Contributors to Sweet and Milky Odor Attributes of Spray-Dried Skim Milk Powder

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Contributors to sweet and milky odor attributes of spray-dried skim milk powder have been investigated. Spray-dried skim milk was homogenized with water, and the volatiles were isolated by simultaneous steam distillation extraction (SDE). The odor concentrate was fractionated by silica gel TLC. Two polar fractions with sweet and milky odors were analyzed by GC and GC–MS. To elucidate the compounds directly contributing to the characteristic flavor, these fractions were further fractionated by a preparative GC and the separated fractions and peaks were sniffed. Nonanoic acid, decanoic acid, and dodecanoic acid were responsible for a sweet, fatty, and butter-like odor; undecanoic acid contributed a sweet, and butter-like odor; and γ-undecalactone, γ-dodecalactone, a γ-lactone, δ-decalactone, and δ-undecalactone gave a sweet and milky odor.

Keywords: *Volatiles; flavor compounds; skim milk powder*

INTRODUCTION

Skim milk powder is widely used for various food products as a quality modifier; that is, addition of skim milk powder to food products gives characteristic flavor, smooth taste, body, and good taste to the products. The flavor is nevertheless one of the most important quality factors of skim milk powder, but there have been few studies except for our previous papers (Shiratsuchi et al., 1994a,b); however, extensive works have been carried out and reviewed on the flavor of fluid milk or milk fat (Kinsella et al., 1967; Forss, 1971, 1979; Badings and Neeter, 1980; Osawa 1987; Coulibaly and Jeon, 1992; Moio et al., 1993). We have investigated, therefore, contributors to sweet and milky odors in skim milk powder, which is important for the manufacture of high-quality food products.

MATERIALS AND METHODS

Materials. The commercially processed spray-dried skim milk powder was obtained from Meiji Milk Products Co., Ltd. (Tokyo, Japan). The silica gel 60 F_{254} plate for thin-layer chromatography (TLC) of odor concentrate was from E. Merck (Darmstadt, Germany). Diethyl ether, *n*-pentane, and anhydrous sodium sulfate were from Nakarai Tesque, Inc. (Kyoto, Japan). γ-Nonalactone, γ-undecalactone, γ-dodecalactone, γ-tetradecalactone, and various volatile fatty acids were from Tokyo Kasei, Inc. (Tokyo, Japan). δ-Octalactone, δ-nonalactone, δ-decalactone, δ-undecalactone, δ-dodecalactone, and δ-tetradecalactone were obtained from Ogawa Co., Ltd.

Isolation and Fractionation of Volatile Flavor Compounds. The skim milk powder (300 g) was homogenized in 900 mL of deionized water using a high-speed blender, and the homogenate was placed in a 2000-mL round-bottom flask. Volatiles were separated with 80 mL of diethyl ether from the homogenate by simultaneous steam distillation–extraction under reduced pressure (approximately 150 mmHg) for 1 h, using a modified Likens–Nickerson apparatus (SDE method).

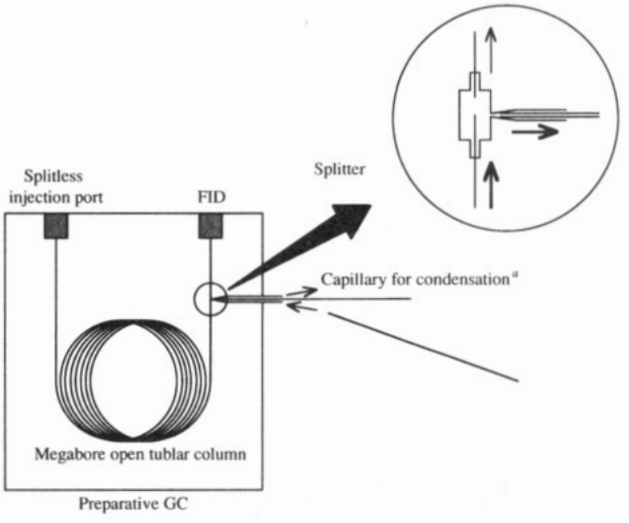

Figure 1. Scheme of preparative GC. [a]The capillary (0.53 mm i.d. × 25 cm) was coated with a film (1 μm) of chemically bonded Carbowax 20M.

