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Off-Flavor Compounds in Spray-Dried Skim Milk Powder

Hideki Shiratsuchi,*,† Mitsuya Shimoda,† Kazuhiro Imayoshi,† Katsuhiko Noda,‡ and Yutaka Osajima†

Department of Food Science and Technology, Faculty of Agriculture, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812, Japan, and Central Research Institute, Meiji Milk Products Company, Ltd., 1-21-3 Sakae-Cho, Higashimurayama-Shi, Tokyo 189, Japan

Off-flavor of a spray-dried skim milk powder has been investigated. Commercially processed spray-dried skim milk was homogenized with water, and the volatiles were isolated by simultaneous steam distillation–extraction under reduced pressure using diethyl ether as a solvent. The odor concentrate was analyzed by gas chromatography and gas chromatograph–mass spectrometry. To elucidate the compounds contributing to the off-flavor, the concentrate was fractionated by silica gel TLC. A slightly polar fraction with cowhouse-like odor, which was similar to the odor attribute of off-flavor, was further fractionated by a preparative GC. Tetradecanal, having a content about 10 times higher in the off-flavored skim milk than in a normal one, was responsible for a sickening and aldehydic odor. β-Ionone and benzothiazole were 76 and 37 times higher in the off-flavored skim milk, respectively, and the former imparted a fragment and haylike odor and the latter a sulfuric and quinoline-like odor.

INTRODUCTION

The acceptance of food products depends to a major extent on their flavor quality. Skim milk powder, widely used as a raw material for various food products, is manufactured through many processes such as separation of milk fat, heat treatment, concentration, and (spray) drying. Hence, flavor quality of skim milk powder is greatly affected not only by the quality of raw milk but by changes of flavor during many processes. Nowadays, an off-flavor is sometimes detected in imported skim milk powder, and this fact has became a serious obstacle to the use of skim milk powder. Many studies 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) have carried out a comparative study on volatile compounds in raw milks from four animal species; however, there have been few studies on skim milk powder except for our previous paper (Shiratsuchi et al., 1994). For fluid milk and their products, Azzara and Campbell (1992) reviewed off-flavors due to cow's diet, environment, heat treatment, bacterial spoilage, storage, and oxidation of lipids but provided little information on off-flavors of skim milk powder. The aim of this study is to relate the off-flavor to the difference of composition of volatile compounds in a skim milk powder.

MATERIALS AND METHODS

Materials. For the purpose of this study we used two kinds of commercially processed spray-dried skim milk powder, obtained from Meiji Milk Products Co., Ltd. (Tokyo, Japan). One was pasteurized milk with off-flavor, the odor attribute of which could be described as cowhouse-like odor (off-flavored skim milk), and the other one was ultra-high-temperature-sterilized milk with normal flavor (normal skim milk). Silica gel 60 F_{254} plates for thin-layer chromatography (TLC) of odor concentrates were from E. Merck (Darmstadt, Germany). Diethyl ether and anhydrous sodium sulfate were from Nakarai Tesque, Inc. (Kyoto, Japan), tetradecanal was from Aldrich (Milwaukee, WI), and 2-ethylhexyl acetate, β-ionone, and benzothiazole were from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan).

Isolation of Volatile Flavor Compounds. The skim milk powdre (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 and extraction under reduced pressure (approximately 80 mmHg) for 1 h, using a modified Likens–Nickerson apparatus (SDE method). After addition of 30 μL of 0.1% 2-ethylhexyl acetate, the extract was dried over anhydrous sodium sulfate for 3 h and concentrated to about 100 μL. A series of procedures were repeated seven times for the GC and GC–MS analysis and 70 times for the fractionation of odor concentrate.

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 (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 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.

Fractionation of Odor Concentrates. The odor concentrates from 21 kg of the normal and off-flavored skim milk powder were fractionated by silica gel TLC (solvent, diethyl ether/n-pentane 20:80). The slightly polar fraction with cowhouse-like odor, which seemed to contribute directly to the off-flavor, was eluted from silica gel support and further fractionated by preparative GC (Shimoda et al., 1993). 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. Helium carrier gas

* Author to whom correspondence should be addressed.
† Kyushu University.
‡ Meiji Milk Products Co., Ltd.

