

## Evaluation of Mixed Probiotic Starter Cultures Isolated from Kimchi on Physicochemical and Functional Properties, and Volatile Compounds of Fermented Hams

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### Abstract

The objective of this study was to investigate the effects of mixed starter cultures isolated from kimchi on physicochemical properties, functionality and flavors of fermented ham. Physicochemical properties, microbial counts, shear force, cholesterol contents and volatile compounds of fermented ham were investigated during processing (curing and ripening time). Curing process for 7 d increased saltiness, however, decreased hunter color values (L, a, and b values). Ripening process for 21 d increased most parameters, such as saltiness, color values, weight loss, shear force and cholesterol content due to the drying process. The mixed starter culture had higher lactic acid bacteria than the commercial one. While eight volatile compounds were identified from fermented hams during curing process, total fifty-eight volatile compounds were identified from fermented hams during ripening process. The main volatile compounds were alcohols, esters and furans. However, no differences in volatile compounds were observed between two batches. Fermented hams (batch B) manufactured with probiotic starter culture (LPP) had higher sensory score in texture, color and overall acceptability than counterparts (batch A), while the opposite trend was observed in flavor. Therefore, mixed probiotic starter culture isolated from kimchi might be used as a starter culture to be able to replace with commercial starter culture (LK-30 plus) for the manufacture of fermented ham.

**Keywords:** kimchi, physico-chemical properties, functionality, volatile compounds, fermented ham

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### Introduction

Fermented ham is a traditional meat produced mainly in Europe and North America, South America, Asia, Oceania and Africa. Traditional fermentation procedure of fermented ham involves the application of mixture of salt and nitrite, and the addition of spices like pepper, all spice and coriander for enhancing flavor and curing or ripening procedure during several weeks or months (Campbell-Platt and Cook, 1995).

Starter cultures having various functional properties are used to reduce ripening time, add functionality and enhance flavor or safety of fermented meat products. In the previous studies, Vural (1998) who studied on the effects of three different starter cultures in Turkish semi-

dry fermented sausages, and reported that the combination of *Pediococcus acidilactici*, *Staphylococcus xylosus* (SX) and *P. pentosaceus* (PP), and the combination of *S. carnosus* (SC) and *Lactobacillus pentosus* (LP) in fermented sausages significantly reduced the pH, increased the lactic acid content and improved the characteristics of sausage, such as color, appearance, flavor and general acceptability as compared to control (no starter culture). Park *et al.* (1997) studied the physicochemical properties of fermented sausages manufactured with starter cultures of SC plus LP, SC plus PP, and SX plus PP had higher L values, hardness and sensory scores than those of sausages prepared with SC plus LP.

The antimicrobial activities of various starter cultures has been studied in fermented meat products. Among them, Sameshima *et al.* (1998) reported that *L. rhamnosus*, *L. paracasei* and *L. sake* isolated from the intestinal tracts inhibited the growth of *Staphylococcus aureus* in fermented sausage. The antibacterial activity of *L. sake* had antimicrobial activity for *Listeria monocytogenes* has

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been documented (Papamanoli *et al.*, 2003). Kaban and Kaya (2006) reported that mixed starter cultures (*Pediococcus acidilactici* + *Lactobacillus curvatus* + *Staphylococcus xylosus* and *Lactobacillus sakei* + *Staphylococcus carnosus*) in Turkish dry-fermented sausage had significant inhibition effects on the growth of *S. aureus*.

Starter cultures are used to improve the sensory quality or flavor profile of fermented meat products. Enzymatic and chemical reactions, such as proteolysis and lipolysis that developed during curing or ripening time have been attributed to the generation of flavor precursors, starter cultures and endogenous are important in these reaction (Martin *et al.*, 2006; Toldra, 1998).

Garriga *et al.* (1996) conducted sensory evaluation of fermented sausages manufactured with various *Lactobacillus* strains as starter cultures and reported that *L. plantarum* CTC305 had the highest acidic taste that was unacceptable by the panelists, whereas *L. curvatus* CTC 371 could improve odor intensity, aged flavor and atypical flavor as compared to control. Martin *et al.* (2006) reported that fungi such as *Penicillium chrysogenum* and *Debaryomyces hansenii* have a positive impact on the volatile compounds of ripened pork loins, being they were responsible for higher levels of long chain aliphatic and branched hydrocarbons, furanones and long chain carboxylic acids.

These starter cultures mentioned above are mainly lactic acid bacteria (LAB), which are isolated from various sources that included the intestinal tracts, feces, fermented products. Since the interest in isolation of starter cultures has greatly increased, many researchers tried to investigate various sources having potentiality to be used as starter cultures. Among those, kimchi is Korean traditional foods prepared by fermented with LAB originating from raw materials including cabbage, radish, garlic, onion and ginger. LAB is present in properly fermented kimchi at  $10^7$ - $10^9$  colony forming unit (CFU)/g, which depends on temperature and humidity of fermentation (Lee *et al.*, 1992; Lee *et al.*, 2006). Thus, the objective of this study was to investigate the effects of combined starter culture isolated from kimchi on physicochemical properties, functionality and flavor profile of fermented ham, as compared to those with commercial starter culture.

