Study of dairy flavor by sensory-directed analytical techniques and sensory evaluation

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Chapter 1. General Introduction

Utilization of milk, also called dairy farming, first originated in Western Asia as early as 7000 B.C. and developed through the domestication of sheep and goats. This meant that humankind became able to repeatedly utilize the animal protein without depending on hunting. Dairy farming is significant as it converts plant resources into available food and benefits the soil by recycling livestock excrement. The domestication of cattle began around 6000 B.C. and their milk was used before 4000 B.C. In Europe, dairy farming has been conducted over thousands of years for manufacturing butter and cheese. In the 19th century, the habit of drinking milk spread into key urbanized countries in Europe and the United States, although problems occurred with food fraud and hygiene for milk in the United Kingdom and the United States (1, 2). The handling of these problems led to the evolution of food chemistry, food analysis, and food engineering. Today, dairy farming is performed in many areas of the world, and cattle which have a longer secretion period and an abundance of milk as a result of breeding dominate dairy livestock.

Ancient Japanese dairy products, known as "*So*" and "*Raku*," are nutritious food which have been consumed by aristocrats since the Kofun period. Their manufacture was lost during the Nanboku-chō period. Western culture then flowed into Japan at a later stage of the Edo period, around the 1860s, and the production of raw milk, condensed milk, butter, and cheese began at this time in Japan. After World War II, rehydrated skim milk for nutritional enhancements was provided by the United States, and the provision of milk for school lunch was popularized. At the same time, milk production increased along with a high economic and population growth in Japan, and Western-style eating habits were established.

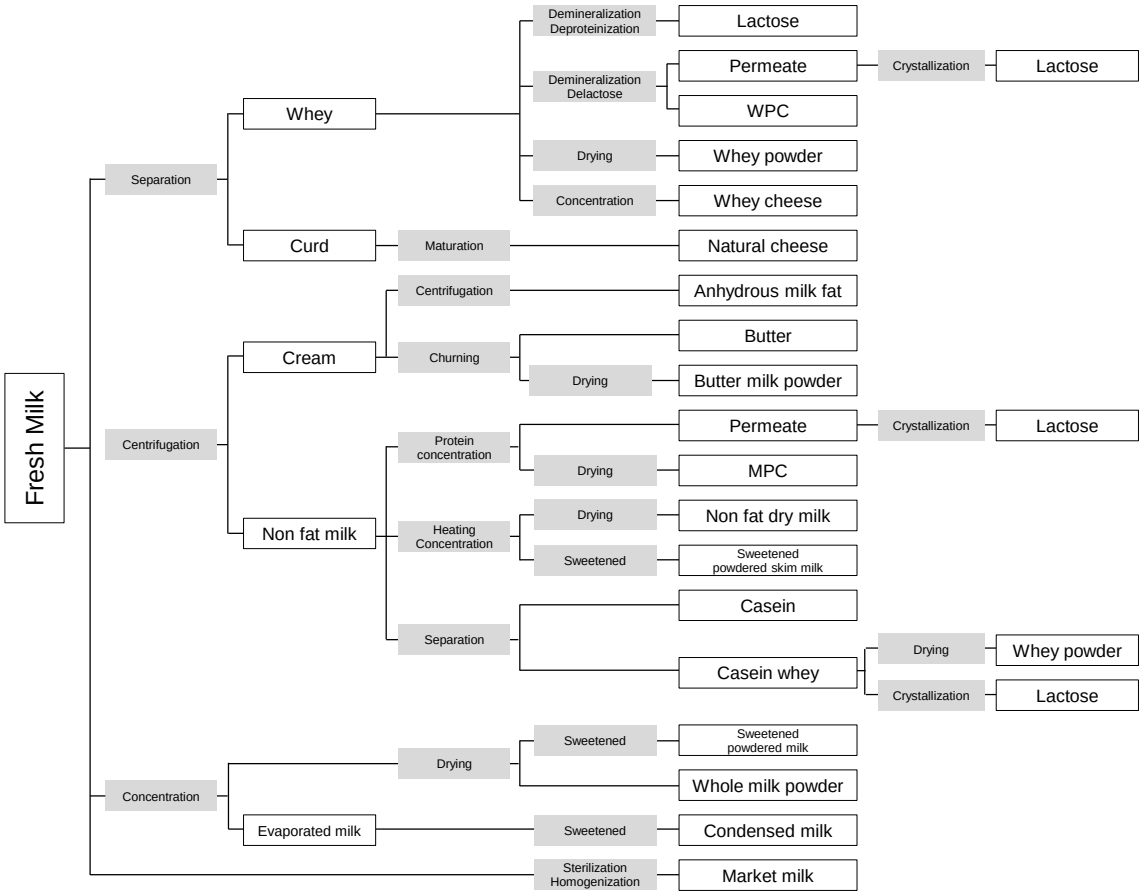


Figure 1.1. An outline of manufacturing process of dairy products.

During a long history, dairy products came to be consumed in various forms such as butter, cheese, yogurt, and powdered milk. Because dairy products include proteins, lipids, and sugars, they are considered to be nutritionally balanced. Nowadays, the process of dairy production is highly mechanized and sanitized. An outline of the manufacturing process of dairy products is shown below (Figure 1.1).

Generally, scientific analyses are conducted to detect substances or to differentiate the chemical composition, qualitatively or quantitatively. When the technique of separation is different, different results are obtained even if the sample used is the same. In the case of flavor, this is due to a complex mixture of various compounds. An analysis of dairy flavor started in the

1950s and has been expanding with the development of analytical methods (3). Dairy products comprise a variety of ingredients, mainly amino acids, sugars, and lipids with trace amounts of flavor compounds. Because a wide variety of dairy products are manufactured (Figure 1.1), dairy flavors are different in individual products owing to the variations in the manufacturing process. The breeding environment, feed, and climate are also known to affect milk flavor (4). Furthermore, dairy flavor compounds are widely ranging from highly-volatile compounds to relatively high boiling point compounds such as fatty acids. The separation and concentration techniques for delicate dairy flavors should be first selected to suit the target compound and then the physical properties. The chemical nature and concentration range of the flavor compound should also be taken into consideration. Additionally, for understanding the overall flavor, it is important not only to observe individual compounds by instrumental analysis but also to confirm their contribution to actual food systems by combining data analysis with sensory evaluation. An outline of the flavor analysis techniques is shown below (Figure 1.2). In the following sentences, the techniques employed in this thesis are described.

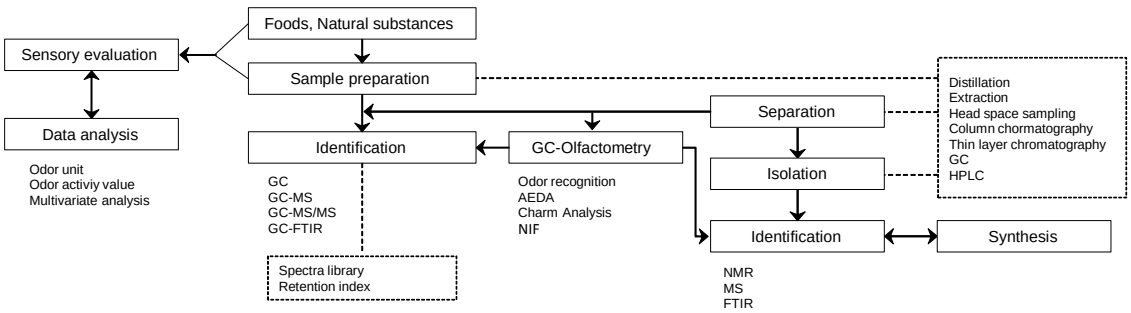


Figure 1.2. Outline of flavor analysis.

Distillation techniques have often been used to isolate volatile compounds in food. In the flavor industry, steam distillation is used to obtain essential oils from herbs, such as mint. Steam distillation allows distilling the high-boiling compounds at a temperature below the boiling point. A simultaneous distillation-extraction (SDE) technique has been commonly used to isolate flavor compounds for the purpose sample pretreatment. The heat-sensitive food requires a careful operation because this method directly heats the sample (5). In recent years, high vacuum distillation was improved and is now a suitable method for foods wherein isolating flavor was difficult and was designated as solvent-assisted flavor evaporation (SAFE) (6). This method prevents aroma degradation using a short distillation pass of compact apparatus and the liquid nitrogen trap.

Solid-phase extraction is another potent technique for flavor analysis. The principle of this method is to absorb flavor compounds in the stationary phase and then collect them through interactions with a solvent. The stationary phase and the solvent are required to be selected according to the nature of the flavor compound. For the purpose of collecting all flavor compounds, a porous polymer such as Tenax TA or Porapak Q is used. A silica gel chromatography is used in isolation on the compounds' polarity. Size-exclusion chromatography (SEC) can be separated by molecular size or, in some cases, by molecular weight.

Gas chromatography (GC) is an essential tool for complicated flavor analysis. The flavor compound is separated in the GC column and is detected by a variety of detectors. In particular, GC-mass spectrometry (GC-MS) can identify the compound by a spectrum library and is a useful tool for flavor analysis. The odor activity of the individual flavor compound cannot be evaluated by conventional GC. However, GC-olfactometry (GC-O) may detect the odor-active compounds because humans directly assess the GC eluates (7). The comparison of obtained GC-O results to conventional GC results using retention indices assigns the relative

importance of identified volatile compounds and discriminates them to be odor active. Various GC-O analyses have been developed to evaluate the relative importance of odorants. These methods are classified into three categories that are dilution technique, detection frequency technique, and direct intensity technique. The dilution technique is used to assess a series of prepared diluted extracts by GC-O. The odorant detected at the maximum dilution of an extract is interpreted to contribute the most. In this technique, aroma extract dilution analysis (AEDA) and charm analysis are well known. The detection frequency technique has 6–12 assessors that carry out GC-O on the same extract and the odorant to be detected at a particular retention index is counted. The odorant most frequently detected is the most important. In this technique, the nasal impact frequency (NIF) is well known.

The sensory evaluation of foods is a scientific technique that applies the results obtained through the use of the sensory organs, particularly gustatory, olfactory, and visual, to the principles of experimental design and statistical analysis. An analytical sensory evaluation, where the training and management of descriptive panelists is important, is carried out for the purpose of understanding the sample feature. In the sensory evaluation, it is necessary to select the most appropriate method depending on the purpose. It is also essential to pay proper attention to the rules of sample presentation, including the way in which questions are asked and the choice of evaluation terms and scale.

Large amounts of data can be accumulated by instrumental analysis and sensory evaluation. The application of statistical and multivariate analysis to the accumulated data enables the extraction of information. For example, it is possible to detect significant differences between samples, compounds, or sensory attributes, and to understand the mutual relationship between samples using multivariate analyses, such as principal component analysis (PCA) or cluster analysis.

Our surrounding environment continues to change and influence the food industry, including (8): shorter food product life cycles through the development of the distribution industry; natural taste and gourmet trends; increased demand of dairy products caused by the global population growth; population decline in Japan and aging of the population; a decrease in milk production by domestic dairy farmers in Japan; a reduction of milk production caused by global warming; an inflow of overseas dairy products facilitated by the promotion of free trade; food waste; food sustainability; and growing health and wellness as represented in the metabolic syndrome.

In order to address the above concerns, it is necessary to effectively utilize milk resources. Dairy flavor is a key factor in food products because the variety of dairy products is not only used as a raw material in foods but also are directly consumed. In particular, a dairy flavor that can reproduce a natural flavor may play a more important role in milk alternatives to solve the shortage of milk resources due to population growth and global warming. Further, an understanding of flavor changes through loss and generation in the dairy production process and flavor differences between regions is essential for the palatable development of dairy products. In terms of dairy product palatability, *Koku* is a very important concept. The Japanese people often use the word *Koku* to represent a thick, deep taste. The concept of *Koku* is complex wherein various concepts and terms are blended together. The sensation of *Koku* is found in many dairy products such as cheese and cream. An investigation of *Koku* would contribute to the improvement of dairy product palatability.

A study of dairy flavor has been undertaken by many researchers, and most of them were overseas study examples such as United States and European countries (9, 10). However, there are not many studies focused on the dairy products that are manufactured and consumed in Japan. In this thesis, the focus is on the key flavor compounds of dairy products that are available in Japan. Studies were performed to combine the sensory-directed analytical techniques in the sensory evaluation. Skim milk powder (SMP) is an important dairy product because its production is increasing in response to global demand (Figure 1.3.A and B). In Japan, approximately 90% of the domestic SMP is produced in the Hokkaido area as a specific dairy product stipulated by the Japanese law. In chapter 2, the flavor of SMP used as a raw material in various kinds of food was analyzed by comparing it with UHT milk. The characteristics were elucidated by the identification of character impact compounds and aroma simulation experiment. The demand for whole milk powder (WMP) in China (Figure 1.3.D) and the production amount in New Zealand has increased (Figure 1.3.C). In chapter 3, the aroma impact compounds, properties, and flavor of WMPs made in Japan, Oceania, and China were compared. Recently, the production of sweet cream in Japan tends to increase with the conversion of production items from butter to cream and the application to various kinds of processed foods as a food material. In chapter 4, the characteristics of *Koku* impact compounds of sweet cream were identified from the viewpoint that *Koku* is a unique flavor term of Japan. Finally, these data are summarized in chapter 5.

