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Physico-chemical and Microbial Properties of Sausages Affected by Plant Scale and Cooking Treatments during Refrigerated Storage

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Abstract

Purpose: The objective of this study was to examine the effect of plant scale and cooking conditions on the quality characteristics of sausages during refrigerated storage. **Methods:** Sausages used in this study were classified into two groups: those submitted to 1st cooked treatments and those submitted to 2nd cooked treatments. The pH, volatile basic nitrogen (VBN), gas production ratio, and microorganisms were measured in triplicate. **Results:** The change of quality in the products was assessed every 7 days by measuring pH, VBN levels, total microbes, coliform bacteria, *Escherichia coli*, and pathogenic bacteria in the products. Pathogenic bacteria such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium perfringens*, and *E. coli* were not detected in the sausages with 1st cooked treatments. The results showed that the pH of the sausages decline as storage time increased. The pH value of the sausages with 2nd cooked treatments changed gradually. VBN levels were generally lower in products with 2nd cooked treatments than in those with 1st cooked treatments, but they varied with the type of products. On the 35th day, the number of total microbes ranged between 6.13-7.12 log CFU/g in products with 1st cooked treatments and 3.44-6.92 log CFU/g in products with 2nd cooked treatments, showing fewer bacteria in the latter products. **Conclusions:** 1st cooked treatments were effective in microbial control, but 2nd cooked treatments could prolong the shelf life of the sausages, indicating a need for differential management of each product.

Key words: sausage, cooking condition, pathogenic bacteria, quality, storage

가열조건 및 공장 규모에 따른 소시지의 냉장저장 중 이화학적 및 미생물적 품질특성

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Abstract

목적: 본 연구는 육제품 제조 시 가열조건 및 육가공장 규모에 따른 육제품의 저장 중 이화학적 및 미생물학적 변화에 대해 알아보고자 실시하였다. **연구방법:** 가열조건은 1차 및 2차 가열조건으로 하였고, 육가공장 규모는 대, 중, 소로 구분하여 이화학적 및 미생물학적 변화로 pH, VBN, 일반세균, 대장균군, 대장균 및 병원성 세균에 대해 7일 간격으로 측정하였다. **결과:** 육제품의 가열조건에 따른 병원성 세균(*Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium perfrengens* 및 *Escherichia coli*)에 대해 측정한 결과 1차 가열 후 모두 음성으로 나타났다. pH는 초기 1차 및 2차 가열 후 각각 6.17-6.55 및 6.17-6.56의 범위로 가열조건에 따른 차이를 보이지 않았다. 그러나 저장 35일에는 1차 가열 제품은 5.13-6.00의 낮은 pH를 보인 반면 2차 가열 제품의 경우 5.42-6.19 범위로 1차 가열제품보다 높은 경향을 보였다. VBN 함량은 초기 3.89-7.77 mg%의 범위를 보였고 저장 35일에는 9.38-13.05 mg%로 점차 증가하는 경향을 보였다. 가열조건에 따라서는 2차 가열 후 감소하는 경향을 보였으나 육가공장 규모에 따라 다르게 나타났다. 일반세균수는 초기 1.39-2.96 log CFU/g이였고 저장 35일에 1차 및 2차 가열 후 각각 6.13-7.12 log CFU/g 및 3.46-6.92 log CFU/g으로 2차 가열 후 세균수가 적게 나타났다. 또한 육가공장 규모에 따라서는 이화학적 및 미생물학적으로 차이가 나타나지 않았다. **결론:** 따라서 1차 가열로 병원성 미생물 제어는 가능하나 2차 가열로 인하여 저장기간을 연장시킬 수 있을 것으로 보이며 제품의 특성에 맞는 관리가 필요할 것으로 사료된다.

