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Note

Characterization of Off-Flavors in Porcine Liver Collected by SDE

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The odor-active volatile compounds of porcine liver were collected by a simultaneous steam distillation–solvent extraction method (SDE). The extracted compounds were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The key aroma compounds of off-flavor in porcine liver were characterized using gas chromatography-olfactometry (GCO) technique. The volatiles identified included aldehydes (1.89%), ketones (0.62%), alcohols (0.61%), furans (0.11%), thiazoles (0.21%), phenols (0.31%), pyrazines (0.18%), esters (12.31%) and acids (80.06%). 1-Octen-3-one and hexanol are considered to be responsible for the intense and weak metallic odor, respectively, and other identified major volatiles with their odor note given in parenthesis were as follows : (*E,E*)-2,4-heptadienal (fishy), (*E*)-2-octenal (tallowy), (*E*)-2-nonenal (cardboard-like), (*Z*)-4-decenal (cardboard-like) and (*E,E*)-2,4-decadienal (deep-fried). It was also confirmed that the fishy and metallic flavor in liver was not completely removed even after subjecting to heat treatment. The metallic and fishy notes make up the whole liver-like off-flavor in porcine liver.

Keywords: porcine liver, gas chromatography-olfactometry, off-flavor, fishy, metallic

Flavor is one of the most important characteristics of foods and is related to its palatability. As a food, animal liver is an important source of protein, fat, and vitamins. It has higher mineral contents, particularly iron, than most other foods (McNaught, 1948). Liver constitutes a very small portion of meat by-products and meat-products consumed in Japan and Asia. The limited consumption of liver is partially due to its characteristic off-flavor which occurs during cooking. The objectionable odor could be described as intensely fishy and/or metallic. Although some reports on flavor volatiles in liver, for instance, volatile constituents of pressure cooked pork liver (Mussinan & Walradt, 1974) and sheep liver (Lorenz *et al*., 1983) have been made, no detailed information is yet available on the characteristics of off-flavor in porcine liver. Furthermore, several extensive studies were carried out to improve the acceptability of chicken liver by employing specified cooking methods to eliminate undesirable flavor or by masking its unplesant odor (Kimura & Ogawa, 1985; Kimura *et al*., 1990a; Kimura *et al*., 1990b), however, none of these studies addressed the above problems of liver off-flavor. Porcine liver was chosen in this study, because its odor is considerably stronger than that of other animal liver (Mussinan & Walradt, 1974). The objectives of our study were to identify and characterize the volatile components in porcine liver to determine the primary off-flavor believed to contribute to the fishy and/or metallic impression.

Materials and Methods

Materials Fresh porcine liver was purchased from domestic commercial sources (Shibaura Internal Organ Co., Tokyo), and kept frozen (−80°C) until required. Gas chromatographic authentic chemicals were purchased from commercial sources (Sigma Chemical Co. (St. Louis, MO) and Wako Pure Chemical Industries, Ltd.) and used without further treatment. 1-Octen-3-one was provided by Takasago Int. Co. (Tokyo).

Collection of volatiles by SDE Porcine liver (100 g) was homogenized with 500 ml of distilled water, transferred to a 3-*l* round-bottomed flask, and simultaneously steam distilled and extracted with 60 ml of diethyl ether (previously redistilled) for 120 min in a Likens-Nickerson apparatus (SDE method) (Nickerson & Likens, 1966). The obtained porcine liver ether extract was dried with anhydrous sodium sulfate and concentrated to 1 ml on a Yamato evaporator and then further concentrated to 0.2 ml with a gentle nitrogen stream. The porcine liver volatile concentrate thus obtained was analyzed with GC, GCO and GC/MS.

GC-Olfactometry analysis (GCO) The GCO system consisted of a Shimadzu GC-9A gas chromatograph equipped with a flame ionization detector (FID), and an olfactometer (a sniffing port). Separations of the volatile compounds were done on a CP-WAX 52 CB fused silica capillary column (30 m×0.25 mm i.d. 0.25 μm film thickness, Chrompack). The sniffing port was supplied with humidified air at 30 ml/min. GCO-SDE sample sniffing was performed by two trained panelists.