The condenser of the SDE apparatus was cooled with a mixture of water and diethylene glycol at −5 °C. The extract was dried over anhydrous sodium sulfate for 3 h and concentrated to about 100 μL. Seventy samples of the skim milk powder were successively treated. The concentrates were put together, and further concentration was conducted to about 2 mL. The odor concentrate from 21 kg of the skim milk powder, which had a typical skim milk odor, that is, sweet, milky, powdery, and peanut-like odors, was fractionated by silica gel TLC (solvent, diethyl ether/*n*-pentane 20:80). The two polar fractions with sweet and milky odor, which seemed to contribute directly to the characteristic flavor of skim milk powder, were eluted with diethyl ether (30 mL) from the silica gel support scrapped from the TLC plate and concentrated to about 100 μL.

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 m × 0.25 mm i.d. fused silica capillary column, coated with cross-linked polyethylene glycol 20M (PEG 20M), film thickness 0.25 μm

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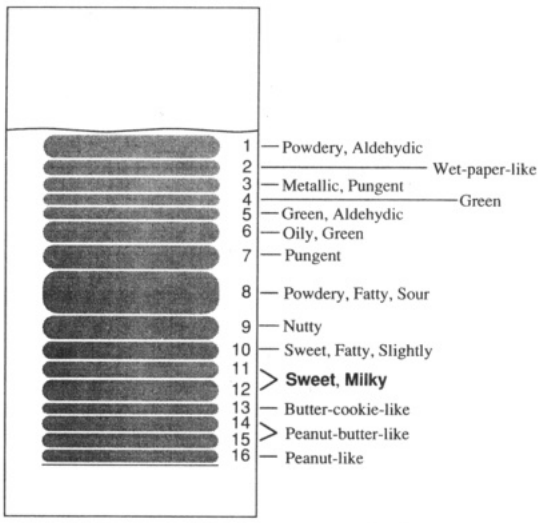

Figure 2. Thin-layer chromatogram of odor concentrate of skim milk powder.

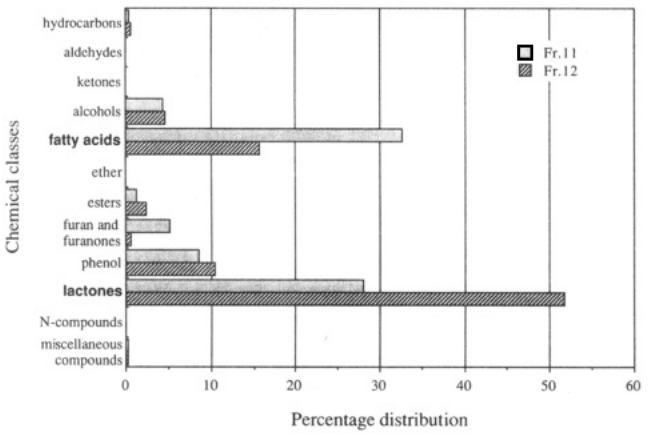

Figure 3. Percentage distribution of chemical classes in fractions 11 and 12 of the thin-layer chromatogram.

(DB-Wax; J&W Scientific, Folsom, CA). The oven temperature was programmed from 50 to 230 °C at 2 °C/min (60-min hold). The injector and detector temperatures were 200 and 250 °C, respectively. The helium carrier gas flow rate was 22 cm/s with an injection splitter at a split ratio of 30:1. 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 eV and ion source temperature of 200 °C. The scanning rate was 1 scan/s. The mass spectra of the unknown compounds were compared with those in the NIST data base of the Automass 50 system and other published spectra (*Eight Peak Index of Mass Spectra*, 1983; *Wiley/NBS Registry of Mass Spectral Data*, 1989).