주제어: 소시지, 가열조건, 병원성 미생물, 품질특성, 냉장저장

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Ⅰ. Introduction

An increase in income and change in consumer taste and dietary life has resulted in increased consumption of meat products. Although meat products are considered a good source of protein in food, they can easily spoil and cause food poisoning under optimal conditions for microbial growth (Smith JL 1991). Major pathogenic microorganisms that contaminate meat and meat products include *Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium perfringens*, and *Escherichia coli* O157:H7 (Borch E & Arinder P 2002, Kim JY et al. 2005). In Korea, "Standard for Processing and Ingredient Specification of Livestock Products (2013)" classifies meat products into heat-treated meat products and others. It also defines which bacteria are pathogenic. According to the common standard, six food-poisoning bacteria such as *Salmonella*, *S. aureus*, *L. monocytogenes*, *E. coli* O157:H7, *V. parahaemolyticus*, and *C. perfringens* should be examined to be negative, while coliform bacteria should also be negative in heat-treated meat products. Distribution channels and shelf life affect microbial counts, pH, and volatile basic nitrogen (VBN) levels in meat products. Change in pH is reportedly brought about by exposure to alkaline conditions caused by the type of *Lactobacillus* in the product, amount of carbohydrate present, changes in protein buffer, dissociation of electrolytes, and amino acid degradation. VBN levels in the muscle protein during shelf life are reportedly increased by degradation of amino acids, low molecular inorganic nitrogen, and production of ammonia (Davies A & Board R 1998). There have been studies on the effects of factors such as materials, preservatives, packaging, and storage conditions on the change in quality of meat products. Roller S et al. (2002) reported the effects of chitosan, carnosine, and sulfite on the microbial control and prevention of quality deterioration in pork sausage. Lee YW & Kim JG (1996) reported the effects of shelf life on the increase in total microbial count, VBN, lipid oxidation, and decrease in the flavor of ham and sausage. Mendonica AF et al. (1989) reported that adding potassium sorbate, sodium acetate, and sodium chloride to pork chops resulted in a decrease in microorganism count and improvement in color. Zamora MC & Zaritzky NE (1987) reported that treatment of potassium sorbate in refrigerated beef inhibited bacterial growth. Molins RA et al. (1986) also reported that fresh pork treated with 1% sodium orthophosphate lasted twice as long as that in the control group.

Temperature is one of the most important factors that affect quality characteristics of meat products during their shelf life. In Korea, it is recommended that refrigerated meat products should be stored at a temperature between -2°C and 10°C. Most food distributors in Korea store refrigerated meat products at a temperature below 10°C for about 4 weeks. Meanwhile, storage temperature for ready-to-eat (RTE) meat products was set between -2 and 10°C at the Codex Alimentarius Commission in 2007. Recommended storage temperature for RTE meat products was also set at 6°C in Korea in March 2008.

The objective of this study was to examine the impacts of cooking conditions (1st and 2nd cooking) and plant scales (large plant, medium plant, and small plant) on sausage quality. To that end, changes in the pH, VBN, total microbes, coliform bacteria, *Escherichia coli*, and pathogenic bacteria were measured in the sausage during refrigerated storage at 6°C.

Ⅱ. Materials and Methods

1. Materials and experimental treatments

Meat products - sausages - with 1st cooked and 2nd cooked treatments were chosen at three domestic meat processing plants (Large scale processing plants: A, B; Medium scale processing plants: C, D; Small scale processing plants: E, F) and were stored at 6°C in order to assess their quality characteristics. The smoking temperature and time were the common method used in meat processing (Park HG et al. 2003). The sausage with 1st cooked treatments was smoked for 4 hr at 29.44°C and then smoked again for 3 more hours at 54.44°C. The sausage with 2nd cooked treatments was packaged after 1st cooked treatments and it was cooked again for about 15 to 20 min at 85-90°C (Fig. 1).

2. pH

Firstly, 45 mL of distilled water was poured into a 5 g of pulverized sample in order to be homogenized in a ultra-turrax (T25, Janken & Kunkel, Staufe, Germany). Then, pH was measured three times for each sample with a pH meter (340, Mettler-Toledo GmbH, Schwerzenbach, Switzerland).

3. Volatile Basic Nitrogen (VBN)

Firstly, 45 mL of distilled water was poured into a 5 g of pulverized sample in order to be homogenized in a homoge-

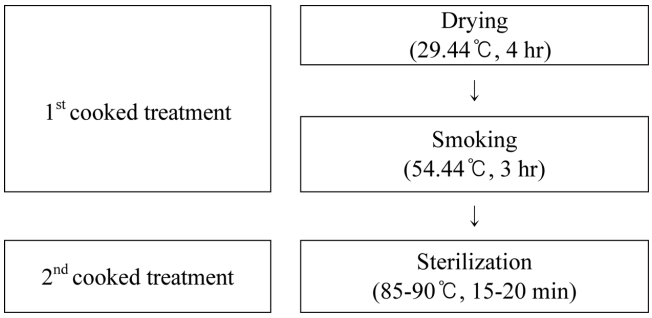

Fig. 1. Cooking treatments for the manufacture of sausages.

nizer (AM-7, Nihonseiki Kaisha Ltd., Tokyo, Japan). Then, filter paper (Whatman No.1, Whatman™, Maidstone, England) was used to filter the sample. Using a Conway container, 1 mL of 0.01 N H_3BO_3 (Daejung Chemical, Seoul, Korea) and 50 μL of the indicator (0.066% methyl red in ethanol:0.066% bromocresol green in ethanol=1:1) were placed in the inner room and 1 mL of the sample and 1 mL of 50% K_2CO_3 (Daejung Chemical, Seoul, Korea) were placed in the outer room and were sealed immediately. Then, they were incubated for 90 min at 37°C and titrated with 0.02 N H_2SO_4 (Daejung Chemical, Seoul, Korea). Distilled water was used instead of sample extract. VBN value was calculated in the following equation (Pearson D 1968).

