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Effect of Different *Pediococcus pentosaceus* and *Lactobacillus plantarum* Strains on Quality Characteristics of Dry Fermented Sausage after Completion of Ripening Period

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Abstract The aim of this study was to evaluate the effect of three different strains of lactic acid bacteria (LAB) starter cultures: *Pediococcus pentosaceus* (KC-13100) (PP), *Lactobacillus plantarum* (KCTC-21004) (LP1), and *L. plantarum* (KCTC-13093) (LP2) on the physicochemical and microbiological characteristics, and sensory quality of dry fermented sausages after 21 days of drying and ripening period. Treatments added with PP and LP2 strains showed a significant higher ($p<0.05$) LAB and total plate counts, and water activity (a_w) of all three treatments was below 0.85 after the completion of the ripening process. A significant variation ($p<0.05$) in pH values of treatments was exhibited due to the difference in acidification capacity of the LAB strains: $LP2<PP<LP1$. Treatments had significant difference ($p<0.05$) in the thiobarbituric acid reactive substances (TBARS) content, in the following order: $LP1>PP>LP2$. Substantial variations ($p<0.05$) in shear force values were detected amongst three batches ($LP2>LP1>PP$). In sensory attributes, PP treated samples had significantly higher ($p<0.05$) color and overall acceptability scores. The current findings proved how important the optimal assortment of starter culture. Inoculation with PP produced importantly beneficial effects on sensory quality improvement of dry fermented sausage.

Keywords dry fermented sausage, starter cultures, lactic acid bacteria (LAB), sensory evaluation, water activity

Introduction

Fermentation is one of the popular techniques amongst the production methods for healthy foods (Pilevar and Hosseini, 2017). Under specific temperature and relative humidity conditions, fermented sausages are produced through the combination of microbiological, biochemical, and physical activities (Casaburi et al., 2007). Due to these important processes phenomena, the changes in sensory attributes of the product occurred during ripening. Meat preservation through fermentation by indigenous species has been used for centuries (Swetwathana and Visessanguan, 2015). *Lactobacillus*

sakei, *Lactobacillus curvatus* and *Lactobacillus plantarum* (LP1) species were the most commonly identified in traditional fermented sausages (Hugas et al., 1993; Kittisakulnam et al., 2017; Talon et al., 2007); other members, such as *Weissella*, *Leuconostoc*, *Lactococcus*, and *Pediococcus* are also found as minority species (Aquilanti et al., 2016). However, the idea of starter culture application to produce dry fermented sausages was first introduced in the 1940s, with Patent US2225783A (Jensen and Paddock, 1940). Higher populations of appropriate microorganisms, regarded as starter cultures, are utilized in the production of dry-fermented sausages (Pilevar and Hosseini, 2017).

Starter cultures or starters are single or combined formulas of desired strains of microorganisms with a certain enzymatic function that, when applied to a substrate at a given concentration, convert it into a food product with particular qualities (Hammes and Hertel, 1998). This concept for meat products can be characterized as productive microorganisms capable of multiplying within meat products, improving their preservation, governing their hygienic safety, and enhancing their market acceptance, conserving or refining their nutritional excellence (Hammes and Hertel, 1998). The utilization of starter cultures in the fermentation process of meat products helps to ensure food safety and standardize the characteristics of the final product (Baka et al., 2011; Bonomo et al., 2011).

In response to the changes in transportation and eating paradigm these days, the application of starter cultures in dry fermented sausages is becoming especially crucial in enhancing safety and shelf life by attaining the required pH and water activity (a_w) and hindering the proliferation of pathogenic and spoilage microorganisms (Ciuciu Simion et al., 2014; Essid and Hassouna, 2013). The selection of starter cultures and environmental factors across fermentation and ripening are the most crucial factors influencing the characteristics and consistency of fermented meat products (Tabanelli et al., 2012; Toldrá, 2006). The application of starter cultures, along with strict temperatures and relative humidity (RH) factors, are among the key drivers of the dynamic phenomena that occur during ripening, the primary tool employed by the fermented sausage industries to enhance the quality and safety of their products (Bassi et al., 2015).

Starter cultures utilized in meat fermentation presently encompass lactic acid bacteria (LAB) and coagulase-negative cocci (CNC). Several species of CNC, such as *Staphylococcus* spp. and *Kocuria* spp., play role in proteolytic, lipolytic, and nitrate reductase activities which promote products' quality of redness and flavor characteristics (Bedia et al., 2011; Capita et al., 2006; Fernández-López et al., 2008; Leroy et al., 2006). Regarding LAB starter cultures, species primarily utilized are *Lactobacillus sakei*, *Lactobacillus plantarum*, *Lactobacillus curvatus*, *Lactobacillus pentosus*, *Lactobacillus casei*, *Pediococcus acidilactici*, and *Pediococcus pentosaceus* (PP; Hugas and Monfort, 1997). According to Montanari et al. (2016), the selection of starter cultures has a significant role in fermentation and the rate of acidification.

