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ARTICLE

Distribution of Microorganisms in *Cheongyang* Red Pepper Sausage and Effect of Central Temperature on Quality Characteristics of Sausage

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Abstract The objective of this study was to provide preliminary data for food industry by investigating the distribution of microorganisms in raw materials and sausage examining the effect of heating temperature on sausage quality. Total microbes in sausage ranged 2.21–3.11 Log CFU/g. *Bacillus pumilus*, *B. licheniformis*, *Staphylococcus saprophyticus*, and *Enterococcus faecalis* were detected on sausage. Total microbes in raw materials was 1.59–7.16 Log CFU/g. Different types of microorganisms were found depending on raw materials, with *B. pumilus* and *B. subtilis* were being detected in both raw materials and sausage. Total microbes in sausage after heating was in the range of 1.10–2.22 Log CFU/g, showing the trend of decrease in total microbe with increasing heating temperature, although the decrease was not significant. With increasing heating temperature, pH and hardness were also increased. The yield of sausage manufactured at 85°C was 95.42% while that manufactured at 65°C was 96.67%. Therefore, decreasing heating temperature during sausage production might increase yield and save energy without microbiological effect.

Keywords sausage, heating temperature, bacterial distribution, physicochemical properties, yield

Introduction

Consumption of meat products in Korea has increased by 2 times with increasing income (Choi et al., 2016). Generally, sausage manufacturing process begins with trimming raw meat followed by curing, grinding, heating, cooling, and packing processes (Choi et al., 2010). The processed meat products have possibility of raw meat contamination in the manufacturing process and contamination during the manufacturing (Sofos et al., 2013). It is concerned that the microorganisms contaminated during the manufacturing process may affect as the cross contamination and the source of the re-contamination. Pathogens that contaminating foods during the manufacturing process can cause cross-contamination or recontamination. Knowing the initial distribution of microorganisms and the initial type of microorganisms after completing the packing process is important concerning food spoilage caused by microorganisms (Björkroth and Korkeala, 1997; Nerbrink and Borch, 1993; Newton and Rigg, 1979). It is known that different kinds of microorganisms growing in the same place can affect each other. This can lead to accelerated food spoilage or deteriorated food quality, especially meat products (Russo et al., 2006).

Heating process of meat products coagulates meat proteins, thereby making products more palatable for consumption through enhancing texture, flavor, and color (Lee et al., 2017). Heating process generally refers to the average central temperature of the product during cooking. It has been reported that the final central temperature varies depending on the composition of products. It can impact on the texture, binding characteristics, and meat emulsion of each product (Choi et al., 2014; Foegeding and Ramsey, 1987; Puolanne and Kukkonen, 1983; Singh et al., 1985).

Currently, food hygiene law in Korea stipulates that meat product should be heated with central temperature of the product at 63℃ or above for 30 min or the equivalent. However, there is a difficult with meat processing technology to apply this standard under hygienic conditions. To control *Escherichia coli* or other bacteria that cause food poisoning, heating process is conducted through smoking, and a second heating is then conducted after packing in some manufacturing plants which may reduce the flavor and texture of the product (Barbosa-Cánovas et al., 2014). According to a research by Choi et al. (2016), it is possible to inactivate or destruct pathogenic microorganisms in meat products by heating only once during the manufacture process.

Thus, the objective of this study was to provide preliminary data for the food industry by investigating the distribution of microorganisms in raw materials, and sausage, and evaluating physicochemical quality of sausage depending on heating temperature.

Materials and Methods

Materials

In this study, sausages containing *Cheongyang* red pepper were used. Pork hind leg (Korea) and back fat (Korea) were used as raw meats. As supplementary materials, purified water, eggs (liquid, Korea), grapefruit extract (imported), garlic powder (Korea), onion powder (Korea), franks seasoning (Korea), curing agents (Korea), starch (wheat, 99.58%, imported), hot seasoning (Korea), sugar (imported), and salt (Korea) were used.