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Table 1. Comparison of Absolute Contents of Volatile Constituents, Aldehydes, Alcohols, Esters, and N-Containing Compounds in Normal and Off-Flavored Skim Milk Powders

peak[a]	compd	Kovats index[b]	content,[c] ppb		ratio[d]
			normal	off-flavored	
	(A) Aldehydes				
14	hexanal	1085	2.32	1.54	0.66
36	octanal	1287	1.47	2.40	1.63
51	nonanal	1391	ne	ne	
54	(*E*)-2-octenal	1435	1.85	3.40	1.84
65	benzaldehyde	1522	10.68	9.18	0.86
66	(*E*)-2-nonenal	1536	1.71	7.07	4.12
74	(*E*,*Z*)-2,6-nonadienal	1590	0.52	1.57	3.05
3A	undecanal	1612	nf	1.52	
80	(*Z*)-2-decenal	1644	2.06	2.43	1.18
85	salicylaldehyde	1674	1.55	3.63	2.34
88	dodecanal	1708	2.14	4.91	2.29
100	?-tridecanal[e]	1821	4.73	3.16	0.67
108	?-tridecanal[e]	1881	0.85	5.43	6.36
109	tridecanal	1886	27.47	12.57	0.46
112	tetradecanal	1927	ne	ne	
120	?-tetradecenal[e]	1997	6.65	8.09	1.22
126	pentadecanal	2042	1.48	3.41	2.30
135	hexadecanal	2137	3.02	1.67	0.55
169	(*Z*)-9-octadecenal	2693	5.10	54.16	10.62
	total		73.64	126.12	1.71
	(B) Alcohols				
5	ethanol	947	10.90	8.72	0.80
10	2-methyl-3-buten-2-ol	1040	2.92	1.87	0.64
1A	2-methyl-1-propanol	1095	nf	1.02	
30	1-pentanol	1252	0.46	0.69	1.49
42	2,2,4-trimethylpentanol	1326	1.26	nf	
45	2,3-dimethyl-2,3-butanediol	1338	1.19	1.24	1.05
52	2-butoxyethanol	1411	ne	ne	
58	1-octen-3-ol	1459	ne	ne	
61	2-ethylhexanol	1491	ne	ne	
69	linalool	1552	1.10	3.49	3.18
71	1-octanol	1565	2.01	3.69	1.83
2A	1-methyl-4-(methylethyl)-(*E*)-2-cyclohexenol	1575	nf	1.11	
79	ethylene glycol	1635	2.70	3.13	1.16
92	1-decanol	1748	ne	ne	
116	1-dodecanol	1977	2.69	4.46	1.66
118	2-tridecanol	1986	3.52	3.71	1.05
119	3,7-dimethyl-1,7-octanediol	1988	2.69	1.61	0.60
128	nerolidol	2050	2.74	2.98	1.09
8A	1-tetradecanol	2204	nf	3.45	
140	δ-cadinol	2212	8.50	3.93	0.46
150	1-hexadecanol	2400	ne	ne	
158	dihydrophytol	2537	2.78	42.93	15.44
163	1-octadecanol	2603	ne	ne	
166	(*E*)-phytol	2646	4.11	tr	
9A	1-docosanol	2979	nf	24.94	
	total		49.57	112.99	2.28
	(C) Esters				
2	ethyl formate	825	3.40	4.73	1.39
3	ethyl acetate	888	9.37	13.96	1.49
60	6-methylheptyl acrylate	1480	1.73	5.44	3.15
96	diethylene glycol butyl ester	1786	ne	ne	
4A	3-hydroxy-2,4,4-trimethyl-2-methylpropanoate[e]	1875	nf	2.72	
5A	2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl 2-methylpropanoate[e]	1894	nf	5.26	
122	methyl myristylate	2008	1.63	4.52	2.76
6A	methyl pentadecanoate	2124	nf	6.57	
142	methyl palmitate	2233	3.91	3.66	0.94
155	methyl stearate	2445	9.27	10.51	1.13
178	dioctyl adipate	2846	3.80	tr	
	total		33.10	57.39	1.73
	(D) N-Containing Compounds				
63	1*H*-pyrrole	1512	0.65	1.96	3.00
77	2-formyl-1-methylpyrrole	1620	1.43	1.54	1.07
78	*N*-methylacetamide	1623	2.34	2.88	1.23
84	cyclohexyl isothiocyanate	1670	16.70	26.78	1.60
90	*N*-butyl-*N*-nitrosobutanamine[e]	1722	2.13	2.72	1.28
94	*N*,*N*-dibutylformamide	1767	1.23	1.59	1.29
98	*N*,*N*-dibutylacetamide	1813	3.74	3.69	0.99

