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Volatile compounds of human milk

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Volatile flavor compounds of human milk have been investigated comparing to commercial cow milk. Volatiles from the milks were isolated by simultaneous steam distillation–extraction (SDE) under reduced pressure using diethyl ether as a solvent. The odor concentrates were analyzed by gas chromatography and gas chromatography–mass spectrometry. Identified compounds from the human milk included 6 esters, 13 ketones, 6 fatty acids, 2 lactones, 24 aliphatic aldehydes, 9 alcohols, 18 hydrocarbons, and 6 miscellaneous compounds. The composition of volatile compounds was quite different from that of the cow milk. Of the volatiles in the human milk the primary important class was aldehydes, and the secondary one was volatile fatty acids.

INTRODUCTION

Human milk is an important medium for early sensory experiences of human infants. Human milk, however, is not a food of invariant flavor but like the milk of other animals human milk is also flavored by ingested compounds such as garlic, mint, vanilla, alcohol, *etc.* It provides the potential for a rich source of varying chemosensory experiences to the infants, although human milk has to have a typical odor which is related to a human metabolism (Mennella, 1995).

Extensive work has been carried out on the effects of heat treatment and storage on the aroma of cow milk (Rerkrai *et al.*, 1987; Badings, 1991; Gaafar, 1991; Contarini, *et al.*, 1997). Detailed information on volatile flavor compounds in the raw milks from cow, ovine, caprine and water buffalo was provided by Moio *et al.* (1993a; 1993b; 1994). A large number of volatile flavor compounds from spray-dried skim milk powder were identified and quantified to make clear the flavor quality of skim milk (Shiratsuchi, *et al.*, 1994a; 1994b; 1995).

To date, no information is available on the volatile flavor compounds of human milk. We have carried out a study on the identification and quantification of volatiles in human milk to investigate the early sensory experiences of human infants.

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MATERIALS AND METHODS

Materials

Fresh human milk (1 L) was obtained from Meiji Milk Products Co., Ltd. (Tokyo) as a mixture of milks from Japanese voluntary mothers. The milk sample was frozen just after collection and stored in a glass bottle at -20°C until it was analyzed (the storage time was about 2 weeks). Ultra-high temperature treated (UHT) cow milk (whole milk) as a reference was purchased from a market.

Isolation of Volatile Flavor Compounds

A milk sample (1 L) was placed in a 2000-mL round bottom flask. Volatiles were separated with 80 mL of diethyl ether by simultaneous distillation–extraction under reduced pressure (approximately 100 mmHg) for 2 h, using a modified Likens–Nickerson apparatus (SDE method). Temperature of the milk was about $62\text{--}65^{\circ}\text{C}$ during the SDE operation. For the cow milk this SDE procedure was repeated 5 times. After addition of 2-ethylhexyl acetate (20 ppb) as an internal standard, the extract solution was dried over anhydrous sodium sulfate for 15 h and concentrated to about $100\mu\text{L}$.

Capillary Gas Chromatography (GC)

Capillary GC analysis was carried out on a Hewlett–Packard Model 5890A gas chromatograph equipped with a flame ionization detector (FID) and connected to a Shimadzu Chromatopak C–R5A integrator. Separation was achieved on a $60\text{ m} \times 0.25\text{ mm}$ i.d. fused silica capillary column, coated with cross-linked polyethylene glycol 20 M, film thickness $0.25\mu\text{m}$ (DB–Wax; J&W Scientific, Folsom, CA). The oven temperature was programmed from 50 to 230°C at $2^{\circ}\text{C}/\text{min}$ (40-min hold). The injector and detector temperatures were 240 and 250°C , respectively. The helium carrier gas flow rate was $28\text{ cm}^3/\text{s}$ with an injection splitter at a split ratio of 20:1. Retention indices were estimated in accordance with a modified Kovats method (Van den Dool and Kratz, 1963).

Capillary Gas Chromatography–Mass Spectrometry (GC–MS)

Electron impact mass spectrometric data were collected on a JEOL Automass 50 mass spectrometer interfaced to a Hewlett–Packard 5890 Series II gas chromatograph. The column and chromatographic conditions were the same as described for GC analysis. The mass spectrometer was operated at an ionization voltage of 70 V and an ion source temperature of 200°C . The mass spectra of unknown compounds were compared with those in the Wiley/NBS Registry of Mass Spectral Data (Wiley/NBS Registry of Mass Spectral Data, 1983).

