

Role for Volatile Branched-Chain and Other Fatty Acids in Species-Related Red Meat Flavors

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Abstract

Species-related meat flavors were investigated for red meats (bovine, porcine, caprine, and ovine). Volatile branched-chain fatty acids (VBCFAs) including 2-methylbutanoic, 3-methylbutanoic, 4-methylpentanoic, 2-ethylhexanoic, 4-methylhexanoic, 4-methyloctanoic, 6-methyloctanoic, 4-ethyloctanoic, 4-methylnonanoic, and 2-ethyldecanoic acids were identified in the meats from cow (bovine), pig (porcine), goats (caprine; American white goat and Korean black goat), and lamb (ovine). Beef flavor of bovine meat was characterized by the basic meaty flavor, lacking in goaty and muttony flavor impacts due to low or absent in 4-methyloctanoic and 4-ethyloctanoic acids. Porcine meat contained the least number of VBCFAs among sample species tested, and 3-methylbutanoic acid contributed to the unclean sweaty odor of pork. Caprine meat from Korean black and American white goats lacked in short VBCFAs (C5, C6, and C7) and contained 4-methyloctanoic and 4-ethyloctanoic acids contributing to the characteristic goaty flavor of caprine meat. Caprine meat flavor was distinctively characterized by 4-ethyloctanoic acid, while 4-methyloctanoic acid provides sweaty-muttony flavor to ovine meat. Although kinds of VBCFAs are same in two different varieties of caprine meats, meat sample from Korean black goat had stronger goaty odor and contained higher concentration of 4-ethyloctanoic acid than the meat sample from American white goat did.

Key words : volatile branched-chain fatty acids, species-related meat flavor, Korean black goat

INTRODUCTION

A complex array of volatile compounds has been identified in red meats, and over 650 have been reported in beef alone¹. However, in spite of the availability of this information, chemical characterization of species-related meat flavors remains incomplete. It is generally agreed that meat flavors are complex sensations, but the overall flavors include a common basic meaty-brothy component along with species-related notes that specifically characterize the flavor of that species. Species-related meat flavors greatly influence the economic value and consumer acceptance of meats from the various species of food animals. Depending on cultural food patterns, species-

related meat flavors may be considered either desirable or undesirable, such as the case for lamb, mutton, and goat meat around the world.

The origin of species-related flavors has been controversial, but Wasserman and Spennelli² have concluded on the basis of sensory data that the lipids of pork and lamb provided the characterizing flavors to their meats. Brennan and Lindsay³ obtained similar results, but noted that beef and pork fats elicited relatively weak species characterizations when evaluated as warm broths. Early work by Hornstein and Crowe^{4,5} demonstrated that significant differences occurred in the long-chain fatty acid composition of lipids from beef, lamb, and pork, but these workers were unable to associate any of these fatty acids with species-related flavors. Later Wong et al.⁶ identified a number of volatile medium-chain fatty acids (C5-C12) in lamb and mutton which included several branched-chain homologues. Based on semi-quantitat-

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²This research was supported by the research grant (300-4410) from Korea Research Foundation.

ive data and sensory assessments, Wong's group^{6,7)} concluded that 4-methyloctanoic and 4-methylnonanoic acids were responsible for the undesirable sweaty or sooty-like flavor of mutton. They further indicated that 4-methyloctanoic acid contributed to the flavor of goat meat.

Little further attention has been given to the role of volatile free fatty acids in species-related meat flavors because sufficiently sensitive analytical methods for the quantitative determination of the volatile branched-chain fatty acids have not been available. Sensitive methods are necessary because some of these compounds are present in very low concentration, and exhibit very potent aromas. For example, flavor thresholds for 4-methyloctanoic and 4-ethyloctanoic acids have been reported at 0.5 ppm⁷⁾ and 0.0018 ppm⁸⁾, respectively. Recently, we have developed a method for the quantitative analysis of volatile fatty acids which provides detection to about 0.5 ppb⁹⁾, and reported distribution of VBCFAs in perinephric fats of various red meat species¹⁰⁾. Given the consumption of the lean meat portion, it is necessary to quantify VBCFAs in lean meats from various animals to investigate their role in characterizing species-related meat flavors.

