir	3.0	54	4.2	0.3
2	RK2: Ryazhenka kefir	2.3	26	<1	0.5
3	UHT milk	≪1	<1	<1	0.5
4	RK3: UHT milk fermented with RK1 inoculate	1.7	21	4.2	0.5
5	RK4: UHT milk fermented with RK1 inoculate (pH 4.1)	1.1	18	1.2	na
6	RK5: UHT milk fermented with RK1 inoculate (pH 5.4)	0.9	19	1.6	na
7	evaporated milk	≪1	18	<1	0.4
8	RK6: evaporated milk fermented with RK1 inoculate	5.6	47	11	0.8
9	RK7: evaporated milk fermented with RK1 inoculate	5.6	56	18	0.5
10	OK1: original kefir	≪1	na	na	na
11	OK2: UHT milk fermented with OK1 inoculate	≪1	2.6	<1	na
12	cream, fresh pasteurized	≪1	0.7	<1	≪1
13	cream, heated (80 °C for 8 h)	≪1	1.8	<1	≪1

[a] Flavor thresholds in water (see text and **Table 3**): dihydromaltol, 250 μg/kg; DMHF, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, 30 μg/kg; EHMF, 5-ethyl-4-hydroxy-2-methyl-3(2*H*)-furanone, 5 μg/kg; maltol, medium average = 10000 μg/kg; na, not analyzed.

In all analyzed samples containing DHM at concentrations above its flavor threshold (OAV > 1), DMHF occurred at 10−20 times higher OAVs and EHMF at OAVs relatively close to those of DHM. A comparison of OAVs indicates a dominant flavor impact of DMHF over DHM among the caramelized-smelling odorants measured in those samples.

Maltol (OAV < 1) did not reach its flavor threshold concentration in any of the measured liquid dairy samples, indicating low to no flavor impact in those samples.

The flavor impact information obtained for the studied cycloenolones via their OAVs is only directional. To demonstrate the actual flavor impact of an odorant, flavor recombination trials with all compounds of high OAVs in a sample, and systematic omission of the odorants in question, need to be performed (*13*). However, those trials are beyond the scope of the present study.

DHM in Foods. On the basis of GC-O data, DHM was found as an important odorant in sweet bell pepper powder (*10*) and identified among the most odor active volatiles in barley malt (*8*). Among other volatile compounds, DHM was quantified in various wines (*21*), and its content was monitored during the aging of sweet fortified wines under aerobic and anaerobic conditions (*22*). Although the aroma impact of the analyzed wine volatiles was discussed using data similar to OAVs, sensory threshold data of DHM were not presented. DHM has been reported to contribute to the "toasty caramel" aroma of heated oak used in winemaking (*11*). Although DMHF and EHMF have been found as key odorants in Swiss cheese (*23*) and later in other fermented dairy products, DHM has not been reported in these products. To the authors' knowledge, DHM was found in this study for the first time as a potent odorant in a dairy product.

DHM Formation. In aqueous Maillard reaction models, DHM was identified from the reaction of D-glucose with L-phenylalanine under anaerobic boiling conditions (*24*) and in heated fructose/cycteamine mixtures (*12*). Using stable isotope labeled 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), an odorless Maillard reaction intermediate from hexoses (*25*). In Ryazhenka, 3-hydroxy-DHM was also found by GC-MS at a peak size smaller than that of DHM (data not shown), which makes the formation of DHM via 3-hydroxy-DHM in Ryazhenka feasible. In fermented foods, the furanones with six and seven carbon atoms, DMHF and EHMF, had been found as important aroma compounds and their formation studied (*26*). EMHF is a biological product in those foods, whereas both the processing and microorganisms can give rise to DMHF. Formation of EHMF was proposed to involve shorter precursors with five and two carbon atoms. The fact that DHEM, a pyranone with seven carbon atoms, has not been found in Ryazhenka or in nature might support the hypothesis of DHM formation from an intermediate with six carbon atoms, such as 3-hydroxy-DHM, and not by condensation of shorter precursors.

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