Manufacturing process for sausage

Raw meats and *Cheongyang* red pepper were cut using a chopper (0.3 cm) respectively. They were then mixed for 20 minutes while maintaining the temperature at 7℃–10℃. The mixed sausage mass was stuffed in casings and heated until central temperature reached at 65℃, 72℃, 78℃, and 85℃. They were then cooled down until central temperature reached 5℃ or lower. They were then vacuum packed. Sausages used in this study were manufactured by M Co. These sausages were similar to commercial products.

Experiment Ⅰ - Microbe assessment of raw materials and sausage

Microbiological assessment of raw materials for sausage manufacturing and cooked sausage

Total microbes, *E. coli*, and coliform bacteria in raw meats and supplementary materials (*Cheongyang* red pepper, eggs, garlic powder, onion powder, franks seasoning, curing agents, starch, sugar, salt, grapefruit extract, and purified water) were assessed for sausage manufacturing. Among raw materials, bacteria were detected in *Cheongyang* red pepper, eggs, garlic powder, onion powder, franks seasoning, curing agents, and starch. Microbial identification for those materials was conducted using mass spectrometry (MS) and microbe of cooked sausage heated until central temperature reached at 80°C assessed same method.

Identification of microorganisms

Among different types of colonies, one colony was selected. A pure single colony was then isolated using streak plate method through repetitive isolation and cultivation. Right after smearing the isolated colony onto a slide (VITEK MS-DS, Austria), 1 μL of matrix reagent (MS-CHCA) was dropped onto the slide to fix the colony. Microorganisms were identified using matrix-assisted laser desorption ionization time-of-flight MS (MALDI-TOF, Biomerieux, France) and *in vitro* diagnostic (IVD) program.

Experiment Ⅱ- Quality characteristics of meat product by central temperature

Quality assessment of sausage by heating temperature

Sausages that were manufactured with same procedure were heated until central temperature reached at 65°C, 72°C, 78°C, and 85°C, respectively. Microbiological and physicochemical properties were evaluated and compared for those sausages.

Total microbes, *E. coli*, and coliform bacteria

For total microbes, sterilized peptone water 225 mL was added to 25 g of sample. The mixture was homogenized and serially diluted by a factor of 1:10. Then 1 mL of each dilution was dispensed into petri dish under sterilizing operation, 20 mL plate count agar medium (Difco, Laboratories, USA) were added to each dishes, and cultured at 37°C for 48 h. The number of colonies was then counted. For *E. coli* and coliform bacteria, 1 mL of each dilution made for total microbes counting was dispensed into 3M Petrifilm Plate (3M, St. Paul, MN, USA) and cultured at 37°C for 24 h. The number of blue colonies with gas was counted for *E. coli* whereas that of red and blue colonies with gas was counted for coliform bacteria.

pH

Sample (5 g) was added to 45 mL of distilled water and the mixture was homogenized using a homogenizer. For each sample, pH was measured three times using a pH meter (Model 13-620-530A, Accumet, Malaysia).

Yield

Yield was calculated by dividing the weight of sample before heating by the weight of sample after heating. It was then converted to percentage.

Salinity and sugar content

Sample (5 g) was added to 45 mL of distilled water and the mixture was homogenized using a homogenizer. Salinity and sugar content were measured three times for each sample using a salinity meter (Atago, PAL-03S, Tokyo, Japan) and a sugar content meter (Atago, PAL-1, Tokyo, Japan), respectively.

Texture profile analysis

Texture profile analysis (TPA) of 1.5×1.5×1.0 cm samples was measured using a texture analyzer (TA-XT2*i*, Stable Micro Systems, England). Hardness (g), springiness, cohesiveness, gumminess (g), and chewiness (g) were assessed. A cylinder with diameter of 20 mm was used as a probe for this measurement under the following conditions: maximum load, 2 kg; head speed, 2.0 mm/sec; distance, 8.0 mm; and force, 5 g (Shim et al., 2018).