The present study quantified the contents of VBCFAs in the meats of cow (bovine), pig (porcine), goat (caprine), and lamb (ovine) and described flavor notes of the red meats. The contents of 4-methyloctanoic and 4-ethyloctanoic acids in two different varieties of meat samples from Korean black goat and American white goat were compared.

MATERIALS AND METHODS

Materials

Samples of red meats which possessed characteristic species-specific flavors were obtained from carcasses of animals in commercial trade at Pusan and Taegu Korea. The samples of meats were powdered by blending with dry ice in a high-speed blender-homogenizer, and then were held at -24°C until analysis was carried out. Samples of goat meats were obtained from a mature male dairy goat (American white goat and Korean black goat). Samples of ovine meat

were obtained from a mature male dairy lamb. Bovine meat sample was obtained from a typical choice market beef steer. Porcine meat sample was obtained from a typical choice market slaughter hog.

Methods of analysis of volatile free fatty acids (VFFAs)

VFFAs in the meat samples were analyzed according to the basic method described by Ha and Lindsay⁹⁾. Five grams of each meat sample containing 6.5 µg of 2-ethylnonanoic acid as internal standard (I.S.), diethyl ether (15ml), and 5.5N H₂SO₄ (0.5ml) were blended with a high speed prob homogenizer (Brinkmann Instruments Co., Westbury, NY, USA). Hexane (20ml) and anhydrous Na₂SO₄ (12.5g) were then added, and the fatty acids in this extract were adsorbed on 5g of deactivated neutral alumina. After twice washing neutral lipids from the alumina with 10ml portions of hexane : diethyl ether (1 : 1, v/v), fatty acids were desorbed with 5ml of formic acid in diisopropyl ether (6%, v/v). After removal of excess organic solvent, VFFAs were simultaneously distilled and extracted (SDE) for 20min with diethyl ether after acidifying (0.25ml of 5.5N H₂SO₄) aqueous solution (25ml) with diethyl ether using a micro-SDE apparatus (Chrompack Inc., Bridgewater, NY, USA). The diethyl ether extract containing VFFAs was brought to near dryness using a nitrogen stream, and butyl esters were prepared using BF₃/butanol (14%).

Butyl esters were extracted into pentane, and were separated using a bonded phase polyethylene glycol capillary column (Supelcowax 10, 60m × 0.32mm i.d., 0.25µm coating thickness, Supelco Inc., Bellefonte, PA) with HP 5890 (Hewlett Packard, TX, USA) Gas chromatography equipped with a flame ionization detector (temperature, 250°C; hydrogen, 30ml/min; air, 300ml/min). Helium was used for the carrier gas (2ml/min) and make-up gas (30ml/min). The column oven temperature was programmed from 50 to 230°C at 2°C/min after 15 min hold at 50°C, and the injector temperature was 250°C.

Quantification of fatty acids was performed by comparing the peak area of each butyl ester peak with that of I.S. (2-ethylnonanoic acid), and emplo-

ying method correction factor for each fatty acid. At least two injections were made for each sample during gas chromatographic analysis, and the coefficient of variability was less than 20 for each of the fatty acids⁹. The identities of VFFAs were verified by mass spectral analysis and retention indices of the butyl esters of VFFAs¹⁰. Flavor and aroma assessments of fatty acids were carried out by the authors, and descriptions of the aroma properties of individual fatty acids reported elsewhere¹².

RESULTS AND DISCUSSION

Several VBCFAs including 2-methylbutanoic, 3-methylbutanoic, 4-methylpentanoic, 2-ethylhexanoic, 4-methylhexanoic, 4-methyloctanoic, 6-methyloctanoic, 4-ethyloctanoic, 4-methylnonanoic, and 2-ethyldecanoic acids were identified from the cooked meat portion of bovine, porcine, caprines (Korean black goat and American white goat), and ovine. VBCFAs contained in red meat samples above their threshold values were listed in Table 1 and their aroma characteristics of the authentic compounds were described in Table 2.

2-Methylbutanoic, 3-methylbutanoic, 2-ethylhexanoic, 6-methyloctanoic, and 4-methylnonanoic acids were present in caprine meat sample. The concentrations of 2-methylbutanoic and 3-methylbutanoic acids were higher than their reported threshold values.