$$\text{VBN (mg \%)} = (a-b) \times f \times 0.02 \times 14.007/S \times 100 \times 100$$

a: Volume (mL) of 0.02 N H_2SO_4 solution in the experiment
b: Volume (mL) of 0.02 N H_2SO_4 solution in the control experiment in which equivalent amount of distilled water was used in place of sample solution
f: Standard index of 0.02 N H_2SO_4
S: Weight of the sample

4. Gas production ratio

Products with slime which was produced during the storage and products swollen due to gas production were selected and analyzed in percentage terms.

$$\text{Gas production ratio (\%)} = \text{Number of swollen products} / \text{Number of total products} \times 100$$

5. Total microbes, *Escherichia coli* and coliform bacteria

Packaging of the products was sterilized with 70% ethanol and then 25 g of the sample was cut off aseptically in order to be homogenized in a 225 mL of sterilized peptone water (Hansol tech, Seoul, Korea) and decimally diluted step by step. Plate count agar medium (Petrifilm, 3M Science, Seoul, Korea) was used to cultivate the sample at 35°C for 48 hr to determine the total microbial count. Petrifilm (3M Science, Seoul, Korea) was used to cultivate the diluted sample. Colonies with blue bubbles were counted as *E. coli* and those with purple bubbles and blue bubbles were count as coliform bacteria.

6. Pathogenic bacteria

Pathogenic bacteria assessment were performed according to the method of "Standard for Processing and Ingredient Specification of Livestock Products (2013)."

1) *Listeria monocytogenes* (*L. monocytogenes*)

A 10 g of the sample was seeded onto 90 mL of Fraser broth (Oxoid, Lenexa, KS, USA) and cultivated at 35±1°C for 24-48 hr as a qualitative test for *L. monocytogenes* in the sample. Enriched media was streak inoculated in Listeria selective agar (Oxoid, Lenexa, KS, USA) for culturing it at 35±1°C for 48 hr. Then, the colonies surrounded by dark brown or black circles, which is the typical shape of Listeria were selected. Colonies cultured in isolation were moved to tryptic soy agar (Difco, NJ, USA) in order to be cultured at 37°C for 18-24 hr and then identified with Vitek2 compact instrument (BioMerieux, Marcy l'Etoile, France). As a qualitative test, 10 g of the selected sample and 90 mL of diluted solution were mixed and decimally diluted. Then, 1 mL of diluted solution by each step was cultured in Listeria selective agar (Oxoid, Lenexa, KS, USA) in at 35±1°C for 48 hr and then moved to tryptic soy agar in order to be cultured at 37°C for 18-24 hr and then identified with Vitek2 compact instrument (BioMerieux).

2) *Staphylococcus aureus* (*S. aureus*)

A 10 g of the sample was seeded onto 90 mL of tryptic soy broth (Difco, NJ, USA) with 10% NaCl (Daejung Chemical, Seoul, Korea) and cultivated at 35-37°C for 18 hr as a qualitative test for *S. aureus* in the sample. Enriched media was smeared in Baird-Parker agar (Oxoid, Lenexa, KS, USA) for culturing it at 37°C for 24 hr. Then, 1.0-1.5 mm thick, black, shiny, convex colonies with a 2-5 mm sized opaque region were selected. Colonies cultured in isolation were moved to tryptic soy agar in order to be cultured at 37°C for 18-24 hr and then identified with

Vitek2 compact (BioMerieux) instrument. As a qualitative test, 10 g of the selected sample and 90 mL of diluted solution were mixed and decimally diluted. Then, 1 mL of diluted solution by each step was cultured in Baird-Parker agar at 37±1°C for 48 hr and then moved to tryptic soy agar in order to be cultured at 37°C for 18-24 hr and then identified with Vitek2 compact (BioMerieux) instrument.

3) *Clostridium perfringens* (*C. perfringens*)

A 10 g of the sample was seeded onto the lower part of 90 mL of cooked meat medium (Oxoid, Lenexa, KS, USA) and anaerobically cultivated at 35°C for 18-24 hr as a qualitative test for *C. perfringens* in the sample. Enriched media was smeared in Perfringens agar (Oxoid, Lenexa, KS, USA) for anaerobically culturing it at 37°C for 18-24 hr. Colonies cultured in isolation were moved to tryptic soy agar to be cultured at 37°C for 18-24 hr and then identified with Vitek2 compact instrument (BioMerieux). As a qualitative test, the sample was cooked at 70°C for 20 min and then 10 g of the selected sample and 90 mL of diluted solution were mixed and decimally diluted. Then, 1 mL of diluted solution by each step was cultured in Perfringens agar at 35°C for 48 hr and then moved to tryptic soy agar in order to be cultured at 37°C for 18-24 hr and then identified with Vitek2 compact instrument (BioMerieux).

