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Studies on the Key Aroma Compounds in Soy Milk Made from Three Different Soybean Cultivars

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ABSTRACT: An investigation by aroma extract dilution analysis (AEDA) of the aroma concentrate of soy milk made from a major Japanese soybean cultivar, Fukuyutaka (FK), revealed 20 key aroma compounds having flavor dilution (FD) factors of not less than 64. Among them, 2-isopropyl-3-methoxypyrazine, *cis*-4,5-epoxy-(*E*)-2-decenal, *trans*-4,5-epoxy-(*E*)-2-decenal, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone, and 2′-aminoacetophenone were identified as the key aroma compounds in soy milk for the first time. (*E*,*E*)-2,4-Decadienal exhibiting a fatty note and *trans*-4,5-epoxy-(*E*)-2-decenal exhibiting a metallic/sweet note were detected as having the highest FD factors of 4096, followed by hexanal (green), (*E*)-2-nonenal (fatty), and (*E*,*E*)-2,4-nonadienal (fatty) having FD factors of 1024. Although all of these compounds might be generated from lipids, various aroma components, which were thought to be generated from amino acids, sugars, and ferulic acid, were detected having FD factors of 64−256. Investigation by comparative AEDA experiments of the soy milk aroma concentrates of two cultivars for soybean curd and soy milk, FK and Vinton81 (VT), and one cultivar for boiled beans, Miyagishirome (MY), revealed that most of the key aroma compounds were common to all of them, but 2-isopropyl-3-methoxypyrazine, exhibiting a pea-like/earthy note, was detected only in FK and VT. In addition, a sensory experiment revealed that the pea-like/earthy notes in FK and VT were significantly stronger than that in MY. These results demonstrated that a pea-like/earthy note contributed by 2-isopropyl-3-methoxypyrazine might be one of the essential characteristics to describe soy milk aromas.

KEYWORDS: soy milk, aroma extract dilution analysis, 2-isopropyl-3-methoxypyrazine, 2-alkyl-3-methoxypyrazine

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.), containing high amounts of protein, carbohydrates, and quality lipids, has been used as popular foodstuff from a nutritional viewpoint since ancient times in East Asia. Over the past 50 years, especially after the 1990s, various soybean cultivars have been developed for special soy foods, such as soybean curd (tofu), soy milk (tonyu), soybean paste (miso), green soybeans (edamame), boiled beans (nimame), fermented soybeans (natto), soy sauce (shoyu), soybean sprouts (moyashi), and roasted soybean flour (kinako) in addition to vegetable soybean oil.[1] In recent years, soybean has attracted attention all over the world due to the functionality of its healthy ingredients. In particular, the consumption of soy milk has been increasing as one of the most easily digestible soy foods in the world.

Soy milk is an easily digestible soy food as well as a half-finished product of soybean curd. After soaking in sufficient water for half a day, soybeans are comminuted in about 6−10 parts of water, and the obtained suspension is then boiled for several minutes. Soy milk is obtained by filtration of the boiled suspension as a white emulsified liquid. Soybean curd is obtained by coagulating soy milk at about 70 °C with a coagulant. High-quality soybean curd and soy milk retain many physical properties of the soybeans, such as high protein content, moderate ratio of β-conglycinin (7S) and glycinin (11S), high sugar content, moderate seed size, and light yellow hilum.[2−7]

Whereas soy milk resembles bovine milk in appearance, its typical green off-odor limits consumer use. The off-odor components in soy milk are thought to be decomposition products from soybean lipid by autoxidation, photo-oxidation, and an enzymatic reaction, especially by lipoxygenase and hydroperoxide-lyase. Many investigations have been reported for more than 30 years to specify the off-odor components generated from lipids, their formation mechanisms, their differences in quantity among the several cultivars of soybeans, and their changes under different heat sterilization conditions.[8−13] To reduce those off-odor components generated by lipoxygenase in soybean curd and soy milk, improved soybean cultivars lacking lipoxygenase have been developed in recent years.[14] Although soybean curds made from these cultivars have a reduced green note off-odor, some papers have suggested that they were inferior to those made from normal cultivars in quality due to a less sweet taste and mouthfeel sensation.[15,16] These papers suggest that the preference of soybean curd is influenced not only by the off-odor components generated from the lipids but by other desirable compounds. In addition, the key compounds contributing to the soybean curd and soy milk aromas have not yet been fully clarified.