However, none of these VBCFAs are considered as having characteristic beef flavor. Being compared with other species like ovine and caprine, beef flavor is characterized by the basic meaty flavor compounds from reaction flavor compounds^{1,13} of amino acids, carbohydrates, and lipids, because of lacking in goaty and muttoney flavor impact due to low in 4-methyloctanoic and 4-ethyloctanoic acids.

Porcine meat contained the least number of VBCFAs among four sample species tested in this study, and they were 2-methylbutanoic, 3-methylbutanoic, and 6-methyloctanoic acids. None of these VBCFAs possessed characteristic pork flavor, although lipoly-

Table 2. Aroma properties of volatile branched-chain fatty acids (VBCFAs) that present in red meat samples above their thresholds

VBCFAs	Aroma properties ^a
2-Methylbutanoic acid	rotten fruit, estery, sweat (5ppm) ^b
3-Methylbutanoic acid	sweat socks, fatty acid-like (5ppm)
4-Methylpentanoic acid	cheese-like, waxy (5ppm)
2-Ethylhexanoic acid	phenolic, waxy (5ppm)
4-Methylhexanoic acid	woody, sweet-sweaty (10ppm)
4-Methyloctanoic acid	waxy, goaty (1ppm)
6-Methyloctanoic acid	phenolic, wet woody, slightly sweet (2.5ppm)
4-Ethyloctanoic acid	goaty (5ppb)
4-Methylnonanoic acid	waxy-sweet, soapy, fatty acid-like (1ppm)
2-Ethyldecanoic acid	musty, musky, sweet, woody (1ppm)

^aReported in reference 12

^bThe concentration close to their thresholds

Table 1. The concentrations of volatile branched-chain fatty acids (VBCFAs) isolated and identified from meat samples of various species

VBCFAs	Meats					Aroma/Flavor thresholds (ppm)
	Bovine	Porcine	Caprine 1 ^a	Caprine 2 ^b	Ovine	
	$\mu\text{g/g}$					
2-Methylbutanoic acid	5.24	6.72	– ^c	–	7.25	1.6 ^d
3-Methylbutanoic acid	0.24	9.86	–	–	0.31	0.07 ^e
4-Methylpentanoic acid	–	–	–	–	0.82	0.61 ^e
2-Ethylhexanoic acid	1.26	–	–	–	–	82.4 ^e
4-Methylhexanoic acid	–	–	–	–	1.27	7.3 ^e
4-Methyloctanoic acid	–	–	1.21	0.29	3.21	0.02 ^e
6-Methyloctanoic acid	0.05	1.25	2.15	1.29	1.27	4.1 ^e
4-Ethyloctanoic acid	–	–	0.57	0.97	0.18	0.0018 ^f
4-Methylnonanoic acid	0.07	–	–	–	0.74	0.65 ^e
2-Ethyldecanoic acid	–	–	10.38	0.17	–	0.02 ^e

^aAmerican white goat ^bKorean black goat ^cNot detected
^dKeith and Powers¹⁴ ^eBrennand et al.¹² ^fBoelens et al.⁶

zed porcine fat produced sweaty-odorlike 3-methylbutanoic acid. The origin of VB-CFAs in meat lipids has been explained that rumen microorganisms synthesize short branched-chain fatty acid utilizing propionyl Co-A as substrate and then elongate to the long chain methyl branched chain fatty acids¹⁵. Considering this, it is not surprising that porcine meat sample obtained from monogastric animal does not have much branched-chain fatty acids (BCFAs). However, fermented feeds could easily serve as a source of 2-methylbutanoic and 3-methylbutanoic acids, which derived from leucine and isoleucine, respectively.

Goat meats were obtained from two different varieties of caprine (Korean black goat and American white goat). Both of the samples were lacking in short VBCFAs (C5, C6, and C7) and contained 4-methyloctanoic acids and 4-ethyloctanoic contributing to the characteristic goaty flavor of caprine meat. Especially, 4-ethyloctanoic acid was the important goaty compound present in both of the goat meats. In our previous report¹⁰, the mature male goat perinephric fat from American white goat contained 4-ethyloctanoic acid at level (13ppm) well above threshold level (1.8~6 ppb) and we stated that this acid is probably most important for the goaty flavor notes of mature goats. Since we consume the meat portion, it was necessary to examine the concentration of this fatty acid in meat if the content of this fatty acid in meat portion is high enough to determine the species-specific meat flavor.