6. Statistical analysis

All tests were performed at least three times for each experimental condition and mean values are reported. One-way ANOVA was performed for all variables using the general linear model (GLM) procedure of the SAS (Statistics Analytical System, ver. 9.12, SAS Inst., Inc., Cary, NC, USA) statistical package. Duncan's multiple range test ($p<0.05$) was used to determine the differences between treatment means. Statistical analysis of each parameter combined the data from three batches.

Ⅲ. Results and Discussion

1. pH

The pH changes in sausages stored at 6°C are shown in Table 1-2. The pH in products with 1st cooked treatments ranged between 6.17-6.55, whereas the pH in products with 2nd cooked treatments ranged between 6.17-6.56, showing a marginal difference between the two groups in the early stage. With time, pH in both the groups slightly significantly decreased. However, on day 35, pH levels significantly ranged between 5.13-6.00 and 5.42-6.19 in the two groups, respectively, showing a higher pH of the products with 2nd cooked treatments ($p<0.05$). Among sausages with 1st cooked treatments, the pH dropped below 6.0 on the 14th day for product C, on the 21st day for products B, E, F, and G, and on the 28th day for product A. However, among sausages with 2nd cooked treatments, the pH did not fall below 6.0 in products A and B, even by the 35th day. The pH did fall below 6.0 in products C, D, E and F, but not until the 28th day. Generally, pH of sausages depends on quality of meat material and additives, and gradually decreases with time, owing to in-

Table 1. pH changes in the sausages with 1st cooked treatments during refrigerated storage

Plant scale	Storage (days)					
	0	7	14	21	28	35
A[1)]	6.17±0.02^{Ca}	6.21±0.05^{Da}	6.01±0.04^{CDb}	6.04±0.04^{Bb}	5.62±0.01^{Bc}	5.25±0.02^{Bd}
B	6.19±0.00^{Cb}	6.27±0.01^{CDa}	6.03±0.02^{Cc}	5.87±0.02^{Cd}	5.43±0.04^{Ce}	-
C	6.22±0.01^{Bca}	6.25±0.01^{CDa}	5.96±0.04^{Db}	5.63±0.04^{Dc}	5.31±0.04^{Dd}	5.29±0.02^{Bd}
D	6.27±0.04^{Bb}	6.37±0.04^{Ba}	6.36±0.02^{Aa}	6.34±0.01^{Aa}	6.26±0.01^{Ab}	6.00±0.02^{Ac}
E	6.55±0.03^{Aa}	6.61±0.03^{Aa}	6.13±0.03^{Bb}	5.69±0.04^{Dc}	-	-
F	6.26±0.04^{Ba}	6.31±0.01^{BCa}	6.05±0.03^{Cb}	5.87±0.03^{Cc}	5.56±0.01^{Bd}	5.13±0.03^{Ce}

1st cooking condition - drying: 29.44°C, 4 hr; smoking: 54.44°C, 3 hr.

1) A, B: sausage manufactured from large scale processing plants; C, D: sausage manufactured from medium scale processing plants; E, F: sausage manufactured from small scale processing plants.

A-D Means within a column with different letters are significantly different ($p<0.05$).

a-e Means within a row with different letters are significantly different ($p<0.05$).

Table 2. pH changes in the sausages with 2nd cooked treatments during refrigerated storage

Plant scale	Storage (days)					
	0	7	14	21	28	35
A[1)]	6.25±0.01^{Bbc}	6.25±0.08^{Cbc}	6.32±0.01^{Bab}	6.39±0.04^{Aa}	6.09±0.04^{Ad}	6.18±0.01^{Ac}
B	6.19±0.01^{Cb}	6.27±0.01^{Ca}	6.21±0.01^{Cb}	6.13±0.02^{CDc}	6.14±0.03^{Ac}	6.19±0.01^{Ab}
C	6.17±0.01^{Cb}	6.25±0.02^{Ca}	6.22±0.01^{Cab}	6.18±0.02^{BCb}	5.77±0.05^{Cd}	5.52±0.03^{Cd}
D	6.28±0.03^{Bb}	6.47±0.02^{Ba}	6.45±0.04^{Aa}	6.10±0.03^{Dc}	5.95±0.01^{Bd}	5.60±0.01^{Be}
E	6.56±0.01^{Ab}	6.71±0.03^{Aa}	6.26±0.05^{BCc}	6.20±0.03^{Bc}	5.95±0.01^{Bd}	5.42±0.02^{De}
F	6.27±0.04^{Bb}	6.41±0.02^{Ba}	6.03±0.02^{Dc}	6.01±0.02^{Ec}	5.77±0.02^{Cd}	5.43±0.01^{De}