Table 1 (Continued)

peak[a]	compd	Kovats index[b]	content,[c] ppb normal	content,[c] ppb off-flavored	ratio[d]
115	benzothiazole	1962	ne	ne	
123	methyl *N,N*-dimethyldithiocarbamate	2012	1.63	1.44	0.88
134	2-*tert*-butylindole	2131	13.75	15.94	1.16
7A	2,3-dimethylquinoline	2151	nf	3.60	
136	2-oxo-1-methyl-3-isopropylpyrazine[e]	2160	2.68	2.18	0.81
137	2-undecanone *O*-methyloxime[e]	2163	4.42	6.71	1.52
154	indole	2435	2.78	8.44	3.03
164	2-pentadecanone *O*-methyloxime[e]	2619	4.54	19.09	4.21
165	*p*-phenylaniline	2636	ne	ne	
177	2-heptadecanone *O*-methyloxime[e]	2827	10.48	14.40	1.37
181	*N,N*-dibutylhexanamide	2869	19.46	16.12	0.83
	total		96.75	137.84	1.42

[a] Components numbered 1A, 2A, and so on were detected in the off-flavored skim milk alone. [b] Modified Kovats indices calculated for DB-Wax capillary column on the GC system. [c] Symbols: ne, not evaluated since the peak contained more than one component; nf, not found; tr, trace. [d] Ratios of the content in off-flavored skim milk powder against that in normal one. [e] Tentative identification by mass spectrum alone.

