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Evaluation of Curing and Flavor Ingredients, and Different Cooking Methods on the Product Quality and Flavor Compounds of Low-fat Sausages

Seung Seok Yoo[1], Sung Ho Kook, Sung Yong Park, Jae Han Shim[2] and Koo Bok Chin*

Department of Animal Science, Institute of Agricultural Science and Technology Chonnam National University, Gwangju 500-757, Korea

[1]Department of Culinary Science, Honam University, Gwangju 506-714, Korea

[2]Division of Applied Bioscience and Biotechnology, Institute of Agricultural Science and Technology, Chonnam National University, Gwangju 500-757, Korea

Abstract The effects of ingredients added (first cutting ingredients vs. both first and second cutting ingredients), and cooking method (smoking vs. boiling) on product quality and volatile compounds were examined for low-fat and regular-fat sausages. Regular-fat sausages had slightly higher pH values (6.2-6.3) than those (6.1-6.2) of low-fat counterparts. However, the pH values of the sausages were not significantly affected by the different ingredients and cooking methods ($p>0.05$). Approximately 30 volatile compounds were identified from these comminuted sausages. The headspace concentrations of 4-methyl-1-[1-methylethyl]-3-cyclohexen-1-ol, α- terpenyl acetate, eugenol, trans-caryophyllene and myristicine were lower in low-fat and regular-fat sausages containing the first cutting ingredients alone, than in those with both cutting ingredients. The volatile compounds of the smoked comminuted sausages were mostly phenols and hetero-compounds, and a lot of volatile compounds were shown before the retention time (RT) of 30 min. However, not many volatile compounds were detected in the boiled sausages prior to the RT of 30 min.

Keywords: product quality, volatile compounds, low-fat and regular-fat sausages, ingredients, cooking method

Introduction

Fat contributes to food by providing better mouthfeel, texture and flavor and also plays an important role in flavor release (1). Chevance and Farmer (2) reported that the release of monoterpene hydrocarbons, sesquiterpene hydrocarbons, terpenes containing oxygen, cyclopentenones, phenyl propanoids and phenols was greatly increased with decreasing fat content. However, the demand for low-fat and no-fat meat products has been increased due to "health-concerns". In addition, reducing fat may continue to be important for healthy life styles. When fat is reduced or removed from a formulation, the ingredients available to replace it are water, protein, carbohydrate, minerals, and air. As a result, low-fat meat products can be successfully produced with fat replacement to compensate for consumer palatability. Chevance *et al.* (3) reported that the addition of carbohydrate fat replacers to low-fat meat products could assist the flavor characteristics of low-fat meat products by delaying the release of odor compounds.

Several ingredients, not only the curing ingredients such as salt, phosphate, sodium nitrite and sodium ascorbate, but also flavor enhancers including milk and soy proteins and various spices, have been used for the manufacture of meat products. They were commonly added to the sausage mixture in two stages. The purpose of the addition of the first cutting additives, including salt, cure blend, sodium tripolyphosphate and sodium erythorbate, was to extract salt soluble proteins and to improve functional properties, especially water holding capacity (WHC) and cooking loss (CL, %), thereby improving emulsion stability (4). The second cutting additives, which are flavor enhancers including non-fat dry milk, maltodextrin and spices, were added to improve taste, flavor and mouthfeel of comminuted sausages. Each additive may exhibit its characteristics into the gas phase concerned with flavor release. Salt and sugar play an important role in the flavor release of foods (5). Chevance and Farmer (6) identified the major volatile compounds in frankfurters and reported that aldehyde, ketones, furanthiols and alicyclic sulfur compounds were derived from meat fractions while most terpenes were derived from the spices.

There were several cooking methods to cook the meat products. Smoked-cooking is a typical cooking method for the manufacture of meat products to improve flavor, texture and microbial safety. Some volatile compounds such as phenols and terpenes were derived from the smokiness and spiciness of frankfurters (6). Poligne *et al.* (7) reported that a major quantity of the volatile compounds detected in the processed meat was derived from the smoking process, especially several smoke-borne molecules such as furan, nitrogen and phenol compounds. Mottram (8) investigated the effect of cooking conditions (grilled, boiled and roasted) on the formation of volatile compounds in pork and reported that well done grilled pork contained 66 heterocyclic compounds including pyrazines, thiazoles, thiophenes, furans, and pyrroles.