Under the conditions of fermentation and maturation of the sausages, the growth of the LAB strains is decisive in order to be regarded as a possible starter. An important feature is the ability of starter strains to rapidly acidify, as it enhances taste, safety, aroma and bacteriostatic or bactericidal properties (Leroy et al., 2006, Zagorec and Champomier-Vergès, 2017). Two recognized LAB strains with functional acidification properties during meat fermentation are *Lactobacillus plantarum* and PP (Cocconcelli, 2007). The 16S rDNA sequence analysis study between the two strains showed that there was more than 99% sequence similarity between the two strains (Bacha et al., 2010). *L. plantarum* (LP2) has had considerable beneficial effects on quality improvement, such as increased acidifying activity and improved food quality, especially the taste and odor of the product when compared to the commercial starter culture (Van Ba et al., 2018). Similarly, PP had the highest effect on the sensory quality of the products (Ho et al., 2009). Klingberg et al. (2005) identified *L. plantarum* and *L. pentosaceus* strains as promising candidates for probiotic meat starter cultures. The report of Bacha et al. (2010) indicates that starter cultures of *P. pentosaceus* and *L. plantarum* were initially formulated for products with shorter curing times at higher fermentation

temperatures. To date, comparative studies between *P. pentosaceus* and *L. plantarum* strains on the quality characteristics of dry fermented sausages have not been explored. We believe that evaluating the technological properties of individual strains helps to select and allow high-quality products to be manufactured on an industrial scale by using them as a single strain or as multiple strains. Therefore, the objective of this research was to investigate the effect of three different starters of LAB starter cultures: *P. pentosaceus* (KCTC-13100), *L. plantarum* (KCTC-21004), and *L. plantarum* (KCTC-13093) on the physicochemical, microbiological, and sensory quality of dry fermented sausages.

Material and Methods

Starter culture preparation

The three different starter cultures from LAB strains employed in manufacturing of three different types of dry fermented sausages in the present study were obtained from the Microbial Resource Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea as lyophilized stocks. The LAB strains were: *P. pentosaceus* (KCTC-13100) (PP), *L. plantarum* (KCTC-21004) (LP1), and *L. plantarum* (KCTC-13093) (LP2). MRS broth (Difco, Becton, NJ, USA) was used for the enrichment of the starter cultures and incubated at 37°C for 24 h. The formulated suspension blended into the sausage batter was at one mL/kg and each strain was maintained to have approx. 7 Log CFU/g. The viable cell count in the starter cultures suspensions was performed using a hemocytometer (Marienfeld-Superior, Paul Marienfeld GmbH & Co.KG, Germany) supported with computer magnification system.

Dry fermented sausages manufacture and sampling

Pork sausages with low-temperature fermentation were produced in the pilot meat processing center, Animal Resources Department, Daegu University. Fresh loin pork meat used for the study was purchased from the local market of Geyongsan, Korea. The lean meat was stored in a refrigerator until use after removing the excess fat and connective tissues. Chilled pork samples and pork fat were cut into small cubes and minced twice using a meat mincer (SF-2002, SamwooDew, Daegu, Korea). The basic sausage formulation included lean pork meat (80%), pork fat (20%), ice (12%), NPS (a mixed salt of NaCl and nitrite, 97:3) (0.34%), NaCl (1.70%), sodium ascorbate (0.20%), sugar (0.50%), glucose (0.50%) and spices (0.40%). After the ingredients were thoroughly mixed, the batter was divided into three batches (4 kg each) and randomly assigned into three different treatments of starter cultures: PP, LP1 and LP2. The ultimate starter cultures (LAB) concentration attained a value of $\sim 10^7$ CFU/g when applied to the meat batters. The batters and respective starter cultures were completely homogenized using rotary food mixer (Spar Food Machinery MFG, Taiwan) and stuffed into collagen casings (IKJIN, Seoul, Korea), 24 mm diameter and 150 mm length, with vacuum filling machine (RVF 327, Düker-REX Fleischereimaschinen GmbH, Laufach, Germany). Fermentation and ripening of sausages were done in digital chamber unit (SMK-2000SL, Metatek, Seoul, Korea) equipped with temperature and RH control system. In the fermentation period of the first seven days, the temperature was maintained at 23°C and RH was alternated to 90%–95%. In the ripening period, the next 21 days following the fermentation period, the temperature was maintained at 15°C and RH was ranged from 70%–75%. After the completion of the ripening period, dry fermented sausages were withdrawn from each batch, and physicochemical and microbiological and sensory qualities were analyzed. All analyses were carried out in triplicate for each batch.

Microbial quality analysis

Microbiological quality characteristics were conducted by enumeration of total plate count (TPC) and LAB. About 25 g

portion of a sample from each dry fermented sausage was taken aseptically with a sterile spoon, mixed with 225 mL of 0.1% peptone water, and homogenized in a Stomacher Lab Blender (model 400 Circulator, Seward Laboratory, New York, NY, USA) for 30 seconds. Serial 10-fold dilutions (10^{-1} to 10^{-7}) were prepared by diluting one mL of the sample in nine mL of 0.1% sterile peptone water. Enumerations of the grown colony of microorganisms were conducted after incubating samples with their respective selective medium: Plate Count Agar (PCA; Difco) was used for total microbial counts and Lactobacillus MRS agar (Difco) for LAB. Plates from different and appropriate dilutions were incubated in triplicate at 37°C for 48 h (Drosinos et al., 2005). The average numbers of colonies per countable plate were counted and the total numbers of colonies per gram (CFU/g) were determined, and then data were presented in Log CFU/g.

Determination of pH

The pH values of dry fermented sausages were analyzed using a digital pH meter (Mettler Toledo, Columbus, OH, USA). Three grams of sample was homogenized with 30 mL of distilled water for 1 minute using a homogenizer (Model Polytron® PT 2500 E Stand Dispersion Device, Kinematica AG, Switzerland). The electrode was dipped into the suspension and the pH value of the sample was recorded.