## Materials and methods

### Materials and brine solution

Domestic pork loins from the same origin of animals slaughtered the previous day were purchased from a

wholesale meat market, and the excess fat was trimmed. As a commercial starter culture, LK-30 plus (*Lactobacillus sake* + *Staphylococcus carnosus* + *Micrococcus varians*) of Gewuerzmüller Inc., German) was used for comparison with a novel mixed starter culture isolated from kimchi. It was compared of lactic acid bacteria isolated from kimchi, including *Lactobacillus plantarum* L155 having cholesterol assimilation activity, *Lactobacillus plantarum* L167 producing angiotensin converting enzyme (ACE) inhibition peptides and *Pediococcus damnosus* L12 producing bacteriocin, was used as a novel mixed starter culture (LLP) as previously reported by Han *et al.* (2006). The formulation of curing solution is summarized in Table 1.

### Experimental design and sample preparation

Two experiments, 1 and 2, were designed. The first experiment (1) was carried out to compare physicochemical and textural properties between commercial starter culture (LK-30 plus) and a novel starter culture (LLP). Experiment 2 compared physicochemical and textural properties between no starter culture and LLP, in fermented ham. After loins were cut into approximately 250-300 g of strips, each strip was stuffed into a net casing. In experiment 1, the loin strips were divided into two batches (A and B). Batched containing LK-30 plus and LLP of approximately  $10^6$  cells/g were divided into batch A and B, respectively. After the inoculated strips were cured into brine solution (Table 1) at  $8\pm 1^\circ\text{C}$  for 7 d, then were dried at  $8\pm 1^\circ\text{C}$  for 1 d. Then they were cold-smoked on smoke chamber (NU-vu, ES-13, Food System, Canton, MA, USA), and were held at  $15^\circ\text{C}$  and a relative humidity of 85% on Temperature & Humidity chamber (Myung Technology, Korea) for about 3 wk.

### pH and proximate analysis

pH values were measured five times using solid pH meter (Mettler Toledo, MP120, Switzerland), which was calibrated with a buffer (pH 4 and 7). Moisture, crude fat and crude protein contents were determined according to AOAC (1995), using the dry-oven ( $102^\circ\text{C}$ , 16 h) method,

**Table 1. The composition of brine for curing**

Ingredients	Weights (g)
Water	1,000
Salt	150
Sugar	70
NaNO <sub>2</sub>	0.4
NaNO <sub>3</sub>	0.7
White pepper	3.0

**Table 2. Gas chromatography mass spectrometry operation conditions for the analysis of volatile compounds in fermented hams**

Type	Hewlett Packard 6890 GC-FID and GC-MS system
Column (HP-5)	30 m length × 0.25 mm i.d. × 0.1 μL film thickness
Detector	Flame ionization detector (FID) 5973 mass selective detector (MSD)
Carrier gas	Helium
Injector type	Splitless mode
Purge time	45 min
Purge flow	15 mL/min
Flow rate	1 mL/min
Inlet temperature	250°C
Detector temperature	270°C in FID Initial temp : 40°C Initial holding time : 5 min Rate : 4°C/min until 160°C Rate : 10°C/min until 250°C Final temp : 250°C Final holding time : 6 min
5973 mass selective detector	
Ion source temperature	178°C
Ionization energy	70 eV
Scan	2.0 Scans/s
Filament emission	1 mA
Library	Wiley 77n MS library

soxhlet fat extraction and BUCHI protein analyzer (Kjeltec Auto System, B-322, Switzerland), respectively.

#### Color (L, a and b) and saltiness measurements

Hunter L (lightness), a (redness) and b (yellowness) values were measured five times using Chroma meter (CR-10, Minolta Corp. Ramsey, Japan). Saltiness of samples was measured five times using salt manager (SATO SK-10S, Japan).

#### Allo-kramer shear forces

Allo-kramer shear forces (kgf/g) were measured using Instron Universal Testing Machine (Model 3344, USA). Fermented ham samples were measured using a 10-blade Allo-kramer shear probe with a 200 mm/min cross speed (Lee and Chin, 2011).

#### Weight loss (WL, %)

Weight loss (WL, %) of samples was calculated as follows:  $WL (\%) = (A - B) \times 100 / A$  (A = initial sample weight; B = sample weight during ripening).