Statistical analysis

Statistical analyses were performed using general linear model (GLM) procedure of statistics analytical system (SAS) program, version 9.12 (SAS Inst., Inc., Cary, NC, USA). Comparison between groups of mean was conducted by Duncan's multiple range test ($p<0.05$). All process performed at least three times.

Results and Discussion

Experiment Ⅰ-Microbiological assessment of raw materials and sausage

Distribution of microorganisms in raw materials of sausage can affect their quality. Such information can be used to set the condition for manufacturing sausages. Thus, total microbes, *E. coli*, and coliform bacteria in raw materials used for sausage manufacturing were primarily assessed in this study. In addition, bacteria detected in total microbes were isolated and identified (Table 1). Total microbes of raw meats were 3.45 Log CFU/g. In raw materials, *Cheongyang* red pepper showed the highest total microbes (7.16 Log CFU/g), followed by garlic powder (6.14 Log CFU/g) and onion powder (4.17 Log CFU/g). No bacteria were detected in purified water, grapefruit extract, or curing agents. Coliform bacteria were detected in eggs and onion powder while *E. coli* was only detected in eggs. Except these materials, no coliform bacteria or *E. coli* were detected in other materials.

According to results of bacterial identification among bacteria isolated from raw materials, only *Pseudomonas fluorescens* was identified in raw meats. It has been reported that *P. fluorescens* is the main spoilage microorganism when meats are stored aerobically in a refrigerator (Dainty and Mackey, 1992). However, it was thought that *P. fluorescens* does not affect the quality of sausage since it will be destructed by heat treatment considering that the thermal death point of this bactera is at 42°C. Detected microorganisms in raw materials varied depending on materials, with *Staphylococcus xylosus*, *Citrobacter koseri*, and *P. aeruginosa* being the main organisms detected. *Bacillus* species bacteria were detected in *Cheongyang* red pepper, eggs, garlic powder, and onion powder. *Bacillus* species bacteria can be easily found on soil as well as foods including grains and farm products. Spores may be found in processed food such as cocoa, spice, dried food, and herb (Priest, 1989). Most spices used for food have various function, including antimicrobial activities. However, it has been reported that controlling hygiene and quality of foods is important since foods are likely to become contaminated during various manufacturing processes (Kwon et al., 2006). Banerjee and Sarkar (2003) and McKee (1995) have reported that *Bacillus cereus*, *B. subtilis*, *Clostridium perfringens*, and *Listeria monocytogenes* were present in spices used for manufacturing meat products. In general, initial distribution and type of microorganisms in sausage mixture are determined by raw meats. When other raw materials and curing agents are added, water activity is decreased and oxygen is consumed, resulting in altered growth of microorganisms (Lüecke, 1985). Therefore, controlling hygiene and sanitation might be essential since levels of contamination in raw materials can crucially affect the quality of sausage.

Meat products are good sources of protein. However, they can easily go bad and deteriorate than other foods. Initial bacterial count is important in that it affects the quality of products as well as their storage period (Kim et al., 2012; Newton

Table 1. Microbiological assessment of raw materials

Variables	Total microbes (Log CFU/g)	*Escherichia coli* (Log CFU/g)	Coliform bacteria (Log CFU/g)
Raw meat	3.45±0.03	ND	ND
Purified water	ND	ND	ND
Grapefruit extract	ND	ND	ND
Mixing curing	ND	ND	ND
Hot seasoning	ND	ND	ND
Sugar	ND	ND	ND
Salt	ND	ND	ND
Cheongyang red pepper	7.16±0.04	ND	ND
Egg	3.95±0.05	2.31±0.01	2.38±0.00
Garlic powder	6.14±0.09	ND	ND
Onion powder	4.17±0.10	ND	1.80±0.14
Frank seasoning	3.36±0.10	ND	ND
Starch	1.59±0.11	ND	ND