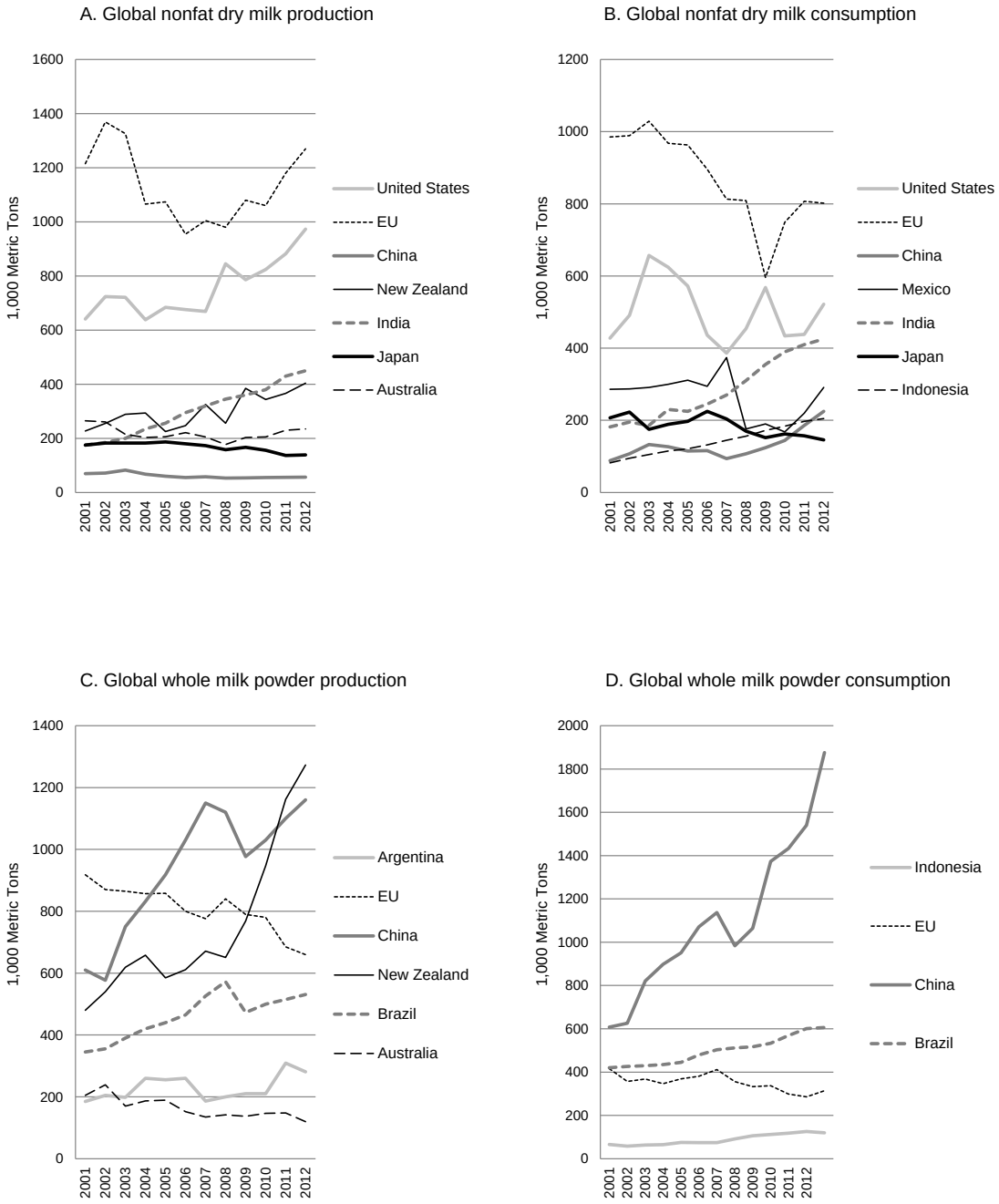


Figure 1.3. Global powdered milk production and consumption.

A and B (upper): skim milk powder, C and D (lower): whole milk powder. Data was extracted from USDA statistics 2001–2014 of "*Dairy: World Markets and Trade*."

Chapter 2. Character Impact Odorants of High-heat Skim Milk Powder Isolated by 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 (11) and fodder consumed (4), and the flavor profile of dairy products seems to affect the diversity, the features, and the consumer preferences of each country. For instance, ultra-high temperature pasteurized (UHT) milk is said to be preferred over low-temperature long-time (LTLT) milk in Japan (11), and accounts for 93% of the pasteurized milk consumed.

Skim milk powder (SMP) 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 SMP is classified into the following 3 types based on the whey protein nitrogen index (WPNI) (12), 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) (13). Based on the removal of the moisture and fat, and on the subsequent decrease in its volume and weight, the following advantages of SMP have been demonstrated. Firstly, the decrease in the volume enables the adjustment of the concentration and flavor upon dissolving. Secondly, during long-term storage, the low moisture and low lipid content prevents microbe proliferation and lipid oxidation. SMP with these advantages has been used for the foods such as milk beverage, yogurt, ice cream, confectionery, and bread. Especially, high-heat SMP (HHSMP) is known to be suitable for confectionery and bread. Generally, SMP is dissolved in water prior to

use. When dissolved in water, HHSMP possesses a characteristic aroma that differs from UHT milk, and this is an important factor influencing consumer preference.

The aroma of SMP has been the subject of extensive investigation. Shiratsuchi *et al.*, for example, have reported on a series of studies that analyzed the flavor compounds and cowhouse-like odor of SMP (14–17). Recently, by using aroma extract dilution analysis (AEDA) (18), Karagül-Yüceer *et al*. reported the clarification of flavor compounds in US SMP derived using different heat treatments (19) and identified the major flavor compounds and attributes of stored SMP (20). In a subsequent study, those authors found 2-methyl-3-furanthiol in medium-heat SMP and high-heat short-time pasteurized milk (HTST) as a related compound of vitamin-like attribute (21). In addition, it was known that this compound has been isolated from whey protein concentrate for the first time in dairy products (22).

On the other hand, Karagül-Yüceer *et al*. reported that the compound corresponding to cooked/sulfurous attribute was not identified in HHSMP, though the attribute exhibited a high score (19). The following report of those authors had vagueness that advanced the verification of the cooked/sulfurous attribute by using dimethyl sulfide not reported in SMP (23). 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.

Material and Methods

Materials. A 20-kg sample of HHSMP was obtained from Morinaga Milk Industry Co., Ltd. The sample was received by overnight shipment after checking the quality of aroma, taste, and appearance. This HHSMP was packed in an aluminum-polyethylene laminated bag sealed and stored at -25 °C until required. The moist 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 °C for 3 sec was purchased from a domestic market, and used immediately. The composition of milk was 3.5 % fat and 8.3 % non-fat 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.

Authentic chemicals for co-injection into a gas chromatograph and for the sensory evaluations were purchased from reliable commercial sources. The following compounds were synthesized according to literature procedures: 2-acety-1-pyrroline (24), 2-acetyl-2-thiazoline (25), 3-methylnonane-2,4-dione (26), and (*Z*)-6-dodecen-4-olide (27).

Separation of the Volatiles from the Milk Sample. The heat step of simultaneous distillation-extraction (SDE) is known to generate by-products 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 non-volatiles containing 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 Corporation, 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 × 3.0 cm i.d. glass column was used after substituting 99.5 % ethanol by 400 mL or more distilled water per 60 mL 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 non-volatiles by SDE using a modified Likens-Nickerson apparatus attached to the addition funnel (Figure 2.1). The eluate was fed drop-wise 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 °C (when the extract was dropped) to approximately 80 °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, 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 (19, 20). 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 3 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 °C to 210 °C or from 40 °C 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/sec. 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-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 applied using 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 °C to 210 °C at a rate of 3 °C/min for all runs. The injector, detector, and the sniffing port temperatures were 250 °C, 300 °C, and 250 °C, respectively. Flow rate of the helium carrier gas was 27 cm/sec. GC-O was conducted by three assessors.

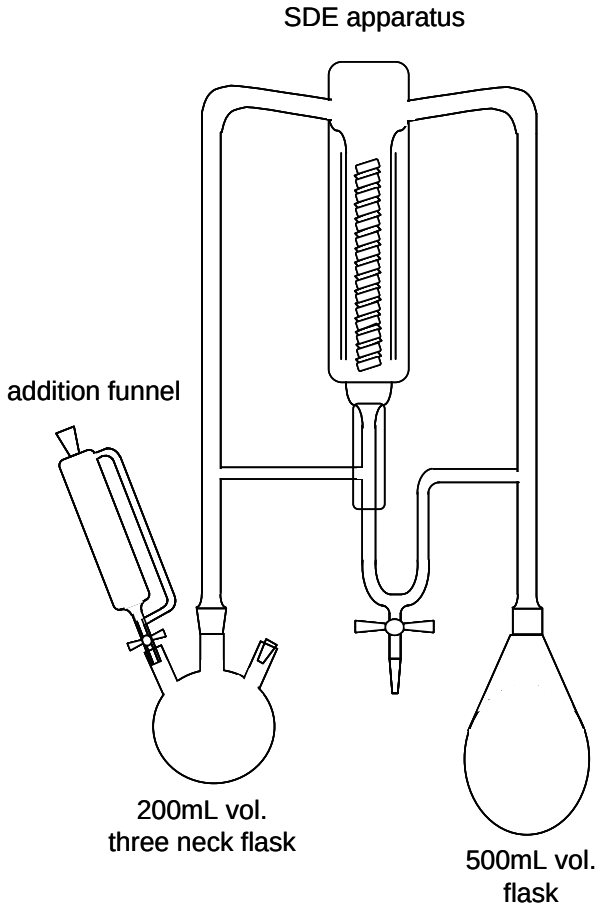


Figure 2.1. Modified SDE apparatus used in chapter 2. Reprinted with permission from (28). Copyright 2008 American Chemical Society.

GC-Atomic Emission Detector. 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 with a film thickness of 0.25 μm (Agilent Technologies, Inc.). 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 serial 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 based on 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 analysis 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 comparing it to that of the authentic standard.

Quantification of Components. The compound, which odor activity value (OAV) (29) had shown one or more, was 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 single ion monitoring (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 2.3. 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 the dairy sample. The sensory profiles of the dairy sample were determined by 10 experienced assessors. The assessors, who had completed more than 60 h of training course in many aspects of sensory analysis (recognition, description and discrimination tests), were recruited from the laboratory of the Ogawa & Co., Ltd. The assessors were aged from 25 to 52, and were 4 females and 6 males. The aroma attributes defined by the assessors are listed in Table 2.1. The terms used to describe the aroma attributes as papery, brothy, and vitamin-like were also referred to the literature (19, 20). The assessors were trained in the reference samples shown in Table 2.1. The assessors, who were asked to rank the intensity of the odor attributes, evaluated them ortho-nasally. 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 % non-fat milk solids (equivalent to UHT milk), and purchased UHT milk. Each sample was assigned a 3-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 8 compounds in 1 L of low-odor reconstituted HHSMP (low-odor matrix). For the aroma simulation experiment, the low-odor matrix was necessary in order 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. An 86-g of HHSMP was dissolved in 1L of odor free distilled water. The reconstituted HHSMP was passed through a column packed with 60mL

of SEPABEADS SP70. The column effluent was collected, and odor free nitrogen gas was blown into the effluent in order to replace oxygen for 5 minutes. 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. This amount was 1.5 times amount that of quantification value.

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. Then, the sensory properties of HHSMP and the UHT milk were evaluated using the evaluation terms (Table 2.1) collected from the assessors in advance. The mean value of the sensory score for both samples is shown in Figure 2.2. The sensory scores were analyzed using a *t* test; therefore, the results reveal the properties of both samples. The sensory evaluation 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.

Table 2.1. Preparation of reference samples for sensory evaluation. Reprinted with permission from (28). Copyright 2008 American Chemical Society.

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 one-half of a sample with distilled water
heated/sweet	canned evaporated milk	dilute one-tenth of a sample with distilled water
papery/woody	brown paper towel	soak 2 g of brown paper towel in 100 mL distilled water for 2 h, then remove
animal	unflavored gelatin	dissolve 5 g of gelatin in 100 mL distilled water and melt in microwave oven; refrigerate until evaluation
brothy	extract of meat (dry)	dilute one-thousandth of a sample with distilled water
mushroom like/metallic	mushroom broth	remove sliced mushrooms from broth and 3 in 100 with distilled water
vitamin-like	thiamin nitrate	dissolve 0.05 g of thiamin nitrate in 100 mL distilled water and adjust to pH 2.0 with 1 M hydrochloric acid

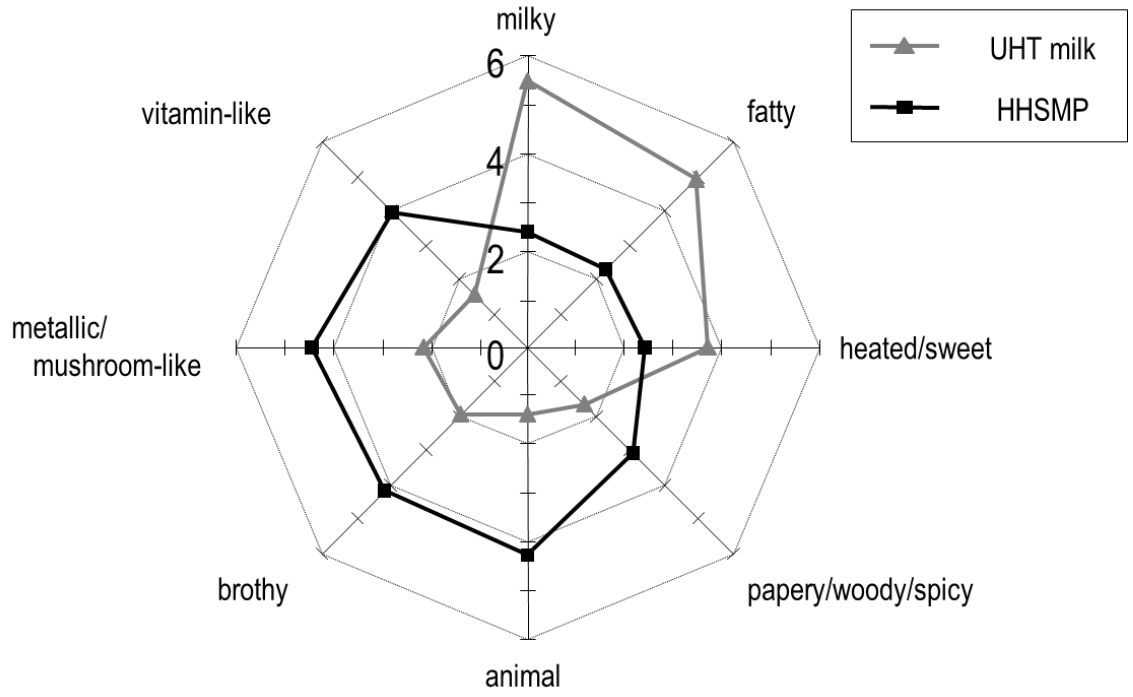


Figure 2.2. Sensory profile of UHT milk and HHSMP. Reprinted with permission from (28). Copyright 2008 American Chemical Society.

