

Character Impact Odorants of High-Heat Skim Milk Powder Isolated by Simultaneous Distillation–Extraction

NORIAKI KOBAYASHI,^{*,†} YASUMICHI MIZOTA,[‡] KENJI KUMAZAWA,[†] AND OSAMU NISHIMURA[†]

Research Laboratory of Health Materials, Ogawa & Company, Ltd., 15-7 Chidori Urayasushi, Chiba 279-0032, Japan, and Food Research and Development Institute, Morinaga Milk Industry Company, Ltd., 5-1-83 Higashihara, Zamashi, Kanagawa 228-8583, Japan

To identify the character impact odorant of high-heat skim milk powder (HHSMP), a comparative study using ultrahigh-temperature (UHT) milk was performed. Aroma concentrate was prepared by column adsorption combined with simultaneous distillation–extraction. Aroma extract dilution analysis (AEDA) revealed 58 aroma peaks with flavor dilution (FD) factors ranging from 10 to 3000; from these, 41 compounds were identified and 7 compounds were tentatively identified (FD factor \geq 300). Among these HHSMP and UHT milk components, methyl 2-methyl-3-furyl disulfide and bis(2-methyl-3-furyl) disulfide, which appeared to be generated during the processing of each product, were identified. When the results of the AEDA of both samples were compared, it was considered that the characteristic aroma of HHSMP was not explained by a single compound but instead formed from a mixture of several types of compounds contained in common with the UHT milk. The contribution of these compounds to the aroma of HHSMP was confirmed by an aroma simulation experiment.

KEYWORDS: AEDA; bis(2-methyl-3-furyl) disulfide; methyl 2-methyl-3-furyl disulfide; high-heat skim milk powder; UHT milk; simultaneous distillation–extraction

INTRODUCTION

Aroma is one of the important factors that influence the quality of dairy products, and an original aroma can easily be changed by factors such as heating, hydrolysis, oxidation, and contamination with other aromas. The aroma of dairy products is known to differ according to the pasteurization method (1) and fodder consumed (2), and the flavor profile of dairy products seems to affect the diversity, features, and consumer preferences of each country. For instance, ultrahigh-temperature (UHT) pasteurized milk is said to be preferred over low-temperature–long-time milk (LTLT) in Japan (1) and accounts for 93% of the pasteurized milk consumed.

Skim milk powder is one of the main dairy products and is generally manufactured from raw milk through a continuous process entailing the removal of fat, pasteurization, concentration, and drying. The skim milk powder is classified into the following three types based on the whey protein nitrogen index (WPNI) (3), which is correlated to the pasteurization conditions: low heat (80 °C, 20 s), medium heat (105 °C, 2 s), and high heat (120 °C, 2 s) (4). On the basis of the removal of the

moisture and fat and the subsequent decrease in its volume and weight, the following advantages of skim milk powder have been demonstrated. First, the decrease in the volume enables the adjustment of the concentration and flavor upon dissolving. Second, during long-term storage, the low moisture and lipid contents prevent microbe proliferation and lipid oxidation. Skim milk powder with these advantages has been used for foods such as milk beverage, yogurt, ice cream, confectionery, and bread. Especially, high-heat skim milk powder (HHSMP) is known to be suitable for confectionery and bread. Generally, it is dissolved in water prior to use. When dissolved in water, HHSMP possesses a characteristic aroma that differs from that of UHT milk, and this is an important factor influencing consumer preference.

The aroma of skim milk powder has been the subject of extensive investigation. Shiratsuchi et al., for example, have reported a series of studies that analyzed the flavor compounds and cowhouse-like odor of skim milk powder (5–8). Recently, by using aroma extract dilution analysis (AEDA) (9), Karagül-Yüceer et al. reported the clarification of flavor compounds in U.S. skim milk powder derived using different heat treatments (10) and identified the major flavor compounds and attributes of stored skim milk powder (11). In a subsequent study, those authors found 2-methyl-3-furanthiol in medium-heat skim milk powder and high-heat–short-time pasteurized milk (HTST) as a related compound of vitamin-like attribute (12). In addition,

* Author to whom correspondence should be addressed (telephone +81-47-30-51-408; fax +81-47-30-51-424; e-mail kobayashi.noriaki@ogawa.net).

[†] Ogawa & Company.

[‡] Morinaga Milk Industry Company, Ltd.

Table 1. Preparation of Reference Samples for Sensory Evaluation

attribute	reference sample	preparation method
milky	3.5% UHT milk	dilute one-third of a sample with distilled water
fatty	4.4% high-fat milk	dilute half of a sample with distilled water
heated/sweet	canned evaporated milk	dilute $1/10$ of a sample with distilled water
papery/woody	brown paper towel	soak 2 g of brown paper towel in 100 mL of distilled water for 2 h, then remove
animal	unflavored gelatin	dissolve 5 g of gelatin in 100 mL of distilled water and melt in microwave oven; refrigerate until evaluation
brothy	extract of meat (dry)	dilute $1/1000$ of a sample with distilled water
mushroom-like/metallic	mushroom broth	remove sliced mushrooms from broth and dilute $3/100$ of a broth with distilled water
vitamin-like	thiamin nitrate	dissolve 0.05 g of thiamin nitrate in 100 mL of distilled water and adjust to pH 2.0 with 1 M hydrochloric acid

it was known that this compound has been isolated from whey protein concentrate for the first time in dairy products (13).

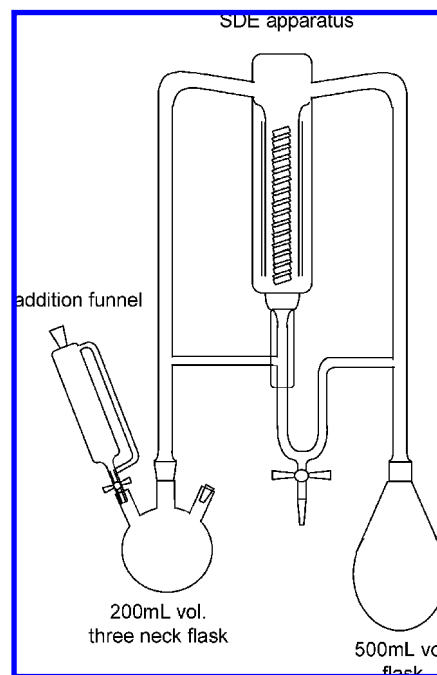
On the other hand, Karagül-Yüceer et al. reported that the compound corresponding to the cooked/sulfurous attribute was not identified in HHSMP, although the attribute exhibited a high score (10). The subsequent report by those authors had a vagueness that advanced the verification of the cooked/sulfurous attribute by using dimethyl sulfide not reported in skim milk powder (14). From these observations, the contribution of 2-methyl-3-furanthiol and dimethyl sulfide in cooked/sulfurous attribute was suggested. However, it is still uncertain whether this sensory attribute is expressed only by these compounds or whether there are other important contributors. The compound that exhibits the sensory attribute of HHSMP has therefore not been sufficiently clarified.