Consumers can perceive volatile aroma compounds from food through the interaction of binding, partitioning and releasing between volatile and food matrix. Changes

*Corresponding author: Tel: 82-62-530-2121; Fax: 82-62-530-2129
E-mail: kbchin@chonnam.ac.kr
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in particle diameter had significant effect on the thermodynamic component of aroma release, whereas no influence of the lipid fraction and emulsifier fraction was observed (9). Stephen Elmore *et al.* (10) suggested that several volatile compounds, which have been found at elevated levels in cooked meat from animal fed supplements high in *n*-3 acids, were formed when methyl alpha-linoleate reacted with cysteine and ribose. In addition, the lipid type, such as oil and fat, affected the perception of flavor. The liquid lipid fraction of the sample was found to absorb aroma compounds, whereas samples with higher solid content showed higher release (11). Although the effect of volatile compounds differs based on the type and structure of lipid, and the temperature, the lipid added to foods has many properties, such as the source of flavor, precursor, flavor masking, mouthfeel and solvent (12).

The objective of study was to analyze the volatile aroma compounds of comminuted sausages as affected by the ingredients (first vs. first and second cutting ingredients) and cooking method (boiling vs. cooking) using gas chromatography-mass spectrophotometry (GC-MS), followed by extraction with simultaneous distillation and solvent extraction (SDE) method.

Materials and Methods

Chemicals Sodium sulfate (anhydrous granule type) was purchased from Junsei Chemical Co. (Tokyo, Japan). Dichloromethane (HPLC grade) was purchased from Merck (Darmstadt, Germany). 1-Heptanol and n-hexadecane, used as internal standards for measuring the relative amounts of volatile compounds, were purchased from Sigma Chemical Co. (St. Louis, MI, USA).

Preparation of comminuted sausages Low-fat and regular-fat sausages were manufactured according to the described by Chin *et al.* (13). The formulation of these sausages is listed in Table 1. These sausages were manufactured with two types of cutting ingredients: (first vs. first and second). Then, the sausage batters were stuffed into 26-mm-diameter cellulose casings, and cooked to an internal temperature of 71.7°C in a smoke chamber (Nu-Vu, ES-B, Food System, USA) as described by Chin *et al.* (13), or in a water bath (WB-3070, New Power Eng. Co. Ltd., Korea) to reach the internal temperature of 71.7 °C. Each cooked sausage was immediately chilled with ice, vacuum packaged and stored at 4°C until analyzed.

pH and proximate analysis Proximate analysis, such as moisture, fat and protein content (%), was determined by AOAC (14) method. pH values of the homogenized sausages were measured by a digital pH meter (Mettler-Toledo, Model 340, Schwarzenbach, Switzerland), which was calibrated with a buffer of pH 4 and 7 (Thermo Orion, USA) and randomly repeated five times.

Simultaneous distillation and solvent extraction (SDE) SDE was performed with the Likens-Nickerson apparatus (LN), which enabled the continuous steam distillation for isolation of the volatile compounds in the sausages (15). Sausages (100 g) were homogenized with distilled water (900 mL) and dichlormethane (50 mL) was used as a solvent. They were then placed round bottom flasks of volume 100 mL and 2 L, and heated for 3 hrs at 100 and 50°C, respectively, for continuous simultaneous extraction. Two milligrams of each 1-heptanol and hexadecane were added to the flask mixtures before heating as an internal standard for measuring the relative amounts of volatile compounds. The moisture in the collected volatile compounds was eliminated by anhydrous sodium sulfate and the remaining compounds were concentrated to 1 mL with Kuderna-Danish apparatus (KD) and nitrogen gas for the quantitative analysis of volatile compounds.

Gas Chromatography and Mass Spectrophotometry (GC-MS) The volatile compounds isolated by SDE method were quantitatively analyzed by gas chromatography (HP 6890, Hewlett-Packard, Palo Alto, USA) under operating conditions that were described in our

Table 1. The formulation of comminuted sausages

	Ingredients					
	Meat	Fat	FR*	Water	NMI*	Total
Regular-fat Sausage	55	15	0	24	6	100
Low-fat Sausages	55	0	2.5	36.5	6	100
	NMI					Amount (%)
The first cutting additives	Salt (NaCl)					1.40
	Cure blend (Prague powder)					0.256
	Sodium tripolyphosphate					0.30
	Sodium erythorbate					0.05
The second cutting additives	Sugar (Sucrose)					1
	Non-fat dry milk					1
	Malto-dextrin (DE=18.5)					1
	Mixed spice (Spice #5)					1
	Fat replacer					(2.5)
Total						6 (8.5)