Determination of water activity (a_w)

a_w of sausages was analyzed using a_w measuring device (LabMaster- a_w , Novasina AG, Switzerland) at 25°C after the samples were prepared by slicing the core of samples about 4 mm cubes.

Instrumental color analysis

Analysis of sausages color was performed after cutting the samples into two cm slices thickness and reading was performed from the inner surface of the sausages. Five spectral data were measured for each sample using a portable chromameter (CR-400, Konica Minolta, NJ, USA) after calibrating with the manufacturer supplied white calibration plate ($Y=92.80$, $x=0.3136$, and $y=0.3194$). For color analysis, an average score for L^* , a^* , and b^* was taken from the mean of five random readings and expressed as L^* (lightness), a^* (redness), b^* (yellowness) using the CIE color system.

Volatile basic nitrogen (VBN) analysis

Measurements of the VBN content of samples were determined using the Conway micro diffusion method (Conway, 1950). Two Conway's tools per each sample were used after cleaning with a neutral detergent. A sealing agent (vaseline) was applied to the edge of the outer ring of each unit. Three grams of the sample was homogenized with 30 mL of distilled water at $1,000\times g$ for 1 min using a homogenizer. The homogenate was filtered using Whatman no. 1 filter paper (GE Healthcare Life Sci., Pittsburg, PA, USA). The filtrate (1 mL) was pipetted to the outer chamber of a Conway micro diffusion unit, and 1 mL of 0.01 N boric acid (H_3BO_3) and 100 μL of Conway indicator (0.066% bromocresol green:0.066% methyl red, 1:1) were pipetted to the inner chamber. Then, 1 mL of 50% potassium carbonate (K_2CO_3) was added to the outer chamber of the Conway unit and sealed immediately. Incubation of the materials was performed for 2 h at 37°C. After the addition of 0.02 N sulfuric acid (H_2SO_4) to the inner chamber of the Conway unit, the VBN contents were measured. Total volatile basic nitrogen (VBN) values were expressed in mg%.

Thiobarbituric acid reactive (TBARS) analysis

Analysis of lipid oxidation was performed by analyzing the TBARS (Pikul et al., 1989). Sausage sample (5 g) was homogenized with a 50 μ L of BHA (7.2% in ethanol) and 15 mL of distilled water and then centrifuged at 1,500 \times g for 15 min using a centrifuge (Hanil Science Industrial, Incheon, Korea). The supernatant (2 mL) was mixed with 4 mL thiobarbituric acid solution (20 mM TBA in 15% Trichloroacetic acid, TCA) followed by heating in a water bath at 90°C for 30 min and then cooling to room temperature. Therefore, TBARS were extracted from cooled samples. The absorbance of each sample was measured at 532 nm using a spectrophotometer (Multiskan Go, Thermo Fisher Scientific, MA, USA). TBARS, mg malonaldehyde per kg, of the sausage was calculated by multiplying the optical density of the reading with a K factor of 5.2.

Texture profile analysis (TPA)

TPA of sausage samples was examined by a TPA measurements device (TA 1, Lloyd Instruments, Largo, FL, USA). Briefly, four lots of cubic shape (1cm long, 1cm thick and 1cm wide) samples from each dry fermented sausage were subjected to the analysis. Two compression cycle tests were applied by compressing 80% of the original portion. During TPA analysis, the treatments had $a_w < 0.85$. Hence, 80% compression was employed to differentiate the products in their characteristics of TPA attributes. Between the two consecutive compression cycles, 20 s was elapsed. By applying a 1 N load cell at a crosshead speed of 2 mm/s, deformation curves for force-time were developed. The following parameters were determined (Bourne, 1978): hardness (kgf), the maximum force needed to deform the sample; springiness (m), the capacity of the sample to recover its original form after the applied force was removed; cohesiveness, degree of the sample deformation before rupture; and chewiness (kgf), the amount of work needed to masticate the sample before swallowing. For shear force determination, five consecutive slices (3 cm thick) of sausages were selected at random from each treatment and critiqued perpendicular to the cross-section using a 10-blade Lloyd shear probe attachment on a texture analyzer with a 200 mm/min cross speed. The maximum shear force estimate was recorded and calculated as kg force/g.

Sensorial analysis

Sensory analysis was performed using descriptive sensory analysis (scoring method) for color, aroma, sourness, and overall acceptability attributes of dry fermented sausages. Seven trained panelists were involved in the sensory evaluation who are in the Department of Animal Resources, Daegu University. The panelists were trained with sensory quality attributes of dry fermented sausages for 2 weeks prior to the actual evaluation. They were trained using a 5-point hedonic scale. The intensity or degree assigned to express the attributes was from 1 'the least quality/intensity' to 5 'the highest quality/intensity' that corresponds to 'very pale to very dark', 'very weak fermented aroma to very strong fermented aroma' and 'light sour to strong sour' for color, aroma and sourness sensory characteristics of samples, respectively. Seven different type of sausages were used during the training sessions (one from Korean company and other 6 imported products from different companies of three countries). The panel were given 3 slices of samples (3 mm thickness) on white plastic dishes during the judgment. All samples were separately coded with three digits and were randomly served to avoid carry-over effects. Cold water was also provided for rinsing their mouths before each sample was tested. The sensory evaluation procedure was approved by the life management committee of Daegu University, IRB number (1040621-201905-HR-004-02).