The objectives of the present investigation were to clarify the key aroma compounds in soy milk by the application of aroma extract dilution analysis (AEDA) to a soy milk aroma concentrate made from a major Japanese soybean cultivar for soybean curd and soy milk, Fukuyutaka (FK). Moreover, to clarify the relationship between the key aroma compounds and soybean cultivars, a comparison of the AEDA experiments of the soy milks made from three different cultivars, FK, Miyagishirome (MY), which is one of the major Japanese cultivars for boiled beans, and

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Vinton81 (VT), which is one of the major American cultivars for soybean curd and soy milk, was investigated.

MATERIALS AND METHODS

Materials. *Soybean Samples.* FK was purchased from Suda Shoji Co., Ltd. (Tokyo, Japan), MY was purchased from Suzuya Co., Ltd. (Sendai, Japan), and VT was purchased from Muso Co., Ltd. (Osaka, Japan).

Chemicals. Hexanal, 1-octen-3-ol, methional, (*E*)-2-nonenal, (*E*,*E*)-2,4-nonadienal, (*E*,*E*)-2,4-decadienal, 4-nonanolide, 2′-aminoacetophenone, 2-*sec*-butyl-3-methoxypyrazine, and 2-isobutyl-3-methoxypyrazine were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). 1-Octen-3-one, 2-isopropyl-3-methoxypyrazine, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 2-methoxy-4-vinylphenol, and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone were obtained from Sigma-Aldrich Japan Co., Ltd. (Tokyo, Japan). The following compounds were synthesized according to the literature procedures: 2-acetyl-1-pyrroline[17] and *cis*-, *trans*-4,5-epoxy-(*E*)-2-decenal.[18]

Preparation of the Soy Milk Aroma Concentrate. Soybeans (40 g) were homogenized with 200 mL of distilled water by a mixer after soaking in distilled water for 12 h. Another 200 mL of distilled water was then added, and the mixture was boiled for 3 min. After cooling, the soy milk was obtained by filtration of the suspension, followed by centrifugation (3000 rpm × 10 min, 5 °C). The obtained soy milk was passed through the glass column (100 mm × 10 mm i.d.) filled with 5 mL of SP700 resin (Mitsubishi Chemical Co., Ltd.), which was conditioned with distilled water before use, followed by washing with distilled water (50 mL) and eluting with 20 mL of dichloromethane. The dichloromethane fraction was dried with an excess amount of anhydrous sodium sulfate and then distilled by solvent assisted flavor evaporation (SAFE) (40 °C, <5.0 × 10^{-3} Pa).[19] The soy milk aroma concentrate was obtained by concentrating the obtained dichloromethane layer by rotary evaporation, followed by nitrogen steam evaporation to about 100 μL.

Identification of the Key Aroma Components in the Soy Milk Aroma Concentrate. One thousand grams of FK, after soaking in distilled water for 12 h, was homogenized with 5000 mL of distilled water by a mixer. Another 5000 mL of distilled water was then added, and the mixture was boiled for 3 min. After cooling, the soy milk was obtained by filtration of the suspension, followed by centrifugation (3000 rpm × 10 min, 5 °C). The soy milk was passed through SP700 resin, followed by washing with distilled water (500 mL) and eluting with 1000 mL of dichloromethane. The dichloromethane fraction was dried with an excess amount of anhydrous sodium sulfate and then distilled by SAFE (40 °C, <5.0 × 10^{-3} Pa). The soy milk aroma concentrate was obtained by concentrating the obtained dichloromethane layer by rotary evaporation, followed by nitrogen steam evaporation to about 100 μL. After replacement of the solvent from dichloromethane to *n*-pentane, 100 μL of the soy milk aroma concentrate was separated by silica gel chromatography using a glass column (200 × 10 mm i.d.) filled with a *n*-pentane slurry of 3 g of Wakogel C-300 (40−64 mm, Wako Pure Chemical Industries, Ltd., Osaka, Japan). Separation was performed with 20 mL of *n*-pentane (fraction I), followed by 20 mL of a *n*-pentane/diethyl ether mixture (9:1) (fraction II), 20 mL of a *n*-pentane/diethyl ether mixture (1:1) (fraction III), 20 mL of diethyl ether (fraction IV), and 20 mL of ethyl acetate (fraction V). The respective fractions were concentrated by nitrogen steam to about 50 μL. Identification of each key aroma compound was performed by comparing its Kovats GC retention index (RI) and mass spectrum with the authentic compound by gas chromatography−mass spectrometry (GC-MS), in addition to comparison of its RI and odor quality with the authentic compound by gas chromatography−olfactometry (GC-O).