Variables	Microbial species	Confidence value (%)
Raw meat	*Psedomonas fluorescens*	99.9
Cheongyang red pepper	*Bacillus pumilus*	99.9
	Bacillus subtilis	99.9
	Pseudomonas aeruginosa	99.9
	Serratia marcescens	99.9
	Staphylococcus xylosus	99.9
	Citrobacter koseri	99.9
	Enterobacter asburiae	99.9
Egg	*Bacillus licheniformis*	99.9
	Serratia liquefaciens	99.9
	Staphylococcus xylosus	99.9
	Staphylococcus sciuri	99.9
	Citrobacter koseri	99.9
Garlic powder	*Bacillus pumilus*	99.9
	Salmonella gallinarum	99.9
Onion powder	*Bacillus badius*	99.9
Frank seasoning	*Pseudomonas aeruginosa*	99.9
Starch	*Pseudomonas aeruginosa*	99.9
	Klebsiella oxytoca	99.9

ND, not detected.

and Rigg, 1979). Table 2 shows total microbes and type of isolated bacteria in sausage. Total microbes were in the range of 2.21–3.11 Log CFU/g. *E. coli* and coliform bacteria were detected in some products. Compared with the result in raw

Table 2. Microbiological assessment of sausage

Variables	Total microbes (Log CFU/g)	*Escherichia coli* (Log CFU/g)	Coliform bacteria (Log CFU/g)
Cheongyang red pepper sausage (Unit: Log CFU/g)	2.21–3.11	ND–1.30	ND–2.00
Variables	Microbial species	Confidence value (%)	
Cheongyang red pepper sausage	*Bacillus pumilus*	99.9	
	Bacillus subtilis	99.9	
	Staphylococcus saprophyticus	99.9	
	Enterococcus faecalis	99.9	

ND, not detected.

materials, this might be caused by cross-contamination between raw materials or during the manufacturing process. Regarding the microbiological quality of the sausage, therefore, it is considered that hygienic production of the raw materials, the preprocessing method, and appropriate control according to the heat treatment are required.

In this study, MS was used to identify isolated bacteria in sausage. *B. pumilus*, *B. subtilis*, *S. saprophyticus*, and *E. faecalis* were detected in these products (Table 2). *S. marcescens*, *B. cepacia*, *S. xylosus*, *C. koseri*, *P. aeruginosa*, and *E. asburiae* were detected in raw materials (Table 1), while they were not detected in sausage, suggesting that those bacteria might have been destructed by heating process. However, *B. pumilus* and *B. subtils* detected in raw materials were not completely destructed by heating process, indicating that proper control of raw materials is needed during the preprocessing and manufacturing process. In addition, *S. saprophyticus* and *E. faecalis* detected in raw materials were detected in sausage. They might contaminate sausage through cross-contamination.

Weissella viridescens and *Leuconoctoc camosum* are known to be related to emulsified meat products. It has been reported that *W. viridescens* can survive heating process, thereby being related to contamination of raw meats. Samelis et al. (1998) have reported that products can be exposed to *Leu. camosum* during processing treatment or if air can penetrate into packaged products. *Bacillus* species can be found in soils as well as various food ingredients It can survive in poor environment by forming spores with thermal death point of above 100℃. This species is also widely spread in meat products. It has been reported that *B. subtilis*, *B. pumilus*, and *B. amyloliquefaciens* are present in sausage (Borch et al., 1988). Among *Bacillus* species, *B. mycoides*, *B. thuringiensis*, *B. circulans*, *B. lentus*, *B. polymyxa*, *B. carotarum*, and *B. cereus* are known to show toxicity which might cause, but not necessarily, food poisoning. However, controlling *Bacillus* species by heating might cause negative effect on the quality of sausage.