The purpose of this study was to identify the aroma compound that expresses the character of HHSMP as compared to UHT milk, which is representative of general dairy products in Japan.

MATERIALS AND METHODS

Food Samples. A 20 kg sample of HHSMP was obtained from Morinaga Milk Industry Co. The sample was received by overnight shipment after the quality of aroma, taste, and appearance had been checked. This HHSMP was packed in an aluminum–polyethylene laminated bag sealed and stored at $-25\text{ }^{\circ}\text{C}$ until required. The moisture content of HHSMP was 3.8%, and the WPNI was 1.2 mg/g. The HHSMP is produced in Japan. A UHT milk sample processed at $120\text{ }^{\circ}\text{C}$ for 3 s was purchased from a domestic market and used immediately. The composition of milk was 3.5% fat and 8.3% nonfat milk solid. Reference samples for the sensory evaluation (3.5% UHT milk, 4.4% high-fat milk, canned evaporated milk, unflavored gelatin, and boiled mushrooms) were purchased from a domestic market.

Chemicals. Authentic chemicals for co-injection into a gas chromatograph and for the sensory evaluations were purchased from the following reliable commercial sources: compounds 2–4, 14, 19, 22, 25, 29, 31, 32, 37, 41, 42, 44, 46, 51, 53, 54, and 57 (Table 3) and 2-methylpentanoic acid were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan); compounds 10, 17, 28, 36, and 38, and a

**Figure 1.** Modified SDE apparatus used in this study.

meat extract were obtained from Sigma-Aldrich Co. (St. Louis, MO); compounds 8 and 35 were obtained from Acros Organics (Geel, Belgium); compound 16 was obtained from Oxford Chemicals, Ltd. (Essex, U.K.); compounds 23, 27, 30, 34, 39, 40, 45, 47, and 50 were obtained from Soda Aromatic Co., Ltd. (Tokyo, Japan); and compound 24, 2-octanol, thiamin nitrate, and 99.5% ethanol were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). The following compounds were synthesized according to literature procedures: 2-acetyl-1-pyrroline (15), 2-acetyl-2-thiazoline (16), 3-methylnonane-2,4-dione (17), and (Z)-6-dodecen-4-olide (18).

Separation of the Volatiles from the Milk Sample. The heat step of simultaneous distillation–extraction (SDE) is known to generate byproducts such as amino–carbonyl reactant. Therefore, an aroma extract that excluded proteins and sugars of the precursor as much as possible using adsorption resin was prepared. Moreover, the aroma compounds were separated by using SDE from the nonvolatiles contained in the extract. A 100 g sample of the HHSMP was reconstituted in 900 mL of distilled water and blended using an electric mixer (M Technique Co., Ltd., Osaka, Japan) at 3600 rpm for 1 min. The internal standard compounds, 2-octanol (10 μg) and 2-methylpentanoic acid (200 μg), were added to HHSMP, and the sample was passed through a column packed with 60 mL of SEPABEADS SP70 (Mitsubishi Chemical Corp., Tokyo, Japan). The resin did the soaking preservation with 99.5% ethanol after more than 5 times of 99.5% ethanol washing by Soxhlet extractor. The resin packed into a 40 cm \times 3.0 cm i.d. glass column was used after substituting 99.5% ethanol by 400 mL or more of distilled water per 60 mL of resin. The adsorbed components were eluted with dichloromethane (400 mL), and the eluate was dried over anhydrous sodium sulfate. The volatiles in the eluate were separated from the nonvolatiles by SDE using a modified Likens–Nickerson apparatus attached to the addition funnel (Figure 1). The eluate was fed dropwise into the steam from the funnel (flow = approximately 10 mL/min), and the extraction was continued for 1 h. The temperature at the time of the distillation was from approximately $60\text{ }^{\circ}\text{C}$ (when the extract was dropped) to approximately $80\text{ }^{\circ}\text{C}$ (at the ends). The distillate was dried over anhydrous sodium sulfate and the solvent reduced in volume to approximately 5 mL using a rotary evaporator and then subsequently concentrated with a nitrogen stream to approximately 100 μL . Also, the same procedure was carried out on the UHT milk. The resulting concentrate was used as the sample for the AEDA and instrumental analysis. For further identification of the odorants, the methods described by Karagül-Yüceer et al. were used (10, 11). The procedures described above were repeated and the volatile fractions were collected (a total of 500 g of HHSMP was used).

The volatile fraction was extracted with sodium bicarbonate solution (0.5 M NaHCO₃; 3 × 50 mL) and washed with saturated brine (3 × 50 mL). The bottom (dichloromethane) phase containing the weak acidic/neutral/basic volatile was dried over anhydrous sodium sulfate, and the solvent was reduced in volume to approximately 5 mL using a rotary evaporator and then concentrated by a nitrogen stream to approximately 100 μL. The pooled aqueous phase containing the acidic volatile was acidified with 1 mol/L hydrochloric acid to pH 1.5–2.0 and then extracted three times with 50 mL of dichloromethane and dried over anhydrous sodium sulfate. The solvent was then reduced in volume to approximately 5 mL using a rotary evaporator and subsequently concentrated by a nitrogen stream to approximately 100 μL.

GC-MS. The system consisted of an Agilent model 6890N gas chromatograph coupled to an Agilent model 5973N series mass selective detector (MSD). The column used was a 60 m × 0.25 mm i.d. DB-Wax (Agilent Technologies, Inc.) with a film thickness of 0.25 μm. The oven temperature was programmed from 80 to 210 °C or from 40 to 210 °C at a rate of 3 °C/min. The injector temperature was 250 °C. Flow rate of the helium carrier gas was 26 cm/s. An injection volume of 1.0 or 0.2 μL was applied using the split (the split ratio was 1:30) or splitless technique. The MSD conditions were as follows: capillary direct interface temperature, 220 °C; ionization voltage, 70 eV (EI); mass range, 33–300 amu; and an ion source temperature of 150 °C. The GC-MS was operated in the total ion mode or in the selected ion monitoring (SIM) mode.