#### Microbial test

Total plate count, deMan, Rogosa and Sharpe (MRS)

and violet red bile (VRB) agar were used to determine total bacteria counts, lactic acid bacteria (LAB) and *Enterobacteriaceae*, respectively. In brief, homogenized samples (10 g) were mixed with 90 mL of sterile distilled water (DW) and after shaking well, serial dilutions were prepared. Aliquots (0.1 mL) of each dilution was spread on plate count, MRS and VRB agar. They were incubated at 37°C for 2 d, counted and expressed as Log CFU/g.

#### Cholesterol content

Cholesterol content of samples was measured according to the method of Searcy and Bergquist (1960). After a 4 mL-aliquots of fat extract were dried under nitrogen in a 60°C water bath, fat residues were saponified with 10 mL of 15% ethanolic KOH. Then, 5 mL of double distilled water was added and allowed to cool down to the room temperatures, as previously reported by Kim *et al.* (2014).

#### Volatile compounds analysis

Volatile compounds of samples were extracted using solid-phase microextraction (SPME) technic. Homogenized samples (10 g) and internal standard (1 mL, 200 ppm chlorobenzene in methanol, Supelco, USA) were transferred into a 40 mL amber screw top vial with hole cap PTFE/silicone septa (Supelco, USA). Before extraction of volatile compounds, fiber (75 mm, Carboxen-polydimethylsiloxane, Supelco, USA) was conditioned by heating in a gas chromatograph injection port equipped flame ionization detector (GC-FID) at 250°C for 1 h. Vials including samples were preheated for equilibration at 50°C for 30 min, and SPME fiber for extraction of volatile compounds was exposed to the headspace above the sample at 50°C for 30 min. After injection SPME fiber in GC-FID injection port, volatile compounds extracted from sample were isolated from SPME fiber at 250°C for 5 min. Qualitative analysis of volatile compounds was performed by GC-MS (mass spectrometry). The operation conditions of GC-FID and GC-MS were listed in Table 2. Quantitative analysis of volatile compounds was calculated as follows:

Concentrations (mg/kg) = each peak area × 20 / peak area of internal standard

#### Sensory evaluation

Three-week ripened hams were sliced and evaluated by seven panelists using 8-point hedonic scale (1, worst; 8, best). Panelist evaluated sensory parameters such as fla-

vor, texture, juiciness, color, saltiness and overall acceptability.

### Statistical analysis

The experiment was replicated three times. Data were analyzed using two-way analysis of variance (ANOVA) in SPSS 12.0 software for Windows, as factors for starter cultures and curing (0, 3, and 7 d) or ripening time (0, 7, 14, and 21 d). Means were separated by the Duncan's multiple range test ( $p < 0.05$ ).

## Results and Discussion

### Proximate composition (%) of fermented hams as affected by starter culture and processing time (Curing and ripening)

Since interactions between starter cultures and processing time (curing or ripening time) were not observed in all parameters, the data were pooled with the results presented in Tables 3-7. As shown in Table 3, moisture, fat and protein contents (%) were affected by processing time due to the evaporation of moisture during processing time. As a result, moisture content was decreased, while fat and protein contents were increased with increased processing time ( $p < 0.05$ ). These results might be partially due to the release of moisture in fermented loin hams due to relatively lower humidity and air circulation during ripening time. However, proximate compositions were not affected by starter cultures (A vs B) ( $p > 0.05$ ). This result was supported by Cenci-Goga *et al.* (2012) in which the control and selected dairy-origin starter (SDS) added fermented sausages were changed in the physicochemical characteristics during fermentation or ripening time. Cagno *et al.* (2008) reported that the three Italian sausages had 41-47% moisture, 18-25% fat and 25-37% protein with pH of 5.99-6.62, which was higher than our results. The two type of the Italian fermented sausages were similar moisture content to our products with higher fat content. However, the protein contents of our fermented ham were higher than those of the Italian fermented sausages.

### Effect of different starter cultures on pH, saltiness, color values, microbial counts and cholesterol contents (mg/100 g) of fermented hams during curing time

The results of physicochemical, microbial and functional properties as affected by starter cultures and curing time in fermented hams are shown in Table 4. Starter cultures didn't affect pH, microbial properties and cholesterol contents during curing time ( $p > 0.05$ ), however, saltiness were increased, but Hunter color values (L, a, and b) were decreased with increased curing time ( $p < 0.05$ ). These results indicated that brine solution permeated into the fermented hams, resulting in these parameter during curing time. However, pH values were ranged from 5.50 to 5.56, which didn't a marked change during curing time. This result is in agreement with the results of Krockel (1995) who reported that pH values of fermented hams were not remarkably reduced during curing procedure. Thus, curing process of fermented ham for 7 d affected the saltiness and color values of fermented ham ( $p > 0.05$ ).