Statistical analysis

Statistical data analysis for the three replicates was carried out Variance (ANOVA) using SAS software version 9.4 (SAS

Institute, Cary, NC, USA). A significance level of $p < 0.05$ was used for all evaluations. Differences among the means were compared according to Duncan's Multiple Range Test.

Results and Discussion

For the production of dry fermented sausages, the selection and use of effective starter culture are very crucial among quality determinant factors in order to achieve customer interest, safety, and storage stability of the products (Leroy, 2013; Toldrá, 2006). Table 1 presents the microbiological quality characteristics of dry fermented sausage from different strains of LAB starter cultures after the ripening period. Statistical analysis revealed a substantial difference ($p < 0.05$) in LAB and TPCs among different strains of LAB starter cultures. Similarly, Essid and Hassouna (2013) illustrated that the application of the selective starter substantially influences the total viable, staphylococci, LAB, and Enterobacteriaceae counts. Moreover, treatments showed a similar trend in total plat count and LAB count, and the total viable number in all batches was very close to LAB counts. Both LAB and total plat counts were significantly higher ($p < 0.05$) in PP and LP2 treatments as compared to LP1. For the development of the physicochemical characteristics of fermented sausages, such as texture, taste, hygiene, and safety-related properties, the improved growth performance of LAB is very critical (Essid and Hassouna, 2013). The comminuted sausage meat system contains several sugars, which originate from the meat content as well as from the nonmeat ingredients (Bacha et al., 2010). The prospective difference in the utilization of available fermentable carbon sources mainly added sugar and glucose in the sausage formulation by different strains of LAB during the early stage of fermentation might be the main reason for the substantial difference in LAB counts of the current finding. Regarding the TPC, it speculates the growth characteristics of all groups of microorganisms using general-purpose media, PCA, including LAB. During the fermentation of sausages, the lowered pH, and lactic acid and other metabolites are produced to inhibit the growth of other groups of microorganisms including molds (Bacha et al., 2010). As result, the growth of LAB in PCA as dominant bacteria is expected. In the present study, a significant positive correlation was exhibited between TPC and LAB counts ($r = 0.992$, $p < 0.05$), (data not presented).

The effect of different strains of LAB starter cultures on the pH, a_w , VBN, and TBARS values of dry fermented sausage after the ripening period is presented in Table 2. Valyasevi et al. (2001) described that LAB are the major producers of lactic acid responsible for the decrease in pH and the increase in acidity during the fermentation. In the current study, a significant variation ($p < 0.05$) in pH values of treatments was exhibited due to the difference in acidification capacity of different strains of LAB starter cultures. In pH values of treatments: PP < LP2 < LP1 having 4.91, 5.05, and 5.23. As compared LP1 treatment,

Table 1. Effect of different strains of LAB starter cultures on total plate count (TPC) and LAB counts of dry fermented sausage (Log CFU/g) after drying and riping period

Parameter	Treatments ¹⁾			SEM
	PP	LP1	LP2	
TPC	9.82 ^a	8.59 ^b	9.70 ^a	0.20
LAB	9.75 ^a	7.86 ^b	9.72 ^a	0.29

¹⁾ Treatments are different strain of LAB starter culture used in the present study: PP, *Pediococcus pentosaceus* (KCTC-13100); LP1, *Lactobacillus plantarum* (KCTC-21004); LP2, *Lactobacillus plantarum* (KCTC-13093). n=3.

^{a,b} Means with different superscript are significantly different ($p < 0.05$). LAB, lactic acid bacteria.

Table 2. Effect of different strains of LAB starter cultures on pH, water activity (a_w), VBN, and TBARS values of dry fermented sausages after drying and ripening period

Attributes	Treatments ¹⁾			SEM
	PP	LP1	LP2	
pH	4.91 ^c	5.23 ^a	5.05 ^b	0.01
a_w	0.83 ^a	0.78 ^b	0.74 ^c	0.00
VBN (mg %)	14.01 ^a	11.63 ^b	14.05 ^a	0.24
TBARS (mg MDA/kg)	0.83 ^b	0.90 ^a	0.74 ^c	0.01

¹⁾ Treatments are as described in the Table 1. PP, *Pediococcus pentosaceus* (KCTC-13100); LP1, *Lactobacillus plantarum* (KCTC-21004); LP2, *Lactobacillus plantarum* (KCTC-13093). n=3.

^{a,b} Means with different superscript are significantly different ($p < 0.05$).

LAB, lactic acid bacteria; VBN, volatile basic nitrogen; TBARS, thiobarbituric acid reactive substances.

the lower pH values exhibited in PP and LP2 inoculated lots may have resulted from better adaptation and fast multiplication of the LAB starter cultures (Table 1) to the meat environment and the meat processing conditions which may have mainly contributed to the acidification of the products. The samples with the lower pH values corresponding with treatments those having higher LAB counts at the end of the fermenting/ripening process. The rapid growth of LAB is important because it leads to the carbohydrate breakdown and buildup of organic acids, primarily lactic acids (Nie et al., 2014; Zhao et al., 2011). In the production of sausages, a lower pH value is important because it helps to prevent the growth of undesirable microorganisms and enhance the redder color of the products (Lorenzo et al., 2014a; Lorenzo et al., 2014b). The lower pH plays a crucial role in the development of the distinctive flavor, color, and aroma, and microbiological consistency of the fermented sausages (Hammes et al., 1990; Hugas and Monfort, 1997).