Preparation of the Solvent Extract of Raw Soybeans. Soybeans (40 g) were milled by a coffee grinder and then extracted with 400 mL of dichloromethane by a Soxhlet apparatus for 4 h. The obtained dichloromethane layer was dried with an excessive amount of anhydrous sodium sulfate and then distilled by SAFE (40 °C, <5.0 × 10^{-3} Pa). The aroma concentrate of raw soybeans was obtained by concentrating the distillate by rotary evaporation, followed by nitrogen steam evaporation to about 100 μL.

Gas Chromatography−Olfactometry. An Agilent model 6850 series gas chromatograph equipped with a thermal conductivity detector (TCD) was used. A fused silica column (30 m × 0.25 mm i.d. coated with a 0.25 μm film of DB-Wax, J&W Scientific; or 30 m × 0.25 mm i.d. coated with a 0.25 μm film of DB-1, J&W Scientific) was used with splitless injection (1.0 μL). The valve-delay time was 1 min. The column temperature was programmed from 40 to 210 °C at the rate of 5 °C/min for all runs. The injector and detector temperatures were 250 and 230 °C, respectively. Helium was used as the carrier gas at a flow rate of 1 mL/min. A glass sniffing port was connected to the outlet of the TCD and heated by a ribbon heater over 210 °C. Moist air was pumped into the sniffing port at 100 mL/min to quickly remove the odorant eluted from the TCD out of the sniffing port. The soy milk aroma concentrates and the Soxhlet extracts of FK, MY, and VT were applied to GC-O analysis. Determination of the odor qualities detected by sniffing was achieved by triplicate experiments for each subject.

Aroma Extract Dilution Analysis. The original odor concentrate of the soy milk aroma concentrate was stepwise diluted with dichloromethane to 1:4, 1:16, 1:64, 1:256, 1:1024, and 1:4096, and aliquots (1 μL) of each fraction were analyzed by capillary GC on a DB-Wax and DB-1 column. Two subjects performed the GC-O experiments, and the key aroma compounds were determined in triplicate experiments by each subject with not less than twice detections. AEDA was performed three times with respect to each sample by two subjects. The detection of each compound was defined as not less than twice detections by both subjects, and the Flavor Dilution (FD) factor of each compound was determined as a maximum dilution degree of detection.

Gas Chromatography−Mass Spectrometry. An Agilent 7890A gas chromatograph coupled to an Agilent model 5975C inert XL series mass spectrometer was used. The column was a 60 m × 0.25 mm i.d. DB-Wax fused-silica capillary column (J&W Scientific) with a film thickness of 0.25 μm. The column temperature was programmed from 80 or 40 to 210 °C at the rate of 3 °C/min. The injector temperature was 250 °C. The flow rate of the helium carrier gas was 1 mL/min, and the split ratio was 1:30 or splitless. The mass spectrometer was used under the following conditions: ionization voltage, 70 eV (EI); ion source temperature, 150 °C.

Sensory Evaluation of the Soy Milks Made from Three Cultivars. Seventeen subjects were recruited from Ogawa & Co., Ltd. They were trained for 6 months with in-house programs of recognition, description, and discrimination tests involving about 300 odorants. The respective soy milk samples (20 mL) were poured into a plastic cup at 25 °C to the sensory panels, who were asked to score the strength of the pea-like/earthy note on a scale from 1 (weak) to 7 (strong). The quality of the evaluation was defined as the following aromas: 2-isopropyl-3-methoxypyrazine for pea-like/earthy note, *trans*-4,5-epoxy-(*E*)-2-decenal for metallic note, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone for caramel-like note, hexanal for green note, (*E*,*E*)-2,4-decadienal for fatty note, and methional for cooked potato-like note. Two-way layout analysis of variance without replication was applied to the sensory evaluation results performed by a seven-point scoring method.