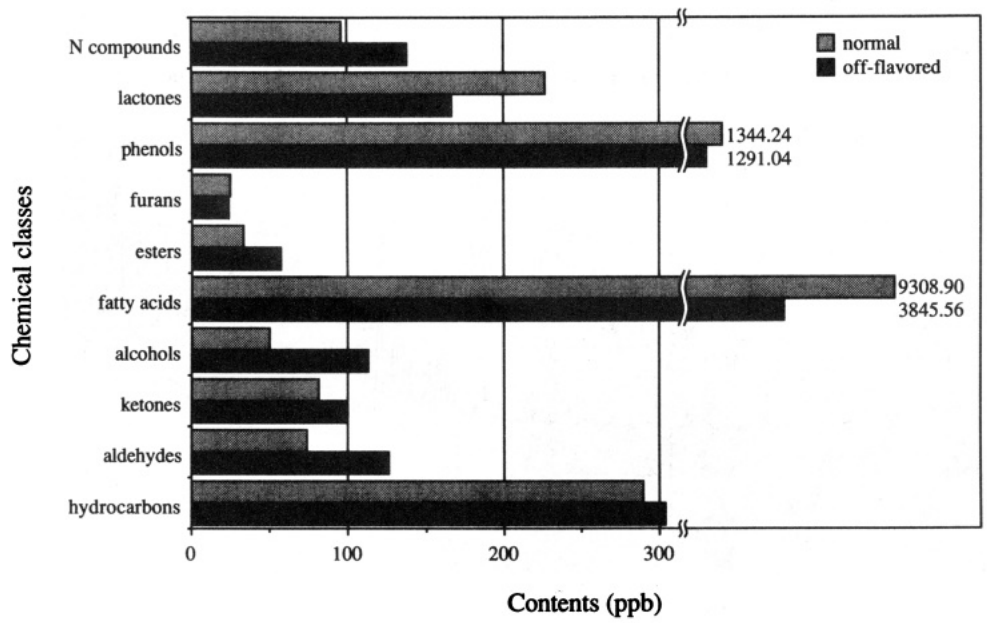

Figure 1. Levels of volatile compounds grouped according to chemical class in the normal and off-flavored skim milk powders.

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 with a split ratio of 20:1. The separation port was specifically 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. The separated compounds were eluted on a filter paper so that their odors could be sniffed or in a small glass tube (2 cm × 0.8 cm i.d.) for splitless injection into a GC–MS, with a drop of diethyl ether.

RESULTS AND DISCUSSION

The yields of total volatiles were 0.0007% (w/w, relative to the skim milk powder used) from normal skim milk and 0.0012% from off-flavored skim milk. In a previous paper (Shiratsuchi et al., 1994), we reported the identification and quantification of volatile compounds in a normal skim milk powder and this paper showed that nine peaks were newly identified in the off-flavored one alone (1A–9A; Table 1). Moreover, 14 peaks were newly detected in the off-flavored skim milk powder (not contained in this paper). The absolute levels of the volatile compounds from normal and off–flavored skim milk powders grouped in chemical classes are listed in Figure 1.

A comparison of the levels of chemical classes in both skim milk samples shows that fatty acids and lactones were contained at higher amounts in the normal skim milk powder; fatty acids were found significantly higher in the normal one, being more than 2 times higher. On the contrary, the contents of aldehydes, alcohols, esters, and N-containing compounds were higher in the off-flavored skim milk powder. The quantitative values of the aldehydes are listed in Table 1A. Several aldehydes such as octanal, (*E*)-2-octenal, (*E*)-2-nonenal, (*E,Z*)-2,6-nonadienal, salicylaldehyde, dodecanal, a tridecenal isomer, pentadecanal, and (*Z*)-9-octadecenal were significantly abundant in the off-flavored one. The level of (*Z*)-9-octadecenal was more than 10 times higher than that in normal skim milk powder. Moreover, undecanal was detected in the off-flavored skim milk alone. The aldehydes could contribute to off-flavor of the skim milk powder, because the off-flavored skim milk had a slightly aldehydic odor. Table 1B shows the difference of quantitative values of alcohols between normal and off-flavored skim milk. 1-Pentanol, linalool, 1-octanol, 1-dodecanol, and dihydrophytol were significantly abundant in the off-flavored skim milk powder. Furthermore, 2-methyl-1-propanol, 1-methyl-4-(methylethyl)-(*E*)-2-cyclohexenol, 1-tetradecanol, and 1-docosanol were detected in the off-flavored one alone. Even with the high odor thresholds, the difference of composition of the alcohols between off-