Therefore, since bacilli detected in the result of this study do not contain toxicity to cause food poisoning, it is thought that hygiene control must be provided from the production of raw materials to the distribution, considering the quality of the product in storage.

ExperimentⅡ- Quality assessment of sausage according to heating temperature

When manufacturing heated meat products, heating temperature affects the texture and sensory characteristics depending on the level of protein thermal denaturation (Moon, 2013). Heating temperature is also an important factor in the manufacturing process since it can impact heating time, yield, and energy efficacy for manufacturing the product. Heating times taken until central temperatures reached 65℃, 72℃, 78℃, and 85℃ were 22, 29, 30, and 35 min, respectively. This is because it takes longer time to reach higher target temperature in the center of the product. Yields of sample heated until central temperatures reached 65℃, 72℃, 78℃, and 85℃ were 96.67%, 95.99%, 96.04%, and 95.42%, respectively (Fig. 1), indicating that yields tended to increase when heating temperature was decreased. Compared to manufacturing sausage at 85℃, yield of sausage manufactured at 78℃ was increased by 0.62%. However, the yield of sausage manufactured at 65℃ was increased by 1.25% which was the highest among the four conditions ($p<0.05$). When manufacturing meat products, as the temperature in the range of 45℃–75℃ is increased, loss of weight is increased due to decrease in emulsified volume of meat protein and decreased binding capacity of water and fat (Acton, 1972; Jones and Mandigo, 1982). Jones and Mandigo (1982) have suggested that this may be caused by structural changes along with fat expansion and protein denaturation. Therefore, lowering the temperature during manufacturing process not only can increase yield, but also can reduce considerable energy consumption considering the scale of production.

Table 3 shows pH, salinity, and sugar content of sausage in response to heating temperature. The pH tended to increase when central temperature was increased from 65℃ to 78℃. However, the pH of sausage heated at 85℃ was lower than that at 78℃. pH of sausage heated at 78℃ was 6.49, which was significantly different, while there was no statistically differences among others. Lakkonen et al. (1970) have reported that pH is generally increased when heat treatment is performed for meat. Chin et al. (2006) have reported that the pH of commercial sausages is in the range of 5.5–6.4. Meanwhile, for heated meat, Moon (2013) has reported that higher heating centeral temperature leads to higher pH of the heated meat, regardless of parts of meat. It has also been reported that the pH of meat emulsion is more likely to be influenced by mixing of raw materials rather than composition of emulsion such as fat, protein, or moisture content (Gregg et al., 1993). Based on these results, it

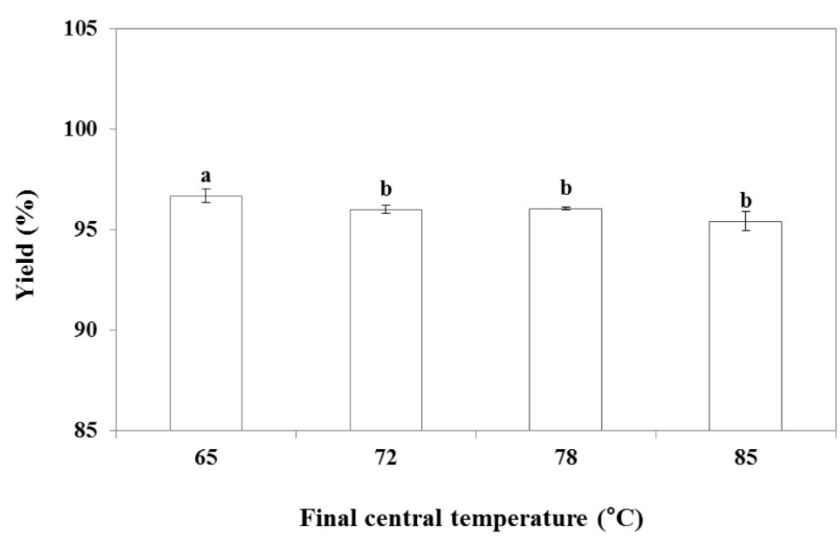

Fig. 1. Yield of sausage according to final central temperature. ^{a,b} Means with different letters are significantly different (p<0.05).