It is well known that a food product's shelf-life stability is usually dependent on the a_w ; a_w at the end of ripening will improve the excellence of fermented sausage and prolong its shelf life. Inoculation of different strain starter cultures resulted in a significant variation ($p < 0.05$) in the a_w value of ripened sausage samples (Table 2). On the base of a_w values, treatments are ordered as follows: LP2 < LP1 < PP with the corresponding values of 0.74, 0.78, 0.83, respectively. Our findings disagree with those previous studies reports which found no significant difference in a_w value of treatments with different starter cultures across the ripening period (Chen et al., 2020; Van Ba et al., 2018). At the end of the ripening process, the a_w of all three batches was below 0.85. This result also disagrees with Van Ba's (Van Ba et al., 2017a; 2017b) findings which reported higher a_w values (0.85–0.88) for the same product type that could be attributed to elevated moisture contents of the samples in their studies. The denaturation of sarcoplasmic proteins as result of the drop in pH during fermentation and degradation of protein caused by microorganisms involved in fermentation likely to decrease the water holding capacity; this occurrence in turn responsible in lowering the a_w as the moisture vanished during drying. Despite all treatments in current study exhibited pH values of ≤ 5.05 (Table 2), there was no significant correlation between a_w and pH value of treatments ($r = -0.547$, $p > 0.05$) (data not presented). Fermented sausages are categorized in final products as “semi-dry” and “dry” based on a_w . The sausages are categorized as “semi-dry” with a_w between 0.90 and 0.95 and are labeled “dry” with a_w below 0.90 (Lücke, 1998; Wang et al., 2015). The sausages fermented by three strain of LAB starter cultures in the present study belonged to the dry fermented sausage according to these classification criteria.

The content of VBN consists of NH_3 , H_2S , and $\text{CH}_3\text{CH}_2\text{SH}$, etc., which are produced by spoilage bacteria or endogenous enzymes from the decomposition/degradation of proteins (Huang et al., 2014). These low molecular non-protein nitrogen compounds could possibly be formed from protein degradation during fermentation (Ruiz-Capillas and Jiménez-Colmenero,

2005) that impart to the VBN content of the product. Consequently, VBN is commonly used as an important indicator showing the shelf life and microbial consistency of processed meat products (Van Ba et al., 2018). Our findings show that the quality of VBN varies significantly ($p < 0.05$) among treatments at the end of the ripening period, ranging from 11.63 mg% to 14.05 mg% for LP1 and LP2 treated samples, respectively (Table 2). VBN values ranging from 7 to 18 mg % was reported by Lin and Lin (2002) in Chinese style dry-cured sausage during the ripening period. In the current study, the VBN content was much lower compared to the levels documented by Rai et al. (2010) for the same product type (20–25 mg percent). The findings may be attributed to the inoculated bacteria's ability to neutralize the VBN content with their organic acids (e.g. lactic acid) or bacteriocin production (Yin et al., 2002). Ruiz-capillas and Jiménez-colmenero (2005) documented that low molecular non-protein nitrogen compounds could be possibly resulted from protein degradation during fermentation. The present study exhibited that VBN values to have a significant correlation with LAB counts ($r = 0.945$, $p < 0.05$) and a significant negative correlation with pH value ($r = -0.867$, $p > 0.05$) of treatments (data not presented).

Analysis of TBARS is used as an important indicator for the development of secondary lipid oxidation products, primarily malondialdehyde, which can lead to oxidized fat off-flavor. Lipid oxidation in meat products may change their nutritive values, colors, and flavors (Kim et al., 2015), and associated with health risks (Grun et al., 2006). At the end of the ripening process, treatments inoculation with different strains of LAB starter cultures had a significant difference ($p < 0.05$) in the TBARS content (Table 2), in the following order: LP1 > PP > LP2. This result is concordant with that of Bingol et al. (2014), who found a difference in TBARS values and contemplated the variation in the result as it could be from lactic activities of starter cultures that importantly decrease the pH value. The current finding disagrees with Yim et al. (2017) who demonstrated no significant difference in TBARS value between the fermented sausages treated with the commercial cultures mix and the commercial cultures mix + *Lactobacillus plantarum* at the end of the ripening process. TBARS values of fermented sausages in the range of 0.6–2.8 mg MDA / kg are acceptable (Marco et al., 2006). In this study, TBARS values of all treatments did not exceeded the stated range.

Customers pay a considerable attention to its color when determining meat products, which, like visual perception, is primarily caused by the existence of pigments but also depends on the tissue composition and meat structure. The color of meat products is, therefore, one of the mainly important quality parameters that govern the response and decision at the retail outlet of the product. Color depends on a variety of factors in the case of dry-fermented sausage, such as the composition of the sausage, the fat-lean ratio, the quantity and kind of spices and the additives and technical operations applied (Pérez-Alvarez and Fernandez-Lopez, 2011). In most instances, however, sausages become red (cured color) during the fermentation stage due to the development of nitrosomyoglobin resulting from the combination of nitric oxide (NO), produced by the bacterial conversion of nitrate to nitrite, and myoglobin (Cavalheiro et al., 2013). The color traits lightness, redness, and yellowness of fermented sausages at the end of the ripening process are presented in Table 3. Treatments added with PP and LP2 exhibited a higher value of L* (Lightness), whereas the lowest L* values of sausages were in batches inoculated with LP1 starter cultures ($p < 0.05$). Samples inoculated with LP2 had elevated b* (Yellowness) value than other strains treated samples. Redness (a*) is the most important trait for determining the degree of products oxidation, and the lower redness value in meat is contemplated as a sign of oxidation (Ergezer et al., 2018). In the present study, the addition of different strains of LAB starter cultures did not have a substantial difference ($p > 0.05$) in the redness (a*) values of dry fermented sausages at the end of the ripening process.