RESULTS AND DISCUSSION

The recoveries of total volatiles were 1800 ppb and 190 ppb for the human and cow milk, respectively. The volatile concentrate from the human milk developed oily and aldehydic odors, on the contrary, the concentrate from the cow milk had sweet and milky odors. Total of 109 peaks from two milk samples were definitely or tentatively identified (Table 1). The area of the peaks identified represented 94 and 93% of the chromatogram

Table 1. Comparison of volatile compounds in human and cow milk.

Kovats index ^a	compounds	contents (ppb)	
		in human milk	in cow milk
Esters			
889	ethyl acetate	— ^b	20.1
1638	ethyl decanoate	tr ^c	—
1806	methyl dodecanoate	4.1	—
1839	ethyl dodecanoate	2.4	—
2005	methyl tetradecanoate	1.3	—
2216	methyl hexadecanoate	6.1	—
	total	13.9	20.1
Ketones			
840	acetone	—	1.8
890	2-butanone	—	9.0
951	2,3-butanedione	—	1.0
960	2-pentanone	—	2.1
1047	2,3-pentanedione	tr	tr
1177	2-heptanone	1.8	10.5
1190	cyclopentanone	—	0.1
1239	6-methyl-2-heptanone ^d	tr	—
1255	3-octanone	5.6	—
1308	4-octen-3-one	4.2	—
1339	6-methyl-5-hepten-2-one ^d	1.6	—
1392	2-nonanone	tr	6.4
1411	3-octen-2-one	2.8	tr
1469	<i>p</i> -menthan-3-one ^d	1.0	—
1503	2-decanone	—	0.1
1517	3,5-octadien-2-one	1.0	—
1598	2-undecanone	2.5	—
1705	2-dodecanone	—	tr
1816	2-tridecanone	1.6	2.2
2028	2-pentadecanone	—	1.5
2211	2-heptadecanone	—	tr
	total	22.1	34.7
Acids			
1452	acetic acid	tr	—
1625	butanoic acid	—	0.4
1852	hexanoic acid	—	1.9
1962	heptanoic acid	—	tr
2065	octanoic acid	47.8	10.6
2174	nonanoic acid	3.1	0.7
2277	decanoic acid	445.3	37.0
2389	undecanoic acid	33.4	0.7
2493	dodecanoic acid	868.8	20.6
	total	1398.4	71.9
Lactones			
2053	γ -nonalactone	—	0.7
2112	δ -nonalactone	—	1.2
2151	γ -decalactone	tr	—
2226	δ -decalactone	—	3.4
2397	γ -dodecalactone	—	tr
2442	δ -dodecalactone	4.6	—

Table 1. Continued. (1)

		total	4.6	5.3
		Aliphatic aldehydes		
975	<i>n</i> -pentenal	4.7	—	
1086	<i>n</i> -hexanal	69.4	1.4	
1134	(<i>E</i>)-2-pentenal	0.6	—	
1191	<i>n</i> -heptanal	3.2	—	
1224	(<i>E</i>)-2-hexenal	3.8	tr	
1243	(<i>E</i>)-4-heptenal	tr	—	
1291	<i>n</i> -octanal	5.6	—	
1305	(<i>Z</i>)-4-heptenal	1.1	—	
1325	(<i>E</i>)-2-heptenal	12.3	0.2	
1394	<i>n</i> -nonanal	tr	—	
1431	(<i>E</i>)-2-octenal	14.0	—	
1442	(<i>Z</i>)-2-octenal	tr	—	
1464	(<i>E,Z</i>)-2,4-heptadienal	4.7	—	
1493	(<i>E,E</i>)-2,4-heptadienal	2.3	—	
1504	<i>n</i> -decanal	tr	0.1	
1537	(<i>E</i>)-2-nonenal	6.7	—	
1558	(<i>Z</i>)-2-nonenal	0.8	—	
1588	(<i>E,Z</i>)-2,6-nonadienal	2.0	—	
1644	(<i>Z</i>)-2-decenal	2.1	0.6	
1681	(<i>E,Z</i>)-2,4-nonadienal	tr	—	
1701	(<i>E,E</i>)-2,4-nonadienal	4.4	—	
1751	(<i>E</i>)-2-undecenal	3.7	—	
1766	2,4-decadienal	6.3	—	
1814	(<i>E,E</i>)-2,4-decadienal	13.7	0.2	
		total	161.4	2.5
		Alcohols		
917	2-propanol	2.6	—	
1006	2-butanol	—	1.6	
1251	<i>n</i> -pentanol	1.4	—	
1313	(<i>E</i>)-2-penten-1-ol	2.0	—	
1319	(<i>Z</i>)-2-penten-1-ol	3.0	—	
1450	7-octen-4-ol	6.4	—	
1456	3-methylhexanol	tr	—	
1490	2-ethylhexanol	0.7	0.2	
1617	2-octen-1-ol	tr	—	
1788	4,8-dimethylnonanol	1.7	2.6	
		total	17.8	4.4
		Hydrocarbones		
850	(<i>E</i>)-2-octene	—	1.8	
1000	<i>n</i> -decane	5.2	—	
1043	toluene	3.3	1.7	
1138	ethylbenzene	2.7	—	
1156	1,4-dimethylbenzene	—	0.4	
1200	<i>n</i> -dodecane	5.3	—	
1203	<i>d</i> -limonene	5.1	0.7	
1206	<i>n</i> -propylbenzene	tr	3.2	
1221	<i>m</i> -ethyltoluene	tr	tr	
1231	<i>p</i> -ethyltoluene	tr	0.7	
1225	<i>o</i> -ethyltoluene	tr	1.1	
1278	1,2,4-trimethylbenzene	1.0	2.3	