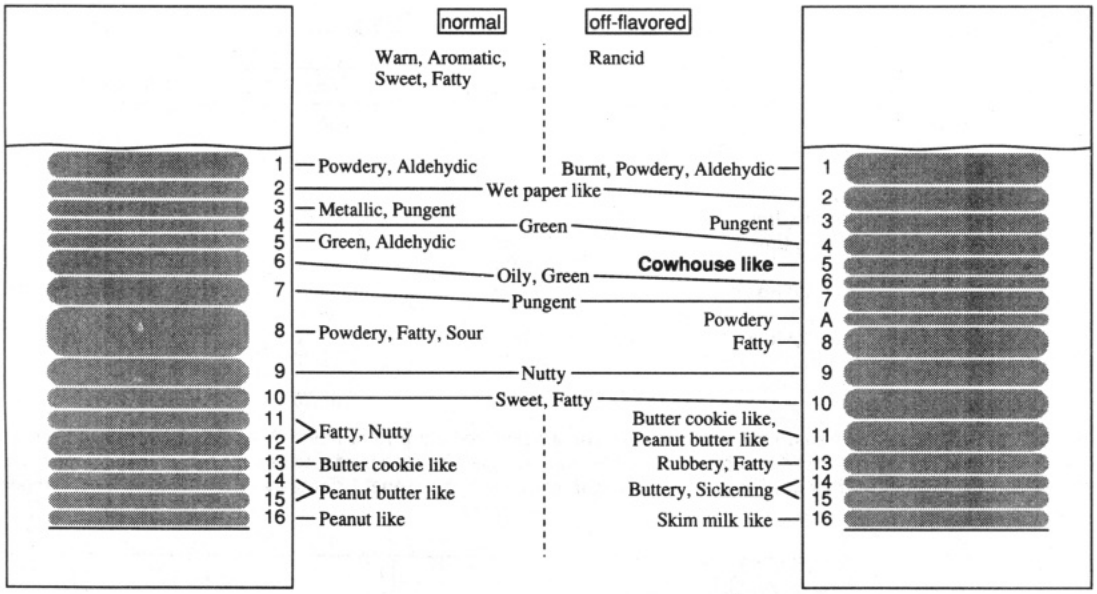

Figure 2. Thin-layer chromatograms of odor concentrate of normal and off-flavored skim milk powders.

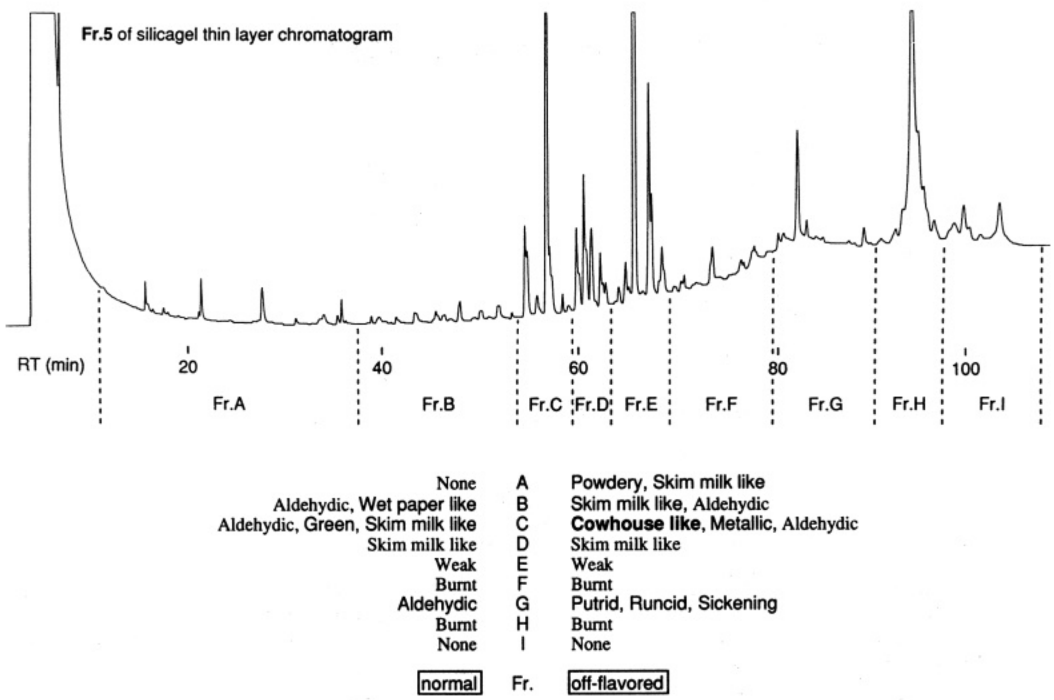

Figure 3. Sniffing of the fractions obtained by preparative gas chromatography on fraction 5 of the thin-layer chromatogram. For more information, see Materials and Methods.