RESULTS AND DISCUSSION

Identification of the Key Aroma Compounds in Soy Milk Made from FK. Soy milk made from FK had a roast, green, and fatty aroma. The obtained soy milk aroma concentrate had the

Table 1. Key Aroma Compounds (Structures Given in Figure 1) in the Soy Milks Made from Three Different Cultivars

no.	retention index DB-WAX	DB-1	odor qualities	compound	fraction[a]	FD factor Fukuyutaka	Miyagishirome	Vinton81	ref
1	1052		leafy, green	hexanal	II	1024	64	256	8
2	1297		mushroom-like	1-octen-3-one	III	64	64	<64	14
3	1336		popcorn-like	2-acetyl-1-pyrroline	III	64	<64	<64	13
4	1431	1075	pea-like, earthy	2-isopropyl-3-methoxypyrazine[b]	II	64	nd[c]	<64	
5	1446		mushroom-like	1-octen-3-ol	II	64	<64	64	9
6	1449		cooked potato-like	methional	III	256	256	1024	13
7	1532		sweet, fruity	(*E*)-2-nonenal	II	1024	64	256	8
8	1652		green	unknown		256	<64	<64	
9	1698		fatty	(*E*,*E*)-2,4-nonadienal	II	1024	4096	4096	8
10	1763		green, fatty	(*E*,*Z*)-2,4-decadienal	II	256	256	<64	8
11	1807		fatty	(*E*,*E*)-2,4-decadienal	II	4096	4096	4096	8
12	1964		sweet, caramel-like	3-hydroxy-2-methyl-4-pyranone	IV	256	<64	<64	13
13	1986		sweet, metallic	*cis*-4,5-epoxy-(*E*)-2-decenal	III	64	<64	64	
14	2000		sweet, metallic	*trans*-4,5-epoxy-(*E*)-2-decenal	III	4096	256	1024	
15a	2029		fatty, milky	4-nonanolide	III	64[d]	<64[d]	256[d]	8
15b	2029		sweet,caramel-like	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	IV	64[d]	<64[d]	256[d]	46
16	2075		sweet, fatty	unknown		64	64	64	
17a	2191		spicy	2-methoxy-4-vinylphenol	III	256[e]	256[e]	1024[e]	13
17b	2191		caramel-like	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	IV	256[e]	256[e]	1024[e]	
18	2218		fruity, grape-like	2′-aminoacetophenone	III	64	64	<64	

[a] Each fraction was obtained by silica gel chromatography as described under Materials and Methods. [b] The compound was tentatively identified by GC-O analysis equipped with DB-WAX and DB-1 column by comparison to the standard compounds. [c] Not detected. [d] The FD factor was represented as a mixture of 4-nonanolide and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone. [e] The FD factor was represented as a mixture of 2-methoxy-4-vinylphenol and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone.

same characteristics when it was dipped in the *mouillette*, which is a filter paper for sensory evaluation, and sniffed.

To evaluate the key aroma compounds in soy milk, AEDA was applied to the soy milk aroma concentrate made from FK, and 20 compounds were detected with FD factors of not less than 64 (Table 1). Among them, 2-isopropyl-3-methoxypyrazine (**4**), *cis*-4,5-epoxy-(*E*)-2-decenal (**13**), *trans*-4,5-epoxy-(*E*)-2-decenal (**14**), 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (**17b**), and 2′-aminoacetophenone (**18**) were identified as the key aroma compounds in the soy milk for the first time.

(*E*,*E*)-2,4-Decadienal (**11**), exhibiting a fatty note, and *trans*-4,5-epoxy-(*E*)-2-decenal (**14**), exhibiting a metallc/sweet note, were detected with the highest FD factor of 4096, followed by hexanal (**1**) (green), (*E*)-2-nonenal (**7**) (fatty), and (*E*,*E*)-2,4-nonadienal (**9**) (fatty) with FD factors of 1024. As reported in many previous studies, these compounds generated from lipid might be important for the soy milk and soybean curd aroma.[8,10−16] With FD factors of over 64, methional (**6**) (cooked potato-like), 2-methoxy-4-vinylphenol (**17a**) (spicy), 1-octen-3-one (**2**) (mushroom-like), 1-octen-3-ol (**5**) (mushroom-like), 2-acetyl-1-pyrroline (**3**) (popcorn-like), 2-isopropyl-3-methoxypyrazine (**4**) (pea-like, earthy), and 2′-aminoacetophenone (**18**) (grape-like) were detected as the most potent odorants except for the flavor components exhibiting fatty, green, and sweet notes.