1st cooking condition - drying: 29.44°C, 4 hr; smoking: 54.44°C, 3 hr.
2nd cooking condition - sterilization: 85-90°C, 15-20 min.
[1)] A, B: sausage manufactured from large scale processing plants; C, D: sausage manufactured from medium scale processing plants; E, F: sausage manufactured from small scale processing plants.
[A-E] Means within a column with different letters are significantly different (p<0.05).
[a-e] Means within a row with different letters are significantly different (p<0.05).

creased microbial growth. Results from this study were similar to those reported by Lee YW & Kim JG (1996). Sterilizing effects of heat treatment resulted in a lower pH of products with 1st cooked treatments compared to that of products with 2nd cooked treatments.

2. Volatile Basic Nitrogen (VBN)

Changes in VBN levels in sausages stored at 6°C are shown in Table 3-4. VBN levels in products with 1st cooked treatments ranged between 3.89-7.20 mg%, whereas these levels in products with 2nd cooked treatments ranged between 6.07-7.77 mg%, showing significantly higher VBN levels in the latter products, except for that in product E in the early stage (p<0.05). However, with time, VBN levels in both groups were slightly but significantly increased regardless of the frequency of heat treatment. On the 35th day, VBN level was significantly lower in the products with 2nd cooked treatments than in those with 1st cooked treatments (p<0.05). Cresopo FL et al. (1978) reported that VBN levels in meat increased with time, which corroborates with the results from this study. "Standard for Processing and Ingredient Specification of Livestock Products (2013)" defined that the VBN level in meat material and packaged meat should be 20 mg%;

Table 3. VBN level changes in the sausages with 1st cooked treatments during refrigerated storage (Unit:mg%)

Plant scale	Storage (days)					
	0	7	14	21	28	35
A[1)]	4.95±0.95^{BCd}	6.81±0.54^{BCc}	9.49±0.97^{Ab}	8.34±0.96^{ABb}	12.08±0.95^{Aa}	11.58±0.96^{a}
B	7.17±1.65^{Ab}	9.28±2.01^{Aab}	8.72±1.70^{Aab}	8.38±0.97^{ABab}	10.97±0.00^{Aa}	-
C	6.08±0.96^{ABc}	8.75±1.37^{ABb}	8.84±0.00^{Ab}	9.43±1.66^{Aab}	11.14±1.93^{Aab}	12.05±1.19^{a}
D	5.08±0.98^{BCc}	5.53±0.00^{Cc}	3.92±1.68^{Bc}	8.26±0.95^{ABb}	8.45±1.54^{Bb}	10.93±0.95^{a}
E	3.89±0.00^{Cb}	5.00±0.96^{Cb}	4.97±0.96^{Bb}	7.29±0.00^{Ba}	-	-
F	7.20±0.00^{Ab}	7.91±0.98^{ABbc}	5.03±0.97^{Bd}	9.49±0.97^{Ab}	13.51±0.00^{Aa}	12.69±0.96^{a}

1st cooking condition - drying: 29.44°C, 4 hr; smoking: 54.44°C, 3 hr.
[1)] A, B: sausage manufactured from large scale processing plants; C, D: sausage manufactured from medium scale processing plants; E, F: sausage manufactured from small scale processing plants.
[A-C] Means within a column with different letters are significantly different (p<0.05).
[a-d] Means within a row with different letters are significantly different (p<0.05).

Table 4. VBN level changes in the sausages with 2nd cooked treatments during refrigerated storage (Unit:mg%)

Plant scale	Storage (days)					
	0	7	14	21	28	35
A[1)]	6.07±0.96^{cd}	6.43±0.84^{BCc}	5.03±0.01^{BCd}	8.85±0.96^{b}	10.50±0.96^{ABCa}	11.13±0.02^{Ba}
B	7.77±0.96^{b}	9.19±0.83^{Aab}	5.26±0.48^{BCc}	10.25±1.17^{a}	9.70±2.29^{BCab}	8.64±0.31^{Dab}
C	6.61±0.95^{bc}	6.64±2.09^{BCbc}	4.49±0.97^{Cc}	7.76±0.00^{b}	12.63±0.95^{Aa}	13.05±0.00^{Aa}
D	6.68±0.96^{c}	6.67±0.96^{BCc}	7.80±0.97^{Abc}	8.85±0.03^{b}	11.09±0.08^{ABa}	11.10±0.96^{Ba}
E	6.10±0.96^{b}	8.33±0.96^{ABa}	8.94±1.68^{Aa}	8.26±0.95^{a}	8.27±0.40^{Ca}	9.38±0.96^{Ca}
F	7.72±0.95^{c}	8.36±0.97^{ABbc}	7.45±0.52^{Ac}	8.34±0.96^{bc}	10.50±0.23^{ABCa}	9.52±0.97^{CDab}