Preparative GC and Sniffing. The odor extracts from two polar functions with sweet and milky odors were further fractionated by preparative GC (Shimoda et al., 1993) to detect contributors to the odor attributes. For preparative GC, a Shimadzu GC8A gas chromatograph equipped with a 60 m × 0.75 mm i.d. cross-linked PEG 20M (film thickness, 1 μm) megabore open tubular glass column (Supelcowax 10; Supelco, Bellefonte, PA) and FID was used. The oven temperature was programmed from 50 to 220 °C at 2 °C/min. The helium carrier gas flow rate was 25 cm/s with a splitless injection. Short capillaries (25 cm × 0.53 mm i.d.) with a chemically bonded PEG 20M film were used for trapping separated fractions or components. The trapping port was especially designed so that a large negative temperature gradient could be produced in the middle of the capillary, and the capillary could be exchanged in a very short time (Figure 1). The

Table 1. Identification and Peak Area Percentages of Volatile Compounds of Principal Chemical Classes in Fractions 11 and 12 of the Thin-Layer Chromatogram

compd	rel area,[a,b] % fraction 11	fraction 12
Alcohols		
ethanol	2.59	3.65
2-butanol	tr	nf
2-methyl-3-buten-2-ol	0.08	tr
2-pentanol	nf	tr
1-butanol	0.21	0.16
1-pentanol	0.32	0.10
3-methyl-2-buten-1-ol	0.43	0.15
1-hexanol	tr	nf
2-butoxyethanol	nf	0.12
1-heptanol	0.10	nf
2-ethylhexanol	0.06	tr
isooctanol	0.06	nf
1-octanol	tr	nf
ethylene glycol	0.28	0.24
α-terpineol	0.06	nf
benzenemethanol	0.12	nf
1,2,3-propanetriol	tr	nf
1-octadecanol	0.05	0.20
Fatty Acids		
acetic acid	tr	0.29
propanoic acid	0.09	0.08
butanoic acid	0.68	0.28
hexanoic acid	1.30	0.79
heptanoic acid	tr	nf
octanoic acid	5.59	3.12
nonanoic acid	0.18	0.10
decanoic acid	12.67	5.82
undecanoic acid	nf	tr
dodecanoic acid	8.40	2.28
tetradecanoic acid	3.56	2.91
hexadecanoic acid	0.13	nf
Furan and Furanones		
dihydro-5-methyl-2(3*H*)-furanone	0.05	nf
dihydro-4-methyl-2(3*H*)-furanone	nf	0.08
2-furanmethanol	4.86	0.25
dihydro-4,5-dimethyl-2(3*H*)-furanone	0.05	tr
dihydro-5-ethyl-2(3*H*)-furanone	nf	0.07
dihydro-5-pentyl-2(3*H*)-furanone	0.23	0.24
Lactones		
δ-octalactone	nf	0.27
γ-nonalactone	0.13	nf
δ-nonalactone	nf	tr
δ-decalactone	0.14	10.65
γ-undecalactone	0.10	nd
δ-undecalactone	tr	0.39
γ-dodecalactone	9.38	0.27
γ-lactone[c]	1.08	nf
δ-dodecalactone	4.70	25.00
γ-tetradecalactone	0.67	0.18
δ-tetradecalactone	11.76	14.93
N-Containing Compounds		
2,3-dihydro-1*H*-indene	nf	tr
N,*N*-dibutylformamide	tr	tr
N,*N*-dibutylacetamide	0.05	nf
2-*tert*-butylindole	tr	nf
3-ethyl-3-methyl-2,5-pyrrolidinedione	nf	tr
Miscellaneous Compounds		
2-ethoxybutane	nf	tr
1-ethoxybutane	nf	tr
2-methyl-1,3-dioxolane	tr	nf
octamethylcyclotetrasiloxane	0.11	0.24
cyclohexyl isothiocyanate	0.09	tr
benzothiazole	tr	nf

[a] Relative percentage of total peak area (solvent excluded). [b] Symbols: nf, not found; tr, less than 0.04%. [c] Tentative identification by mass spectrum alone.