Regarding the TPA traits, inoculation of different strains of LAB starter cultures significantly influenced ($p < 0.05$) the hardness, cohesiveness, and chewiness values of dry fermented sausages after the ripening process (Table 4). The highest

Table 3. Effect of different strains of LAB starter cultures on color values of dry fermented sausages after drying and riping period

Parameter	Treatments ¹⁾			SEM
	PP	LP1	LP2	
L* (lightness)	52.89 ^a	48.59 ^b	52.71 ^a	2.74
a* (redness)	6.16	5.93	5.91	0.87
b* (yellowness)	9.58 ^b	9.56 ^b	11.78 ^a	0.92

¹⁾ Treatments are as described in the Table 1. PP, *Pedococcus pentosaceus* (KCTC-13100); LP1, *Lactobacillus plantarum* (KCTC-21004); LP2, *Lactobacillus plantarum* (KCTC-13093). n=3.

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

LAB, lactic acid bacteria.

Table 4. Effect of different strains of LAB starter cultures on textural properties analysis (TPA) and shear force values of dry fermented sausage after drying and riping period

Attributes	Treatments ¹⁾			SEM
	PP	LP1	LP2	
Hardness (kgf)	3.82 ^b	5.83 ^a	2.34 ^c	1.00
Springiness	0.94	0.89	0.88	0.07
Cohesiveness	0.30 ^b	0.29 ^b	0.48 ^a	0.06
Chewiness (kgf)	1.06 ^b	1.46 ^a	0.96 ^b	0.21
Adhesiveness	0.71	0.79	1.21	1.22
Shear force (kgf)	1.52 ^c	3.80 ^b	6.58 ^a	0.73

¹⁾ Treatments are as described in the Table 1. PP, *Pedococcus pentosaceus* (KCTC-13100); LP1, *Lactobacillus plantarum* (KCTC-21004); LP2, *Lactobacillus plantarum* (KCTC-13093). n=3.

^{a-c} Means with different superscript are significantly different (p<0.05).

LAB, lactic acid bacteria.

values of hardness and chewiness were exhibited in LP1 treated samples and the addition of LP2 gave the highest score for the cohesiveness trait. Samples from PP and LP1 strains treated sausages exhibited a similar (p>0.05) cohesiveness property. In the hardness value of sausages: LP1>PP>LP2, and treatments are ranked as follows based on the chewiness characteristic: LP1>PP, LP2. However, no differences were observed among treatments (p>0.05) in springiness and adhesiveness profiles. Significant differences (p<0.05) in shear force values of fermented sausages were observed after the ripening process, in the shear force values treatments: LP2>LP1>PP. This finding may be associated with differences in moisture content among treatments (Table 2) or maybe due to differences in biochemical processes that have affected the evaporation of water from the products during the drying process.

Measuring the sensory quality attributes is the most important approach for predicting oxidative stability, product shelf-life, and acceptability of consumers. The sensory attributes (color, aroma, sourness, and overall acceptability) of dry fermented sausage from different strains of LAB starter cultures were evaluated by the trained panel after the completion of the ripening process and shown in Table 5. In the ripening process, bacterial starter cultures have a considerable role in the acidification and development of fermented sausages with new and distinct quality attributes that contributes to the sensory acceptability and physical properties (Bassi et al., 2015). The fermentation of carbohydrates is primarily carried out by LAB that dominate the process of fermentation and produce lactic acid and other flavoring compounds (Ravyts et al., 2012). Dry fermented sausage flavor is affected by various processing components such as different formula of ingredients (especially

Table 5. Effect of different strains of LAB starter cultures on sensory properties of dry fermented sausages after drying and riping period

Attributes	Treatments ¹⁾			SEM
	PP	LP1	LP2	
Color	4.12 ^a	3.87 ^a	2.53 ^b	0.51
Aroma	4.23	3.50	3.42	0.66
Sourness	2.68	2.90	2.98	0.64
Overall acceptability	4.00 ^a	3.33 ^b	3.31 ^b	0.37

¹⁾ Treatments are as described in the Table 1. PP, *Pediococcus pentosaceus* (KCTC-13100); LP1, *Lactobacillus plantarum* (KCTC-21004); LP2, *Lactobacillus plantarum* (KCTC-13093).

^{a-c} Means with different superscript are significantly different ($p < 0.05$).

LAB, lactic acid bacteria.

spices), starter cultures, processing circumstances (like smoking), etc (Kaban and Kaya, 2009; Leroy et al., 2006). In the current study, all three treatments varying in the type starter cultures inoculated exhibited similar scores ($p > 0.05$) in sourness, desirable sour flavor of dried meat products, and aroma sensory attributes; the panelists did not perceive any distinct acid taste and aroma difference. However, treatments had difference in color and overall acceptability scores and the samples added with PP strain had substantially higher ($p < 0.05$) ratings both in color and overall acceptability attributes as compared to other strains treated sausages.