Karagül-Yüceer *et al.* reported that medium and HHSMP indicated high in cooked/sulfurous attribute (19). This was a tendency different from 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 (30). Both feature of cooked/sulfurous and potato/brothy 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 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 (5, 31). 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 (32). 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 non-volatiles such as fat by using SDE. Bicchi *et al*. applied a similar two-step procedure in order to analyze the aroma of honey (33). 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 (32). The aroma concentrate that we obtained exhibited the feature accepted in HHSMP and the 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 factor ranging from 10 to 3000, 44 were common to both of them (Table 2.2). 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 be 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 (Nos. **6**, **7**, **10**, **13**, **14**, **17**, **18**, **42**, and **53**). Moreover, using selected ion monitoring, 6 trace compounds (Nos. **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 GC-AED. These compounds were common with the result of AEDA applied to HHSMP (19) and UHT milk (34–36) in many points.

In this 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 the 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.

Table 2.2. Odor compounds in HHSMP and UHT milk (Log10 FD ≥1.0). Reprinted with permission from (28). Copyright 2008 American Chemical Society.

No.	Compounds	Odor descriptor	RI[b]	Identification	$\log_{10}$ FD[e]	
					HHSMP[f]	UHT[g]
1	2-acetyl-1-pyrroline	nutty	1347	RI, Odor, MS, AED[c]	1.5	1.5
2	nonanal	dusty	1398	RI, Odor, MS	1.5	1.5
3	1-octen-3-ol	fatty, oily	1442	RI, Odor, MS	1.5	2.0
4	methional	potato-like	1456	RI, Odor, MS, AED[c]	1.5	2.0
5	(*E*,*Z*)-2,4-heptadienal[a]	green	1466	RI, Odor, MS	1.5	1.5
6	3,5-octadien-2-one[a]	citrus-like	1582	RI, MS	1.0	1.5
7	(*E*,*Z*)-2,4-nonadienal[a]	citrus-like, oily	1657	RI, Odor, MS	n.d.[h]	1.5
8	methyl 2-methyl-3-furyl disulfide	canned corn-like, cooked	1670	RI, Odor, SIM[d], AED[c]	2.5	2.5
9	unknown	rice bran-like, bitter	1681	-	1.5	n.d.[h]
10	(*E*,*E*)-2,4-nonadienal	oily	1702	RI, Odor, MS	1.5	1.5
11	3-methylnonane-2,4-dione	green, fishy	1720	RI, Odor, SIM[d]	2.0	1.5
12	unknown	green, oily, nutty	1729	-	n.d.[h]	2.0
13	2-acetyl-2-thiazoline	nutty, oily	1756	RI, Odor, MS, AED[c]	1.0	2.0
14	(*E*)-2-undecenal	fatty, milky	1761	RI, Odor, MS	n.d.[h]	1.5
15	unknown	nutty, cooked	1786	-	1.5	1.5
16	furfuryl methyl disulfide	green, nutty	1800	RI, Odor, SIM[d], AED[c]	1.5	1.5
17	(*E*,*E*)-2,4-decadienal	plastic-like, oily	1808	RI, Odor, SIM[d]	1.5	1.5
18	tridecanal[a]	oily, green	1826	RI, MS	1.5	2.0
19	hexanoic acid	soapy	1873	RI, Odor, MS	1.5	1.5
20	unknown	lactone-like	1884	-	n.d.[h]	1.5
21	unknown	nutty, green	1919	-	1.5	1.5
22	benzothiazole	rubber-like	1947	RI, Odor, MS, AED[c]	2.0	2.0
23	5-octanolide	lactone-like	1963	RI, Odor, MS	n.d.[h]	1.5
24	phenol	phenol-like	2001	RI, Odor, MS	1.5	1.5
25	2-methylphenol	smoky	2009	RI, Odor, MS	1.5	1.5
26	unknown	nutty, rice bran-like	2017	-	2.0	n.d.[h]
27	4-nonanolide	peach, lactone-like	2022	RI, Odor, MS	n.d.[h]	1.5
28	4-hydroxy-2,5-dimethyl -3(2*H*)-furanone(furaneol)	sweet	2034	RI, Odor, SIM[d]	1.5	2.0
29	octanoic acid	acid-like	2065	RI, Odor, MS	1.5	1.5
30	5-nonanolide	lactone-like	2078	RI, Odor, MS	n.d.[h]	1.5
31	4-methylphenol	smoky	2087	RI, Odor, MS	2.5	2.5
32	3-methylphenol	smoky	2103	RI, Odor, MS	1.5	2.0
33	unknown	peach, lactone	2131	-	n.d.[h]	1.5
34	4-decanolide	peach, lactone	2140	RI, Odor, MS	n.d.[h]	1.5
35	bis(2-methyl-3-furyl)disulfide	nutty, rice bran-like	2147	RI, Odor, SIM[d]	2.0	2.0
36	4-ethylphenol	barny, spicy	2164	RI, Odor, MS	1.5	1.5
37	nonanoic acid	lactone-like	2178	RI, Odor, MS	1.5	1.5
38	3-ethylphenol	barny, spicy	2183	RI, Odor, MS	1.5	1.5
39	5-decanolide	lactone-like	2188	RI, Odor, MS	3.0	3.5
40	3-hydroxy-4,5-dimethyl -2(5*H*)-furanone(sotolon)	spicy, rubber-like	2200	RI, Odor, SIM[d]	1.5	n.d.[h]
41	2-aminoacetophenone	foxy, plaster-like	2207	RI, Odor, MS	3.0	3.0
42	methyl anthranilate	oily, smoky, vanillin	2237	RI, Odor, MS	1.0	1.5
43	α-cadinol[a]	woody	2264	RI, MS	2.0	1.5
44	decanoic acid	acid-like	2283	RI, Odor, MS	2.0	2.0
45	5-undecanolide	lactone-like	2307	RI, Odor, MS	1.0	2.5
46	9-decenoic acid	metalic, acid	2362	RI, Odor, MS	1.5	1.5
47	4-dodecanolide	lactone, sweet	2368	RI, Odor, MS	2.0	2.5
48	unknown	rice bran-like	2377	-	1.5	1.5
49	(*Z*)-6-dodecen-4-olide	lactone	2388	RI, Odor, MS	3.0	3.5
50	5-dodecanolide	lactone-like	2420	RI, Odor, MS	1.5	3.0
51	indole	peach, smoky, fatty	2434	RI, Odor, MS	1.5	2.0
52	(*Z*,*Z*)-6,9-dodecadien-4-olide[a]	lactone-like	2470	RI, MS	1.5	1.5
53	skatol	fecal, mothball-like	2476	RI, Odor, MS	1.0	2.0
54	dodecanoic acid	acid-like	2511	RI, Odor, MS	2.0	2.0
55	unknown	citrusy, acid-like	2518	-	n.d.[h]	2.0
56	11-dodecenoic acid[a]	acid-like	2540	RI, MS	1.5	1.5
57	vanillin	vanilla	2554	RI, Odor, MS	1.5	n.d.[h]
58	unknown	nutty, smoky, green	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 comparing it to that of the authentic standard. [c] Atomic emission detector. [d] Selected ion monitoring. [e] Flavor dilution factor. [f] HHSMP. [g] UHT milk. [h] Not detected.

Based on previously reported observations, the existence of these compounds was not surprising. For instance, in the study of Drake *et al*. (21), the compounds corresponding to vitamin-like attribute identified in evaporated milk was not found in evaporated milk. On the other hand, based on 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 (19). The compound corresponding to this attribute was not identified in this study, or in a related study (23). In addition, the reference sample of cooked/sulfurous attribute was skim milk heated at 85 °C for 30 min (19). 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 (37). Thus, such a compound might contribute in part to the cooked/sulfurous attributes in previous reports. 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*. (38). 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 HTST milk.

Further, 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 H_2S (39). However, the 2-mercapto-3-pentanone of the major reactant was not detected in this study. Thus, it is expected that the possibility of the generation of 2-MFT and their derivatives that originate from the interaction of aroma compounds under SDE is low. Additionally, the maintenance of the feature of original sample in the aroma concentrate obtained by the abovementioned method

indicates the low possibility of these disulfides generation with SDE. Considering the heating in the production 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*. (40), and the subsequent formation of disulfides are suspected. Since 2-MFT was observed in HTST milk and medium-heat SMP (21), 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*. (32) and Hofmann *et al*. (41) reported that the dimerization of the 2-MFT advanced under low temperature (-15 °C 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 this 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. Firstly, 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 No. **23**, **27**, **30**, **34**, **45**, and **50** (Table 2.2). 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 2.2). In the production process of SMP, the removal of fat and the dispersion of aroma during drying could have influenced these results. Secondly, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (**40**) and vanillin (**57**) were detected only in HHSMP (Table 2.2). Vanillin in dairy product is known to originate from plant lignin, and forms by temporal change and/or pasteurization (42). 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. Thirdly, 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. Based on the comparative results of the AEDA, the characteristic features of HHSMP was considered to be conferred by 2 or more aroma compounds that are contained in common with the UHT milk. An aroma simulation experiment was conducted in order to confirm this supposition.

Calculation of OAV. In order to determine the contribution of the characteristic aroma of HHSMP in the matrix, the OAV was calculated using the procedure reviewed by Grosch (43). 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 2.3. The quantification results are shown in Table 2.4. On the quantitative analysis using SDE, it was necessary to pay attention to the recovery rates of aroma compound. Núñez *et al*. reported a poor recovery rate when using SDE (44). In the present study, the tendency for low recovery rates of acids observed (Table 2.3), and the quantification value was corrected by the recovery rate from water. Since 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.

Based on the above calculation, 18 compounds that exhibited an OAV > 1 were identified (Table 2.4). Karagül-Yüceer *et al*. indicated that the threshold values of free fatty acids were higher in reconstituted skim milk than those of in water (23). Based on previously reported observation, the calculation of OAV was done by using the especially high threshold

reported. The 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.

Table 2.3. Masses (*m*/*z*) used, response factor, and recovery rate of the selected compounds. Reprinted with permission from (28). Copyright 2008 American Chemical Society.

No.	Compound[a]	Selected ions	Respnse factor[d]	Recovery rate[e]
1	2-acetyl-1-pyrroline	83[c], 111	1.7	0.6
2	nonanal	57, 70[c]	2.6	0.9
8	methyl 2-methyl-3-furyl disulfide	113, 160[c]	0.9	0.6
10	(*E*,*E*)-2,4-nonadienal	67, 81[c]	1.3	1.0
11	3-methylnonan-2,4-dione	43, 99[c]	0.9	1.1
17	(*E*,*E*)-2,4-decadienal	81[c], 95	1.3	1.0
19	hexanoic acid	60, 73[c]	0.8	1.0
22	benzothiazole	108, 135[c]	0.6	1.1
29	octanoic acid	60[c], 73	0.9	2.0
31	*p*-cresol	77, 108[c]	0.9	0.9
35	bis(2-methyl-3-furyl) disulfide	113, 226[c]	0.9	1.1
38	3-ethylphenol	107, 122[c]	0.8	1.1
39	5-decanolide	71, 99[c]	1.1	0.5
41	2-aminoacetophenone	120, 135[c]	1.1	0.9
44	decanoic acid	60[c], 73	0.7	2.2
47	4-dodecanolid	85[c]	0.9	1.1
49	(*Z*)-6-dodecen-4-olide	85[c]	0.9	1.1
50	5-dodecanolide	71, 99[c]	0.9	1.0
54	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.

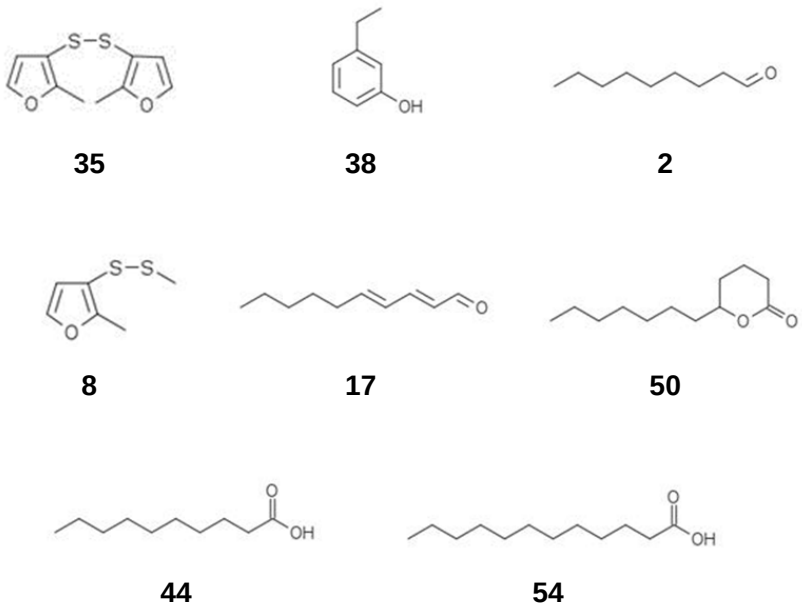
Moreover, the result of chapter 2 was similar to the report by Karagül-Yüceer *et al*. of the contributions of acids and (*E*,*E*)-2,4-decadienal (23). The omission test of these author pointed out that 5-decanolide and 2-aminoacetophenone did not influence reproduction of the entire aroma. These compounds did not show anticipated OAV compared with the height of the FD factor, and it was supported that the importance of these compounds to the entire aroma were not high.

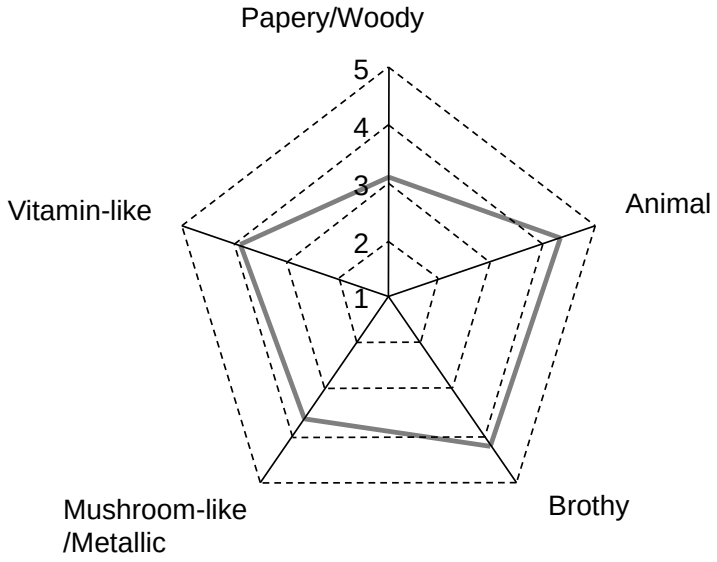
Reconstitution Experiment. The aroma simulation experiment was conducted in order to determine whether the characteristic aroma of HHSMP confirmed by the sensory evaluation is attributable to the odor-active compounds that had the highest OAVs. The experiment was conducted as follows. The compounds for the aroma model were selected from compounds which would have significant influences indicating OAV above 10. And, an aroma model comprising the following 8 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 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 were treated independently. The average score of each attribute is shown in Figure 2.3.