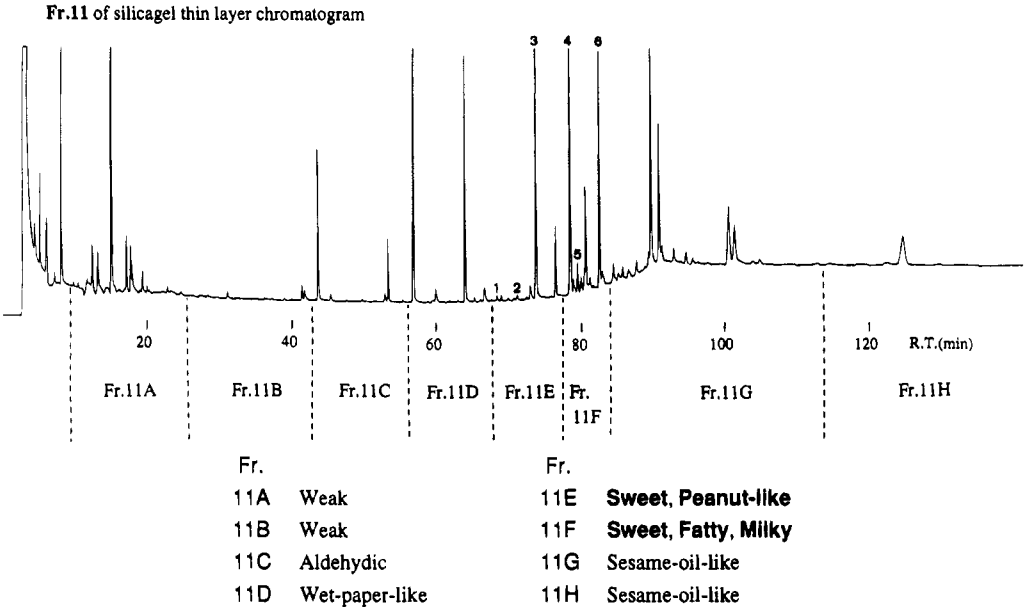

Figure 4. Sniffing of the fractions obtained by preparative GC on fraction 11 of the thin-layer chromatogram. For more information, see Materials and Methods and Table 2.

Table 2. Sniffing of Fraction 11E and 11F Compounds by Preparative Gas Chromatography

peak[a]	compd	odor characteristics	content,[b] ppb
		Fraction 11E	
1	nonanoic acid	sweet, butter-like	19.8
2	γ-undecalactone	sweet, milky	8.5[c]
3	decanoic acid	sweet, fatty, butter-like	1586.6
		Fraction 11F	
4	γ-dodecalactone	sweet, milky	38.0
5	γ-lactone[d]	sweet, milky	16.1
6	dodecanoic acid	sweet, fatty, butter-like	1928.2

[a] Peak number in Figure 4. [b] Contents in odor concentrate before any fractionation which were related to the weight of skim milk powder. [c] Approximate evaluation since the peak contained more than one component. [d] Tentative identification by mass spectrum alone.

separated compounds were eluted from the capillary column with a drop of ethyl ether on a filter paper for sniffing. To identify the compound, the ether eluate was splitless-injected to GC–MS.

RESULTS AND DISCUSSION

A thin-layer chromatogram of the odor concentrate is shown in Figure 2. Among these fractions, fractions 11 and 12 had sweet and milky odors, which prompt remembrance of the flavor of skim milk. It was considered, therefore, that possible contributors to skim milk flavor were included in these fractions, and concentrates of volatile compounds from them were applied to GC and GC–MS analysis. The distribution in chemical classes of the identified compounds from fractions 11 and 12 on TLC is shown in Figure 3. Table 1 lists identification and peak area percentages of volatile compounds of principal chemical classes.

Most of the hydrocarbons, aldehydes, and ketones were present in extremely low level (relative area ≤ 0.1%). They inherently must not exist in these polar fractions because of their low polarities. Actually, these compounds were contained in less polar fractions at relatively high level (not shown in this paper). There would be little contribution to skim milk flavor from their odor attributes and contents.