GC-Olfactometry (GC-O). The GC-O system consisted of an Agilent model 6850 gas chromatograph equipped with a thermal conductivity detector (TCD) and a sniffing port. A glass sniffing port was connected to a 0.5 m × 0.53 mm i.d. fused silica capillary tube from the outlet of the TCD. The capillary tube, which was housed in a 1.0 mm i.d. copper tube, was heated by a ribbon heater. On the sniffing port, moist air at ~100 mL/min was supplied to the odorant that eluted out of the sniffing port. The aroma concentrate (1.0 μL) was injected using a splitless technique. The column used was a 30 m × 0.25 mm i.d. DB-Wax with a film thickness of 0.25 μm (Agilent Technologies, Inc.). The oven temperature was programmed from 80 to 210 °C at a rate of 3 °C/min for all runs. The injector, detector, and sniffing port temperatures were 250, 300, and 250 °C, respectively. Flow rate of the helium carrier gas was 27 cm/s. GC-O was conducted by three assessors.

GC Atomic Emission Detector (GC-AED). This system consisted of an Agilent model 6890N gas chromatograph coupled to an Agilent model G2350A series atomic emission detector (AED). The column used was a 30 m × 0.25 mm i.d. DB-Wax (Agilent Technologies, Inc.) with a film thickness of 0.25 μm. The GC was used under the same conditions as the GC-MS. The AED conditions were as follows: transfer line and cavity temperatures were both 250 °C. The GC-AED employed hydrogen/oxygen reagent gas mixtures with detection at 193 nm, carbon; 181 nm, sulfur; and 174 nm, nitrogen.

AEDA. The original aroma concentrate of HHSMP and the UHT milk were both serially diluted with ethanol to 1:10, 1:30, 1:100, 1:300, 1:1000, and 1:3000. Each dilution level corresponded to a log₁₀ flavor dilution factor (FD factor): 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5, respectively. Aliquots (1.0 μL) of each fraction were analyzed by GC-O using the DB-Wax column.

Identification of Compounds. Each compound, excluding the exceptions mentioned below, was identified by comparing it with an authentic standard on the basis of the following criteria: matching retention index on the same column, mass spectra, and description of odor quality. Compounds **8**, **11**, **16**, **28**, **35**, and **40** were identified on the basis of the following criteria by comparing each with an authentic standard: matching retention index, monitoring selected ions of the compound, and odor quality. Compounds **5–7**, **18**, **43**, **52**, and **56** were tentatively identified on the basis of the matching retention index and mass spectra of the in-house library. For the heteroatomic aroma compounds, element information derived from the GC-AED was used for identification. The retention indices (RI) of GC-O and GC-AED analyses were calculated using an *n*-alkane ladder. The RI of GC-O was calculated from the elution time of the odorant by using the elution time of *n*-alkane. Each RI was subsequently confirmed by comparing it to that of the authentic standard.

Quantification of Components. Compounds having odor activity values (OAVs) (19) of one or more were quantified. The quantification of each compound was performed using the same GC-MS conditions as described above with the exception that the MSD was operated in the SIM mode. The response factors of the compounds selected for quantification were the ratios of the peak area of the mixture containing a known amount of compound and the internal standards. The recovery rate of the selected compound was obtained by collecting a known amount of compound and internal standards that were added to odor-free water using the same method described above. The selected ions used for the SIM, response factors, and recovery rates of the selected compounds are shown in **Table 3**. 2-Methylpentanoic acid was used as the internal standard for the acids, and 2-octanol was used as that for other compounds. The quantitative values were finally obtained by multiplying the response factor and the recovery rate by the interpolation value.

Sensory Profiles of Dairy Samples. The sensory profiles of the dairy samples were determined by 10 experienced assessors. The assessors, who had completed more than 60 h of a training course in many aspects of sensory analysis (recognition, description, and discrimination tests), were recruited from the laboratory of Ogawa & Co., Ltd. The assessors were aged from 25 to 52 and included four females and six males. The aroma attributes defined by the assessors are listed in **Table 1**. The terms used to describe the aroma attributes as papery, brothy, and vitamin-like were also referred to the literature (10, 11). The assessors were trained in the reference samples shown in **Table 1**. The assessors, who were asked to rank the intensity of the odor attributes, evaluated them orthonasally. The respective odor intensity was scored on a scale of 1 (considerably weak) to 7 (strong). The dairy samples used for evaluation were HHSMP, adjusted to 8.6% nonfat milk solids (equivalent to UHT milk) and purchased UHT milk. Each sample was assigned a three-digit random number and presented in 30 mL covered plastic cups at 25 °C. The statistical analyses were performed using SPSS version 11.0J or Microsoft Excel 2002.

Aroma Simulation Experiment. An aroma model of HHSMP was prepared by dissolving the following eight compounds in 1 L of low-odor reconstituted HHSMP (low-odor matrix). For the aroma simulation experiment, the low-odor matrix was necessary to exclude the influence of the other aromas. The low-odor matrix was prepared by the column adsorption method. A concrete method was as follows. Eighty-six grams of HHSMP was dissolved in 1 L of odor-free distilled water. The reconstituted HHSMP was passed through a column packed with 60 mL of SEPABEADS SP70. The column effluent was collected, and odor-free nitrogen gas was blown into the effluent to replace oxygen for 5 min. This low-odor matrix was adjusted at the time of use and used after being verified as low-odor. The Brix scale values before and after the adjustment were, respectively, 8.9 and 8.5. The aroma model contained 1 μg of bis(2-methyl-3-furyl) disulfide, 300 μg of 3-ethylphenol, 4.7 mg of nonanal, 10 μg of methyl 2-methyl-3-furyl disulfide, 100 μg of (*E,E*)-2,4-decadienal, 5.9 mg of 5-dodecanolide, 10.8 mg of decanoic acid, and 11.4 mg of dodecanoic acid in 100 mL of ethanol. An additional amount of the aroma model was empirically adjusted until the reconstituted aroma exhibited a similarity to the reconstituted HHSMP. Addition amount of aroma model was 1.5 mL to 1 L of the low-odor matrix.