GC and GC-MS analyses Separation of volatile compounds was achieved on a CP-WAX 52 CB (30 m×0.25 mm i.d. 0.25 μm, Chrompack) fused silica capillary column attached to a micro-computer controlled Shimadzu GC-9A equipped with a flame ionization detector (FID). Oven temperature was programmed at 60°C held for 4 min and then raised to 200°C at 2°C/min and held for 60 min. Nitrogen was used as carrier gas with a split ratio of 1 : 40. Injector and detector temperatures were 200°C and 220°C, respectively. A multi-functional data processor

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(Shimadzu Chromatopac C-R6A) connected to the gas chromatograph was used for a relative quantitative calculation. GC/MS was conducted using a GCQ (Finnigan MAT) mass selective detector system equipped with a CP-WAX 52 CB capillary column (30 m×0.25 mm i.d., 0.25 μm, Chrompack). GC conditions were almost the same as those described for GC. The MS analyses conditions were as follows: capillary direct interface temperature was 200°C; ion source temperature, 200°C; ionization energy, 70 eV; mass range, 20–450 *m/z*; electron multiplier voltage, 1600 V.

Compound identification Identifications of the volatile compounds were made by comparing their retention time and mass spectral patterns (Eight Peak index of Mass Spectra, 1983; MacLafferty & Stauffer, 1989) with those of authentic compounds. In some cases only a tentative identification was made following a NIST mass spectra library search.

Results and Discussion

The volatile compounds were collected by SDE and the yield of total volatiles, estimated by the addition of measured amount of internal standard to the porcine liver, was 13.8 mg%. The overall flavor of the concentrates was characteristic and representative of boiled meat aroma, mild sulfurous odor, fishy and/or metallic off-flavor, although their individual odor qualities varied. Also, it was found that the fishy off-flavor in porcine liver subjected to heat treatment during collection of volatiles (SDE, about 100°C) was not completely removed. The odor of the liver was thought to be fishy, metallic and liver-like.

To identify the volatile compounds responsible for the porcine liver off-flavor, GC and GC-MS were used. Odor characterization was also carried out using GCO. Table 1 shows the identified flavor compounds and the odor description. Description was done by sniffing the corresponding peaks of each compound at the sniffing port of the GC, according to their elution on a CP-WAX 52CB column. The results of quantitative analysis (calculated as % peak area using GC-FID with CP-WAX 52 CB column) of the volatile compounds are also shown in Table 1. Results of the quantitative analysis of the major classes of compounds in volatiles are as follows: acids (80.06%), esters (12.31%), aldehydes (1.89%), ketones (0.62%), alcohols

Table 1. Volatile compounds in porcine liver collected by SDE and identified using GC/GC-MS and characterized by GCO.