In conclusion, the addition of different strains of LAB starter cultures had a significant effect on LAB and total plat counts, a_w , VBN and TBARS value, and the sensory attributes (color and overall acceptability) of dry fermented sausages after the ripening process. The inoculation with *P. pentosaceus* (KCTC-13100 strain) produced importantly beneficial effects on quality improvement of dry fermented sausage in terms of LAB counts, instrumental color, and sensory evaluation; it is a potential candidate for use as starter cultures in the production of quality dry fermented sausage.

Conflict of Interest

The authors declare no potential conflict of interest.

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Author Contributions

Conceptualization: Kang SN. Data curation: Seleshe S. Formal analysis: Seleshe S. Methodology: Seleshe S, Kang SN. Software: Seleshe S. Validation: Seleshe S, Kang SN. Investigation: Kang SN. Writing -original draft: Semeneh S, Kang SN. Writing -review & editing: Seleshe S, Kang SN.

Ethics Approval

The sensory evaluation procedure was approved by the life management committee of Daegu University, IRB number

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References

- Aquilanti L, Garofalo C, Osimani A, Clementi F. 2016. Ecology of lactic acid bacteria and coagulase negative cocci in fermented dry sausages manufactured in Italy and other Mediterranean countries: An overview. *Int Food Res J* 23:429-445.
- Bacha K, Jonsson H, Ashenafi M. 2010. Microbial dynamics during the fermentation of *Wakalim*, a traditional Ethiopian fermented sausage. *J Food Qual* 33:370-390.
- Baka AM, Papavergou EJ, Pragalaki T, Bloukas JG, Kotzekidou P. 2011. Effect of selected autochthonous starter cultures on processing and quality characteristics of Greek fermented sausages. *LWT-Food Sci Technol* 44:54-61.
- Bassi D, Puglisi E, Cocconcelli PS. 2015. Comparing natural and selected starter cultures in meat and cheese fermentations. *Curr Opin Food Sci* 2:118-122.
- Bedia M, Méndez L, Bañón S. 2011. Evaluation of different starter cultures (*Staphylococci* plus lactic acid bacteria) in semi-ripened Salami stuffed in swine gut. *Meat Sci* 87:381-386.
- Bingol EB, Ciftcioglu G, Eker FY, Yardibi H, Yesil O, Bayrakal GM, Demirel G. 2014. Effect of starter cultures combinations on lipolytic activity and ripening of dry fermented sausages. *Ital J Anim Sci* 13:3422.
- Bonomo MG, Ricciardi A, Salzano G. 2011. Influence of autochthonous starter cultures on microbial dynamics and chemical-physical features of traditional fermented sausages of Basilicata region. *World J Microbiol Biotechnol* 27:137-146.
- Bourne MC. 1978. Texture profile analysis. *Food Technol* 32:62-72.
- Capita R, Llorente-Marigómez S, Prieto M, Alonso-Calleja C. 2006. Microbiological profiles, pH, and titratable acidity of chorizo and salchichón (two Spanish dry fermented sausages) manufactured with ostrich, deer, or pork meat. *J Food Prot* 69:1183-1189.
- Casaburi A, Aristoy MC, Cavella S, Di Monaco R, Ercolini D, Toldrá F, Villani F. 2007. Biochemical and sensory characteristics of traditional fermented sausages of Vallo di Diano (Southern Italy) as affected by the use of starter cultures. *Meat Sci* 76:295-307.
- Cavalheiro CP, Piovesan N, Terra LM, Lovato M, Terra NN, Fries LLM. 2013. Colorimetric and sensory characteristics of fermented cured sausage with Brazilian ostrich meat addition. *Food Sci Technol (Campinas)* 33:660-665.
- Chen X, Mi R, Qi B, Xiong S, Li J, Qu C, Qiao X, Chen W, Wang S. 2020. Effect of proteolytic starter culture isolated from Chinese Dong fermented pork (Nanx Wudl) on microbiological, biochemical and organoleptic attributes in dry fermented sausages. *Food Sci Hum Wellness* 10:13-22.
- Ciucu Simion AM, Vizireanu C, Alexe P, Franco I, Carballo J. 2014. Effect of the use of selected starter cultures on some quality, safety and sensorial properties of Dacia sausage, a traditional Romanian dry-sausage variety. *Food Control* 35:123-131.
- Cocconcelli PS. 2007. Starter cultures: Bacteria. In *Handbook of fermented meat and poultry*. Toldrá F, Hui YH, Astiasarán I, Nip WK, Sebranek JG, Silveira ETF, Stahnke LH, Talon R (ed). Blackwell, Ames, IA, USA. pp 137-158.
- Conway EJ. 1950. *Microdiffusion analysis and volumetric error*. Crosby, Lockwood and Son, London, UK.
- Drosinos EH, Mataragas M, Xiraphi N, Moschonas G, Gaitis F, Metaxopoulos J. 2005. Characterization of the microbial