RESULTS AND DISCUSSION

Sensory Evaluation. The purpose of this comparative sensory evaluation was to identify the characteristic attribute of HHSMP as compared to UHT milk, which is representative of general dairy products in Japan. Although we searched the literature regarding these aromas, we were unable to find any previous studies that have compared the sensory attributes of HHSMP and UHT milk. The sensory properties of HHSMP and the UHT milk were evaluated using the evaluation terms (**Table 1**) collected from the assessors in advance. The mean value of the sensory score for both samples is shown in **Figure 2**. The sensory scores were analyzed using a *t* test; therefore, the results reveal the properties of both samples. The sensory evaluation

Table 2. Odor Compounds in High-Heat Skim Milk Powder and Ultrahigh Temperature Milk (\log_{10} FD \geq 1.0)

no.	odorant	odor descriptor	LRI ^b	confirmation	\log_{10} FD ^e	
					HHSMP ^f	UHT ^g
1	2-acetyl-1-pyrroline	popcorn-like	1347	LRI, odor, MS, AED ^c	1.5	1.5
2	nonanal	fatty, dusty	1398	LRI, odor, MS	1.5	1.5
3	1-octen-3-ol	mushroom-like, fatty	1442	LRI, odor, MS	1.5	2.0
4	methional	boiled potato	1456	LRI, odor, MS, AED ^c	1.5	2.0
5	(E,Z)-2,4-heptadienal ^a	green	1466	LRI, odor, MS	1.5	1.5
6	3,5-octadien-2-one ^a	citrus-like	1582	LRI, MS	1.0	1.5
7	(E,Z)-2,4-nonadienal ^a	fatty, citrus-like	1657	LRI, odor, MS	nd ^h	1.5
8	methyl 2-methyl-3-furyl disulfide	cooked, canned corn-like	1670	LRI, odor, SIM ^d , AED ^c	2.5	2.5
9	unknown	rice bran-like, bitter	1681		1.5	nd
10	(E,E)-2,4-nonadienal	fatty, oily	1702	LRI, odor, MS	1.5	1.5
11	3-methylnonane-2,4-dione	green, fishy	1720	LRI, odor, SIM ^d	2.0	1.5
12	unknown	fatty, oily	1729		nd	2.0
13	2-acetyl-2-thiazoline	popcorn-like	1756	LRI, odor, MS, AED ^c	1.0	2.0
14	(E)-2-undecenal	fatty, milky	1761	LRI, odor, MS	nd	1.5
15	unknown	roast, cooked	1786		1.5	1.5
16	furfuryl methyl disulfide	roast, green	1800	LRI, odor, SIM ^d , AED ^c	1.5	1.5
17	(E,E)-2,4-decadienal	deep-fried, fatty	1808	LRI, odor, SIM ^d	1.5	1.5
18	tridecanal ^a	oily, fatty	1826	LRI, MS	1.5	2.0
19	hexanoic acid	soapy, cheesy	1873	LRI, odor, MS	1.5	1.5
20	unknown	coconut-like	1884		nd	1.5
21	unknown	roast, green	1919		1.5	1.5
22	benzothiazole	rubber-like	1947	LRI, odor, MS, AED ^c	2.0	2.0
23	5-octanolide	coconut-like	1963	LRI, odor, MS	nd	1.5
24	phenol	phenol-like	2001	LRI, odor, MS	1.5	1.5
25	2-methylphenol	smoky	2009	LRI, odor, MS	1.5	1.5
26	unknown	rice bran-like	2017		2.0	nd
27	4-nonanolide	peach, fruity	2022	LRI, odor, MS	nd	1.5
28	4-hydroxy-2,5-dimethyl-3(2H)-furanone (Furaneol)	sweet, cotton candy	2034	LRI, odor, SIM ^d	1.5	2.0
29	octanoic acid	waxy, soapy	2065	LRI, odor, MS	1.5	1.5
30	5-nonanolide	coconut-like	2078	LRI, odor, MS	nd	1.5
31	4-methylphenol	cowy, smoky	2087	LRI, odor, MS	2.5	2.5
32	3-methylphenol	cowy, smoky	2103	LRI, odor, MS	1.5	2.0
33	unknown	peach, sweet	2131		nd	1.5
34	4-decanolide	peach, fruity	2140	LRI, odor, MS	nd	1.5
35	bis(2-methyl-3-furyl) disulfide	vitamin, rice bran-like	2147	LRI, odor, SIM ^d	2.0	2.0
36	4-ethylphenol	barny, spicy	2164	LRI, odor, MS	1.5	1.5
37	nonanoic acid	waxy, soapy	2178	LRI, odor, MS	1.5	1.5
38	3-ethylphenol	barny, spicy	2183	LRI, odor, MS	1.5	1.5
39	5-decanolide	coconut-like, butter	2188	LRI, odor, MS	3.0	3.5
40	3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon)	spicy, rubber-like	2200	LRI, odor, SIM ^d	1.5	nd
41	2-aminoacetophenone	foxy, plaster-like	2207	LRI, odor, MS	3.0	3.0
42	methyl anthranilate	smoky	2237	LRI, odor, MS	1.0	1.5
43	α -cadinol ^a	woody	2264	LRI, MS	2.0	1.5
44	decanoic acid	waxy, soapy	2283	LRI, odor, MS	2.0	2.0
45	5-undecanolide	coconut-like, butter	2307	LRI, odor, MS	1.0	2.5
46	9-decenoic acid	metallic, soapy	2362	LRI, odor, MS	1.5	1.5
47	4-dodecanolide	paech, sweet-floral	2368	LRI, odor, MS	2.0	2.5
48	unknown	rice bran-like	2377		1.5	1.5
49	(Z)-6-dodecen-4-olide	sweat, berry, sweet-floral	2388	LRI, odor, MS	3.0	3.5
50	5-dodecanolide	coconut-like, butter	2420	LRI, odor, MS	1.5	3.0
51	indole	mothball-like, smoky	2434	LRI, odor, MS	1.5	2.0
52	(Z,Z)-6,9-dodecadien-4-olide ^a	powdery, sweet-floral	2470	LRI, MS	1.5	1.5
53	skatol	fecal, mothball-like	2476	LRI, odor, MS	1.0	2.0
54	dodecanoic acid	waxy, soapy	2511	LRI, odor, MS	2.0	2.0
55	unknown	citrusy, acid-like	2518		nd	2.0
56	11-dodecenoic acid ^a	waxy, soapy	2540	LRI, MS	1.5	1.5
57	vanillin	vanilla	2554	LRI, odor, MS	1.5	nd
58	unknown	powdery, smoky	2596		2.0	2.0

^a Tentatively identified compound. ^b Linear retention index on DB-Wax column. The retention index (RI) of GC-O was calculated from the elution time of the odorant by using the elution time of *n*-alkane. Each RI was subsequently confirmed by comparing it to that of the authentic standard. ^c Atomic emission detector. ^d Selected ion monitoring. ^e Flavor dilution factor. ^f High-heat skim milk powder. ^g UHT milk. ^h Not detected.

results for HHSMP were significantly high in animal, brothy, metallic/mushroom-like, and vitamin-like attributes ($p < 0.001$); these sensory attributes indicated the character of HHSMP. In contrast, the sensory evaluation results for the UHT milk were significantly high in milky and fatty attributes ($p < 0.001$); these sensory attributes indicated the character of the UHT milk. Karagül-Yüceer reported that medium- and high-heat SMP indicated high in cooked/sulfurous attribute (10). This was a

tendency different from that of low-heat SMP. The reference sample showing this attribute was the heated skim milk at 85 °C for 30 min. In addition, Drake et al. reported that HHSMP had received the influence of the potato/brothy attribute from the result of the principal component analysis (20). Both cooked/sulfurous and potato/brothy features appeared to be common to the aroma characteristic of sulfur compounds. In the present study, it is thought that the high score in HHSMP of brothy