Table 2.4. Concentration and odor threshold of odorants in HHSMP and their OAV. Reprinted with permission from (28). Copyright 2008 American Chemical Society.

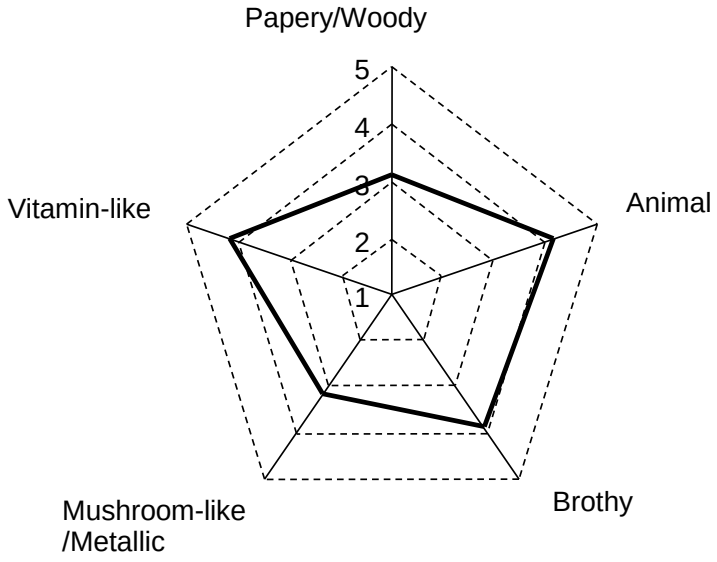
No.	compound	concn[a] (μg/L)	SD[b] (μg/L)	threshold (μg/L)[c]	OAV[h]
35	bis(2-methyl-3-furyl)disulfide	0.01	0.001	0.00002[d]	377
38	3-ethylphenol	2.9	0.1	0.05[d]	57
2	nonanal	47	6	1[d]	47
8	methyl 2-methyl-3-furyl disulfide	0.11	0.01	0.004[d]	27
17	(*E*,*E*)-2,4-decadienal	1.0	0.1	0.07[d]	15
50	5-dodecanolide	58	1	4.6[g]	13
44	decanoic acid	108000	10800	10000[d]	11
54	dodecanoic acid	114000	17600	10000[e]	11
7	(*E*,*E*)-2,4-nonadienal	0.09	0.01	0.01[f]	9
19	hexanoic acid	45000	520	1000[d]	9
49	(*Z*)-6-dodecen-4-olide	5.4	0.4	0.7[g]	8
41	2-aminoacetophenone	1.5	0.1	0.2[d]	7
29	octanoic acid	116000	10100	3000[d]	6
1	2-acetyl-1-pyrroline	0.6	0.2	0.1[d]	6
47	4-dodecanolide	18	1	7[d]	3
39	5-decanolide	55	2	30[g]	2
11	3-methylnonane-2,4-dione	0.04	0.001	0.03[d]	1
31	4-methylphenol	2.8	0.4	2.7[d]	1

[a] Mean value obtained by analyzing 3 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.* (45). [e] Leffering *et al.* (46). [f] Van Gemert (47). [g] Karagül-Yüceer *et al.* (48). [h] OAVs were calculated by dividing the concentrations by the respective odor thresholds in water. It was confirmed that the S/N ratios of SIM mode on the trace compounds were approximately 5.





A: aroma simulation model



B: reconstituted HHSMP

Figure 2.3. Sensory profile of the aroma simulation model (A) and reconstituted HHSMP (B). Reprinted with permission from (28). Copyright 2008 American Chemical Society.

The test for equality did not indicate a significant difference between the samples (χ^2 (4) = 1.23, p = 0.87). Further, 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 was 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-3furyl disulfide and bis(2-methyl-3-furyl) disulfide corresponded to the brothy and the vitamin-like attributes. This fact is also guessed from the relation to the description of above-mentioned Bading (37) and Bendall *et al* (38). Successively, it appeared that the fatty odor of (*E*,*E*)-2,4-decadienal corresponded to the papery and the 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 based on OAV and each sensory attribute was observed, thus the purpose of the experiment was achieved. The possibility for 8 compounds including the 2-MFT derivative that exhibits the feature of HHSMP was suggested from these results.

Chapter 3. Aroma Impact Compound Analysis of Seven Whole Milk Powders Using GC-O with Detection Frequency Method

Introduction

Because of its powdered form, whole milk powder (WMP) is easy to handle and transport, and has good storage stability. It is not only consumed in its production region but is also distributed worldwide as a food commodity. Between 2009 and 2012, WMP production increased in Asia and the Oceania region, particularly in China and New Zealand (49). During this period, China was the world's largest consumer of WMP and New Zealand was the world's largest supplier; meanwhile, Japan's WMP production showed a gradual decreasing tendency (50).

The flavor of WMP is an important factor that determines its deliciousness and quality. In order to control WMP's quality, factors leading to flavor change and formation of off-flavor during the storage period have been studied. Flavor changes in WMPs with butylated hydroxyanisole/butylated hydroxytoluene and those in stored WMPs were detected by sensory evaluation (51), and their related volatile compounds were analyzed by headspace gas chromatography (52). Additionally, the correlation between the flavor properties of stored WMP and the aroma compounds was reported (53). The hexanal contents of dried dairy products such as WMPs were measured using GC after performing simple steam distillation, and the correlation between the peroxide value and the hexanal content was determined (54). The volatile compounds of WMPs produced throughout the year were analyzed first by using an electric nose (E-nose) and solid phase micro extraction GC (SPME-GC), and then by applying multivariate analysis to the data. As a result, WMPs manufactured in different seasons were classified based on some guiding components extracted from the data thus obtained (55). Meanwhile, the flavor and flavor stability of SMP and WMPs were examined by the

GC-Olfactometry (GC-O) intensity method (42). Although it is said that the flavor of dairy products differs based on the climate and feed of a region, and each producing region imparts a characteristic flavor to its WMP, so far no report has analyzed WMPs based on these criteria.

Creating GC-O profiles for food samples, such as WMPs produced in different regions, and classifying the samples by multivariate analysis of their profiles are time consuming and effort intensive. The GC-O detection frequency method has many advantages such as repeatability, short analysis time, and reducing the training needs of panelists (56, 57).

The purpose of chapter 3 was to identify the common components and characteristic compounds of WMPs produced in three regions of Asia and Oceania: Japan, New Zealand, and China, by applying multivariate analysis to the results of the GC-O detection frequency method.

Material and Methods

Materials. WMPs produced in Japan (■1 and ■2) were purchased from a domestic market of the country, those produced in New Zealand (◆1 and ◆2) were obtained from a reliable commercial source, and WMPs produced in China (●1, ●2 and ●3) were purchased from one of its domestic markets. All WMPs were packed in aluminum-polyethylene laminated bags sealed and stored at -20 °C until use. In conducting sensory analysis, WMPs were used by the best-before date.

Preparing aroma concentrates. To separate the volatile components from WMPs, the solvent-assisted flavor evaporation (SAFE) method was used (6). The SAFE method was employed in this study because of its usefulness for the careful isolation of volatiles from oil-rich samples. Each WMP (36 g) was dissolved in distilled water, mixed well, and distilled under the following conditions: water bath temperature, 50 °C; vacuum pressure, 0.1 mPa; distillation time, 1 h; and the traps were cooled with liquid nitrogen. An internal standard

compound (2-octanol, 10 μg) was added to each distillate and extracted thrice with 100 mL of dichloromethane. Each extract was dried and the solvent was reduced to approximately 5 mL using a rotary evaporator under 550 mmHg and subsequently concentrated under a nitrogen stream to almost 100 μL. By adjusting the weight of each WMP for aroma extraction and the final volume of each aroma concentrate, the evaluated samples were prepared under standardized conditions for GC-O.

GC-MS. In order to identify the volatile components of WMPs, an Agilent Model 6890N gas chromatograph coupled to an Agilent Model 5973N series MSD was used. A DB-WAX column (60 m × 0.25 mm i.d.) with a film thickness of 0.25 μm (Agilent Technologies, Inc.) was used. The oven temperature was programmed from 80 to 210 °C at a rate of 3 °C/min. The injector temperature was 250 °C, and the carrier gas flow rate (helium) was 1 mL/min. The 1.0 μL injection volume was applied in splitless mode. The MSD conditions were as follows: capillary direct interface temperature, 220 °C; ionization voltage, 70 eV (EI); mass range, 33–300 amu; and ion source temperature, 150 °C.

Three-port GC-O system. The three-port GC-O system was comprised of an Agilent Model 6890 GC equipped with a flame ionization detector (FID) and connected to a box heater with three sniffing ports (Figure 3.1). Using this instrument, three sniffers can simultaneously sniff odorants separated on a GC column. A DB-WAX column (30 m × 0.53 mm i.d.) with a film thickness of 1.00 μm (Agilent Technologies, Inc.) was used. The oven temperature was programmed from 40 to 210 °C at a rate of 5 °C/min for all runs. The aroma concentrate (2.0 μL) was injected into the injector at 250 °C in splitless mode. The most appropriate carrier gas flow rate (helium) was calculated for the entire GC-O system to be 6.3 mL/min. The FID conditions were as follows: temperature, 250 °C; hydrogen flow, 40 mL/min; air flow, 450 mL/min; and makeup gas flow, nitrogen, 25 mL/min. Approximately one-twentieth of the

column flow was diverted to the FID (Figure 3.1, Crosspiece 1). The remaining flow was directed to Crosspiece 2.

The flow entering Crosspiece 2 was divided into three equal parts (2 mL/min) toward three thermostated lines held at 300 °C. These lines were made of fused silica capillary tubing of identical size (0.59 m, 0.15 mm i.d.). The box heater and the tube connected to the GC were maintained at 280 °C. At the end of the tube, a glass sniffing port was attached, which was replaced with a new one.

Panelists. The number of panelists for the sniffing experiments was 13, who were recruited from the laboratory of Ogawa & Co., Ltd. The panelists had completed more than 60 hours of a training course in a variety of aspects of sensory analysis (recognition, description and discrimination tests), and had previously experienced other GC-O sessions. To avoid physical and mental fatigue, each panelist participated in only one GC-O session per half-day, and the sniffing time was limited to 60 min per session.

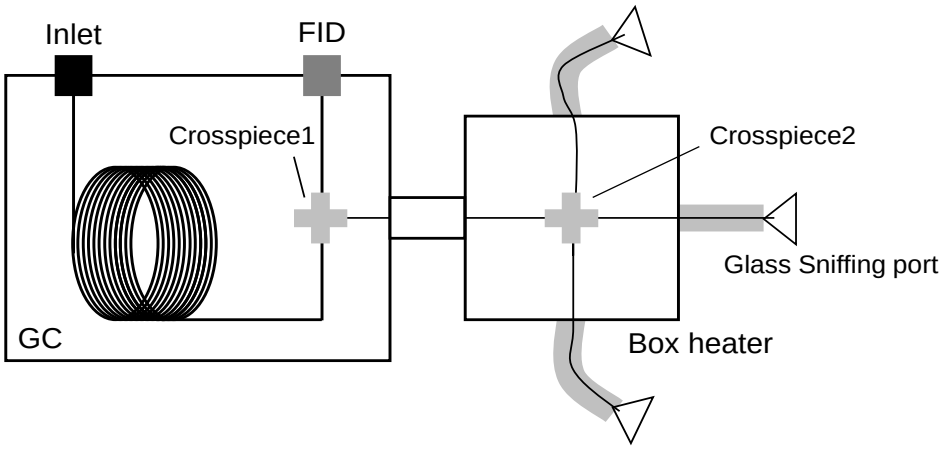


Figure 3.1. Three-port GC−O system.

Detection frequency method. A total of nine values were gathered per sample in performing three sniffing analyses by three different panelists, who recorded the time when the aroma concentrate was eluted and the aroma description. Detection frequency was summed based on the aroma quality detected at the same time by each panel. For converting the frequency data to binary data when the summed frequency was six or more, the converted binary data was assigned to a value of 1, and the rest was assigned to 0. The matrix thus converted was used in the next step.

Multivariate analysis. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were performed using SPSS ver. 11.0J.

Results and Discussion

Seven samples of aroma concentrates, two produced in Japan (samples ■1 and ■2), two in New Zealand (samples ◆1 and ◆2) and three in China (samples ●1, ●2, and ●3) reproduced well the original WMP's sensory properties. Samples ■1 and ■2 had a mild dairy flavor with a pleasant milk-like aroma. Samples ◆1 and ◆2 had a sweet and cheesy dairy flavor with a slightly green or hay-like nuance. Samples ●1, ●2, and ●3 had a buttery, oily, and cheesy flavor, with a fatty and fermented nuance. These aroma concentrates were used for the GC-O and GC-MS analyses.

In the present study, the GC-O detection frequency method was employed using the improved instrument, allowing three panelists to simultaneously sniff (Figure 3.1). The suitable number of panelists for the detection frequency method has been reported as eight to ten people (57). Debonneville *et al*. concluded that the multisniffing device was also suitable for the GC-surface nasal impact frequency (SNIF) method, another detection frequency method (56). The number of panelists who identified the odor by GC-O was counted, and the odor

descriptions were recorded. Forty-one aroma-active compounds were perceived from all seven WMPs. Among these, 20 compounds were identified by comparing their retention indices, mass spectra, and odor descriptions with those of authentic compounds. Sixteen compounds were tentatively identified by comparing their retention indices and odor descriptions with those of the authentic compounds. Each compound shown in Table 3.1 has been reported from dairy products. The compounds that showed high detection frequency in this study corresponded partly to those of Whetstine *et al*. (42), which were found in American WMP.

When a GC-O dilution method, such as CHARM (Combined Hedonic Aroma Response Measurement) and AEDA, is used in the research of key aroma compounds, a dilution series of an extract is prepared per one sample, usually between 8 and 12 in total, and GC-O is applied to each diluted extract. Also, two or more rounds of sniffing are required for reliable detection. On the other hand, when the GC-O detection frequency method is used, GC-O by one time per one sample without dilution is carried out. In chapter 3, using the three-port GC-O system permitted simultaneous data acquisition of three GC-O data, resulting in a shorter GC run time than the conventional dilution method.