### Effect of starter culture and ripening time on pH, saltiness, color, microbial counts, shear force value (kgf/g), weight loss (%) and cholesterol contents (mg/100 g) of fermented hams

Table 5 shows physicochemical, microbial and functional properties as affected by starter cultures and ripening time of fermented loin hams. No differences as affected by starter cultures were observed in all parameters ( $p > 0.05$ ), except for increased total bacterial counts (TPC) and lactic acid bacteria (LAB), in which both TPC and LAB was more detected in fermented hams inoculated probiotic starter culture (batch B) than counterparts (batch A). pH value of batch B (5.38) was lower than one of batch A (5.42) ( $p < 0.05$ ). This result might be partially due to higher level of LAB during ripening. Shear value was increased during ripening due to drying of moisture form the fermented ham ( $p < 0.05$ ), however, no differences were observed between two batches ( $p > 0.05$ ). Li *et al.* (2013) reported that dry fermented sausage containing ground de-

**Table 3. Proximate composition (%) of fermented hams as affected by starter culture and processing time**

	Batches <sup>1</sup>		Curing and ripening time (d)		
	A	B	Curing 0	Ripening 0	Ripening 21
Water	61.97	61.72	73.09 <sup>a</sup>	70.46 <sup>a</sup>	41.98 <sup>b</sup>
Fat	2.94	3.48	2.73 <sup>b</sup>	1.64 <sup>b</sup>	5.26 <sup>a</sup>
Protein	26.64	26.07	18.37 <sup>b</sup>	20.32 <sup>b</sup>	40.37 <sup>a</sup>

<sup>a-c</sup>Means with same row with same superscripts are not different ( $p > 0.05$ ).

<sup>1</sup>Batches: A=fermented ham with commercial starter culture (LK-30 plus); B=fermented ham with probiotic starter culture (*Lactobacillus plantarum* L155 + *Lactobacillus plantarum* L167 + *Pediococcus damnosus* L12).

**Table 4. pH, saltiness, color values, microbial counts and cholesterol contents (mg/100 g) of fermented hams as affected by starter cultures and curing time**

Curing	Batches <sup>1</sup>		Curing time (d)		
	A	B	0	3	7
pH	5.52	5.56	5.56	5.56	5.50
Saltiness	1.70	1.68	0.22 <sup>b</sup>	2.29 <sup>a</sup>	2.56 <sup>a</sup>
Lightness (L)	54.04	54.07	56.52 <sup>a</sup>	52.72 <sup>b</sup>	52.92 <sup>b</sup>
Redness (a)	4.42	3.81	5.08 <sup>a</sup>	3.67 <sup>b</sup>	3.58 <sup>b</sup>
Yellowness (b)	2.88	2.15	5.19 <sup>a</sup>	1.19 <sup>b</sup>	1.04 <sup>b</sup>
TPC <sup>c</sup>	7.00	7.16	6.99	7.20	7.04
MRS <sup>d</sup>	6.82	6.96	6.79	6.99	6.90
VRB <sup>e</sup>	<2	<2	<2	<2	<2
Cholesterol	54.29	52.98	55.10	56.53	49.60

<sup>a,b</sup>Means with same row with same superscripts are not different ( $p>0.05$ ).

<sup>1</sup>Batches are same as Table 3.

<sup>c</sup>TPC: Total plate count.

<sup>d</sup>MRS: Lactobacilli de Man, Rogosa, Sharpe Agar.

<sup>e</sup>VRB: Violet Red Bile Agar.

**Table 5. pH, saltiness, color, microbial counts, shear force value (kgf/g), weight loss (%) and cholesterol contents (mg/100 g) of fermented hams as affected by starter culture and ripening time**

Ripening	Batches <sup>1</sup>		Ripening time (d)			
	A	B	0	7	14	21
pH	5.42	5.38	5.43	5.34	5.39	5.44
Saltiness	4.47	4.20	2.55 <sup>c</sup>	3.61 <sup>b</sup>	5.40 <sup>a</sup>	5.78 <sup>a</sup>
Lightness	51.91	51.78	56.81 <sup>a</sup>	51.91 <sup>b</sup>	49.47 <sup>b</sup>	49.19 <sup>b</sup>
Redness	3.92	3.68	2.99 <sup>b</sup>	3.87 <sup>a</sup>	4.14 <sup>a</sup>	4.19 <sup>a</sup>
Yellowness	1.93	2.03	1.24 <sup>b</sup>	2.23 <sup>a</sup>	2.49 <sup>a</sup>	1.96 <sup>a</sup>
TPC <sup>d</sup>	7.00 <sup>b</sup>	7.90 <sup>a</sup>	6.98	7.71	7.82	7.30
MRS <sup>e</sup>	6.86 <sup>b</sup>	7.77 <sup>a</sup>	6.70	7.64	7.76	7.16
VRB <sup>f</sup>	<2	<2	<2	<2	<2	<2
Weight loss	39.85	42.27	-	33.32 <sup>b</sup>	43.35 <sup>a</sup>	46.52 <sup>a</sup>
Shear force	9.00	9.09	-	5.25 <sup>c</sup>	9.34 <sup>b</sup>	12.56 <sup>a</sup>
Cholesterol	70.16	67.95	48.20 <sup>b</sup>	59.90 <sup>b</sup>	81.85 <sup>a</sup>	79.82 <sup>a</sup>

<sup>a-c</sup>Means with same row with same superscripts are not different ( $p>0.05$ ).