The result of this study (Table 1) clearly showed that 4-ethyloctanoic acid in goat meat has an important role for the species-specific meat flavor. Yet this fatty acid still comes from the marbled fat of meat portion and the origin of species-specific meat flavor is still fat fraction of the animal meat. Recently, 4-ethyloctanoic acid has also been reported to provide the characterizing goatiness to the flavors of fresh goat cheese¹⁶. Sugiyama et al.^{17,18} have identified 4-ethyloctanoic acid as a major characterizing odorant in the sebaceous secretion obtained from the neck hair of mature male goats, but they did not find the compound in similar collections from the female goats. However, the data obtained from the analysis of female

goat meat¹⁰ and fresh goat cheese¹⁶ showed that 4-ethyloctanoic acid can also be synthesized in mammary glands of female goats.

Caprine meat also contained 4-methyloctanoic acid which possesses a sweaty or mutton-like flavor note. Wong et al.⁷ stated that 4-methyloctanoic acid contributed to the flavor of caprine meat, and the current data confirmed that adequate concentration of this compound occurred in caprine meat to contribute a species characterizing flavor to the meat from mature males. Although, they believed that 4-methyloctanoic acid is responsible for the sweaty note of caprine fat, this fatty acid may more likely provide supporting flavor notes to the 4-ethyloctanoic acid in caprine meat flavors. Notably, the mature caprine meat contained 2-methylbutanoic and 3-methylbutanoic acids below the threshold levels. Thus, the aroma of the medium-chain fatty acids would not be modified by the effects of these shorter chain fatty acids in caprine meat.

Ovine meat samples had the largest number of VBCFAs, including short and medium branched-chain fatty acids. Unlike the caprine meat samples, the ovine sample contained the short chain fatty acids at the levels that could affect the mutton-like flavor. These short-chain fatty acids should contribute sweet, butyric-like, and sweat-like flavor notes modifying goaty-muttony flavor notes from 4-ethyloctanoic and 4-methyloctanoic acids. In addition to the VBCFAs, some volatile phenolic compounds also had important sheepy odor contributable to the mutton flavor and we reported this elsewhere¹⁹.

3-Methylbutanoic and 2-methylbutanoic acids in animal meat lipids can originate from branched-amino acids such as leucine and isoleucine, from microbial metabolism in the gastrointestinal tract, and *in vivo* biosynthesis in various tissues. Kako et al.¹³ reported the identification of 4-methylpentanoic acid in pork, but this acid was not found in the porcine perinephric fat¹⁰ and the porcine meat sample included in the current study.

2-Methylbutanoic, 3-methylbutanoic, and 4-methylpentanoic acids have been identified in the pig wastes²⁰⁻²³, and the concentrations of fatty acids have been related to the balance of intestinal micro-

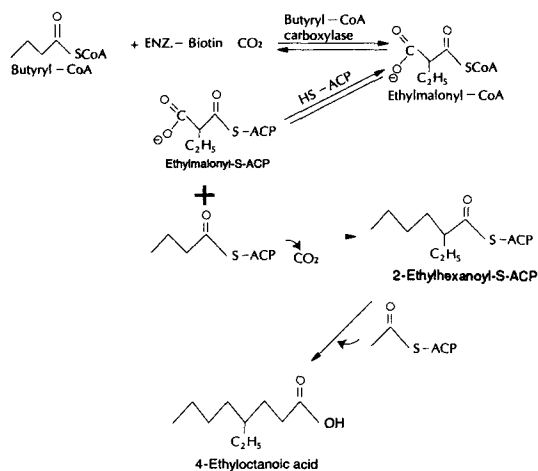


Fig. 1. A proposed biosynthetic mechanism for the production of 4-ethyloctanoic acid.

flora of pigs²³). Elongation of 2-methylbutanoic and 3-methylbutanoic acids in a variety of animals yields corresponding series of branched medium- and long-chain fatty acids. Secretions from human sebaceous glands contain a large number of long chain methyl and dimethyl fatty acids²⁴), and multimethyl branched-long-chain fatty acids have been identified in the uropygial gland of the goose²⁵). Long chain branched fatty acids are probably best known in ruminant milkfat and meat lipids where they are quite abundant²⁶⁻³¹).