Pollien *et al*. described that when the NIF method is used as the detection frequency method, a frequency difference of 30% would generally indicate a significant concentration difference (57). This observation is consistent with the fact that the Weber ratio of a typical discrimination threshold of odor is 30% (58). Therefore, it was considered that if the panelists could detect over 30% of the aroma concentrates, the components could be detected. Additionally, it was thought that the compound could be recognized as the apparent impact compound for the whole aroma of the WMP if panelists could recognize over 60% of the aroma concentrates. Based on this idea, among the detection data shown in Table 3.1, detection values of 6 or more were converted to 1 and detection values of 5 or less were converted to 0. Multivariate analysis was applied to this matrix.

Table 3.1. Aroma impact compounds in WMP.

No.	RI[a]	Compounds	Odor descriptor	Sample / Frequency of detection ●1	●2	●3	■1	■2	♦1	♦2	Earlier identification in dairy product[b]	Identification[c]
1	998	diacetyl	buttery	n.d.[f]	5	5	n.d.	n.d.	2	n.d.	1b, 2	STD, RI
2	1107	unknown	fruity, esteric	2	3	2	3	4	1	2	–	–
3	1233	(*Z*)-4-heptenal	fatty, fishy	2	4	4	1	3	5	2	1a, 1b, 2	STD, RI
4	1262	unknown	fatty	2	2	1	1	1	4	2	–	–
5	1288	octanal	citrus	4	n.d.	1	n.d.	1	n.d.	2	1a, 1b	STD, RI, MS
6	1295	acetoin	buttery	3	5	5	2	3	3	3	4	STD, RI, MS
7	1299	1-octen-3-one	mushroom	9	7	6	1	3	6	3	1a, 1b	STD, RI
8	1317	unknown	fatty, powdery	2	5	4	3	5	1	5	–	–
9	1341	2-acetyl-1-pyrroline	popcorn, nutty	7	6	7	8	6	8	8	1a, 1b, 2, 3	STD, RI
10	1361	unknown	buttery	2	5	4	3	6	2	n.d.	–	–
11	1361	(*Z*)-1,5-octadien-3-one	geranium-like	2	2	n.d.	n.d.	n.d.	2	5	1b	STD, RI
12	1441	(*E*)-2-octenal	fatty, green, nutty	4	4	3	6	4	4	5	1a, 1b	STD, RI, MS
13	1450	1-octen-3-ol	mushroom	6	5	6	4	5	5	5	3	STD, RI, MS
14	1461	methional	cooked potato-like, meaty	7	7	9	5	6	9	6	1a, 1b, 2	STD, RI
15	1553	(*E*)-2-nonenal	fatty, green	9	7	7	2	2	3	4	1a, 1b, 2	STD, RI, MS
16	1607	(*E*,*Z*)-2,6-nonadienal	oily, cucumber-like	5	4	1	2	5	3	3	1b, 2	STD, RI
17	1643	butanoic acid	cheese, butter-like	8	9	9	4	7	4	4	1a, 1b, 2	STD, RI, MS
18	1687	3-methylbutanoic acid	sweaty, cheesy	3	7	7	2	n.d.	n.d.	1	1a, 1b	STD, RI, MS
19	1728	(*E*,*E*)-2,4-nonadienal	oily	3	5	2	1	n.d.	4	3	1a, 1b, 3	STD, RI
20	1741	3-methylnonan-2,4-dione	green, fruity, hay-like,	5	7	7	1	4	8	7	3	STD, RI
21	1778	(*E*)-2-undecenal	green, cardbord-like	1	3	4	1	2	2	3	3	STD, RI
22	1791	2-acetyl-2-thiazoline	popcorn	6	5	5	4	4	2	5	1a, 1b, 2	STD, RI
23	1841	unknown	oily	3	6	7	4	2	2	4	–	–
24	1863	hexanoic acid	fatty, cheesey-sweaty	9	9	8	5	5	6	5	1a, 1b, 2, 3	STD, RI, MS
25	1942	4-octanlide	peach	4	2	4	2	1	3	2	1a, 1b, 5	STD, RI
26	1996	5-octanolide	coconut	7	5	3	4	5	4	4	1a, 1b, 2, 3	STD, RI, MS
27	2020	4,5-epoxy-(*E*)-2-decenal	metallic	5	5	7	6	3	6	4	2	STD, RI
28	2047	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (furaneol)	sweet, cotton candy	9	6	8	4	3	9	6	3	STD, RI
29	2074	octanoic acid	sweaty, waxy	4	4	4	2	2	2	3	1a, 1b, 2, 3, 5	STD, RI, MS
30	2166	4-decanolide	peach	4	4	5	3	4	4	4	1a, 1b, 3, 5	STD, RI, MS
31	2184	3-hydroxy-4,5-dimethyl-2(*H*)-furanone (sotolon)	spicy, sweet	5	4	5	3	4	4	4	1a, 1b, 3	STD, RI
32	2192	5-decanolide	creamy, milky	9	9	9	9	8	9	8	1a, 1b, 2, 3, 5	STD, RI, MS
33	2216	2-aminoacetophenone	grape, stale	5	2	4	4	4	5	4	1a, 1b, 3	STD, RI
34	2291	decanoic acid	fatty, waxy, cheesy	6	7	6	5	5	5	5	3, 5	STD, RI, MS
35	2335	5-undecanolide	sweet	5	5	5	4	3	4	4	3	STD, RI, MS
36	2384	4-dodecanolide	fruity, peach-like	5	8	7	6	6	5	5	1a, 1b, 2, 3, 5	STD, RI, MS
37	2408	(*Z*)-6-dodecen-4-olide	dairy, creamy	7	5	6	3	5	4	4	1a, 1b, 2, 3	STD, RI, MS
38	2435	5-dodecanolide	sweet	3	3	4	3	3	3	3	1a, 1b, 2, 3, 5	STD, RI, MS
39	2454	indole	fecal	3	4	4	2	2	3	2	3	STD, RI, MS
40	2482	dodecanoic acid	fatty, waxy	6	6	5	3	4	4	4	3, 5	STD, RI, MS
41	2524	skatol	fecal	4	1	1	2	3	4	3	1a, 1b, 3	STD, RI, MS

[a] Retention index on DB-WAX column. [b] Previously reported compounds in dairy products, for example: Number [Dairy product/(reference)]; 1a [WMPs/(42)], 1b [SMPs/(42)], 2 [UHT milk/(59)], 3 [skim milk and UHT milk/(28)], 4 [various milks/(60)], 5 [UHT milk/(61)]. [c] Method of identification: STD, by comparison of retention time and odor description of an authentic compound; RI, by comparison of literature retention index; MS, by comparison of mass spectrum with that of an authentic sample.

Table 3.2. Results of principal component analysis.

PC	Eigenvalue	Proportion of PC (%)	Cumulative variance (%)
1	3.7	53.3	53.3
2	1.2	16.5	69.8
3	0.7	10.5	80.4
4	0.6	8.2	88.6
5	0.4	6.3	94.9
6	0.2	2.8	97.8
7	0.2	2.2	100.0

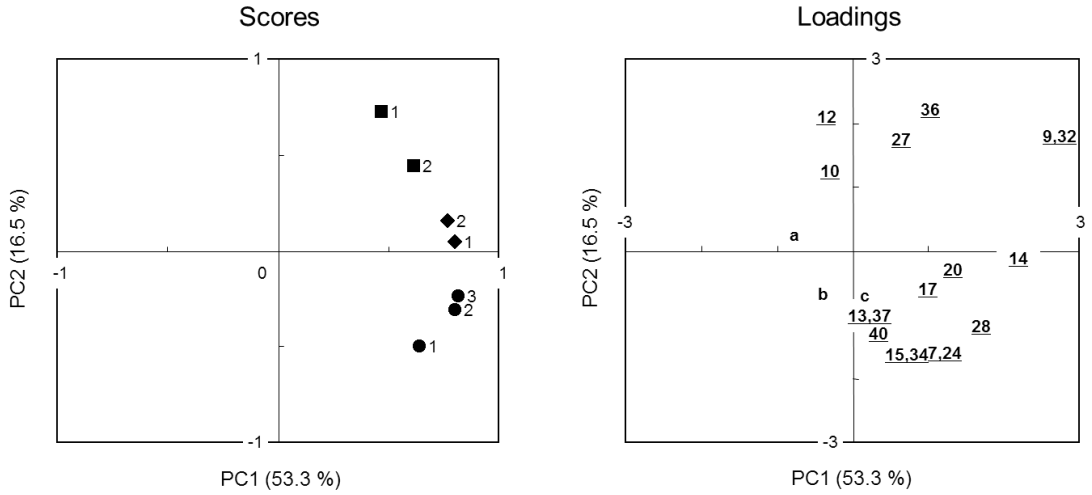


Figure 3.2. Principle component analysis of WMP Aroma.

The left diagram shows a scattered plot of PCA scores, reflecting the distribution of each sample. The right diagram shows a scattered plot of loadings, reflecting the distributions in the left diagram. The underlined numbers in the right diagram are the same as those in Table 1. Letter "a" means compounds Nos. **1**–**6**, **8**, **11**, **16**, **19**, **21**, **25**, **29**–**31**, **33**, **35**, **38**, **39**, and **41** in Table 3.1; "b" means compounds Nos. **22** and **26** in Table 3.1, and "c" means compounds Nos. **18** and **23** in Table 3.1.

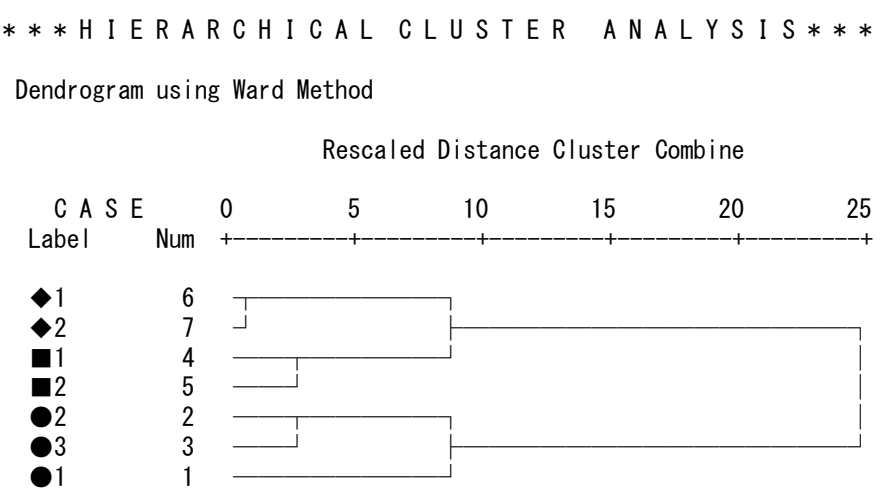
Based on the results of PCA, the total variables were aggregated to 53.3% of the first principal component (PC1) and 16.5% of the second principal component (PC2) (Table 3.2). Because the PC1 and PC2 scores explained approximately 70% of the total variance of WMPs and the eigenvalue of PC3 was below 1 (Table 3.2), this PCA result was employed to examine the relations between the aroma impact compounds and the WMP's sensory properties. Although the PC1 scores explained approximately 53% of the total variance, WMPs were not classified based on individual production regions. Therefore, the PC1 scores can be used to explain the property of WMPs based on the aroma of dairy products. In contrast, the PC2 scores indicated the property of the production region based on the scattering plot of each WMP (Figure 3.2, left). The HCA result with Ward's method also showed three clusters of each production region (Figure 3.3).

Table 3.3. PCA loadings of identified compounds.

		Loading	
No.	Compounds	PC1	PC2
7	1-octen-3-one	1.0692	- 1.5845
9	2-acetyl-1-pyrroline	2.5642	1.7874
10	unknown	- 0.2888	1.2850
12	(*E*)-2-octenal	- 0.3698	2.1093
13	1-octen-3-ol	0.0607	- 1.1368
14	methional	2.1727	- 0.1159
15	(*E*)-2-nonenal	0.5236	- 1.6602
17	butanoic acid	0.9962	- 0.5813
20	3-methylnonan-2,4-dione	1.3344	- 0.2840
24	hexanoic acid	1.0692	- 1.5845
27	4,5-epoxy-(*E*)-2-decenal	0.6320	1.7530
28	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (furaneol)	1.7002	- 1.1948
32	5-decanolide	2.5642	1.7874
34	decanoic acid	0.5236	- 1.6602
36	4-dodecanolide	1.0219	2.2328
37	(*Z*)-6-dodecen-4-olide	0.0607	- 1.1368
40	dodecanoic acid	0.0673	- 1.2282
	a	- 0.7613	0.2060
	b	- 0.3956	- 0.7048
	c	0.1579	- 0.7494

The numbers above are the same as that in Table 3.1 and letters "a," "b," and "c" shown in Figure 3.2.

Figure 3.3. Dendrogram of seven WMP samples by HCA.



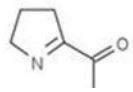
Seventeen compounds (Compounds **7**, **9**, **10**, **12**, **13**, **14**, **15**, **17**, **20**, **24**, **27**, **28**, **32**, **34**, **36**, **37**, and **40**), for which the absolute values of PC1 or PC2 loading were more than 0.99, were shown to have a significant effect on the plot of each WMP (Table 3.3). Among these, compounds **7**, **9**, **14**, **17**, **20**, **24**, **28**, **32**, and **36**, for which the PC1 loading values were more than 0.99, are well-known aroma compounds of dairy products. In particular, 2-acetyl-1-pyrroline (**9**) is known to have a popcorn-like and nutty sweet odor, and 5-decanolide (**32**) is known to have a creamy and milky odor. Each compound is well known as an aroma compound of milk. Methional (**14**) is a sulfur-containing compound with a characteristic cooked potato or meaty odor, and is known to contribute to the whole aroma of WMPs. Based on the PC2 loading values, these effective 17 compounds can be divided into three groups: those with 1 or more, those from 1 to -1, and those with -1 or less. Group 1 comprised compounds **9**, **10**, **12**, **27**, **32**, and **36**; Group 2 compounds **14**, **17**, and **20**; and Group 3 compounds **7**, **13**, **15**, **24**, **28**, **34**, **37**, and **40** (Figure 3.2, right).