1st cooking condition - drying: 29.44°C, 4 hr; smoking: 54.44°C, 3 hr.
2nd cooking condition - sterilization: 85-90°C, 15-20 min.
1) A, B: sausage manufactured from large scale processing plants; C, D: sausage manufactured from medium scale processing plants; E, F: sausage manufactured from small scale processing plants.
A-D Means within a column with different letters are significantly different (p<0.05).
a-d Means within a row with different letters are significantly different (p<0.05).

however, there is no standard VBN level for processed sausages. Dierick N et al. (1974) reported that the VBN level was higher in meat parts that contained higher levels of proteins and free amino acids. This finding is different from the results of this study because protein levels in meat and processing conditions for each meat product used in this study were different from those in the study by Dierick N et al. (1974).

3. Gas production ratio

Gas production ratio in sausages stored at 6°C is shown in Table 5. To measure the gas production ratio, products with slime and swollen package were analyzed in terms of percentage. Vacuum packaging of the sausages gives rise to anaerobic conditions in which microorganisms mostly comprising of lactic acid bacteria, produce carbon dioxide that blows up the balloon. Results from this study showed no gas production in any of the products immediately after their manufacture. Subsequently, the gas production ratio varied with each product. No gas production was observed in products with 1st cooked treatments until the 28th day. Products with 1st cooked treatments did not produce gas until the 21st day. Product D did not produce gas even on the 35th day, whereas product E recorded a 100% gas production ratio. Products A and C recorded 100% gas production ratio on the 35th day. Among the products with 2nd cooked treatments, products A, D, E and F did not produce gas until the 35th day. Gas production ratio in products B and C was 76% and 25%, respectively. Gas production ratio was lower in all products with 2nd cooked treatments than in their 1st cooked counterparts, indicating that the second heat treatment killed bacteria that produce gas and it may enhance storability of sausages. Nissen H et al. (1996) reported that anaerobic conditions exist inside

Table 5. Gas production ratio in sausages with cooking treatments during refrigerated storage (Unit: %)

Cooking treatments	Plant scale	Storage (days)					
		0	7	14	21	28	35
1st	A[1)]	0	0	0	0	29	100
	B	0	0	0	0	21	-
	C	0	0	0	0	11	100
	D	0	0	0	0	0	0
	E	0	0	0	0	100	-
	F	0	0	0	0	7	38
2nd	A	0	0	0	0	0	0
	B	0	0	0	0	0	76
	C	0	0	0	0	0	25
	D	0	0	0	0	0	0
	E	0	0	0	0	0	0
	F	0	0	0	0	0	0

1st cooking condition - drying: 29.44°C, 4 hr; smoking: 54.44°C, 3 hr.
2nd cooking condition - sterilization: 85-90°C, 15-20 min.
1) A, B: sausage manufactured from large scale processing plants; C, D: sausage manufactured from medium scale processing plants; E, F: sausage manufactured from small scale processing plants.

the packaging of meat products and that the gas is mostly produced by lactic acid fermentation. The meat with 2nd cooked treatments had a significantly lower gas production ratio owing to inhibition of microbial proliferation.

4. Total microbes, *Escherichia coli* and coliform bacteria

Changes in total microbes in sausages stored at 6°C are shown in Table 6-7. Total microbes in all sausages with 1st cooked treatments ranged between 1.39-2.96 log CFU/g in the early stage, showing about 1 log CFU/g of difference between each product. Among the seven products with 1st cooked treatments, the experiment had to be stopped in four of the products on the 35th day owing to gas production (p<0.05). Number of bacteria in the remaining three products ranged between 6.13-7.12 log CFU/g. However, among products with 2nd cooked treatments, the number of bacteria in six products ranged between 3.46-6.92 log CFU/g, indicating that the 2nd cooked treatments were effective in inhibiting microbial growth. Food sanitary control was required for products A, B and C, since they had more than 6 log CFU/g of bacteria on the 35th day.