<sup>1</sup>Batches are same as Table 3.

<sup>d</sup>TPC: Total Plate Count.

<sup>e</sup>MRS: Lactobacilli de Man, Rogosa, Sharpe Agar.

<sup>f</sup>VRB: Violet red Bile Agar.

ordorized yellow mustard, the potential antioxidant, had lower shear value than the control, and increased the shear value with increased ripening for 28 d. Popp *et al.* (2013) reported that turkey fermented sausages with dark, normal, and pale muscle had similar shear values and concluded that turkey meat could be used to manufacture the fermented sausages without quality defects. Cholesterol contents of batch A and B were 70.16 and 67.95 mg/100 g, respectively, and were similar to each other. Muguerza *et al.* (2001) reported that the cholesterol level was reduced about 12-13% in Spanish fermented sausages with 20-25% replacing level, and up to 22% in sausages with 30% replacing with pre-emulsified olive oil. The cholesterol level of fermented ham was similar to those of 30%

replacing with the olive oil due to the low-fat content in the fermented ham. However, saltiness, Hunter a and b values, weight loss, shear force and cholesterol contents were increased with increased ripening time ( $p<0.05$ ). These results may be due to moisture loss of fermented hams developed in ripening chamber. Olivares *et al.* (2010) evaluated the fat reduction and ripening time on the sensory characteristics of fermented sausages, and concluded that fat reduction affected texture and other parameters, and ripening time also significant affected the quality differences. They also suggested that the rate of processing time was depended on the fat content, and that slow fermentation process was beneficial to produce low-fat fermented sausages without detrimental to the textural appearance.

### Volatile compounds in fermented hams as affected by starter cultures and either curing or ripening time

Total eight volatile compounds were identified from fermented hams during curing process (Table 6). These were not affected by starter cultures and curing time, except for trans-caryophyllene. Among volatile compounds identified, ethanol was the most abundant compounds (more than 90%), followed by 1-hydroxy-2-butanone and hydrazine. These results were similar to the previous report of Rivas-Cañedo *et al.* (2009) who reported that the alcohol, aldehyde and alkanes were the higher level in volatile compounds identified from Spanish dry-fermented sausage ‘salchichon’.

Total fifty-eight volatile compounds were identified from fermented hams during ripening process (Table 7). Among identified compounds, alcohols, esters and furans were the main volatile compounds extracted from fermented hams. Martin *et al.* (2006) studied effects of selected fungal to the volatile compounds on fermented ham and reported that alcohols were the most representative compounds in final products, especially, ethanol. These results are in accordance with our results. Of the 58 compounds, two compounds, (3-methyl-oxiran-2-yl)-methanol and ethyl propanoate, were affected by starter cultures ( $p < 0.05$ ). Among them, the concentrations of ethanol, ethyl acetate and 2-furfural in fermented hams inoculated LK-30 plus were 1397, 459 and 311 mg/kg, those in loin hams inoculated probiotic starter culture were 1579, 538 and 302 mg/kg, respectively. The ratios of these three compounds in batch A and B were approximately 76.2 and 77.8% in total concentration of the identified compounds, respectively. These results suggested that probiotic starter culture (LLP) isolated from kimchi developed

in this study could be used as starter culture due to the similar volatile compounds except for few cases. Rivas-Cañedo *et al.* (2009) conducted the evaluation of volatile compounds in pressure-treated Spanish fermented sausages and reported high levels of alcohol, aldehyde, and alkanes, as compared with the control samples. Olivares *et al.* (2011) also reported that a total of 95 volatile compounds were identified using SPME, GC, and mass spectrophotometry and the volatile compounds of hexanal, 2-nonanal, and 2, 4-nonadienal, ethyl butanoate and 1-octen-3-ol highly affected the consumer preference of aroma in overall quality.