Samples ■1 and ■2 had a mild dairy flavor with a pleasant milk-like aroma. Each positive position of samples ■1 and ■2 on the PC2 scores was affected by

2-acetyl-1-pyrroline (**9**), 5-decanolide (**32**), (*E*)-2-octenal (**12**), 4,5-epoxy-(*E*)-2-decenal (**27**), 4-dodecanolide (**36**), and unknown (**10**) of Group 1. 2-Acetyl-1-pyrroline (**9**) and 5-decanolide (**32**), the constituent compounds of Group 1, were also considered as compounds showing properties of dairy products based on the PC1 loadings. A fatty, green, and nutty odor that (*E*)-2-octenal possesses and a metallic odor that 4,5-epoxy-(*E*)-2-decenal possesses were known as factors indicating butter aroma (62). 4-Dodecanolide possesses a fruity and peach-like odor; it has also been reported as one of the aroma components of ultrahigh temperature (UHT) milk (61). Compound 10 was unknown, but its contribution to the WMP aroma was inferred from the fact that it had a butter-like odor. Each of these compounds was generally recognized as an aroma component of dairy products. Therefore, samples ■1 and ■2 (produced in Japan) were proposed to have a mild dairy aroma without an unpleasant sensation.

Samples ◆1 and ◆2 had a sweet and cheesy dairy flavor with a slight green or hay-like nuance. Their positions on the PC2 scores were affected by the Group-2 compounds. Methional (**14**), butanoic acid (**17**), and 3-methylnona-2,4-dione (**20**), the constituent compounds of Group 2, were also considered as compounds indicating the properties of dairy products based on the PC1 loadings. The green and hay-like odor of 3-methylpentane-2,4-dione (**20**) contributed to the characteristic grassy odor of samples ◆1 and ◆2. Similarly, the sweet odor with a meaty nuance that methional (**14**) possesses and the cheesy odor of butanoic acid (**17**) contributed to the characteristic odor of samples ◆1 and ◆2, respectively. Based on the high PC1 loading, 2-acetyl-1-pyrroline (**9**), furaneol (**28**), and 5-decanolide (**32**) were suggested as contributors of the sweet and milky odor of samples ◆1 and ◆2, even though they were classified to other groups.

Group 1:



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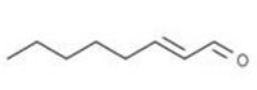

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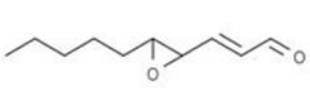

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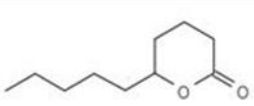

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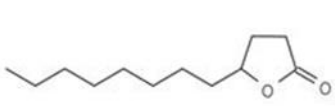

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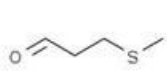
Group 2:



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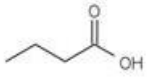

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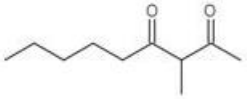

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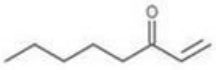

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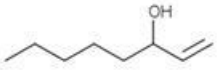

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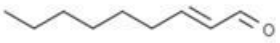

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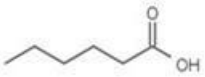

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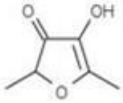

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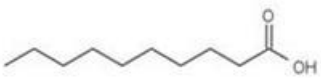

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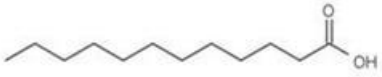

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Samples ●1, ●2, and ●3 had a buttery, oily, and cheesy flavor with a fatty and fermented nuance. Their positions on the PC2 scores were affected by the Group-3 compounds. 1-Octen-3-one (**7**), hexanoic acid (**24**), and furaneol (**28**), present in Group-3 compounds, showed the features of dairy products based on the PC1 loading. Acids such as hexanoic acid (**24**), decanoic acid (**34**), and dodecanoic acid (**40**), and (*E*)-2-nonenal (**15**) possess a fatty, waxy, and sweaty odor. Both 1-octen-3-one (**7**) and 1-octen-3-ol (**13**) possess a deteriorated fat odor with a mushroom nuance. Such compounds in Group 3 probably contributed to the odor perceived in samples ●1, ●2, and ●3.

The seven WMPs, produced in three different regions, were classified into each region by the following experimental steps. Step 1: obtain the sniffing data by using the three-port GC-O based on the detection frequency method; step 2: convert the data obtained in step 1 to binary data; and step 3: apply the binary data obtained in step 2 to multivariate analysis. It was shown that using the three-port GC-O for sniffing is useful for shortening the experiment time, and that the multivariate analysis is useful for considering individual characteristics which different types of foods possess.

As the conclusion of chapter 3, it was found that, depending on the production region, the difference between the whole aromas of different WMPs was caused not by the characteristic compound, but by the balance of aroma impact compounds that commonly occur in WMPs.

Chapter 4. Investigation of *Koku* Impact Compounds in Sweet Cream on the Basis of Sensory Evaluation and Separation Techniques

Introduction

The Japanese word *Koku* is often used to describe a thick, deep taste. This is a complicated term which various concepts were blended together and is conveniently used to describe the tastes of various foods. Yamaguchi presented the general understanding of *Koku* by extracting its common factors which explain the taste of various foods (63). Subsequently, Yamaguchi proposed that *Koku* represents a combined sensation of mixed, multiple components rather than a strong, single component (64). Kobayashi *et al*. revealed that the composite factors of *Koku* of the sweet cream can be expressed by the association of 2 factors, namely, richness in milk-fat and texture and 3 terms, namely, thickness, aftertaste, and volume by the analysis of the elemental terms related to *Koku* using structural equation modeling (SEM), a multivariate analysis technique (Figure 4.1) (65). About the two factors, texture was attributable to kotteri (heavy), stickiness (degree of viscosity), denseness, and nettori (high viscosity); richness in milk-fat was attributable to creaminess, high milk-fat sensation, and fattiness.

Sweet cream consists of many constituents such as lipids, proteins, sugars, and aroma components. The compounds that have an impact upon *Koku* in sweet cream have not been characterized. Recently, long-chain aliphatic lactones in sweet cream have been reported to act upon several kinds of oral senses, which involve perception of creaminess, mouth coating, and fatty mouthfeel at low concentrations (66). However, the formation of lactones under the experimental conditions employed and the compounds other than lactones that contribute to creaminess has not been reported.

In chapter 4, we focused on lipid fractions of sweet cream on the basis of the report of Kobayashi *et al*. (65), and investigated *Koku*-impact compounds in a sweet cream by using fractionation, sensory evaluation and instrumental analysis.

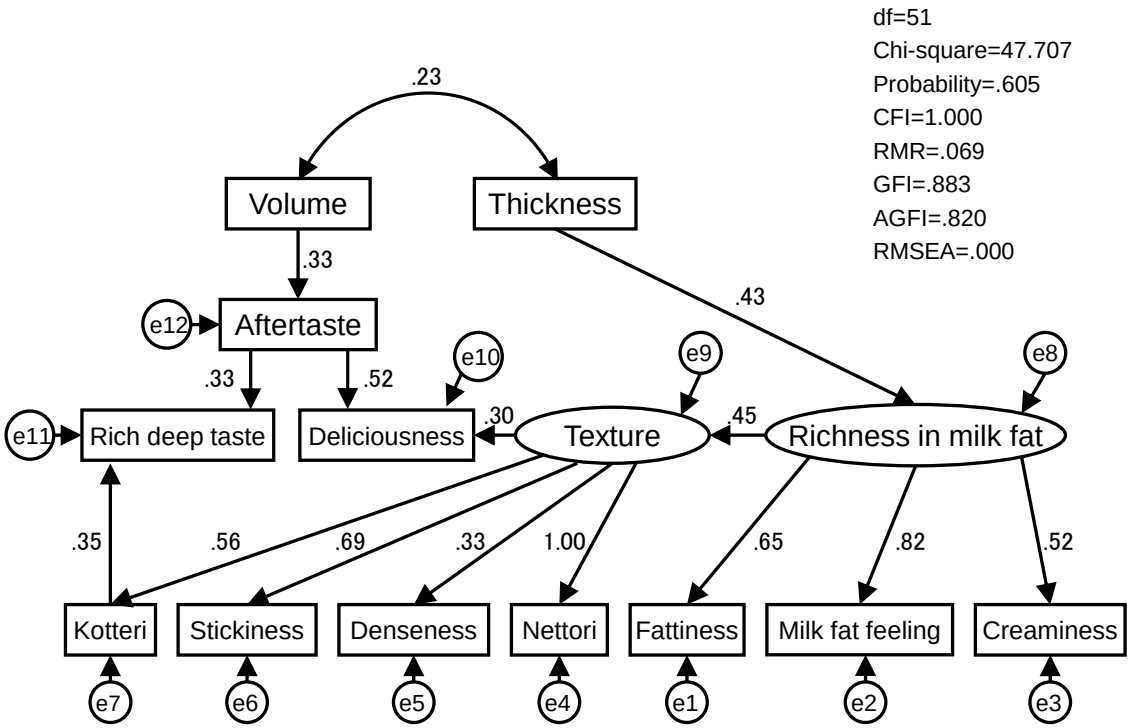

Figure 4.1. Structural equation model for elemental terms related to *Koku*.

This figure is the modified one of reference No. 65. Square boxes mean observation valuable of each term. Ellipse means latent valuable. Circle means residual. Number in the neighbor of the arrowed line is coefficient of correlation value. Chi-square/df, Probability, CFI, RMR, GFI, AGFI and RMSEA are goodness-of-fit indices for SEM.

Material and Methods

Materials. The sample of sweet cream which has a fat content is 47% was purchased at a domestic market and was stored at 4 °C until use. Refined palm oil was used after it was confirmed to be odorless by sensory evaluation and showed absence of long chain aliphatic compounds in GC and GC-MS.

Fractionation of the sweet cream (1) by solvent-assisted flavor evaporation (SAFE). In order to separate volatile from semi-volatile and non-volatile components in sweet cream, SAFE was used (6). Over a period of 30 min, the cream (500 g) was dropped into the SAFE apparatus (sample flask at 50 °C, vacuum at less than 1×10^{-4} Pa). Subsequently, the apparatus was kept for 30 min. The traps were cooled with liquid nitrogen. This operation was performed twice. The SAFE distillate (Figure 4.2) was stored at –20 °C until use. The SAFE residue was used in a subsequent operation.

(2) Acetone extraction. The SAFE residue was extracted twice with 300 ml of acetone. The resulting mixture was separated into two layers by centrifugation (at 3000 rpm, for 5 min, at 10 °C). The lower layer was furthermore extracted three times with 30 mL of acetone and separated into 2 layers. The upper acetone layers were pooled, filtered, and concentrated with a rotary evaporator (sample flask at 35 °C, and vacuum at 280 mmHg). The resulting milk fat (Milk fat Fr.) was stored at -20 °C under a nitrogen atmosphere until use.

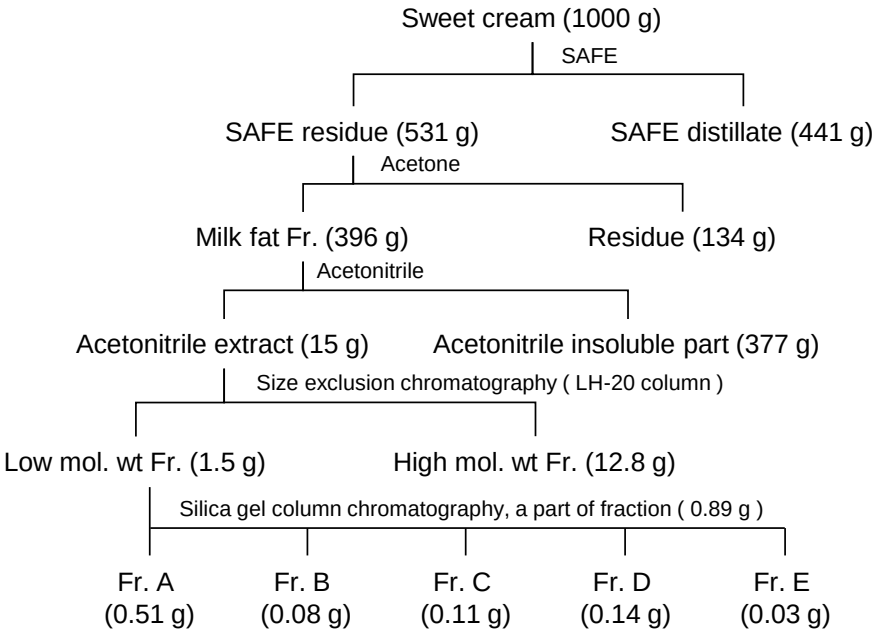

Figure 4.2. Procedure for fractionation of sweet cream.

Table 4.1. Formulations of evaluated samples.

	Weight (g)						
	Base sample	Sample					
Component		a	b	c	d	e	f
Refined palm oil	20.0	20.0	-	20.0	-	20.0	20.0
Lactose monohydrate	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Span 80 (emulsifier)	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Non fat dry milk	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Distilled water	26.8	-	26.8	26.8	26.8	26.8	26.8
SAFE distillate[a]	-	26.8	-	-	-	-	-
Milk fat Fr.[a]	-	-	20.0	-	-	-	-
Acetonitrile extract[a]	-	-	-	0.8	-	-	-
Acetonitrile insoluble part[a]	-	-	-	-	20.0	-	-
Low mol. wt Fr.[a]	-	-	-	-	-	0.1	-
High mol. wt Fr.[a]	-	-	-	-	-	-	0.6
Total	50.0	50.0	50.0	50.8	50.0	50.1	50.6

[a] Fractions shown in Figure 4.2.

(3) Acetonitrile extraction. The Milk fat Fr. was extracted with 300 mL of acetonitrile while stirring the solution over a 30-min period according to the extraction method of Alewijn *et al*. (67). This operation was repeated three times. The upper layers obtained with separate funnels were filtered, and the solvent volume was reduced with a rotary evaporator (water bath at 40 °C, and vacuum at 120 mmHg) and a subsequent high-vacuum transfer (10 min, approximately 1 mmHg). The acetonitrile extract and the acetonitrile insoluble part were stored at -20 °C.