*Fat replacer(FR) = prehydrated Konjac flour: carrageenan: soy protein isolate (1:1:3)
**NMI= non meat ingredients

previous study (16). Linear retention indices were calculated against the *n*-paraffins (C7-C22) as references (17). For GC-MS, HP 6890 GC coupled with a 5973 mass selective detector (Hewlett-Packard Co., Palo Alto, USA) was used to identify the volatiles isolated from each comminuted sausage manufactured under the various conditions. Mass spectra were obtained by electron ionization at 70 eV. The mass range of molecules was tuned from 10 to 500 amu, at a scanning rate of 2 scans/sec. Ion source temperature and filament emission were 178°C and 1 mA, respectively. All mass spectra obtained were recorded on an HP-MS Chemstation data system. The GC column was the same type (HP-5) as used in the GC analysis. Compound identifications were based on comparisons with the Wiley 77n MS library.

Statistical analysis This experiment was replicated in triplicate and data were analyzed by one way analysis of variance (ANOVA) using the SPSS program at a significance level of 0.05%.

Results and Discussion

Experiment I: Effect of cutting ingredients This study was performed to determine the differences of pH, proximate analysis results, and volatile compounds as affected by the cutting ingredients being the first alone or the first and second combined. Mean values of pH and proximate analysis results were not affected by different ingredients ($p>0.05$). In the proximate analysis, low-fat sausages had 76-79% moisture, < 2% fat and 15-17% protein, whereas regular-fat sausages had 62-65% moisture, 17-19% fat and 14-15% protein (Table 2). These results were similar to those of our previous report (18). Thus, regular-fat sausages had slightly higher pH values than those of low-fat counterparts due to the higher fat level.

Approximately 30 volatile compounds were identified from these comminuted sausages. Comminuted sausages manufactured with the first cutting ingredients alone had relatively lower headspace concentrations of 4-methyl-1-[1-methylethyl]-3-cyclohexen-1-ol, α-terpinenyl acetate, eugenol, trans-caryophyllene and myristicine than those with all ingredients (Fig. 1) (Table 3). These results indicated that the second cutting ingredients affected the formation of several volatile compounds of comminuted sausages, leading to the production of more volatile compounds. Chevance *et al.* (3) reported that reduced-fat meat products containing maltodextrin had a relatively low headspace concentration of monoterpene hydrocarbons, compared to regular-fat counterparts. In addition, more than 100 volatile compounds have been identified in the headspace of frankfurter sausages, and most terpenes were derived from the spices, whereas the phenols originated from the smoke ingredients (6). Yoo *et al.* (19) reported that sulfur-containing heterocyclic compounds contributed to meat flavor. Park *et al.* (20) stated that the composition of the characteristic flavor compounds between commercial soybean paste and traditional Korean soybean paste differed, even though the former contained many kinds of flavor component from the latter. These results were partially in accordance with our results that the ingredients affected the volatile compounds of comminuted sausages. Thus, the second cutting additives (flavor enhancers and spices) improved the flavor of comminuted sausages and might increase the palatability of the meat products.

Table 2. Proximate composition and pH values of sausages with different ingredients

Treatments	pH	Proximate analysis (%) Moisture	Fat	Protein
Low-fat sausages				
[1]first cutting additives	6.11	78.8	1.8	16.3
[2]both cutting additives	6.15	76.4	1.8	15.5
Regular-fat sausages				
first cutting additives	6.25	64.6	18.2	14.4
both cutting additives	6.23	62.5	17.4	14.2

[1]first cutting additives (FCA) : salt, phosphate, sodium nitrite, sodium erythorbate.
[2]both cutting additives (BCA) : first cutting additives and second additives, such as flavorants.