Table 3. pH, salinity and sugar content of sausage according to heating temperature

Variables	Final central temperature (℃)			
	65	72	78	85
pH	6.34±0.05^{b}	6.41±0.04^{b}	6.49±0.03^{a}	6.25±0.15^{b}
Salinity(%)	8.25±0.50	8.50±0.58	8.50±0.58	8.75±0.50
Sugar(°brix)	7.75±0.50	7.75±0.50	8.00±0.82	8.25±0.50

^{a,b} Means within a row with different letters are significantly different (p<0.05).

can be inferred that pH can be affected by heating temperature during the manufacturing process. No significant difference was observed in salinity (in the range of 8.0%–9.0%) and sugar content (in the range of 7.0–8.0 °brix) according to heating temperature.

Table 4 shows texture of sausage according to heating temperature. Hardness, gumminess, and chewiness tended to increase as heating temperature was increased. However, there were no significant differences. Carballo et al. (1996) have reported that the final central temperature can lead to structural alteration by thermal denaturation, while Puolanne and Kukkonen (1983) have reported that the hardness of emulsion is likely to increase as central temperature is increased since water binding capacity is decreased. This may be related to yield (Fig. 1) which tended to decrease as heating temperature was increased, thereby affecting the texture. Thus, with decreasing heating temperature, the texture of sausage might be softer and the yield of the product is higher.

Microbiological assessment of sausage according to central temperature

Sausages in the manufacturing process were heated until central temperature reached 65℃, 72℃, 78℃, and 85℃, respectively. Results of microbiological assessment are summarized in Table 5. For total microbe, sausages heated at 85℃ showed the lowest value at 1.10 Log CFU/g, while total microbes of sausages heated at 65℃, 72℃, and 78℃ were in the range of 2.01–2.22 Log CFU/g, showing a tendency of total microbe decrease with increasing heating temperature. However, there were no significant differences among these values. Neither *E. coli* nor coliform bacteria was detected regardless of heating temperature.

Conclusion

This study analyzed distribution of microorganisms in raw materials, and sausage and assessed the effect of heating

Table 4. Texture of sausage according to heating temperature

Variables	Final central temperature (℃)			
	65	72	78	85
Hardness (kg)	1.65±0.17	1.77±0.15	1.74±0.15	2.01±0.19
Springiness	0.94±0.02	0.94±0.02	0.96±0.03	0.95±0.02
Cohesiveness	0.82±0.01	0.82±0.01	0.84±0.01	0.82±0.01
Gumminess (kg)	1.35±0.14	1.45±0.13	1.45±0.12	1.65±0.17
Chewiness (kg)	1.27±0.15	1.36±0.13	1.40±0.13	1.56±0.17

Table 5. Microbiological assessment of sausage according to heating temperature

Variables	Final central temperature (℃)			
	65	72	78	85
Total microbes (Unit: Log CFU/g)	2.22±0.04[a]	2.17±0.12[a]	2.01±0.06[a]	1.10±0.17[b]
Escherichia coli (Unit: Log CFU/g)	ND[1)]	ND	ND	ND
Coliform bacteria (Unit: Log CFU/g)	ND	ND	ND	ND

[a,b] Means within a row with different letters are significantly different ($p<0.05$).
ND, not detected.

temperature during sausage manufacturing on microbiological and physicochemical properties of sausage. Many kinds of microbe detected in raw materials and cooked sausage. Although the difference was not founded statistically, total microbe of sausage according to heating temperature was decreased as central temperature was increased numerically. With increasing central temperature, yields tended to decrease. Therefore, lowering heating temperature for sausage manufacturing might be economical and it can enhance energy efficacy without having negative effect on microbiological perspective.

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