Alcohols except for ethanol had very low relative areas (≤0.3%). Because of their high flavor thresholds, it was unlikely that they contributed to the flavor of skim milk.

The levels of fatty acids in each fraction were more than 32 and 15% of total volatiles, respectively; they constituted the chemical family with the highest proportion in fraction 11. Among them, even-carbon-numbered saturated fatty acids, which have buttery, milky, creamy, or waxy odors, were predominant in both fractions. In our previous paper (Shiratsuchi et al., 1994b), we compared the levels of chemical classes in normal skim milk powder, the odor attributes of which were described as sweet, fatty, and milky odor, and those of an off-flavored one with barn-like odor. The comparison showed the content of fatty acids in the normal skim milk powder was 2 times higher than that in the off-flavored one. Therefore, fatty acids in fractions 11 and 12 could be important contributors to the characteristic flavor of skim milk powder.

Ethyl formate and acetate were present in both fractions, while *n*-butyl ether was detected in fraction 11 only. They have relatively low polarity and might be included in these polar fractions due to migration from other fractions in the same manner as hydrocarbons, aldehydes, and ketones. Judging from their odor characteristics, they may be of slight concern in skim milk flavor.

2-Furanmethanol and some 2(3*H*)-furanones, products of sugar degradation and dehydration by Maillard reaction, were detected in both fractions. 2(3*H*)-Furanones are associated with a sweet, nutty, and caramel-like aroma and may be important for the odor of a condensed milk (Osawa, 1987). They might contribute somewhat to the characteristic flavor of skim milk, but their contents were too low to directly contribute to the sweet odor of skim milk.

It was shown that a pronounced amount of lactones was included in each fraction and amounted to over 50% in fraction 12. Contents of γ-lactones in fraction 12 were lower than those in fraction 11; γ-nona- and γ-undecalactone were absent in fraction 12. On the contrary, δ-lactones were abundant in fraction 12; δ-tetradecalactone was present in both fractions at similar proportions. These lactones, produced by heating from 4- and 5-hydroxy fatty acids (Forss, 1979), have milky, buttery, or creamy odors and hence are added to some margarines to simulate butter flavor (Kinsella et al., 1967).

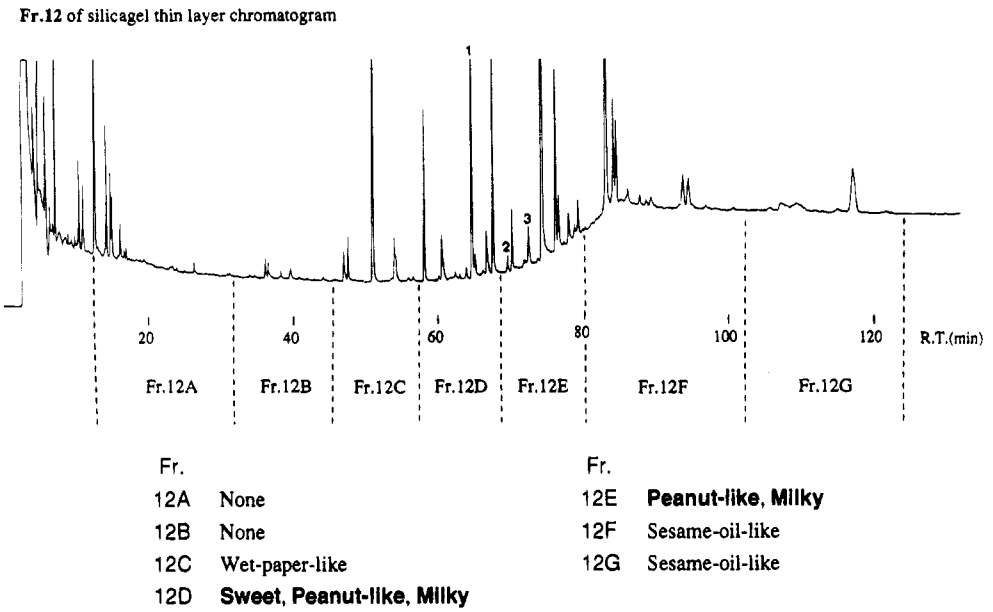

Figure 5. Sniffing of the fractions obtained by preparative GC on fraction 12 of the thin-layer chromatogram. For more information, see Materials and Methods and Table 3.