(4) Size exclusion chromatography. A 3 g portion of the acetonitrile extract dissolved in acetone was placed onto the top of a 50 × 2.0 cm i.d. glass column filled with a slurry of Dextran gel (160 mL, Sephadex LH-20) in acetone. The size exclusion chromatography was carried out according to the method of Winkler *et al*. (68) and was performed using acetone. The injected extract was separated into 45 parts of 3 mL each. Each part was monitored using GC and classified into the following two fractions. (I) The first fraction was designated as the Low mol. wt Fr. This fraction consisted of parts that contained several low molecular weight

compounds (< Mw. 300). (II) The second fraction was designated as the High mol. wt Fr. It consisted of parts that do not contain the low molecular weight compounds. The Low mol. wt Fr. and the High mol. wt Fr. were filtered, and the solvent was removed with a rotary evaporator under the same conditions used for acetone extraction. Both fractions were stored as described above.

(5) Silica gel chromatography. The silica gel column chromatography was performed according to a previously reported procedure for lipid separation (69). A portion of the Low mol. wt Fr. (0.89 g) was dissolved in diethyl ether and applied to a 20.0 × 2.5 cm i.d. glass column filled with the slurry of the silica gel (40 g, Wakogel C-200) in n-pentane. A sequential elution was performed using n-pentane/diethyl ether (95:5, v/v; 200 mL), followed by n-pentane/diethyl ether (90:10, v/v; 200 mL), n-pentane/diethyl ether (80:20, v/v; 200 mL, Fr. B), n-pentane/diethyl ether (60:40, v/v; 200 mL, Fr. C), diethyl ether (v/v; 200 mL, Fr. D) and ethyl acetate (v/v; 200 mL, Fr. E). Eluates with n-pentane/diethyl ether (95:5) and (90:10) had approximately equal chemical compositions from the results of GC-MS analysis. The combination of both eluates was designated as Fr. A. Each fraction was dried over anhydrous sodium sulfate and filtered. The solvent was then removed with a rotary evaporator (water bath at 35°C, and vacuum: fraction A–D at 550 mmHg, and fraction E at 150 mmHg). The concentrated fractions were stored as described above.

Sensory evaluation (1) Assessor. Thirteen assessors (men, 10 and women, 3) were recruited from the laboratory of Ogawa & Co., Ltd. Their age range was between 28–55. Their in-house training courses included more than 60 h of training on many aspects of sensory analysis, including recognition, description, and discrimination tests. The number of participants is described individually in each paragraph below because the number of assessors participating in each experiment was different.

(2) Sample preparation. A control sample for comparative sensory evaluation was required. Sweet cream is not appropriate for a control sample because it essentially contains *Koku* components. It has been known that the expression of taste and flavor differed by food matrices. Considering expression of *Koku* components, preparation of emulsion was essential for a control sample. Additionally, a control sample needed a simple formula because many samples would be prepared based on control sample. From these reason, control sample was prepared by reconstitution of lipids, proteins, sugars and water. These components are major constituent of sweet cream. The components ratio referred to the ratio of sweet cream in "Standard Tables of Food Composition in Japan, Item No. 13014" (70). A lipid used for sample was a refined palm oil that has a similar composition to milk fat. The emulsification condition was at 18,000 rpm for 15 min with a homogenizer. This sample was designated as the base sample and its formula was shown in Table 4.1. The amount of individual fraction added to the evaluation sample was calculated from the fraction weight, and was adjusted to represent the same amount as the original sweet cream. The method of preparing the samples for evaluation is described below.

(3) Sensory evaluation of the SAFE distillate and the Milk fat Fr. The samples for evaluation were prepared by emulsifying the compositions of Sample a and Sample b described in Table 4.1. These two samples and the base sample were presented for sensory evaluation at room temperature in 50 mL glass beakers labeled with a three-digit random number. At the beginning of the evaluation, ten assessors were required to pinch their noses to suppress the aroma effect, to wash their mouths with oolong tea to reduce the influence of the previous sample during the evaluation interval, and to rate the difference of the overall intensity of *Koku* on a scale from -3 (none at all) to 3 (strong). The sample (approximately 1 mL) was dropped on the latter half of tongue with a plastic syringe, briefly swirled in the mouth and evaluated. The evaluation was carried out under yellow light to prevent a color effect with respect to milk fat.

In order to elucidate the differences, Scheffé's paired comparison was used (71) and data analysis was performed using Microsoft Excel 2002. A difference of $p < 0.05$ was considered to be significant.

(4) Sensory evaluation of the Acetonitrile extract and the Acetonitrile insoluble part. The samples for evaluation were prepared by emulsifying the compositions of Sample c and Sample d described in Table 4.1. The evaluation conditions and data analysis were the same as described in section 3 except that fluorescent light was used.

(5) Sensory evaluation of the Low mol. wt Fr. and the High mol. wt Fr. The samples for evaluation were prepared by emulsifying the compositions of Sample e and Sample f in Table 4.1. The evaluation conditions and data analysis were the same as described in section 4 except that 12 assessors participated.

(6) Sensory evaluation of fractions from silica gel chromatography. Fractions from A to E were evaluated by emulsifying with the base sample. The amounts of added fractions per 50 g base sample were as follows: Fr. A, 26 mg; Fr. B, 4 mg; Fr. C, 6 mg; Fr. D, 7 mg; and Fr. E, 2 mg. Nine assessors were asked to rate the overall *Koku* intensity of the five samples on a scale from 0 (none) to 6 (strong). ANOVA was used to elucidate the differences. Data analysis was performed using Microsoft Excel 2002 in accordance with the method of reference (72). The other evaluation conditions were the same as the conditions described in section 4.

(7) Reconstitution experiment. For this reconstitution experiment, the following four samples were prepared: the reconstituted sample, the sample with added amounts of Fr. A and Fr. B, the base sample and the sweet cream with 40% fat. The sample with added amounts of Fr. A and Fr. B was prepared by adding Fr. A (26 mg) and B (4 mg) to the base sample. This sample is designated Sample I. The individual concentrations of the eleven compounds in Table 4.2 were calculated by multiplying the peak area % by the fraction weight. The reconstituted sample was

prepared by adding the eleven compounds to the base sample and these concentrations in the sample were adjusted to the same concentration as the sweet cream. This sample is designated Sample II.

The following 4 attributes were applied to sensory evaluation: thickness, aftertaste, richness in milk-fat, and texture. These words were key element of a structural equation model that was constructed from the words related to sweet cream *Koku* (65), and were considered important for simple and clear expression of *Koku*. On the basis of a standard score of denseness of 13 points in cream cheese (72), a preliminary evaluation of sweet cream was carried out by 6 assessors. The evaluation scores were as follows: thickness, 11.5; aftertaste, 12.5; richness in milk-fat, 10.7; and texture, 8.8, respectively. Based on these four scores, nine assessors rated the intensity of these attributes against the above-mentioned samples on a scale from 0 (none) to 15 (strong). The data analysis was performed by ANOVA (72) using SPSS version 11.0J. The other evaluation conditions were the same as the conditions described in section 4.

(8) Addition test. The compounds identified in the reconstitution experiment were classified into the following 3 groups: long-chain fatty acids, long-chain aliphatic δ-lactones, and cholesterol. The effects of addition of each group of compounds were investigated using a triangle test with 17 assessors. Samples for evaluation were prepared by emulsification of the compounds which were added to the base sample in each group at the concentrations listed in Table 2. The other conditions for evaluation were the same as those described in section 7.

GC. For rapid analysis of the fractions, an Agilent Model 6850 gas chromatograph coupled to an FID was used. The column was a 3.5 m × 0.25 mm i.d. DB-1 with a film thickness of 0.25 μm (Agilent Technologies, Inc.). The oven temperature was programmed to increase from 150 °C to 320 °C at a rate of 20 °C/min. The injector temperature was 350 °C. The flow rate of the nitrogen carrier gas was 1 mL/min for 8.5 min, followed by 20 mL/min at a rate of 5

mL/min. An injection volume of 0.2 μL was applied using the split technique (the split ratio was 1:30). The detector conditions were as follows: detector temperature, 320 °C; hydrogen flow, 30.0 mL/min; air flow, 400.0 mL/min; makeup flow, 25.0 mL/min; makeup gas type, nitrogen.

GC-MS. For the identification of semi-volatiles, an Agilent Model 6890N gas chromatograph coupled to an Agilent Model 5973N series MSD was used. The column was a 60 m × 0.25 mm i.d. HP-5MS with a film thickness of 0.25 μm (Agilent Technologies, Inc.). The oven temperature was programmed to increase from 100 °C to 320 °C at a rate of 4 °C/min. The injector temperature was 320 °C. The flow rate of the helium carrier gas was 1 mL/min. An injection volume of 1.0 μL was applied using the split technique (the split ratios 1:30 and 1:100). The MSD conditions were as follows: capillary direct interface temperature, 220 °C; ionization voltage, 70 eV (EI); mass range, 33–550 amu; and ion source temperature, 320 °C.

Identification of compounds. Identification of long-chain fatty acids, long-chain aliphatic δ-lactones, and cholesterol was based on the comparison of GC retention indices on the HP-5MS column and comparisons of mass spectral peaks with those of authentic standard compounds. The retention indices (RI) of Table 4.2 were calculated using an n-alkane ladder (C10–C32). Long-chain fatty acid monoglycerides were tentatively identified by comparison of mass spectral peaks with those in the Wiley7 library. Other glycerides were estimated by comparing peak patterns of edible oils and their retention times.

Results and Discussion

Separation of *Koku* impact fractions from a sweet cream and sensory evaluation. The fractionation procedure and the mass balance of the sweet cream are shown in Figure 4.2.

In the first step, the sweet cream was separated into the SAFE residue (semi- and non-volatile) and the SAFE distillate (volatile) using SAFE. Lactones of dairy products are known to form quickly at approximately 160 °C. This reaction has been described as an intramolecular reaction of hydroxy fatty acid glycerides (73). The SAFE method used in this experiment has the advantage of providing distillation under the lowest possible temperature. It was thus expected that hydroxyl fatty acid glycerides included in the sweet cream are constrained to convert to the corresponding lactones. Engel *et al*. reported that the compounds with boiling points of 260 °C or higher have low recovery rates according to the original research in the development of SAFE (6). Such compounds can be defined as semi-volatile compounds. Our GC analysis result also indicates that there are few semi-volatile compounds in the SAFE distillate. Additionally, the SAFE residue had few odors that were not similar to those of sweet cream.

The SAFE residue was further separated. Sample a, Sample b and the base sample in Table 4.1 were prepared by the use of resulting fractions and evaluated to confirm whether *Koku* of the sweet cream was derived from the SAFE distillate (volatile fraction) or from the Milk fat Fr. The sensory evaluation results showed that Sample b had significantly stronger *Koku* than that of other two samples on hedonic scale (Figure 4.3.1). The *Koku* sensation is caused by the lipid part of cream and not by volatiles.

Table 4.2. Compounds identified in fractions.

Fraction	No.	Main component	RI[a]	Response factor[b]	Concentration[c] (mg/kg)	Threshold reported[d] (mg/kg)
Fr. A						
	1	decanoic acid	1361	0.51	16.3	250
	2	dodecanoic acid	1562	0.87	21.8	200
	3	tetradecanoic acid	1761	0.66	32.2	5000
	4	hexadecanoic acid	1959	0.67	66.7	15000
	5	octadecanoic acid	2172	0.43	20.3	10000
	6	oleic acid	2151	0.49	101.1	2540
	7	linoleic acid	2139	0.50	10.8	1120
Fr. B						
	8	5-dodecanolide	1708	1.37	1.0	9.3
	9	5-tetradecanolide	1920	1.27	1.3	14.9
	10	5-hexadecanolide	2137	1.16	1.0	91.5
	11	cholesterol	3043	1.37	113.8	-
Fr. C						
		Acylglycerides				
Fr. D						
		Long chain fatty acid monoglycerides (C10–16)				
Fr. E						
		No peak detected by GC				
Internal standard						
		1-chlorooctadecane	2085	1.00	-	-

[a] Retention index on the HP-5MS column. [b] Response factors against the internal standard compound 1-chlorooctadecane. [c] The concentrations were calculated by multiplying the GC–MS peak area percent by the fraction weight. [d] The threshold values reported are shown: compound Nos. **1**–**5** (74), Nos. **6**–**7** (75), and Nos. **8**–**11** (66). The unit of threshold of lactones was modified according to that of the reported data.

Subsequently, by using the acetonitrile extract and the acetonitrile insoluble part, Sample c and Sample d were prepared and evaluated. The sensory evaluation results showed that Sample c has significantly stronger *Koku* than the other two samples (Figure 4.3.2). Moreover, the Low mol. wt Fr. and the High mol. wt Fr. were obtained from the acetonitrile extract by using size exclusion chromatography. The Low mol. wt Fr. mainly contains compounds with a molecular weight of less than 300, and the High mol. wt Fr. predominately consists of triacylglycerides. The sensory evaluation results of Sample e, Sample f and the base sample

showed that Sample e has significantly stronger *Koku* than the other two samples (Figure 4.3.3). Therefore, it was suggested that the sensation of *Koku* in sweet cream is not caused by the triacylglycerides that are the main component of milk fat.

For the purpose of this detailed investigation, the Low mol. wt Fr. was fractionated into five fractions, Fr. A to Fr. E with silica gel chromatography. All fractions were presented for sensory evaluation. The sensory evaluation result indicates that the addition of Fr. A or B significantly affects *Koku* relative to the other samples (Figure 4.4). From the GC-MS analysis, it was found that Fr. A include even-numbered long-chain fatty acids (C10–C18); Fr. B includes cholesterol and even-numbered long-chain aliphatic δ-lactones (C12–C16); Fr. C includes acylglycerides; and Fr. D includes long-chain fatty acid monoglycerides (C10–C16). No peaks were detected in Fr. E. Notably, long-chain fatty acids were found to constitute 43 % of Fr. A, cholesterol constitutes 91 % of Fr. B, and long-chain aliphatic δ-lactones constitute 3 % of Fr. B. The main components identified in Fr. A and B, and their concentrations are shown in Table 4.2.