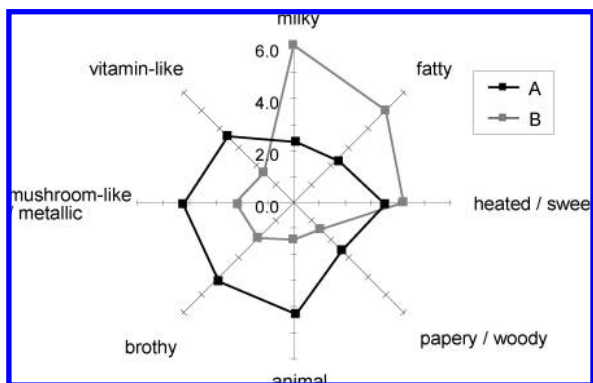


Figure 2. Sensory profile of high-heat skim milk powder (A) and UHT milk (B).

and vitamin-like attributes relates to this fact. Thus, the characteristic aroma attributes of HHSMP were confirmed by the sensory evaluation by comparison with the UHT milk, and it was suggested that these attributes with high scores explain the roughly overall characteristics of HHSMP.

AEDA. Artifacts are known to be generated by the heating step of SDE (21, 22). Moreover, Mottram et al. demonstrated a decreased recovery of thiol owing to the interaction between thiols such as 2-methyl-3-furanthiol (2-MFT) and proteins such as egg albumin (23). In the present study, aroma compounds were separated from the proteins and the sugars of the reaction source by using adsorption resin and isolated from nonvolatiles such as fat by using SDE. Bicchi et al. applied a similar two-step procedure to analyze the aroma of honey (24). These authors emphasized the importance of extracting aroma compounds from the matrix before heating. In addition, after a preliminary extraction of furanthiol, Mottram et al. demonstrated an excellent recovery by separating with SDE (23). The aroma concentrate that we obtained exhibited the feature accepted in HHSMP and UHT milk. Therefore, we assumed that artifact generation and the interaction of the protein had been avoided by this method.

AEDA was used to objectively determine the aroma compounds of HHSMP. The AEDA of the aroma concentrates of HHSMP and UHT milk revealed that of the 48 and 54 aroma peaks, respectively, in the FD factors ranging from 10 to 3000, 44 were common to both of them (Table 2). Thirty-three aroma compounds among the 48 peaks that had been identified this time were initially identified by a GC-MS analysis. The remaining 15 peaks were presumed to be a complicated mixture or too low to be detected quantitatively. Neutral/basic and acid fractionations of the volatile compounds permitted the identification or tentative identification of 9 compounds among those that were present in the mixture (6, 7, 10, 13, 14, 17, 18, 42, and 53). Moreover, using selected ion monitoring, 6 trace compounds (8, 11, 16, 27, 35, and 39) were identified by comparison with the retention indices and odors of the authentic standards. The compound including nitrogen and sulfur was identified by using AED. These compounds were common with the result of AEDA applied to HHSMP (10) and UHT milk (25–27) in many points.

In the present study, methyl 2-methyl-3-furyl disulfide, furfuryl methyl disulfide, and bis(2-methyl-3-furyl) disulfide were found not only in HHSMP but also in UHT milk. The methyl 2-methyl-3-furyl disulfide and bis(2-methyl-3-furyl) disulfide presented a canned corn-like, rice bran-like, and vitamin-like odor.

On the basis of previously reported observations, the existence of these compounds was not surprising. For instance, in the study

Table 3. Masses (m/z) Used, Response Factor, and Recovery Rate of the Selected Compounds

compound ^a	selected ions	response factor ^d	recovery rate ^e
2-acetyl-1-pyrroline	83, ^c 111	1.7	0.6
nonanal	57, 70 ^c	2.6	0.9
methyl 2-methyl-3-furyl disulfide	113, 160 ^c	0.9	0.6
(<i>E,E</i>)-2,4-nonadienal	67, 81 ^c	1.3	1.0
3-methylnonane-2,4-dione	43, 99 ^c	0.9	1.1
(<i>E,E</i>)-2,4-decadienal	81, ^c 95	1.3	1.0
hexanoic acid	60, 73 ^c	0.8	1.0
benzothiazole	108, 135 ^c	0.6	1.1
octanoic acid	60, ^c 73	0.9	2.0
4-methylphenol	77, 108 ^c	0.9	0.9
bis(2-methyl-3-furyl) disulfide	113, 226 ^c	0.9	1.1
3-ethylphenol	107, 122 ^c	0.8	1.1
5-decanolide	71, 99 ^c	1.1	0.5
2-aminoacetophenone	120, 135 ^c	1.1	0.9
decanoic acid	60, ^c 73	0.7	2.2
4-dodecanolide	85 ^c	0.9	1.1
(<i>Z</i>)-6-dodecen-4-olide	85 ^c	0.9	1.1
5-dodecanolide	71, 99 ^c	0.9	1.0
dodecanoic acid	60, ^c 73	0.8	2.6
2-octanol ^b	45 ^c	1.0	
2-methylpentanoic acid ^b	74 ^c	1.0	

^a The compounds for quantification were selected from the compounds that had an OAV of 10 or more. ^b Internal standard. ^c Selected ion for quantification. ^d Response factors were the ratio between the peak area of a known amount compound and that of an internal standard. ^e Recovery rate was obtained by an addition collection experiment.

of Drake et al. (12), the compounds corresponding to vitamin-like attribute identified in evaporated milk were not found in evaporated milk. On the other hand, on the basis of the sensory evaluation of HTST milk and medium-heat SMP, the vitamin-like attribute was not accepted, although 2-MFT that may correspond to the vitamin-like attribute was detected among these samples. Therefore, the possibility that the vitamin-like attribute identified in evaporated milk corresponds to a compound other than 2-MFT was suggested. Moreover, Karagül-Yüceer et al. reported that the cooked/sulfurous attribute of HHSMP indicated a high score (10). The compound corresponding to this attribute was not identified in this study or in a related study (14). In addition, the reference sample of cooked/sulfurous attribute was skim milk heated at 85 °C for 30 min (10). The methyl 2-methyl-3-furyl disulfide and bis(2-methyl-3-furyl) disulfide detected in the present study have the feature of boiled milk as described by Bading (28). Thus, such a compound might contribute in part to the cooked/sulfurous attributes in previous studies. Furthermore, in the present study, brothy and vitamin-like attributes were identified in UHT milk. It is assumed that these relate to the canned corn-like attribute of UHT milk reported by Bendall et al. (29). These observations might be explained by the use in the present study of HHSMP and UHT milk with a heat history that is higher than that of HTST milk.