Judging from their odor attribute, lactones were considered to form an important part of the characteristic flavor of the skim milk powder along with fatty acids. Also in a previous paper (Shiratsuchu et al., 1994b), we found that only fatty acids and lactones were contained in higher levels in a normal one.

2,6-Di-*tert*-butyl-*p*-cresol (BHT) was the sole phenolic compound in both fractions. It was, however, added to diethyl ether as an antioxidant and independent of skim milk flavor.

To clarify the compounds that directly contribute to the characteristic flavor of skim milk powder, further fractionation was carried out by preparative GC. At first, fraction 11 was separated into eight portions (Figure 4). As shown in this figure, fraction 11E had sweet and peanut-like odors, and fraction 11F had sweet, fatty, and milky odors. Because it was considered that the contributors to sweet and milky odor attributes were included in these fractions, every peak in both fractions was further separated, and their odors were sniffed (Table 2). Peak 1, nonanoic acid, peak 2, γ-undecalactone, peak 3, decanoic acid, peak 4, γ-dodecalactone, peak 5, a γ-lactone, and peak 6, dodecanoic acid, had sweet, aldehydic, fatty, and milky odors; peak 2, γ-undecalactone had a sweet, fragrant, and milky odor. On the other hand, fraction 12 was separated into seven portions by preparative GC (Figure 5). Fraction 12D had sweet, peanut-like, and milky odors and fraction 12E peanut-like and milky odors; it was considered that the contributors to the sweet and milky odor attributes were included in these fractions. Peak 1, δ-decalactone, and peak 2, δ-undecalactone, had a sweet, fragrant, and milky odor; peak 3, undecanoic acid, had a sweet, fatty, and butter-like odor (Table 3). The contents of Tables 2 and 3 are the values before any fractionations and related to the weight of skim milk powder. Recovery factors were unknown and considered to be 1.0. Besides the above-mentioned compounds, fatty acids and lactones with large relative areas such as octanoic acid, tetradecanoic acid, δ-dodecalactone, and δ-tetradecalactone were present in these fractions. It was, however, considered that they could not contribute to sweet and milky odor attributes as a result of preparative GC and sniffing.

Table 3. Sniffing of Fraction 12D and 12E Compounds by Preparative Gas Chromatography

peak[a]	compd	odor characteristics	content,[b] ppb
		Fraction 12D	
1	δ-decalactone	sweet, fatty, milky	27.7
		Fraction 12E	
2	δ-undecalactone	sweet, milky	0.7
3	undecanoic acid	sweet, fatty, butter-like	43.4[c]

[a] Peak number in Figure 5. [b] Contents in odor concentrate before any fractionation which were related to the weight of skim milk powder. [c] Approximate evaluation since the peak contained more than one component.

Since nonanoic acid, decanoic acid, undecanoic acid, dodecanoic acid, γ-undecalactone, γ-dodecalactone, a γ-lactone, δ-decalactone, and δ-undecalactone were considered to be contributors to sweet and milky odors of skim milk powder, these four acids and four lactones were added to skim milk in the levels of Tables 2 and 3, which contained 14% skim milk powder. With a panel of 20 untrained persons, a triangle test was done by sniffing the odors of a skim milk control and an amended sample. Sixteen correct responses showed the odor difference between control skim milk and the amended sample was significantly different ($p < 0.05$); all 16 persons appreciated the sweet and milky odors strongly in the amended sample.

Therefore, it was concluded that nonanoic acid, decanoic acid, undecanoic acid, dodecanoic acid, γ-undecalactone, γ-dodecalactone, δ-decalactone, and δ-undecalactone were the contributors to the sweet and milky odors of skim milk powder.

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