Reconstitution experiment. A reconstitution experiment was carried out to confirm that the 11 compounds listed in Table 4.2 contribute to *Koku*. The four samples (Sample I, Sample II, the base sample and the sweet cream with 40% fat) were prepared for sensory evaluation. Kobayashi *et al*. proposed keywords from the structure of sensory terms on sweet cream *Koku* (65). The following four attributes, thickness, aftertaste, richness in milk-fat, and texture, were used for the sensory evaluation. Sample I and Sample II had significantly higher scores in thickness, aftertaste, and richness in milk-fat than the base sample, and tended to approach the score of the sweet cream (Figure 4.5). The increase of the thickness score in Sample I and Sample II was interpreted as an increase in *Koku* because the term *Koku* and thickness were closely related (65). Sample I and Sample II were found to have higher texture scores than that of the base sample. However, a significant difference was not observed. These results suggest

that the addition of the eleven compounds or the Fr. A and B to the base sample tended to enhance the sweet cream *Koku*. Moreover, the addition effects for Sample I and Sample II did not exhibit significant differences. Therefore these effects were estimated to be roughly equal. Consequently, it was shown that the sweet cream *Koku* was reproduced by the eleven compounds shown in Table 4.2.

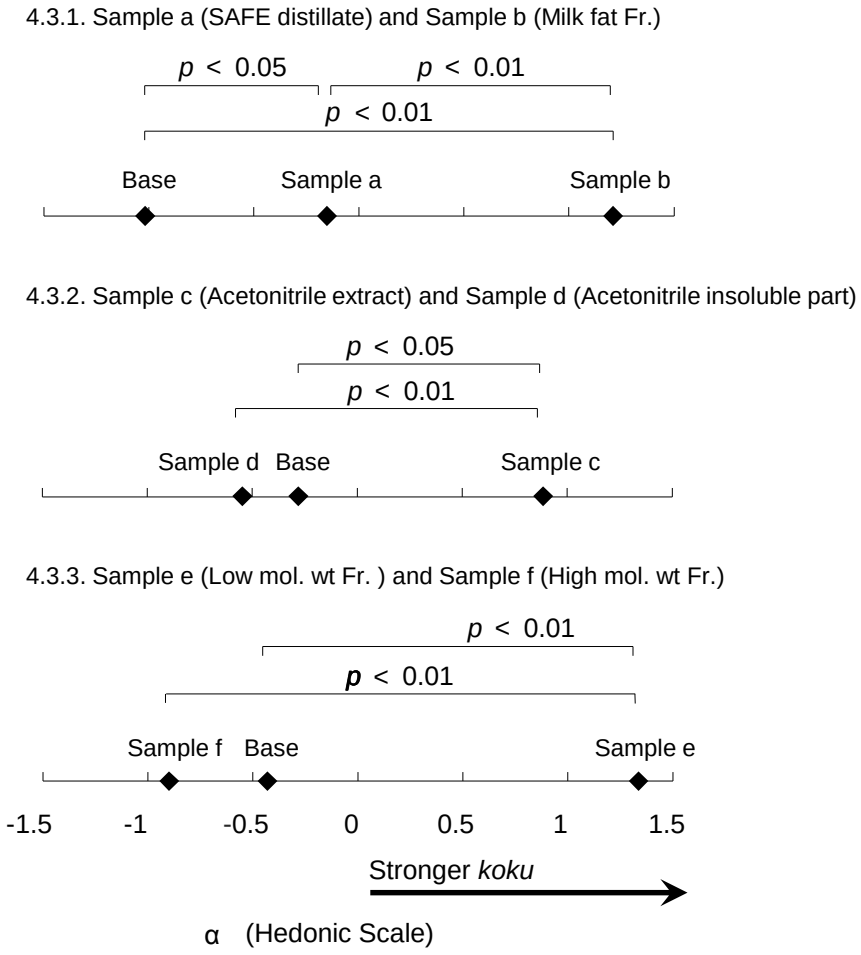

Figure 4.3. Sensory evaluation of the fractions in each separation step.

Details in each sample are shown in Table 4.1. The sensory evaluation scores were statistically analyzed by using Scheffé's paired comparison. "Base" means the base sample as control sample. The horizontal axes in the graphs show the average hedonic scale. The lines on the scale show the significant differences. *Koku* intensity is indicated by arrowed line.

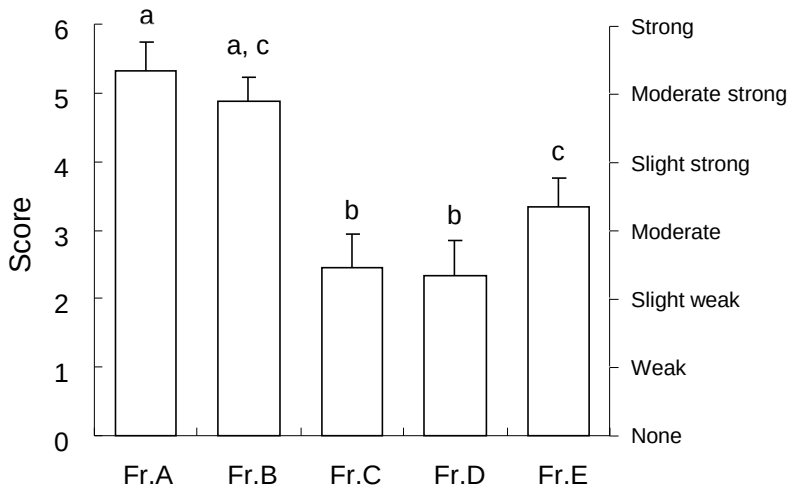

Figure 4.4. *Koku* intensity of the silica gel fractions.

Details on each fraction are shown in Table 4.2. The evaluated scores are expressed as the mean plus standard error (n = 9). Different letters indicate the existence of significant differences ($p < 0.05$). Letter "c" indicates the tendency of difference ($p = 0.05$).

Addition test. The triangle test was employed to test the following three groups: the fatty acid group (Nos. **1**–**7** in Table 4.2), the lactones group (**8**–**10**), and cholesterol (**11**). The results of the test are shown in Table 4-3. Significant differences were identified between the long-chain fatty acids addition sample and the base sample, and between the long-chain aliphatic δ-lactones addition sample and the base sample. There was no significant difference between the cholesterol addition sample and the base sample. The addition test revealed that the long-chain fatty acids and the long-chain aliphatic δ-lactones had pronounced effects on the *Koku*.

Schlutt *et al*. reported that creaminess was reproduced only after addition of an amount of δ-tetradecalactone to sweet cream which exceeds a threshold value (66). Table 4.2 indicates that individual long-chain aliphatic compounds in the fractions are below the threshold level. Our reconstitution experiment suggests that the mixture of long chain aliphatic compounds, which were also below the threshold, contribute to the *Koku* of sweet cream. These observations

indicate that the sweet cream *Koku* may be reproduced not only by a single component above the threshold but also by synergistic or the additive effects of multiple long-chain aliphatic components which are below threshold levels. Long-chain fatty acids were reported to play an important role in the preference of rodents for fat according to the results of the various investigations into the eating-behavior, neuroscience and metabolite determination of lipids in rodents (76). Additionally, Fushiki pointed out the possibility that humans have the ability to taste fatty acids in food, and proposed that such tastes would not be regarded as belonging to the category of classical basic tastes (76). The study of chapter 4 suggests that long-chain fatty acids contribute to the preference of fat not only in rodents but also in humans, and has additionally revealed that long chain fatty acids and lactones reproduce the *Koku* of sweet cream.

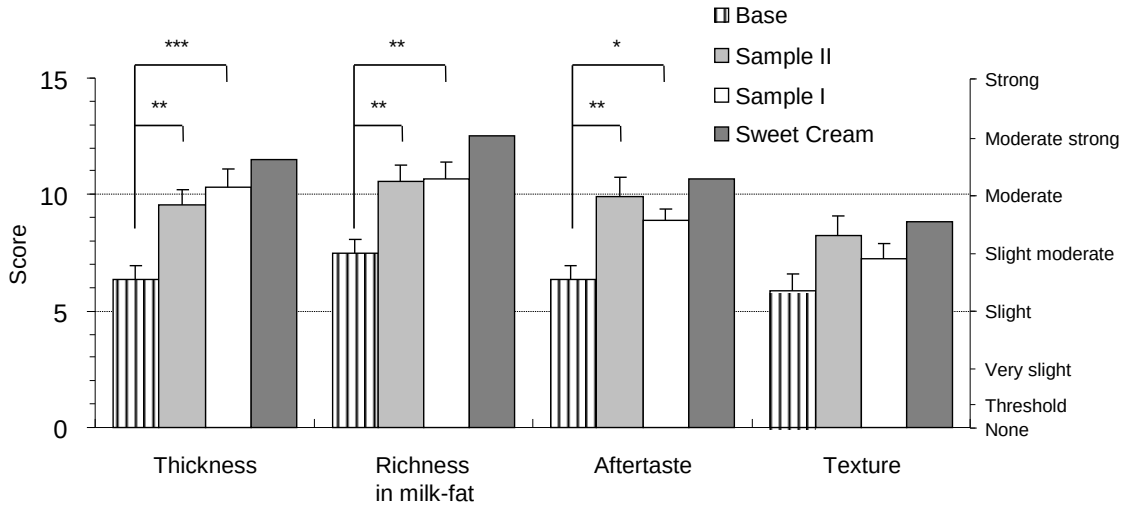

Figure 4.5. Sensory profile of the base sample, Sample I (the sample added Fr. A and Fr. B), Sample II (the reconstituted sample), and sweet cream.

The evaluated scores are expressed as the mean plus standard error (n = 9). Asterisks in the graph indicate significant differences (Tukey's HSD test): ∗, $p < 0.05$; ∗∗, $p < 0.01$; ∗∗∗, $p < 0.001$. Four attributes: thickness, richness in milk-fat, aftertaste, and texture, were the key words used to develop the sensory structure of *Koku* for sweet cream (65).

Table 4.3. Result of the addition test with the triangle test.

	Correct responses/ total responses	p^a
Long chain fatty acids [b]	11/17	0.01
Long chain aliphatic lactones[c]	10/17	0.05
Cholesterol	9/17	NS[d]

[a] The table of reference with respect to the critical number of correct responses in a triangle test was used for the detection of the significant differences (72). [b] Compound Nos. **1**–**7** in Table 4.2. [c] Compound Nos. **8**–**10** in Table 4.2. [d] Not significant.

Chapter 5. Conclusions

The objectives of this thesis were a research assessment of the flavor impact compounds of dairy products consumed in Japan, using an integrated approach of sensory-directed analytical techniques and sensory evaluation. The knowledge of which flavor impact compounds contribute to natural flavor is useful for the development of food palatability, including dairy products. The findings from this thesis are summarized below.

In chapter 2, the comparative study of skim milk powder (SMP) with UHT milk was performed to identify the character impact odorant of high-heat skim milk powder (HHSMP). An aroma concentrate was prepared by column adsorption combined with simultaneous distillation–extraction. AEDA revealed 58 aroma peaks with FD factors ranging from 10 to 3000 and 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 AEDA results of both samples were compared, the characteristic aroma of HHSMP was explained, not by a single compound but by a mixture of several types of compounds in common with UHT milk. The contribution of these compounds to the aroma of HHSMP was confirmed by an aroma simulation experiment.

The main component of SMP is proteins produced by the defatting and drying process. Methyl 2-methyl-3-furyl disulfide and bis(2-methyl-3-furyl) disulfide have a roast meat or sulfurous type odor above a certain level of concentration and were an analog of 2-methyl-3-furanthiol that was found in roast meat. When considering these findings together with the fact that milk is converted from blood and meat, it suggests that the flavor of skim milk powder evokes the existence of protein at the very low concentration of these compounds. This

accomplishment obtained in this study was issued a flavor patent that possesses the fullness of the dairy flavor using these materials (77).

In chapter 3, the common components and characteristic compounds of whole milk powders (WMPs) produced in three different regions of Asia and Oceania were investigated. The volatile components were isolated from seven WMPs produced in Japan, New Zealand, and China. Forty-one aroma-active compounds were detected in these volatile components using GC-O by the detection frequency method. The binary data converted from the detection frequency values were applied to PCA and HCA. HCA results showed three clusters corresponding with the production regions. Based on PCA, approximately 70% of the total variance of WMPs was explained by the PC1 and PC2 scores. PC1 scores examined the property of WMPs based on the aroma of dairy products. PC2 scores indicated the production region based on the scattering of each WMP. Based on PC2 loading values of the aroma-active compounds, it was revealed that, depending on the production region, the differences between the whole aromas of different WMPs was caused not by the characteristic compound but by a balance of aroma impact compounds that commonly occur in WMPs. In this research of aroma impact compounds in WMP, some of the specific flavor compounds in the production areas were able to be elucidated. From another perspective, the combination of the three-port GC-O system using the frequency method with a multivariate analysis shortened the classification of foods based on the aroma impact compounds.

In chapter 4, the thick and deep flavors of sweet cream were studied. These flavors are important factors expressed by the word *Koku* in Japan. To identify compounds having an impact on *Koku*, a sweet cream was fractionated using SAFE, solvent extractions, and chromatography. The resulting fractions were screened by performing sensory evaluations at each step. The *Koku*-containing fractions selected by sensory evaluation were analyzed by

GC-MS and the following main compounds were identified: 7 long-chain fatty acids (decanoic acid, dodecanoic acid, tetradecanoic acid, hexadecanoic acid, octadecanoic acid, oleic acid, and linoleic acid); 3 long-chain aliphatic delta-lactones (5-dodecanolide, 5-tetradecanolide, and 5-hexadecanolide); and cholesterol. Reconstitution experiments performed with these 11 compounds revealed that the long-chain fatty acids and long-chain aliphatic delta-lactones contributed to the *Koku* of sweet cream. Along with the relationships between the terms that represent *Koku* of sweet cream, the elucidation of *Koku* impact compounds in this study contributed to our basic knowledge regarding the palatability of sweet cream. This study suggests the possibility that a person feels a fatty acid and the fatty acid becomes a clue to the taste. Arguments that a fatty acid is a tastant are not yet clear. (78).

The abovementioned results demonstrate that the main components of dairy flavor compounds were derived from amino acids, sugars, and lipids, which are the main components of milk. In addition, the aroma impact compounds of SMP, WMP, and UHT milk in the present thesis were almost common with those of other reported dairy products (9, 10). Furthermore, the characteristics of dairy flavor were influenced by the breeding environment of the cow and manufacturing process of the dairy products. Thus, the characteristics of dairy flavor were interpreted as a different balance of the aroma impact compounds. In this thesis, the characteristics of several dairy products that are produced and consumed in Japan were identified. It is expected that these results will become useful for the development of dairy products with better palatability. It may also contribute to solving such problems as food shortage and food waste.

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Chapter 3.

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Chapter 4.

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