Furthermore, the reasons for considering that these compounds were generated are as follows. Mottram et al. reported the generation of 2-MFT and its derivatives from diacetyl and H₂S (30). However, the 2-mercapto-3-pentanone of the major reactant was not detected in the present study. Thus, it is expected that the possibility of the mutual reaction of the aroma compounds by SDE-generated 2-MFT and their derivatives is low. Additionally, the maintenance of the feature of the original sample in the aroma concentrate obtained by the above-mentioned method indicates the low possibility of these disulfides' generation with SDE. Considering heating in the produc-

tion process of dairy products such as pasteurization, condensation, and drying, the generation of 2-MFT with the pyrolysis of thiamin and/or via the route reported by Mottram et al. (31) and the subsequent formation of disulfides are suspected. Because 2-MFT was observed in HTST milk and medium-heat SMP (12), there is a possibility that more oxidized compounds were generated in UHT milk and HHSMP during the processing of each product. Moreover, Mottram et al. (23) and Hofman et al. (32) reported that the dimerization of the 2-MFT advanced under low temperature (−15 and 6 °C, respectively). The disulfides observed in the present study might have been generated from 2-MFT in the aroma extract. As mentioned above, the disulfides observed in the present study are presumed not to have been artifacts generated by heating in the SDE process and could have been generated in the milk sample.

The FD factors obtained from the AEDA of HHSMP and UHT milk were compared. First, a feature of the aroma in HHSMP is that the FD factors of lactones were lower than those of the UHT milk. In particular, the FD factors of HHSMP were $1/_{10}$ or less than those of the UHT milk for lactones of compounds 23, 27, 30, 34, 45, and 50 (Table 2). It is known that these lactones have a creamy and milky character, and the low evaluation score of the milky attribute corresponded to the low FD factor of the lactones in HHSMP (Figure 2). In the production process of skim milk powder, the removal of fat and the dispersion of aroma while drying could have influenced these results. Second, 3-hydroxy-4,5-dimethyl-2(5H)-furanone (40) and vanillin (57) were detected only in HHSMP (Table 2). However, because the FD factor of these compounds was 1.5, which was not very high, it appears that their influence on HHSMP was low. Third, numerous compounds were found to be common between HHSMP and the UHT milk—in these cases, the FD factors for both samples were almost equal. On the basis of the comparative results of the AEDA, the characteristic features of HHSMP were considered to be conferred by two or more aroma compounds that are contained in common with the UHT milk. An aroma simulation experiment was conducted to confirm this supposition.

Calculation of OAV. To determine the contribution of the characteristic aroma of HHSMP in the matrix, the OAV was calculated using the procedure reviewed by Grosch (33). The OAV is the value obtained by dividing the concentration of the aroma by the threshold value (in the matrix), whereas the FD factor is a relative measurement of the extract in the vapor phase. The OAV is an index that is indicative of the relative contribution of the aroma in the food matrix. Therefore, an aroma compound with a high OAV can be assumed to be an important component of the characteristic flavor.

When OAV was calculated, a quantitative analysis was necessary. Ions used for SIM, response factors, and recovery rates of 19 compounds are shown in Table 3. The quantification results are shown in Table 4. In the quantitative analysis using SDE, it was necessary to pay attention to the recovery rates of aroma compound. Núñez and Bemelmans reported a poor recovery rate when using SDE (34). In the present study, the tendency for low recovery rates of acids was observed (Table 3), and the quantification value was corrected by the recovery rate of water. Because the recovery rate of the compound depends on the matrix, the quantification value that applied the recovery rate from water might be slightly different from that of the matrix.

On the basis of the above calculation, 18 compounds that exhibited an OAV > 1 were identified (Table 4). Karagül-Yüceer et al. indicated that the threshold values of free fatty

Table 4. Concentration and Odor Threshold of Odorants in High-Heat Skim Milk Powder and Their OAVs

compound	concn ^a ($\mu\text{g/L}$)	SD ^b ($\mu\text{g/L}$)	threshold ($\mu\text{g/L}$) ^c	OAV ^h
bis(2-methyl-3-furyl) disulfide ⁱ	0.01	0.001	0.00002 ^d	500
3-ethylphenol	2.9	0.1	0.05 ^d	58
nonanal	47	6	1 ^d	47
methyl 2-methyl-3-furyl disulfide ⁱ	0.11	0.01	0.004 ^d	28
(<i>E,E</i>)-2,4-decadienal	1.0	0.1	0.07 ^d	14
5-dodecanolide	58	1	4.6 ^g	13
decanoic acid	108000	10800	10000 ^d	11
dodecanoic acid	114000	17600	10000 ^e	11
(<i>E,E</i>)-2,4-nonadienal	0.09	0.01	0.01 ^f	9
hexanoic acid	45000	520	5000 ^d	9
(<i>Z</i>)-6-dodecen-4-olide	5.4	0.4	0.7 ^g	8
2-aminoacetophenone	1.5	0.1	0.2 ^d	8
octanoic acid	116000	10100	19000 ^f	6
2-acetyl-1-pyrroline	0.6	0.2	0.1 ^d	6
4-dodecanolide	18	1	7 ^d	3
5-decanolide	55	2	30 ^g	2
3-methylnonane-2,4-dione ⁱ	0.04	0.001	0.03 ^d	1
4-methylphenol	2.8	0.4	2.7 ^d	1

^a Mean value obtained by analyzing three different samples of the same batch.

^b Standard deviation of the mean value of the concentration. ^c Odor threshold in water. ^d Rychlik et al. (35). ^e Leffingwell et al. (36). ^f Van Gemert (37). ^g Karagül-Yüceer et al. (38). ^h OAVs were calculated by dividing the concentrations by the respective odor thresholds in water. ⁱ It was confirmed that the S/N ratios of the trace compounds were approximately 5.

acids were higher in reconstituted skim milk than those in water (14). On the basis of a previously reported observation, the calculation of OAV was done by using the especially high threshold reported. Bis(2-methyl-3-furyl) disulfide with a value of 500 had the highest OAV. Additionally, 3-ethylphenol, nonanal, methyl 2-methyl-3-furyl disulfide, (*E,E*)-2,4-decadienal, 5-dodecanolide, decanoic acid, and dodecanoic acid had OAVs of 10 or more, and these are proposed to be important odor-active compounds for the entire aroma.