Table 1. Continued. (2)

1300	<i>n</i> -tridecane	2.2	tr
1331	1,2,3-trimethylbenzene	1.2	0.4
1400	<i>n</i> -tetradecane	4.9	–
1500	<i>n</i> -pentadecane	11.4	0.1
1600	<i>n</i> -hexadecane	–	2.4
1653	caryophyllene	1.9	–
1700	<i>n</i> -heptadecane	1.8	0.8
1800	<i>n</i> -octadecane	1.0	1.5
1900	<i>n</i> -nonadecane	–	0.8
2000	<i>n</i> -eicosane	1.2	0.3
	total	47.2	18.2
	Miscellaneous		
1512	1 <i>H</i> -pyrrol	tr	tr
1522	benzaldehyde	tr	0.8
1706	α -terpineol	tr	tr
1722	<i>N</i> -butyl- <i>N</i> -nitrosobutanamine ^a	–	tr
1734	methylphenylamine	–	0.3
1764	dimethylphenylmethanol	–	1.4
1770	<i>N,N</i> -dibutylformamide	tr	tr
1813	<i>N,N</i> -dibutylacetamide	tr	tr
1853	geranylacetone	2.4	–
1988	benzothiazole	–	0.3
2093	<i>p</i> -cresol	–	tr
	total	2.4	2.8

^a Modified Kovats indices calculated for DB-Wax capillary column.

^b not detected.

^c trace. ^d Tentative identification by mass spectrum alone.

surfaces obtained from the human and cow milk, respectively (excluding solvent and internal standard). Figures in Table 1 were only approximate concentration since recoveries from the milk samples and FID response factors were not determined for each compound (assume all recoveries and response factors of 1.0). The compounds identified in the human milk included 6 esters, 13 ketones, 6 fatty acids, 2 lactones, 24 aliphatic aldehydes, 9 alcohols, 18 hydrocarbons, and 6 miscellaneous compounds.

Methyl and ethyl esters of fatty acids were identified. Methyl esters of dodecanoic, tetradecanoic and hexadecanoic acids and ethyl esters of decanoic and dodecanoic acid, however, were detected only from the human milk. Large amount of ethyl acetate was found in the cow milk. These are in disagreement with the fact that short-chain and medium-chain fatty acids are abundant in the milks from ruminant animals by the actions of rumen microorganisms (Christie, 1983). Furthermore, it was the most important characteristic that methyl esters were predominant in the human milk. According to Moio, *et al.* (1993a) aliphatic ethyl esters were the most important class of milk volatiles, comprising about half of the neutral volatile fraction of the milks from cow, ovine, caprine and water buffalo. Above all, ethyl esters of butyric, hexanoic, octanoic, and decanoic acids have been reported as the most representative compounds. They also found ethyl acetate only in cow and water buffalo milks. Ethyl esters of butyric, hexanoic and

octanoic acids, however, were not found in the present milk samples (Table 1). Palmquist *et al.* (Palmquist, *et al.*, 1993) demonstrated that the fat composition of cow milk was strongly influenced by the stage of lactation, dietary composition and feeding regimen.