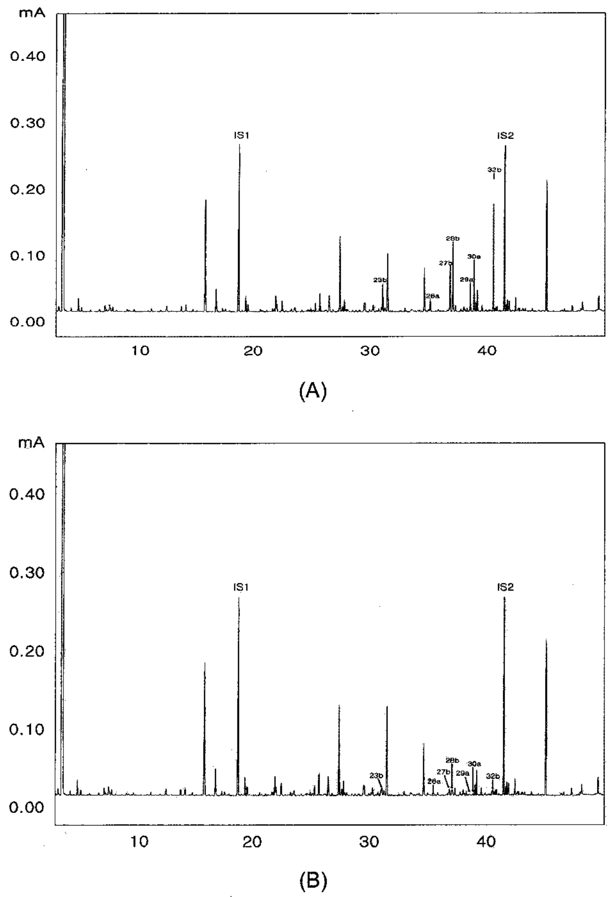

Fig. 1. Gas chromatogram of volatile compounds from comminuted sausages with all cutting ingredients (A) and first cutting additives (B) alone. Number means peak order showed in Table 4: a = no detection between (A) and (B); b = differences were observed between (A) and (B).

Experiment II: Effect of cooking method (smoking vs. boiling) This study was performed to identify the change of volatile compounds of comminuted sausage as affected

Table 3. Quantitative differences of volatile compounds as affected by cutting ingredients (ppm)

Volatile compounds	Peak #	Low-fat FCA*	Low-fat BCA**	Regular-fat FCA	Regular-fat BCA
4-Methyl-1-[1-methylethyl]-3-cyclohexen-1-ol	23	t^{b***}	4.84^{a}	t^{b}	5.44^{a}
5-[2-propenyl] -1,3-benzodioxide	26	t	1.88	t	2.01
α-Terpinenyl acetate	27	1.09^{b}	4.90^{a}	1.31^{b}	5.39^{a}
Eugenol	28	0.98^{b}	3.85^{a}	1.21^{b}	4.25^{a}
2-Methylene-4, 8, 8-trimethyl bicyclononane	29	t	2.42	t	1.75
Trans-caryophyllene	30	t^{b}	8.92^{a}	t^{b}	10.44^{a}
Myristicine	32	0.96^{b}	13.24^{a}	1.34^{b}	10.50^{a}

*FCA: first cutting ingredients; **BCA = Both cutting ingredients; ***T=trace amount
[a,b]Means with same row having same superscript are not different ($p > 0.05$).

by two cooking methods: smoking and boiling. Mean values for pH and proximate composition were not different from previous results of different ingredients (data not shown). The volatile compounds of comminuted sausages produced during smoked-cooking were mostly phenol, aldehyde, and heterocyclic and ketone compounds (Table 4) (Fig. 2). The quantity of volatile compounds detected from comminuted sausages cooked by smoking and boiling became distinctively different before or after the retention time (RT) of 30 min (Fig. 2). Smoked comminuted sausages had the most volatile compounds prior to the RT of 30 min, whereas boiled sausages didn't have the flavor compounds before the RT of 30 min, except for few cases. The volatile compounds on the smoked comminuted sausages prior to the RT of 30 min were mostly phenols and heterocyclic compound, such as furans and furfural which have a five-numbered ring with an oxygen atom, and they play an important role in the flavor development of foods prepared by smoking. The headspace concentrations of 2-methoxy-4-methyl phenol, eugenol, myristicine, pentadecanal and octadecanal extracted from smoked comminuted sausages tended to be