Moreover, the result of the present study was similar to that of Karagül-Yüceer et al. for the contributions of acids and (*E,E*)-2,4-decadienal (14). The omission test of these authors pointed out that 5-decanolide and 2-aminoacetophenone did not influence reproduction of the entire aroma. These compounds in the present study did not show the OAVs anticipated compared with the height of the FD factor, and it was supposed that the importance of these compounds to the entire aroma was not high.

Reconstitution Experiment. The aroma simulation experiment was conducted to determine whether the characteristic aroma of HHSMP confirmed by the sensory evaluation is attributable to the odor-active compounds that had the highest OAVs. The experiment was conducted as follows. The compounds for the aroma model were selected from compounds that would have significant influences as indicated by OAVs above 10. An aroma model comprising the following eight compounds was prepared: bis(2-methyl-3-furyl) disulfide, 3-ethylphenol, nonanal, methyl 2-methyl-3-furyl disulfide, (*E,E*)-2,4-decadienal, 5-dodecanolide, decanoic acid, and dodecanoic acid. An aroma simulation model was prepared by adding the aroma model to a low-odor matrix prepared from HHSMP. The aroma simulation model and the reconstituted HHSMP (as the reference sample) were used for the comparative sensory evaluation. The sensory evaluation was conducted using the following terms that would roughly explain the overall feature of HHSMP; animal, brothy, mushroom-like, vitamin-like, and papery. Sensory evaluation was performed twice, and each set of data was

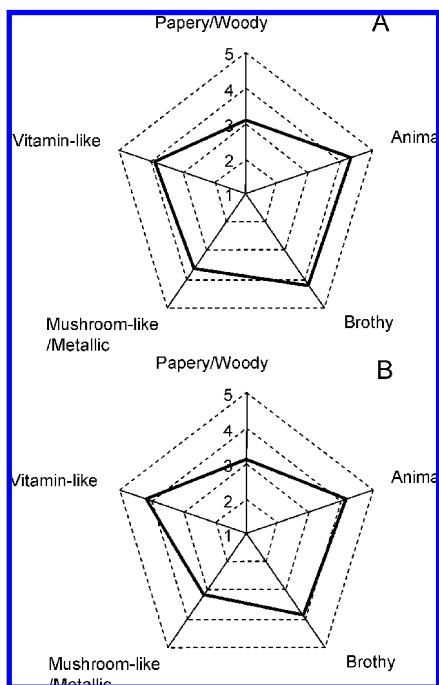


Figure 3. Sensory profile of the aroma simulation model (A) and reconstituted high-heat skim milk powder (B).

treated independently. The average score of each attribute is shown in **Figure 3**.

The test for equality did not indicate a significant difference between the samples [$\chi^2(4) = 1.23, p = 0.87$]. Furthermore, a statistical analysis of each attribute failed to detect any significant differences, although a slightly high score for the metallic/mushroom-like attribute was demonstrated in the aroma simulation model. In addition, the sensory attributes of HHSMP were interpreted to correspond with the compound that showed high OAV. Specifically, it appeared that the cooked, canned corn-like, rice bran-like, and vitamin-like odor of methyl 2-methyl-3-furyl disulfide and bis(2-methyl-3-furyl) disulfide corresponded to the brothy and vitamin-like attributes. It is guessed that this fact is related to the description of the above-mentioned (28, 29). Successively, it appeared that the fatty odor of (*E,E*)-2,4-decadienal corresponded to the papery and mushroom/metallic attribute in the threshold level, and the barny odor of 3-ethylphenol and the acid odor of decanoic acid and dodecanoic acid corresponded to the animal attribute. Thus, the correspondence of each compound selected on the basis of OAV and each sensory attribute was observed; thus, the purpose of the experiment was achieved. However, it appears that a triangle test and/or a matching test would be necessary for an accurate confirmation of the similarity of the entire aromas.

The possibility for eight compounds, including the 2-MFT derivative that exhibits the feature of HHSMP, was suggested from these results. In the future, there might be a necessity for using the latest technique, such as SAFE, to characterize these compounds (generation mechanism, deterioration mechanism, etc.) in dairy products.

LITERATURE CITED

- (1) Iwatsuki, K.; Mizota, Y.; Sumi, M.; Sotoyama, K.; Tomita, M. Effect of pasteurization and UHT processing conditions on the sensory characteristics of milk. *Nippon Shokuhin Kagaku Kogaku Kaishi* **1999**, *46*, 535–542.
- (2) Bendall, J. G. Aroma compounds of fresh milk from New Zealand cows fed different diets. *J. Agric. Food Chem.* **2001**, *49*, 4825–4832.