Compound (amount %)	Conc. (ng/g)[a]	Odor description[b]	ID[c]	Compound (amount %)	Conc. (ng/g)[a]	Odor description[b]	ID[c]
Aldehydes (1.89%)				Thiazoles (0.21%)			
Hexanal	12.9	green, grassy	ms (A, B)	2-Ethylthiazole	3.4	laver-like	ms (A)
Heptanal	54.0	unpleasant	ms (A, B)	2-Acetylthiazole	2.0	nutty	ms (A)
(*E*)-2-Octenal	0.3	nutty, tallowy	ms (A, B)	Benzothiazole	1.3		ms
Methional	5.0	mashed potato	ms (A)	Phenyl-1,2,3-thiazole	13.6		ms
(*E*,*E*)-2,4-Heptadienal	0.1	fishy	ms (A, B)	Phenols (0.31%)			
Decanal	4.0	orange peel	ms (A, B)	Phenol	1.0	phenolic	ms (A, B)
Benzaldehyde	6.8	almond-like	ms (A, B)	*o*-Cresol	27.2		ms (B)
(*E*)-2-Nonenal	1.3	cardboard-like	ms (A, B)	*m*-Cresol	0.6		ms (B)
(*Z*)-4-Decenal	0.6	cardboard-like	ms (A, B)	2,5-di-*tert*-Butylphenol	1.0		ms
Phenylacetaldehyde	51.0	hyacinth	ms (A, B)	Thiophenes (0.17%)			
(*E*,*E*)-2,4-Decadienal	0.6	deep-fried	ms (A, B)	2-Thiopenecarboxaldehyde	1.7		ms
5-Methyl-2-phenyl-2-hexenal	6.8	grapefruit-peel	ms (A)	3-Thiopenecarboxaldehyde	3.7		ms
Pentadecanal	9.5		ms	2.5-Diethylthiophene	6.8		ms
(*Z*)-9-Octadecenal	3.4		ms	2-Phenylthiophene	3.0		ms
Alcohols (0.61%)				5-Methyl-2-thiophene-carboxaldehyde	2.3		ms
Hexanol	9.1	metallic, grassy	ms (A, B)	Pyrroles (0.20%)			
1-Octen-3-ol	15.4	mushroom	ms (A, B)	1-Pentylpyrrol	3.4		ms
6-Methyl-5-hepten-2-ol	0.2	musty, metallic	ms (A, B)	2-Acetylpyrrol	17.0	pungent	ms (A)
Heptanol	6.8	unpleasant	ms (A, B)	Esters (12.31%)			
Furfurylalcohol	10.2	woody	ms (A, B)	Dibutylphthalate	40.8		ms (B)
6-Tetradecanol	17.0		ms	Methyl-6,9-octadecadienoate	1153.0		ms (B)
Phenylethylalcohol	10.2		ms (B)	Indoles (0.31%)			
Ketones (0.62%)				Indole	20.4		ms (B)
2-Heptanone	1.0	green	ms (A, B)	4-Methylindole	10.2		ms (B)
3-Octanone	5.7	varnish, ketone	ms (A, B)	Acids (80.06%)			
2-Octanone	6.4	varnish, walnut	ms (A, B)	Acetic acid	0.2	vinegar	ms (A, B)
Cyclohexanone	22.7	almond	ms (A, B)	Butanoic acid	3.4	buttery	ms (A, B)
1-Octen-3-one	5.4	metallic	ms (A, B)	2-Ethylhexoic acid	3.0		ms
6-Methyl-5-hepten-2-one	3.4	green, estery	ms (A, B)	Nonanoic acid	4.0		ms (B)
2-Nonanone	4.7	ketone	ms (A, B)	Decanoic acid	6.8		ms
3-Nonen-2-one	1.7	orange-peel	ms (A, B)	2-Methyldecanoic acid	2.5		ms
2-Undecanone	2.3	geranium, varnish	ms (A, B)	Benzoic acid	0.3		ms (B)
2-Pentadecanone	0.6	ms		Dodecanoic acid	37.4		ms (B)
Furan (0.11%)				Tetradecanoic acid	615.5		ms (B)
2-Pentylfuran	1.2	greenbean-like	ms (A)	Pentadecanoic acid	136.0		ms (B)
Pyrazines (0.18%)				Hexadecanoic acid	5761.9	waxy	ms (A, B)
2,5-Dimethylpyrazine	5.1		ms	(*z*)-9-Hexadecenoic acid	384.3		ms (B)
2,6-Dimethylpyrazine	5.1		ms	Heptadecanoic acid	125.0		ms (B)
2-Ethyl-6-methylpyrazine	1.0		ms	Octadecanoic acid	29.5		ms (B)
2,5-Diethylpyrazine	1.3		ms	(*z*)-9-Octadecenoic acid	649.6		ms (B)
2,3-Diethyl-5-methylpyrazine	0.1		ms				
2-Methyl-5-propylpyrazine	1.7		ms				
2,3-Dimethyl-isopentylpyrazine	1.7		ms				

[a] Estimated concentrations with respect to the internal standard (cyclohexanol). [b] Odor description using GC-sniffing technique. [c] Identification: ms, tentative identification using mass spectrometry and MS database; (A), odor smelled by the sniffers; (B), injection of standard compound.

(0.61%), thiazoles (0.21%), pyrazines (0.18%), and furans (0.11%) (Table 1). Furthermore, more than 95% of the volatiles with dominating acids and esters were identified. The trace heterocyclic compounds including, pyrazines, thiophenes, thiazoles and furans, have received much attention as producers of characteristic cooked meat flavors (Shibamoto, 1980). Thiophenes are responsible for the mild sulfurous odor of cooked meats (Shibamoto, 1980). Mussinan and Walradt (1974) identified 179 volatile compounds, including 23 furan derivatives in pressure cooked pork liver. Furans may be considered, to some extent, as contributors to some cooked flavor but not as important flavor materials for cooked meats. Also, many of the other well-known products of fat oxidation were identified, e.g., the alcohols, aldehydes, ketones, acids, and esters (Forss, 1973; Sink, 1973).