Hexanal (**1**), 1-octen-3-one (**2**), 1-octen-3-ol (**5**), (*E*)-2-nonenal (**7**), (*E*,*E*)-2,4-nonadienal (**9**), (*E*,*Z*)-2,4-decadienal (**10**), (*E*,*E*)-2,4-decadienal (**11**), *cis*-4,5-epoxy-(*E*)-2-decenal (**13**), *trans*-4,5-epoxy-(*E*)-2-decenal (**14**), and 4-nonanolide (**15a**) might be generated from lipids as discussed in many previous papers.[8−10,12,14−16,18,20−23] 2-Acetyl-1-pyrroline (**3**), 2-isopropyl-3-methoxypyrazine (**4**), methional (**6**), and 2′-aminoacetophenone (**18**) might be formed from the amino acids or metabolites of the raw materials.[24−30] 3-Hydroxy-2-methyl-4-pyranone (**12**) and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (**15b**) might be generated from the Amadori compounds or reducing sugars.[31−35] 3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone (**17b**) is suggested to be generated from the amino acid by heating or from 4-hydroxy-L-isoluecine through the biosynthetic pathway.[36−39] 2-Methoxy-4-vinylphenol (**17a**) might be generated as a thermal degradation product of ferulic acid.[40]

Although most of the previous papers focused on the lipid oxidation products exhibiting green and fatty notes in soy milk, the key aroma components of the soy milk aroma concentrate made from FK consisted of various compounds generated from an amino acid, sugars, and ferulic acid exhibiting various aroma characters.

Comparison of the FD Factors of the Soy Milk Aroma Concentrates Made from Three Soybean Cultivars. To clarify the relationship between the key aroma compounds and soybean cultivar, AEDA experiments were applied to three varieties, FK, MY, and VT (Table 1). The soybean cultivars used for soybean curd and soy milk, such as FK and VT, generally have a high amount of protein and light brown hilum, whereas the cultivar for boiled beans, such as MY, have a lower amount of protein, light yellow hilum, and large seed size.[1] Although almost all of the key aroma compounds were common in them, the FD factors of hexanal (**1**), (*E*)-2-nonenal (**7**), and *trans*-4,5-epoxy-(*E*)-2-decenal (**14**) in MY were lower than those in FK. Considering their generation mechanisms, the linoleic acid content in MY might be lower than that in FK, or the lipoxygenase activity of MY might