BCFAs with substituents at even-numbered carbon atoms in ruminants may result from the incorporation of methylmalonyl-CoA arising from propionate into fatty acids rather than malonyl-CoA, which leads to n-chain fatty acids¹⁵). Thus, 2-methylbutanoic acid can also be synthesized by condensation of methylmalonyl-S-ACP and acetyl-S-ACP. Although various methyl branched-chain fatty acids could be biosynthesized using 2-methylbutanoic and 3-methylbutanoic acids as primers, ethyl branched-chain fatty acids can not be synthesized from these fatty acids. Therefore, ethyl branched-chain fatty acids such as 4-ethyloctanoic acid should be synthesized via ethylmalonyl Co-A mechanism. The proposed biosynthetic mechanism for the 4-ethyloctanoic acid is shown in Fig. 1. The specificity of the synthetase for butyryl-CoA and ethylmalonyl-CoA is believed to determine the amount of 4-

ethyloctanoic acid in depot fat and meat portion. Thus, the presence of elevated levels of 4-ethyloctanoic acid in caprine meat indicated a unique substrate specificity of the acyl-CoA carboxylase for butyryl-CoA in the synthesis of ethylmalonyl-CoA in these species.

In conclusion, 4-ethyloctanoic and 4-methyloctanoic acids were important flavor compounds determining the species-specific meat flavor for the bovine, porcine, caprine, and ovine as being or not being in their meats. The meats from two different varieties of caprine (Korean black goat and American white goat) contained the same kinds of VBCFAs, although there were differences in the concentrations of VBCFAs and the strength of goaty odor between two varieties.

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(Received February 11, 1993)

휘발성 Branched-Chain과 n-Chain Fatty Acids가 육고기의 종을 결정하는 향기 성분으로서의 역할

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요 약

2-Methylbutanoic, 3-methylbutanoic, 4-methylpentanoic, 2-ethylhexanoic, 4-methylhexanoic, 4-methyloctanoic, 6-methyloctanoic, 4-ethyloctanoic, 4-methylnonanoic 및 2-ethyldecanoic acids를 포함하는 다수의 volatile branched-chain fatty acids(VBCFAs)가 쇠고기, 돼지고기, 염소고기(미국산 흰염소고기, 한국산 흑염소고기), 양고기로부터 분리, 동정되었다. 쇠고기 향은 amino acid, carbohydrate, 그리고 lipid의 반응에 의해 생성된 향기성분에 의해 이루어지는 기본 meaty flavor로 결정되어 지는 반면, 염소고기나 양고기의 경우는 쇠고기의 기본향에 4-ethyloctanoic acid와 4-methyloctanoic acid의 독특한 노린내가 더해지므로서 염소고기나 양고기 특유의 향을 내었다. 돼지고기는 3-methylbutanoic acid 함량이 다른 세종류에 비해 높고 3-methylbutanoic acid 특유의 꼬린냄새는 돼지고기의 기름층으로부터 나는 unclean flavor에 중요한 영향을 미치는 것으로 생각된다. 염소고기는 C5-C7 chain length의 volatile branched 및 n-chain fatty acids를 적게 함유하고 있는 반면 4-ethyloctanoic acid는 염소고기의 노린내를 내는 결정적인 화합물이었다. 4-Methyloctanoic acid는 양고기의 sweaty-muttony flavor를 내는 중요한 화합물이었으며 염소고기와는 달리 C5, C6, 그리고 C7 branched-chain fatty acids도 상당량 함유되어 있었다. 한국산 흑염소고기와 미국산 흰염소고기 중에 들어있는 VBCFAs의 종류는 같으나, 한국산 흑염소고기가 미국산 흰염소고기 보다 노린내가 강하며 4-ethyloctanoic acid의 함량도 높았다.