Volatile concentrates of porcine liver exhibited very distinct metallic, fishy and liver-like background notes. Hexanal, 1-octen-3-one, *(E)*-2-octenal, *(E,E)*-2,4-heptadienal, *(E)*-2-nonenal, *(Z)*-4-decenal and *(E,E)*-2,4-decadienal are the main volatile compounds of porcine liver; hexanol and 1-octen-3-ol were also detected. The most potent odorants consisted of 1-octen-3-one (metallic), *(E)*-2-nonenal (cardboard-like), *(Z)*-4-decenal (cardboard-like) and *(E,E)*-2,4-heptadienal (fishy). 1-Octen-3-one and the corresponding alcohol were isolated and characterized as the compounds responsible, respectively, for a metallic and mushroom-like aroma in oxidized dairy products (Stark & Forss, 1962).

The unsaturated aldehydes and ketones have the lowest sensory thresholds and are usually considered the primary sources of oxidized off-flavors (Marsili, 1999). Low threshold value (*T*) for the volatile carbonyls, especially 1-octen-3-one ($T=0.005$ ppb), and *(E)*-2-nonenal (cucumber, cardboard-like, $T=0.08$ ppb), results in odor stronger than their correponding alcohol whose threshold value is 10 ppb (Buttery, 1981). Vinyl ketones have strong flavor and are usually more unpleasant than other unsaturated ketones found in natural products. 1-Octen-3-one was shown to be the compound responsible for the metallic flavor in oxidized butter oil, washed-cream and safflower oil (Stark & Forss, 1962); Hammond and Hill (1964) reported that this compound accounts for the metallic flavor of autoxidized milk fat.

The chemical basis of fishy-like flavor has been associated with the autoxidative deterioration of polyunsaturated fatty acids, particularly ω-3 type fatty acid (McGill *et al*., 1974; Ke *et al*., 1975). The offensive cod-liver-oil-like odor associated with *Synura uvella* (Jüttner, 1981), and the fishy off-flavor of *Uroglena-Americana Chryosophyceae* have been attributed to the formation of (*E,E*)-2,4-heptadienal and (*E,Z*)- and (*E,E*)-2,4-decadienal (Nakahara *et al*., 1988). Hexanal, 2,4-heptadienals and 2,4-decadienals contributed to painty flavors of oxidized fish oils (Karahadian & Lindsay, 1989). The metallic odor was found to be associated with certain vinyl ketones, and the unpleasant fishy character with the class of compounds containing a 2,4-dienal structure (Swoboda & Peers, 1977). Forss *et al*. (1960), Stark and Forss (1962) were unable to identify compounds with fishy aromas in a batch of fishy-flavored butterfat, although they noted that 1-octen-3-one contributed some metallic notes also observed in tainted butter.

To correlate the single aroma impression from the GC result, GCO was used. The following compounds are thought to contribute to the complexity of the porcine liver off-flavor notes. (1) 1-Octen-3-one and hexanol are responsible for the metallic aroma impression. (2) The fishy odor note is attributed to (*E,E*)-2,4-heptadienal. (3) The cardboard-like odor is attributed to (*E*)-2-nonenal and (*Z*)-4-decenal. (4) The green aroma is a by-product of hexanal, 2-heptanone and 6-methyl-5-hepten-2-one. (5) The nutty and almond-like odors are attributed to benzaldehyde and 2-acetylthiazole. (6) Mushroom-like and musty odors are caused by 1-octen-3-ol and 6-methyl-5-hepten-2-ol. Malodorous compounds such as 1-octen-3-one, (*E,E*)-2,4-heptadienal, (*E*)-2-nonenal, and (*Z*)-4-decenal, might have an off-flavor impact on the aroma of porcine liver. Matsutake alcohol (1-octen-3-ol) from *Armillaria matsutake* (Murahashi, 1936) was found in oxidized butter-cream and was believed to be responsible for a mushroom-like flavor in dairy products (Stark & Forss, 1964).

In summary, the significant off-flavor of porcine liver is not just the result of one single odor impression but the combination of two major impressions, metallic and fishy. The relationship between 1-octen-3-one and hexanol and the metallic flavor, (*E,E*)-2,4-heptadienal and fishy, (*E*)-2-nonenal and (*Z*)-4-decenal and cardboard-like were strongly correlated; therefore, it is suggested that these compounds are responsible for the characteristic off-flavor of porcine liver. It was also found that the fishy and metallic flavor in liver was not completely removed by heating, and remained even after being subjected to heat treatment.

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