Many chemical species of ketones, which are considered to be degradation products of hydroperoxides from unsaturated free fatty acids (Hawke, 1966; Dartey and Kinsella, 1971; Forss, 1979) were identified. Total concentration of ketones in the human milk was 22.1 ppb, on the other hand, 34.9 ppb in the cow milk. There was great difference in the composition, that is, methyl ketones such as 2-butanone, 2-heptanone and 2-nonanone were predominant in the cow milk, and ethyl ketones such as 3-octanone and 4-octen-3-one were predominant in the human milk. Methyl ketones are the compounds which play a fundamental role in determining the typical odor of blue cheeses (Gallois and Langlois, 1990). The concentrations of these methyl ketones, however, in the cow milk were extremely low, far below the threshold value (500–8000 ppb) in fresh milk (Badings, 1984). Ethyl ketones have not been found in the milks from ruminant animals (Moio, *et al.*, 1993a). These compounds were found in butter, chicken, beef, pork, *etc.* (Volatile Compounds in Food, 1992). These differences in the composition would be attributed to the differences in the composition of fatty acids and the activities of enzymes such as lipoxygenase, hydroperoxide lyase and hydroperoxide isomerase. Ethyl ketones in the human milk were unlikely to have sensory importance.

The chemical class with the highest proportion was free fatty acids, comprising 84 and 46% of the total volatile fraction of the human and cow milk, respectively. The amount of fatty acids from the human milk comprised to about 20 times as much as that from the cow milk. Since these compounds are known to be released from triglycerides and phospholipids by the action of milk lipase or bacterial lipase in ruminant animals, the activity of milk lipase in the human milk was considered to be higher than that in the cow milk. Octanoic, decanoic and dodecanoic acids were predominant compounds in both the milks. Considering the odor threshold values (200–800 ppb) (Osawa, 1987) these compounds would contribute to buttery, milky and creamy odors only in the human milk. Great difference in the amount of volatile acids in two milk samples would be attributed to the levels of volatile esters.

Among lactones, which are characterized by sweet, milky or creamy odors, δ -deca and δ -dodecalactone are associated with the smell of milk with sweet and body attributes (Osawa, 1987). Recently, it was also reported that δ -deca and δ -undecalactone, and γ -undeca and γ -dodecalactone were the important contributors to the sweet and milky odors of spray-dried skim milk powder (Shiratsuchi, *et al.*, 1995). γ -Nona, δ -nona and δ -decalactone were detected in the present cow milk, on the other hand, δ -dodecalactone was predominant in the human milk. The sensory importance of lactones could not be clarified because the concentrations recovered were very low. The percent recoveries from cow milk were practically less than 5% except for γ -nonalactone (8%). It was considered that the binding of lactones to milk proteins lowered their volatilities. Studies (O'Neill and Kinsella, 1987a; 1987b) on the interaction of volatiles with proteins support this hypothesis.

Aldehydes in Table 1 except for 4-heptenal were oxidation products of oleic, linoleic and linolenic acid. (*E*)- and (*Z*)-4-Heptenal are known to be the degradation product of (*Z,Z*)-11,15-octadecadienoic acid (Belitz and Grosch, 1987). The contributions of

aldehydes to the odors of two milk samples were extremely different. These compounds in the concentrations found in human milk undoubtedly develop oily and aldehydic odors. Because their odor threshold values in aqueous phase are extremely low: hexanal; 4.5 (Buttery, *et al.*, 1987), heptanal; 2.0 ppb (Buttery, *et al.*, 1994), octanal; 0.7 ppb (Karahadian and Johnson, 1993), (*Z*)-4-heptenal; 0.06 ppb (Milo and Grosch, 1993), nonanal; 1.0 ppb (Karahadian and Johnson, 1993), ((*E*)-2-octenal; 3.0 ppb (Cha and Cadwallader, 1998), (*E*)-2-nonenal; 0.08 ppb (Karahadian and Johnson, 1993), (*E,E*)-2,4-nonadienal; 0.06 ppb (Milo and Grosch, 1993), (*E,E*)-2,4-decadienal; 0.07 ppb (Karahadian and Johnson, 1993).

Total concentration of alcohols in the human milk was about 4 times as much as that in the cow milk. Almost all of the compounds were degradation products of unsaturated fatty acids. Considering their quite high odor thresholds in milk (400–600 ppb) it was unlikely that they contributed to the milk odor.

As hydrocarbons 10 aliphatic compounds, 9 aromatic compounds, and 2 terpenes were found. Except for pentadecane in the human milk all compounds were present at low levels less than 10 ppb, so probably did not contribute to the milk odor.

Among miscellaneous compounds the possible contributors to milk odor were not found. Benzothiazole developed the off-flavor in spray dried skim milk powder (Shiratsuchi, *et al.*, 1994b) but it was unlikely to have any sensory importance in the cow milk because of its low concentration.

In conclusion, the results showed that the composition of volatile concentrate from the human milk sample was very different from that of the cow milk sample. In the human milk aldehydic compounds had a first sensory importance and volatile fatty acids had a secondary importance. The sensory contribution of lactones was not made clear although they surely played a role in developing milky odor.

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