Concentrations and type of identified volatile compounds during ripening time were more than those of identified volatile compounds during curing time. These differences may be due to the smoking procedure that was performed prior to ripening. The main volatile compounds derived from smoking were furans and phenols. In the initial ripening time (0 d), compounds such as 2-furfural (1053 mg/kg), ethanol (886 mg/kg), 5-methyl-2-furfural (108 mg/kg), 2-methoxy-phenol (77.9 mg/kg) and 2-(hydroxymethyl)-furan (70.4 mg/kg) were the most representative volatile compounds, and were derived from smoking procedure, except for ethanol. However, since most compounds derived from smoking were markedly decreased with increased ripening time, these compounds might not affect the flavor profiles of final products. Ethanol, which was significantly increased with increased ripening time, might affect the flavor profiles of final products. Ethyl acetate, hexanal and hydrazine were produced 702, 124 and 102 mg/kg in 21 d of ripening time, respectively, and were the most representative volatile compounds in the final products. Although ethyl acetate was not detected during the initial ripening time, it showed the greatest increase during ripening. Ester compounds having fruity smell characteristics were mainly produced by esterification of carboxylic acids and alcohols (Sabio *et al.*, 1998). Therefore, fruity smell characteristics of fermented hams manufactured in this study might have been derived from ethyl acetate. Hexanal is an aldehyde that has a “grassy” odor and formed during the oxidation of linoleic acid via 13-hydroperoxide. Increasing hexanal content indicated that fermented hams were oxidized during ripening time. Because aldehyde compounds like hexanal have lower threshold values, they contribute to flavor profiles of fermented hams (Sabio *et al.*, 1998). Also, significant increases were observed in acetic acid, 3-methoxy-1,2-propanediol, methyl pentanoate, 1-(2-furyl)-ethanone, 3-methyl-2-cyclopenten-1-one, 2-hydroxy-3-

**Table 6. Volatile compounds in fermented hams as affected by starter cultures and curing time**

Volatile compounds	µg/kg			
	Batches <sup>1</sup>		Curing time (d)	
	A	B	0	7
hydrazine	39.9	49.9	38.0	51.8
ethanol	1647	1227	1228	1646
2-nitro-ethanol	18.3	12.3	19.5	11.0
1-hydroxy-2-butanone	40.8	54.7	51.4	44.1
hexanal	10.7	5.19	9.60	6.24
2-methyl-2-cyclopenten-1-one	1.77	1.82	1.65	1.95
2-methoxy-4-methyl-phenol	0.92	0.90	0.98	0.84
trans-caryophyllene	2.70	2.59	0.92 <sup>b</sup>	4.37 <sup>a</sup>

<sup>a,b</sup>Means with a same superscript within a row are not different ( $p > 0.05$ )

<sup>1</sup>Batches are same as Table 3.