Table 6. Total microbial changes in the sausages with 1st cooked treatments during refrigerated storage (Unit: log CFU/g)

Plant scale	Storage (days)					
	0	7	14	21	28	35
A[1)]	2.32±0.03$^{Bf2)}$	3.43±0.02^{Be}	5.68±0.00^{Ad}	5.90±0.00^{Cc}	6.40±0.02^{Ba}	6.25±0.10^{Bb}
B	2.96±0.03^{Ae}	4.36±0.06^{Ad}	5.42±0.04^{Bc}	5.87±0.00^{Cb}	6.18±0.14^{Ca}	-
C	1.85±0.09^{Cf}	3.32±0.09^{Be}	5.20±0.01^{Cd}	6.39±0.04^{Bb}	6.87±0.01^{Aa}	6.13±0.07^{Bc}
D	1.98±0.03^{Cd}	2.19±0.27^{Dd}	4.57±0.09^{Dc}	5.91±0.05^{Cb}	6.24±0.02^{BCa}	6.20±0.04^{Bab}
E	2.22±0.02^{Bd}	3.45±0.12^{Bc}	5.11±0.04^{Cb}	5.80±0.04^{Da}	-	-
F	1.39±0.12^{Dd}	2.63±0.07^{Cc}	2.45±0.17^{Ec}	6.75±0.02^{Ab}	6.99±0.02^{Aa}	7.12±0.05^{Aa}

1st cooking condition - drying: 29.44°C, 4 hr; smoking: 54.44°C, 3 hr.
[1)] A, B: sausage manufactured from large scale processing plants; C, D: sausage manufactured from medium scale processing plants; E, F: sausage manufactured from small scale processing plants.
[A-D] Means within a column with different letters are significantly different (p<0.05).
[a-f] Means within a row with different letters are significantly different (p<0.05).

Table 7. Total microbial changes in the sausages with 2nd cooked treatments during refrigerated storage

(Unit: log CFU/g)

Plant scale	Storage (days)					
	0	7	14	21	28	35
A[1)]	1.78±0.01^{Ce}	2.03±0.11^{Cd}	3.39±0.07^{Cc}	3.76±0.04^{Db}	3.40±0.00^{Ec}	6.66±0.05^{Ba}
B	2.86±0.06^{Ad}	2.09±0.12^{Ce}	1.99±0.12^{De}	4.68±0.16^{ABc}	5.60±0.13^{Bb}	6.92±0.02^{Aa}
C	1.15±0.21^{De}	2.40±0.02^{Bd}	5.26±0.06^{Ab}	4.47±0.03^{BCc}	6.28±0.03^{Aa}	6.27±0.05^{Ca}
D	2.65±0.01^{ABd}	2.71±0.05^{Ad}	3.94±0.05^{Bc}	4.77±0.10^{Ab}	5.17±0.08^{Ca}	5.26±0.08^{Da}
E	2.48±0.01^{Bc}	2.49±0.11^{ABc}	2.18±0.10^{Dd}	4.39±0.12^{Ca}	4.11±0.10^{Db}	3.95±0.03^{Eb}
F	1.15±0.21^{Dc}	2.05±0.14^{Cb}	3.63±0.21^{Ca}	3.54±0.09^{Da}	3.27±0.13^{Ea}	3.46±0.09^{Fa}

1st cooking condition - drying: 29.44°C, 4 hr; smoking: 54.44°C, 3 hr.
2nd cooking condition - sterilization: 85-90°C, 15-20 min.
[1)] A, B: sausage manufactured from large scale processing plants; C, D: sausage manufactured from medium scale processing plants; E, F: sausage manufactured from small scale processing plants.
[A-E] Means within a column with different letters are significantly different (p<0.05).
[a-e] Means within a row with different letters are significantly different (p<0.05).

Table 8. Changes in *Escherichia coli*/Coliform bacteria of sausages with cooking treatments during refrigerated storage

(Unit: log CFU/g)

Cooking treatments	Plant scale	Storage (days)					
		0	7	14	21	28	35
1st	A[1)]	N.D.[2)]	N.D.	N.D./3.08	N.D./2.00	N.D./3.28	N.D./4.00
	B	N.D.	N.D.	N.D.	N.D.	N.D.	-
	C	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	D	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	E	N.D.	N.D.	N.D.	N.D.	-	-
	F	N.D.	N.D./3.91	N.D./4.54	N.D./4.13	-	-
2nd	A	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	B	N.D.	N.D.	N.D.	N.D.	N.D.	-
	C	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	D	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	E	N.D.	N.D.	N.D.	N.D.	-	-
	F	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

1st cooking condition - drying: 29.44°C, 4 hr; smoking: 54.44°C, 3 hr.
2nd cooking condition - sterilization: 85-90°C, 15-20 min.
1) A, B: sausage manufactured from large scale processing plants; C, D: sausage manufactured from medium scale processing plants; E, F: sausage manufactured from small scale processing plants.
2) N.D.: Not detected.