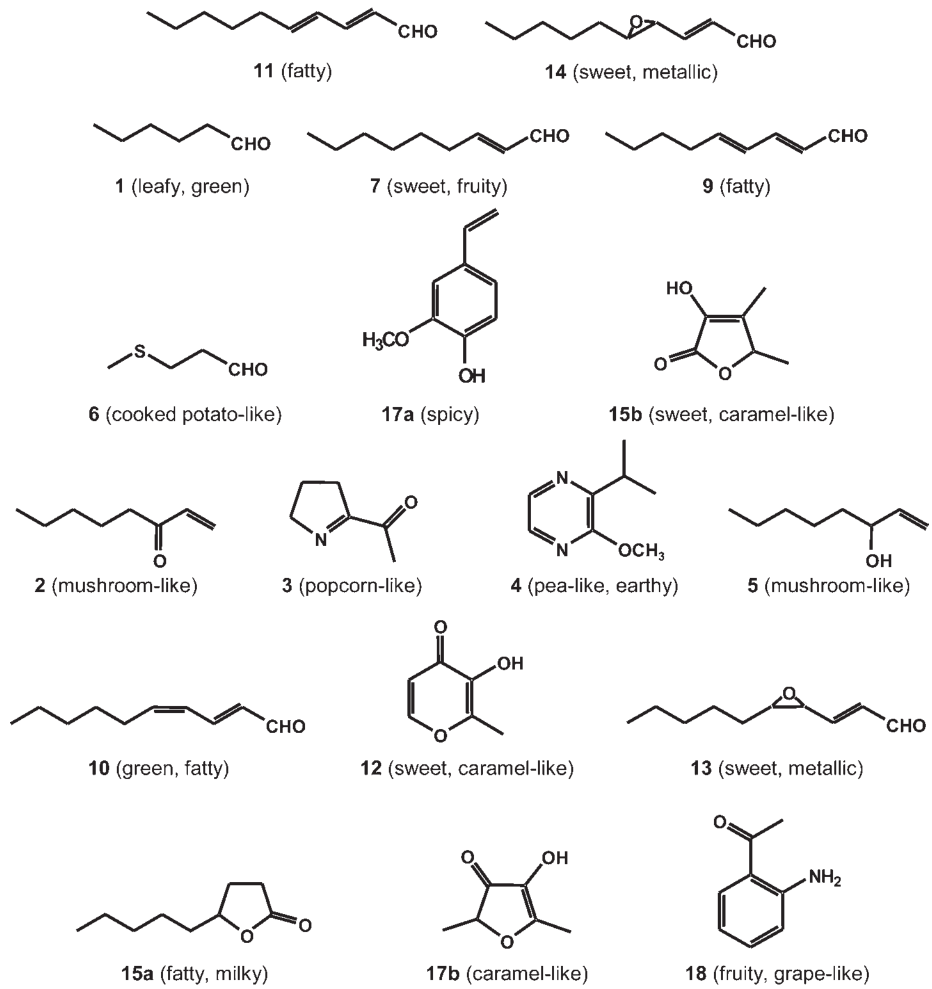

Figure 1. Structures of the key aroma compounds in soy milk.

Table 2. Sensory Evaluation of the Soy Milks Made from Three Different Cultivars

	aroma quality[a] (SD)					
cultivar	pea-like/earthy	metallic	caramel-like	green	fatty	cooked potato-like
Fukuyutaka	4.9 a (±1.2)	4.0 (±1.8)	4.3 (±1.3)	4.2 (±1.2)	5.0 (±1.0)	4.2 cd (±1.1)
Miyagishirome	3.9 b (±1.1)	3.6 (±1.3)	4.0 (±0.9)	4.1 (±1.0)	4.4 (±1.0)	3.9 c (±1.2)
Vinton81	5.4 a (±1.2)	3.8 (±1.4)	4.9 (±1.4)	3.9 (±1.2)	5.4 (±0.5)	4.9 d (±0.6)

[a] Different letters indicate significant differences within the column ($p < 0.05$).

be lower than that of FK. Remarkable differences among the three cultivars were found for 2-isopropyl-3-methoxypyrazine (**4**), exhibiting a pea-like/earthy note. 2-Isopropyl-3-methoxypyrazine (**4**) was detected at the FD factor of 64 in FK, followed by VT at a FD factor of under 64, whereas it was not detected in MY. 2-Isopropyl-3-methoxypyrazine (**4**) might be important to the soy milk aroma because of its detection only in the cultivars for soybean curd and soy milk (FK and VT). 2-Isopropyl-3-methoxypyrazine (**4**) is reported as a potent odorant in various vegetables, wine grapes, raw coffee beans, and various nuts.[41−44] Although its pea-like/earthy note is recognized as a desirable aroma at a moderate concentration, it is recognized as an off-odor note at an excessive concentration.[30]

Sensory Experiment of the Soy Milks. To verify the contribution of the key aroma compounds detected by GC-O to the soy milk aroma, the soy milks made from three cultivars were evaluated in the aroma descriptions of key compounds (Table 2). Whereas FK exhibited a strong fatty note (5.0) and pea-like/earthy note (4.9), VT exhibited a strong caramel-like note (4.9) and cooked potato-like note (4.9) in addition to a fatty note (5.4) and a pea-like/earthy note (5.4). MY exhibited comparatively low scores in all characteristics. In particular, there were significant differences in pea-like/earthy note between FK (4.9) and MY (3.9) as well as between VT (5.4) and MY. These results suggest the importance of the pea-like/earthy note to the soy milk aroma made from FK and VT.

The sensory experiment and the AEDA experiments suggested that 2-isopropyl-3-methoxypyrazine (**4**), exhibiting a pea-like/earthy note, might partly contribute to the soy milk aroma.