**Table 7. Volatile compounds in fermented hams as affected by starter cultures and ripening time**

Volatile compounds	µg/kg					
	Batches <sup>1</sup>		Ripening time (d)			
	A	B	0	7	14	21
hydrazine	78.6	88.9	51.5 <sup>c</sup>	77.8 <sup>b</sup>	104 <sup>a</sup>	102 <sup>ab</sup>
ethanol	1397	1579	886 <sup>b</sup>	1265 <sup>ab</sup>	1723 <sup>ab</sup>	2078 <sup>a</sup>
carbon disulfide	19.0	22.3	t <sup>2</sup>	19.9	44.6	18.2
(3-methyl-oxiran-2-yl)-methanol	18.0 <sup>a</sup>	7.93 <sup>b</sup>	24.4	11.2	8.58	7.65
(2R,3R)-(2-2H1)-1,3-butanediol	25.2	35.8	t	33.6	66.1	22.2
2-nitro-ethanol	61.5	46.8	36.1	42.3	61.3	76.9
acetic acid, ethyl ester	459	538	t <sup>c</sup>	315 <sup>b</sup>	978 <sup>a</sup>	702 <sup>a</sup>
1,2-hydrazinedicarboxaldehyde	2.92	1.53	2.45	1.79	1.84	2.81
acetic acid	4.55	5.01	0.93 <sup>b</sup>	4.75 <sup>a</sup>	6.40 <sup>a</sup>	7.05 <sup>a</sup>
oxo-acetic acid	0.36	0.43	1.59 <sup>a</sup>	t <sup>b</sup>	t <sup>b</sup>	t <sup>b</sup>
1-hydroxy-2-propanone	31.6	31.0	35.7	43.7	32.6	13.3
2-butanamine	1.78	2.33	2.09	1.05	1.78	3.30
propanoic acid, ethyl ester	22.4 <sup>a</sup>	4.11 <sup>b</sup>	4.87	20.6	17.4	10.1
butanoic acid, methyl ester	29.7	19.8	18.9	16.8	32.7	30.7
ethoxy[oxo]acetic acid	2.29	1.35	0.18	2.01	2.82	2.28
3-methoxy-1,2-propanediol	0.58	0.61	t <sup>b</sup>	t <sup>b</sup>	0.91 <sup>a</sup>	1.46 <sup>a</sup>
toluene	18.8	18.9	27.3	22.3	16.1	9.61
1-hydroxy-2-butanone	39.9	48.5	34.2	44.3	56.9	41.7
pentanal	5.94	6.30	0.23	5.56	9.17	9.51
hexanal	63.2	69.2	3.15 <sup>b</sup>	52.9 <sup>ab</sup>	84.6 <sup>a</sup>	124 <sup>a</sup>
butanoic acid, ethyl ester	2.06	2.18	t	1.03	2.64	4.80
3-furfural	2.20	2.78	9.96 <sup>a</sup>	t <sup>b</sup>	t <sup>b</sup>	t <sup>b</sup>
pentanoic acid, methyl ester	5.41	4.24	t <sup>b</sup>	2.76 <sup>b</sup>	7.92 <sup>a</sup>	8.61 <sup>a</sup>
2-furfural	311	302	1053 <sup>a</sup>	99.0 <sup>b</sup>	43.5 <sup>b</sup>	31.5 <sup>b</sup>
2-(hydroxymethyl)furan	25.2	30.1	70.4 <sup>a</sup>	27.0 <sup>b</sup>	9.77 <sup>b</sup>	3.30 <sup>b</sup>
1-(acetyloxy)-2-propanone	2.59	2.53	10.2 <sup>a</sup>	t <sup>b</sup>	t <sup>b</sup>	t <sup>b</sup>
pentanoic acid, ethyl ester	2.29	2.57	0.37	1.91	2.32	5.11
2-methyl-2-cyclopenten-1-one	2.19	1.97	1.10	1.49	2.42	3.30
1-(2-furanyl)-ethanone	1.07	1.02	t <sup>b</sup>	0.26 <sup>b</sup>	1.28 <sup>ab</sup>	2.65 <sup>a</sup>
2,5-hexanedione	1.07	1.07	3.52 <sup>a</sup>	0.76 <sup>b</sup>	t <sup>b</sup>	t <sup>b</sup>
2,5-dimethyl-2,4-hexadiene	0.82	0.89	3.41 <sup>a</sup>	t <sup>b</sup>	t <sup>b</sup>	t <sup>b</sup>
5-methyl-(5H)-furan-2-one	3.82	5.20	18.0 <sup>a</sup>	t <sup>b</sup>	t <sup>b</sup>	t <sup>b</sup>
3-methyl-2-cyclopenten-1-one	2.77	2.90	t <sup>c</sup>	2.08 <sup>bc</sup>	3.10 <sup>b</sup>	6.15 <sup>a</sup>
1-methoxy-1,3-cyclohexadiene	2.41	2.86	10.6 <sup>a</sup>	t <sup>b</sup>	t <sup>b</sup>	t <sup>b</sup>
5-methyl-2-furfural	27.1	31.5	108 <sup>a</sup>	6.34 <sup>b</sup>	1.52 <sup>b</sup>	1.12 <sup>b</sup>
3-methyl-5H-furan-2-one	12.1	14.2	18.9	15.2	11.2	7.14
phenol	12.0	11.2	16.7	12.4	9.53	7.83
delta-3-carene	1.97	1.68	t <sup>b</sup>	2.33 <sup>a</sup>	2.53 <sup>a</sup>	2.43 <sup>a</sup>
2-hydroxy-3-methyl-2-cyclopenten-1-one	9.42	10.1	2.39 <sup>b</sup>	6.85 <sup>b</sup>	9.52 <sup>b</sup>	20.2 <sup>a</sup>
3,6-dihydro-3-methyl-2H-pyran-2-one	2.14	1.16	2.01	1.25	2.20	1.15
2,2,4,6,6-pentamethyl-heptane	3.41	3.67	0.50 <sup>b</sup>	2.22 <sup>b</sup>	3.27 <sup>b</sup>	8.17 <sup>a</sup>
2,4,6-trimethyl-octane	7.78	8.65	0.33 <sup>c</sup>	7.44 <sup>bc</sup>	8.96 <sup>ab</sup>	16.1 <sup>a</sup>
2,3-dimethyl-2-cyclopenten-1-one	3.11	3.18	t <sup>b</sup>	2.20 <sup>b</sup>	3.31 <sup>ab</sup>	7.07 <sup>a</sup>
1-methoxy-1,3-cyclohexadiene	10.8	12.5	19.0 <sup>a</sup>	9.64 <sup>b</sup>	10.4 <sup>b</sup>	7.59 <sup>b</sup>
2-methyl-phenol	2.38	2.63	7.06 <sup>a</sup>	1.89 <sup>b</sup>	0.50 <sup>b</sup>	0.57 <sup>b</sup>
3,6-dimethyl-undecane	9.97	10.9	t <sup>b</sup>	7.81 <sup>b</sup>	9.68 <sup>b</sup>	24.3 <sup>a</sup>
4,8-dimethyl-undecane	7.87	8.08	t <sup>b</sup>	5.12 <sup>b</sup>	7.97 <sup>b</sup>	18.8 <sup>a</sup>
4-methyl-phenol	6.49	7.98	11.6	6.58	5.66	5.14
1-cyclohexen-1-al	8.64	7.64	0.32 <sup>b</sup>	4.43 <sup>b</sup>	8.29 <sup>b</sup>	19.5 <sup>a</sup>
2-methoxy-phenol	47.4	57.5	77.9	55.6	47.6	28.7
nonanal	3.91	4.61	2.42	3.56	4.79	6.27
2,5-dimethyl-phenol	0.95	1.09	t	0.38	0.79	2.90