Changes in *E. coli* and coliform bacterial populations in sausages stored at 6°C are shown in Table 8. *E. coli* and coliform bacteria were not found in both the groups in the early stage. However, with time, coliform bacteria were found in some products. Among products with 1st cooked treatments, 3.18 log CFU/g of coliform bacteria were found in product A by the 14th day and product F showed similar results. However, coliform bacteria were not found in products A and F with 2nd cooked treatments, indicating that 2nd cooked treatments were effective in inhibiting the growth of coliform bacteria. Since additional heat treatment can lengthen the shelf life of products, application of additional heat treatment should be decided on a product-by-product basis. Lamkey JW et al. (1991) reported that the putrefaction by total microbes in meat products during storage was more than 108 CFU/g. All treatments are expected to show putrefaction not less tha n 108 CFU/g during storage (35 days).

5. Pathogenic bacteria

1) *S. aureus, C. perfringens* and *L. monocytogenes*

Changes in pathogenic bacterial populations (*S. aureus*, *C. perfringens*, and *L. monocytogenes*) in sausages stored at 6°C are shown in Table 9. In this study, the test for pathogenic bacteria yielded negative results from the early stage to the 35th day of the products' shelf life. *S. aureus* is a common bacterium found on the skin and noses of humans and animals, and it has the ability to produce enterotoxins that are frequently responsible for food poisoning. *L. monocytogenes* leads to bacterial zoonosis that can cause miscarriage, encephalomeningitis, and septicemia, and even death after ingestion of contaminated food. When poultry and raw meat are contaminated with *C. perfringens*, during the butchering process, it may form spores that can withstand cooking temperatures and act as a source of infection (Kim JY et al. 2005). Studies reported that the limiting water activity (aw) for the growth of *S. aureus* sealed in canned meat at an oxygen concentration of 5.5% was 0.87% at 37°C and 0.91% at 20°C. Farrell GM & Upton ME (1978) seeded *S. aureus* on strips of bacon and stored them at -22°C, 5°C, and 16°C to monitor the number of bacteria in these strips. Number of bacteria decreased in those strips stored at -22°C, whereas the number of bacteria decreased in those strips stored at 5°C until the 13th day but it increased by 108/g on the 35th day.

Mellefont LA & Ross T (2007) reported that the growth

Table 9. Changes in pathogenic bacteria (*Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium perfringens*) of sausages with cooking treatments during refrigerated storage

Cooking treatments	Plant scale	Storage (days)					
		0	7	14	21	28	35
1st	A[1)]	N.D.[2)]	N.D.	N.D.	N.D.	N.D.	N.D.
	B	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	C	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	D	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	E	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	F	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2nd	A	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	B	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	C	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	D	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	E	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	F	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

1st cooking condition - drying: 29.44°C, 4 hr; smoking: 54.44°C, 3 hr.

2nd cooking condition - sterilization: 85-90°C, 15-20 min.

1) A, B: sausage manufactured from large scale processing plants; C, D: sausage manufactured from medium scale processing plants; E, F: sausage manufactured from small scale processing plants.

2) N.D.: Not detected.

of *L. monocytogenes* was inhibited in modified atmosphere packaged (MAP) sliced ham with addition of organic acid salt. Garrido V et al. (2010) seeded *L. monocytogenes* (<10 CFU/g) onto sliced ham to investigate its growth during the product's refrigerated shelf life. The study reported that the bacteria reached the control limit of 100 CFU/g before or on the third day at each temperature. Dickson JS (1991) reported that transfer of *L. monocytogenes* decreased when the initial inoculum was allowed to adsorb to the base tissue prior to contact with the second tissue. The type of base tissue was a factor (p<0.05), with a greater transfer from fat with contact times of less than 1 min and a greater transfer from lean with longer contact times, owing to moisture content. Many studies are currently underway in Korea and internationally, on the effects of processing, packaging methods, and storage temperature on inhibition of pathogenic bacterial growth in sausages. Inhibition of pathogenic bacterial growth was possible in the products used in this study, regardless of heat treatment frequency. Their storability could be evaluated through a microorganism assessment.

Ⅳ. Conclusion

The results of the examination for pathogenic bacteria (*S. aureus*, *C. perfringens* and *L. monocytogenes*) in sausages that were produced from three processing plants in sausages with 1st cooked treatments were all negative. During refrigerated storage, the sausages with 2nd cooked treatments had a higher pH and lower VBN value than those with 1st cooked treatments. Number of total microbes was 1.39-2.96 log CFU/g in the early stage, but increased to 6.13-7.12 log CFU/g in products with 1st cooked treatments, and 3.46-6.92 log CFU/g in products with two heat treatments on the 35th day, showing fewer bacteria in the latter. Thus, 1st cooking treatments were effective in microbial control, but 2nd cooking process could prolong the shelf life of the sausage. The scale of the processing plants was considered, but it did not affect the physicochemical properties and microbial numbers in the meat products.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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