Table 3. FD Factors of 2-Alkyl-3-methoxypyrazines in the Soy Milks Made from Three Different Cultivars

	retention index				FD factor		
no.	DB-WAX	DB-1	odor qualities	compound	Fukuyutaka	Miyagishirome	Vinton81
4	1431	1075	pea-like, earthy	2-isopropyl-3-methoxypyrazine[a]	64	nd[b]	16
19	1492	1151	earthy, musty	2-*sec*-butyl-3-methoxypyrazine[a]	4	nd[b]	nd[b]
20	1525	1158	earthy, musty	2-isobutyl-3-methoxypyrazine[a]	nd[b]	nd[b]	4

[a] The compound was tentatively identified by GC-O equipped with DB-WAX and DB-1 column by comparison to the standard compounds. [b] Not detected.

Table 4. FD Factors of 2-Alkyl-3-methoxypyrazines in Raw Soybeans

	retention index				FD factor		
no.	DB-WAX	DB-1	odor qualities	compound	Fukuyutaka	Miyagishirome	Vinton81
4	1431	1075	pea-like, earthy	2-isopropyl-3-methoxypyrazine[a]	64	nd[b]	16
19	1492	1151	earthy, musty	2-*sec*-butyl-3-methoxypyrazine[a]	4	nd[b]	nd[b]
20	1525	1158	earthy, musty	2-isobutyl-3-methoxypyrazine[a]	4	nd[b]	16

[a] The compound was tentatively identified by GC-O equipped with DB-WAX and DB-1 column by comparison to the standard compounds. [b] Not detected.

Comparison of the FD Factors of 2-Alkyl-3-methoxypyrazines in the Soy Milk Aroma Concentrates and the Extracts of Raw Soybeans. Although the formation mechanism of 2-isopropyl-3-methoxypyrazine (**4**) was still unclear, the proposed mechanisms of 2-alkyl-3-methoxypyrazines were reported by Murray et al.[41] Among them, methylation to hydroxypyrazine with *S*-adenocyl-L-methionine was reported as a model reaction catalyzed by *o*-methyltransferase isolated from young grapevine shoots.[45] The AEDA experiments revealed that 2-*sec*-butyl-3-methoxypyrazine (**19**) was detected in FK and 2-isobutyl-3-methoxypyrazine (**20**) in VT in addition to 2-isopropyl-3-methoxypyrazine (**4**). In contrast, no 2-alkyl-3-methoxypyrazine was detected in MY (Table 3).

To proceed to the discussion about the possible generation mechanism of 2-alkyl-3-methoxypyrazines, the raw soy beans of three varieties were extracted by dichloromethane using a Soxhlet extraction. The AEDA experiments of the individual extracts revealed that 2-isopropyl-3-methoxypyrazine (**4**) was detected in FK and VT, 2-*sec*-butyl-3-methoxypyrazine (**19**) was detected only in FK, and 2-isobutyl-3-methoxypyrazine (**20**) was detected in FK and VT (Table 4). In contrast, no 2-alkyl-3-methoxypyrazine was detected in MY. Because the 2-alkyl-3-methoxypyrazines were detected even in the raw beans of FK and VT as well as in the soy milk aroma concentrates, they might be generated during postharvest drying or raw soybean metabolites as in grapevine fruit. Concerning the metabolites of the raw material, many investigations have been reported for the grapevine fruit on the relationship between the concentration of the 2-alkyl-3-methoxypyrazines and the cultivars, places, and climate.[42] Although the same factors might influence the soybean cultivars, further investigations are needed to clarify the differences in quantity as well as their generation mechanisms.

In conclusion, the AEDA experiments of the soy milk aroma concentrate made from the major Japanese soybean cultivar, Fukuyutaka, revealed that almost all of the key aroma compounds were thought to be generated from lipids, sugars, amino acids, and ferulic acid. Although most of them were common among the two cultivars for the soybean curd and soy milk (FK and VT) and one cultivar for the boiled beans (MY), 2-isopropyl-3-methoxypyrazine was detected only in FT and VT. In addition, FK and VT had a significantly stronger pea-like/earthy note than MY. These results suggested that the 2-alkyl-3-methoxypyrazines exhibiting a pea-like/earthy note might be one of the essential characteristics for soybean curd and soy milk. In addition, the detection of 2-alkyl-3-methoxypyrazines from the raw soybean extracts of FK and VT suggested that the 2-alkyl-3-methoxypyrazines might be generated through a biosynthetic pathway. Further investigations are needed to clarify their importance in the overall soy milk aroma and their quantity differences with regard to several factors, such as soybean variety, growing location, and seasonal fluctuation, in addition to their generation mechanisms in soybeans.

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