- (3) Greenbank, G. R.; Steinbarger, M. C.; Deysher, E. F.; Holm, G. E. The effect of heat treatment of skim milk upon the baking quality of the evaporated and dried products. *J. Dairy Sci.* **1927**, *10*, 335–342.
- (4) Takahashi, K.; Ando, S. In *Science of Milk*; Kaminogawa, S., Ed.; Asakura Publishing Co, Ltd.: Tokyo, Japan, 1996; p 177.
- (5) Shiratsuchi, H.; Shimoda, M.; Imayoshi, K.; Noda, K.; Osajima, Y. Volatile flavor compounds in spray dried skim milk powder. *J. Agric. Food Chem.* **1994**, *42*, 984–988.
- (6) Shiratsuchi, H.; Shimoda, M.; Imayoshi, K.; Noda, K.; Osajima, Y. Off-flavor compounds in spray dried skim milk powder. *J. Agric. Food Chem.* **1994**, *42*, 1323–1327.
- (7) Shiratsuchi, H.; Yoshimura, Y.; Shimoda, M.; Noda, K.; Osajima, Y. Contributors to sweet and milky odor attributes of spray-dried skim milk aroma. *J. Agric. Food Chem.* **1995**, *43*, 2453–2457.
- (8) Shiratsuchi, H.; Yoshimura, Y.; Shimoda, M.; Noda, K.; Osajima, Y. Objective evaluation of off-flavor in spray dried skim milk powder. *Nippon Shokuhin Kagaku Kogaku Kaishi* **1996**, *43*, 7–11.
- (9) Ullrich, F.; Grosch, W. Identification of the most intense volatile flavour compounds formed during autoxidation of linoleic acid. *Z. Lebensm. Unters. Forsch.* **1987**, *184*, 277–282.
- (10) Karagül-Yüceer, Y.; Drake, M. A.; Cadwallader, K. R. Aromatic components of nonfat dry milk. *J. Agric. Food Chem.* **2001**, *49*, 2948–2953.
- (11) Karagül-Yüceer, Y.; Cadwallader, K. R.; Drake, M. A. Volatile flavor components of stored nonfat dry milk. *J. Agric. Food Chem.* **2002**, *50*, 305–312.
- (12) Drake, M. A.; Miracle, R. E.; Caudle, A. D.; Cadwallader, K. R. Relating sensory and instrumental analysis. In *Sensory Directed Flavor Analysis*; Marsili, R., Ed.; CRC Press: New York, 2006; pp 23–53.
- (13) Whetstone, M. E. C.; Croissant, A. E.; Drake, M. A. Characterization of dried whey protein concentrate and isolate flavor. *J. Dairy Sci.* **2005**, *88*, 3826–3839.
- (14) Karagül-Yüceer, Y.; Drake, M. A.; Cadwallader, K. R. Evaluation of the character impact odorants in skim milk powder by sensory studies on model mixtures. *J. Sensory Stud.* **2004**, *19*, 1–13.
- (15) Buttery, R. G.; Ling, L. C.; Juliano, B. O.; Turnbaugh, J. G. Cooked rice aroma and 2-acetyl-1-pyrroline. *J. Agric. Food Chem.* **1983**, *31*, 823–826.
- (16) Cerny, C.; Grosch, W. Evaluation of potent odorants in roasted beef by aroma extract dilution analysis. *Z. Lebensm. Unters. Forsch.* **1992**, *194*, 322–325.
- (17) Guth, H.; Grosch, W. 3-Methylnonan-2,4-dione—an intense odour compound formed during flavour reversion of soya-bean oil. *Fat Sci. Technol.* **1989**, *91*, 225–230.
- (18) Maurer, B.; Hauser, A. Identification and synthesis of new γ -lactones from tuberose absolute (*Polianthes tuberosa*). *Helv. Chim. Acta* **1982**, *65*, 462–476.
- (19) Schieberle, P. New developments in methods for analysis of volatile flavor compounds and the precursor. In *Characterization of Foods: Emerging Methods*; Gaonkar, A. G., Ed.; Elsevier Science: Amsterdam, The Netherlands, 1995; pp 403–431.
- (20) Drake, M. A.; Karagül-Yüceer, Y.; Cadwallader, K. R.; Civile, G. V.; Tong, P. S. Determination of the sensory attributes of dried milk powders and dairy ingredients. *J. Sensory Stud.* **2003**, *18*, 199–216.
- (21) Chaintreau, A. Simultaneous distillation-extraction: from birth to maturity—review. *Flavour Fragrance J.* **2001**, *16*, 136–148.
- (22) Siegmund, B.; Leitner, E.; Mayer, I.; Pfannhauser, W.; Farkaš, P.; Sádecká, J.; Kováč, M. 5,6-Dihydro-2,4,6-trimethyl-4H-1,3,5-dithiazine—an aroma-active compound formed in course of the Likens–Nickerson extraction. *Z. Lebensm. Unters. Forsch.* **1997**, *205*, 73–75.
- (23) Mottram, D. S.; Szauman-Szumski, C.; Dodson, A. Interaction of thiol and disulfide flavor compounds with food components. *J. Agric. Food Chem.* **1996**, *44*, 2349–2351.

- (24) Bicchi, C.; Belliardo, F.; Frattini, C. Identification of the volatile components of some Piedmontese honeys. *J. Apic. Res.* **1983**, *22*, 130–136.
- (25) Czerny, M.; Schieberle, P. Influence of the polyethylene packaging on the adsorption of odour-active compounds from UHT milk. *Eur. Food Res. Technol.* **2007**, *225*, 215–223.
- (26) Iwatsuki, K.; Mizota, Y.; Kubota, T.; Nishimura, O.; Masuda, H.; Sotoyama, K.; Tomita, M. Evaluation of aroma of pasteurized and UHT processed milk by aroma extract dilution analysis. *Nippon Shokuhin Kagaku Kogaku Kaishi* **1999**, *46*, 587–597.
- (27) Colahan-Sederstrom, P. M.; Peterson, D. G. Inhibition of key aroma compound generated during ultrahigh-temperature processing of bovine milk via epicatechin addition. *J. Agric. Food Chem.* **2005**, *53*, 398–402.
- (28) Badings, H. T. Milk. In *Volatile Compounds in Foods and Beverages*; Maarse, H., Ed.; Dekker: New York, 1991; pp 91–105.
- (29) Bendall, J. G.; Olney, S. D. Hept-cis-4-enal: analysis and flavour contribution to fresh milk. *Int. Dairy J.* **2001**, *11*, 855–864.
- (30) Mottram, D. S.; Madruga, S. Some novel meatlike aroma compounds from the reactions of alkanediones with hydrogen sulfide and furanthiols. *J. Agric. Food Chem.* **1995**, *43*, 189–193.
- (31) Mottram, D. S.; Mottram, H. R. An overview of the contribution of sulfur-containing compounds to the aroma in heated foods. In *Heteroatomic Aroma Compounds*; Reineccius, G. A., Reineccius, T. A., Ed.; ACS Symposium Series 826; American Chemical Society: Washington, DC, 2002; pp 73–92.
- (32) Hofman, T.; Schieberle, P.; Grosch, W. Model studies on the oxidative stability of odor-active thiols occurring in food flavors. *J. Agric. Food Chem.* **1996**, 251–255.
- (33) Grosch, W. Evaluation of the key odorants of foods by dilution experiments, aroma models and omission. *Chem. Sense* **2001**, *26*, 533–545.
- (34) Núñez, A. J.; Bemelmans, J. M. H. Recoveries from an aqueous model system using a semi-micro steam distillation–solvent extraction procedure. *J. Chromatogr., A* **1984**, *294*, 361–365.
- (35) Rychlik, M.; Schieberle, P.; Grosch, W. *Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Odorants*; Deutsche Forschungsanstalt für Lebensmittelchemie and Institut für Lebensmittelchemie der Technischen Universität München: München, Germany, 1998; ISBN3-9803426-5-4.
- (36) Leffingwell, J. C.; Leffingwell, D. GRAS flavor chemicals—detection threshold. *Perfum. Flavor.* **1991**, *16*, 1–19.
- (37) Van Gemert, L. J. *Compilations of Odour Threshold Values in Air, Water and Other Media*; Van Gemert, L. J., Ed.; Boelens Aroma Chemical Information Service (BASICS): Zeist, The Netherlands, 2003.
- (38) Karagül-Yüceer, Y.; Vlahovich, K. N.; Drake, M. A.; Cadwallader, K. R. Characteristic aroma components of rennet casein. *J. Agric. Food Chem.* **2003**, *51*, 6797–6801.

Received for review September 18, 2007. Revised manuscript received January 6, 2008. Accepted January 12, 2008.

JF072776Q