**Table 7. Volatile compounds in fermented hams as affected by starter cultures and ripening time (continued)**

Volatile compounds	µg/kg					
	Batches <sup>1</sup>		Ripening time (d)			
	A	B	0	7	14	21
2,6-dimethyl-4H-pyran-4-one	0.22	0.47	1.38 <sup>a</sup>	t <sup>b</sup>	t <sup>b</sup>	t <sup>b</sup>
2-methoxy-4-methyl-phenol	3.18	2.81	5.36	2.37	2.81	1.44
E,Z-3-methyl-2,4-hexadiene	1.19	2.06	0.72	2.24	1.96	1.57
3-hydroxy-4-methoxy-benzaldehyde	0.88	1.65	0.84	1.78	1.26	1.20
trans-caryophyllene	7.23	7.56	4.33	13.0	7.22	5.05
2-methoxy-4-nitro-phenol	3.74	5.15	4.40	4.81	4.39	4.18

<sup>a,b</sup>Means with a same superscript within a row are not different ( $p>0.05$ ).

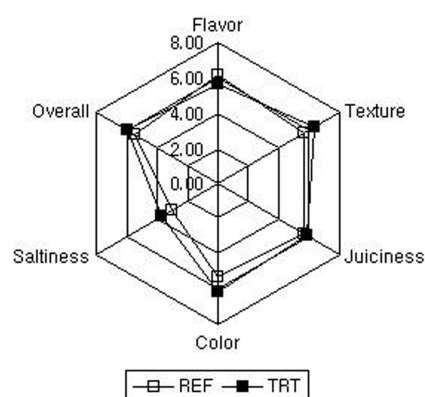
<sup>1</sup>Batches are same as Table 3.

<sup>2</sup>t: trace, not detected.

methyl-2-cyclopenten-1-one, 2,2,4,6,6-pentamethyl-heptane, 2,4,6-trimethyl-octane, 2,3-dimethyl-2-cyclopenten-1-one, 3,6-dimethyl-undecane, 4,8-dimethyl-undecane and 1-cyclohexen-1-al ( $p<0.05$ ). Volatile compounds such as oxo-acetic acid, 3-furfural, 2-furfural, 2-(hydroxy methyl)-furan, 1-(acetyloxy)-2-propanon, 5-hexanedione, 2,5-dimethyl-2,4-hexadiene, 5-methyl-(5H)-furan-2-one, 1-methoxy-1,3-cyclohexadiene, 5-methyl-2-furfural, 1-methoxy-1,3-cyclohexadiene, 2-methyl-phenol, 2,6-dimethyl-4H-pyran-4-one were decreased with increased ripening time ( $p<0.05$ ). Cagno *et al.* (2008) reported that total of 52 volatile compounds were observed in three Italian fermented sausages and the main components of these volatile compound were alcohol, aldehyde and terpenes.

### Sensory evaluation of fermented hams as affected by various starter cultures

As shown in Fig. 1, fermented hams (batch B, TRT) manufactured with probiotic starter culture (LPP) had higher sensory score in texture, color and overall acceptability than counterparts (batch A, REF), while the opposite trend was observed in flavor. These results indicated that mixed probiotic lactic acid bacteria, *Lactobacillus plantarum* L155, *Lactobacillus plantarum* L167 and *Pediococcus damnosus* L12, could be possibility to be used as starter culture in fermented meat products. Cenci-Goga *et al.* (2012) reported that fermented sausages made with selected dairy starter cultures were slightly saltier, juicier and in general more acceptable in consumer sensory evaluation. Olivares *et al.* (2010) reported that the sensory data from consumer sensory of fermented sausages were mainly affected by mostly fat content, indicating that the ripening time should be controlled depending on the fat content.



**Fig. 1. Sensory evaluation of fermented hams as affected by various starter cultures (REF and TRT are same as batch A and B in Table 3)**

### Conclusions

Fermented hams with commercial starter culture (A, LK-30 plus) and mixed starter culture developed in this study (B) were evaluated the product quality during processing time. Curing process increased saltiness, however, decreased hunter color values (L, a, and b values). Ripening process increased most parameters due to the drying process. The mixed starter culture had higher lactic acid bacteria than the commercial one. While 8 volatile compounds were identified from fermented hams during curing process, total 58 volatile compounds were identified from fermented hams during ripening process. The main volatile compounds were alcohols, esters and furans. However, no differences in volatile compounds were observed between two batches. Fermented hams containing mixed probiotic starter culture had higher sensory score in texture, color and overall acceptability than counterparts. In conclusion, mixed probiotic starter culture isolated from kimchi in this study could be used as a starter culture for



the manufacture of fermented ham to replace with the commercial starter culture.

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