flavored and normal skim milk may contribute to the difference of odor characteristics between the two skim milk samples. Table 1C lists the quantitative values of esters. Most of the esters were significantly higher in the off-flavored skim milk powder. In spite of their powerful odor, the esters may not contribute directly to the off-flavor of skim milk powder due to their odor characteristics. Table 1D lists the quantitative values of N-containing compounds. No component was significantly abundant in the normal skim milk powder. The levels of 1*H*-pyrrole, indole, and 2-pentadecanone *O*-methyloxime in the off-flavored skim milk were 3 times higher than those in the normal one. Generally, N-containing compounds have rlatively low odor thresholds and characteristic odor, and so the difference of the levels of these compounds could contribute to development of the off-flavor.

To clarify the compounds that directly contribute to the off-flavor, the fractionation of odor concentrate was carried out by thin-layer chromatography. Chromatograms of the odor concentrates from normal and off-flavored skim milk are shown in Figure 2. The odors of fractions 1, 3, 5, 8, 11, and 13–16 of off-flavored skim milk were different from those of a normal skim milk. Among these fractions, fraction 5 of the off-flavored skim milk had a cowhouse-like odor. However, the corresponding fraction of a normal one had green and aldehydic odor, and the former was similar to the odor attribute of off-flavor. Therefore, fraction 5 of normal and fraction 5 of off-flavored skim milk were then separated in nine portions by preparative GC (Figure 3). As shown in this figure, odors of fractions A–D were different between normal and off-flavored skim milk powders. That is, fraction A of the off-flavored one had powdery and skim-milk-like odors, fraction B had a skim-milk-like odors, fraction B had a skim-milk-like odor, fraction C has cowhouse-like and metallic odors, and fraction D had putrid, rancid, and

Table 2. Determination of Fraction C Compounds by Mass Chromatography

peak	compd	odor characteristics	content, ppb		ratio[a]
			normal	off-flavored	
1	tetradecanal	sickening, aldehydic	3.61	35.00	**9.7**
2	β-ionone	fragrant, haylike	0.01	0.76	**76.0**
3	benzothiazole	sulfuric, quinoline-like	0.06	2.24	**37.3**

[a] Ratios of the content in the off-flavored skim milk powder against that in normal one.

Table 3. Addition of Contributors for Sensory Confirmation of the Off-Flavor

compd	content, ppb
tetradecanal	70.0
β-ionone	1.6
benzothiazole	5.6

sickening odors. Because fraction C of the off-flavored one had a typical cowhouse-like odor, which was similar to the odor attribute of the off-flavor, it was considered that the off-flavor compounds were included in this fraction. Every peak of fraction C was further separated, and their odors were sniffed. Peak 1, tetradecanal, had a sickening and aldehydic odor; peak 2, β-ionone, a fragrant and haylike odor; and peak 3, benzothiazole, a sulfuric acid quinoline-like odor; they all had relatively strong odor. Since they were separated incompletely and could not be determined by FID, comparison of contents of these compounds between normal and off-flavored skim milk powders was carried out by mass chromatography in which each peak area was standardized on internal standard. As a result, their contents in the odor concentrate from off-flavored skim milk powder were about 10, 76, and 37 times higher than those in the normal one, respectively (Table 2). After addition of their authentic compounds to a normal skim milk solution (7% w/v), whose contents are shown in Table 3, the flavor of the skim milk solution was evaluated organoleptically by co-workers. It was confirmed that its flavor was very similar to that of the off-flavored one. Judging from these results, tetradecanal, β-ionone, and benzothiazole were considered to be the compounds contributing to the off-flavor of skim milk powder.

Although the cause for development of off-flavor has not yet been elucidated, an increase of off-flavor was not perceived in the off-flavored skim milk even after storage (for a few months, at room temperature). We are following up this cause by comparing differences of manufacturing processes. In our next paper, the correlation between organoleptic strength of the off-flavor and the contents of the compounds contributing to off-flavor will be discussed.

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