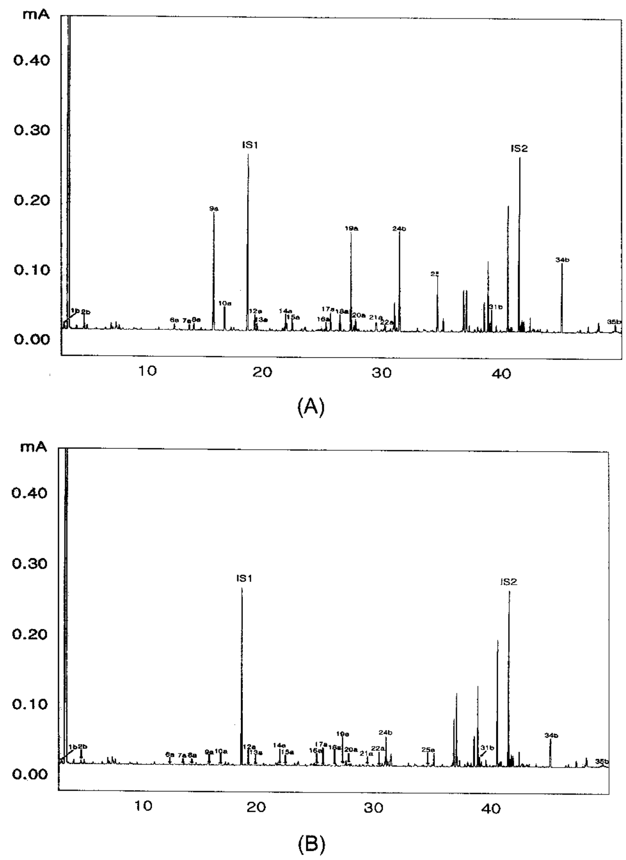

Fig. 2. Gas chromatogram of volatile compounds from comminuted sausages manufactured with smoking (A) and boiling (B). Number means peak order showed in Table 4; a = no detection between (A) and (B); b = differences were observed between (A) and (B).

Table 4. Quantitative analysis of volatile compounds by cooking method (ppm)

Volatile compounds	Peak #	Low-fat Smok	Low-fat Boil	Regular-fat Smok	Regular-fat Boil
Acetone	1	0.69^{b}	0.23^{a}	0.61^{b}	0.22^{a}
2-chloro-2mmethyl butane	3	0.68	-*	0.52	-
Cyclopentanol	6	1.16	-	1.14	-
Octane	7	0.66	-	0.64	-
Hexanal	8	0.79	-	0.54	-
Furfural	9	21.6	-	20.67	-
2-Furan Methanol	10	3.98	-	4.23	-
2-Methyl-2-cyclopentene-1-one	12	1.95	-	1.75	-
2-Acethyl furan	13	1.25	-	1.40	-
5-Methyl furfural	14	3.06	-	3.51	-
Phenol	15	1.83	-	2.28	-
2, 3-Dimethyl-2-cyclopenten-1-one	16	1.10	-	1.25	-
2-Methyl phenol	17	2.60	-	3.42	-
4-Methyl phenol	18	2.95	-	4.00	-
2-Methoxy phenol	19	10.43	-	13.84	-
Nonanol	20	0.64	-	0.57	-
2,4-Dimethyl phenol	21	2.19	-	3.00	-
3,4-Dimethyl phenol	22	0.95	-	1.17	-
2-Methoxy-4-methyl phenol	24	10.08^{b}	1.07^{a}	13.42^{b}	1.03^{a}
4-Ethyl-2-methoxy phenol	25	4.83	-	6.35	-
Eugenol	28	7.99^{b}	6.26^{ab}	7.36^{b}	4.84^{a}
Myristicine	32	12.74^{b}	11.78^{ab}	9.02^{a}	8.33^{a}
Pentadecanal	34	16.85^{b}	12.73^{ab}	7.34^{a}	3.86^{a}
Octadecanal	35	3.14^{b}	1.81^{a}	0.78^{a}	0.30^{a}

smok=smoking; boil=boiling; *- : trace amount.
[a,b]Means with same row having the same superscript are not significantly different ($p > 0.05$).

higher than in the boiled sausages (P<0.05) (Table 4). These results indicated that phenols and a heterocyclic compounds furans were produced by smoking and directly perceived by the olfactory organ because they have low molecular weight and high volatility, as compared with the other volatile compounds after an RT of 30 min. These results were supported by the previous study of Wittkowski *et al.* (21) who reported that the phenol compounds such as guaiacol and its alkyl isomer contributed to the flavor and color of smoked foods. Guillen and Cabo (22) also reported the high stability of lipid oxidation soaked in liquid-smoking solution were observed as compared to the non-smoking counterparts. Poligne *et al.* (7) reported that 69 volatile compounds were identified in the processed pork products and that the cooking-drying-smoking operation enhanced the aromatic spectrum of the product, including phenols, furans, and nitrogen compounds. Mottram (8) investigated the effect of cooking method on volatile compounds and reported that 66 heterocyclic compounds including pyrazines, thiazoles, thiophenes, furans and pyrroles were identified. However, roasting and boiling reduced the heterocyclic compounds, thereby increasing the level of aldehydes and alcohols originating from thermal oxidation of lipids. In conclusion, the present study results indicated that volatile compounds from comminuted sausages were significantly affected by the ingredients added and by the cooking method.

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