- flora from a traditional Greek fermented sausage. *Meat Sci* 69:307-317.
- Ergezer H, Kaya Hİ, Şimşek Ö. 2018. Antioxidant and antimicrobial potential of artichoke (*Cynara scolymus* L.) extract in beef patties. *Czech J Food Sci* 36:154-162.
- Essid I, Hassouna M. 2013. Effect of inoculation of selected *Staphylococcus xylosum* and *Lactobacillus plantarum* strains on biochemical, microbiological and textural characteristics of a Tunisian dry fermented sausage. *Food Control* 32:707-714.
- Fernández-López J, Sendra E, Sayas-Barberá E, Navarro C, Pérez-Alvarez JA. 2008. Physico-chemical and microbiological profiles of “salchichón” (Spanish dry-fermented sausage) enriched with orange fiber. *Meat Sci* 80:410-417.
- Grun IU, Ahn J, Clarke AD, Lorenzen CL. 2006. Reducing oxidation of meat. *Food Technol* 1:36-43.
- Hammes WP, Bantleon A, Min S. 1990. Lactic acid bacteria in meat fermentation. *FEMS Microbiol Rev* 7:165-173.
- Hammes WP, Hertel C. 1998. New developments in meat starter cultures. *Meat Sci* 49:S125-S138.
- Ho TNT, Nguyen NT, Deschamps A, Hadj Sassi A, Urdaci M, Caubet R. 2009. The impact of *Lactobacillus brevis* and *Pediococcus pentosaceus* on the sensorial quality of “nem chua”: A Vietnamese fermented meat product. *Int Food Res J* 16:71-81.
- Huang L, Zhao J, Chen Q, Zhang Y. 2014. Nondestructive measurement of total volatile basic nitrogen (TVB-N) in pork meat by integrating near infrared spectroscopy, computer vision and electronic nose techniques. *Food Chem* 145:228-236.
- Hugas M, Garriga M, Aymerich T, Monfort JM. 1993. Biochemical characterization of lactobacilli from dry fermented sausages. *Int J Food Microbiol* 18:107-113.
- Hugas M, Monfort JM. 1997. Bacterial starter cultures for meat fermentation. *Food Chem* 59:547-554.
- Jensen LB, Paddock LS. 1940. Sausage treatment, US Patent 2,225,783A.
- Kaban G, Kaya M. 2009. Effects of *Lactobacillus plantarum* and *Staphylococcus xylosum* on the quality characteristics of dry fermented sausage “sucuk”. *J Food Sci* 74:S58-S63.
- Kim HW, Hwang KE, Song DH, Kim YJ, Ham YK, Yeo EJ, Jeong TJ, Choi YS, Kim CJ. 2015. Effect of pre-rigor salting levels on physicochemical and textural properties of chicken breast muscles. *Korean J Food Sci Anim Resour* 35:577-584.
- Kittisakulnam S, Saetae D, Suntornsuk W. 2017. Antioxidant and antibacterial activities of spices traditionally used in fermented meat products. *J Food Process Preserv* 41:e13004.
- Klingberg TD, Axelsson L, Naterstad K, Elsser D, Budde BB. 2005. Identification of potential probiotic starter cultures for Scandinavian-type fermented sausages. *Int J Food Microbiol* 105:419-431.
- Leroy F, Geyzen A, Janssens M, De Vuyst L, Scholliers P. 2013. Meat fermentation at the crossroads of innovation and tradition: A historical outlook. *Trends Food Sci Technol* 31:130-137.
- Leroy F, Verluyten J, de Vuyst L. 2006. Functional meat starter cultures for improved sausage fermentation. *Int J Food Microbiol* 106:270-285.
- Lin KW, Lin SN. 2002. Effects of sodium lactate and trisodium phosphate on the physicochemical properties and shelf life of low-fat Chinese style sausage. *Meat Sci* 60:147-154.
- Lorenzo JM, Gómez M, Fonseca S. 2014a. Effect of commercial starter cultures on physicochemical characteristics, microbial counts and free fatty acid composition of dry-cured foal sausage. *Food Control* 46:382-389.
- Lorenzo JM, Sarriés MV, Tateo A, Polidori P, Franco D, Lanza M. 2014b. Carcass characteristics, meat quality and nutritional value of horsemeat: A review. *Meat Sci* 96:1478-1488.
- Lücke FK. 1998. Fermented sausages. In *Microbiology of fermented foods*. vol. 2. Wood BJB (ed). Blackie Academic &

- Professional, London, UK. pp 441-483.
- Marco A, Navarro JL, Flores M. 2006. The influence of nitrite and nitrate on microbial, chemical and sensory parameters of slow dry fermented sausage. *Meat Sci* 73:660-673.
- Montanari C, Bargossi E, Gardini A, Lanciotti R, Magnani R, Gardini F, Tabanelli G. 2016. Correlation between volatile profiles of Italian fermented sausages and their size and starter culture. *Food Chem* 192:736-744.
- Nie X, Lin S, Zhang Q. 2014. Proteolytic characterisation in grass carp sausage inoculated with *Lactobacillus plantarum* and *Pediococcus pentosaceus*. *Food Chem* 145:840-844.
- Pérez-Alvarez JA, Fernandez-Lopez J. 2011. Color characteristic of meat and poultry processing. In *Sensory analysis of foods of animal origin*. Nollet LML, Toldra F (ed). CRC Press, Boca Raton, FL, USA. pp 101-113.
- Pikul J, Leszczynski DE, Kummerow FA. 1989. Evaluation of three modified TBA methods for measuring lipid oxidation in chicken meat. *J Agric Food Chem* 37:1309-1313.
- Pilevar Z, Hosseini H. 2017. Effects of starter cultures on the properties of meat products: A review. *Annu Res Rev Biol* 17:1-17.
- Rai KP, Zhang C, Xia WS. 2010. Effects of pure starter cultures on physico-chemical and sensory quality of dry fermented Chinese-style sausage. *J Food Sci Technol* 47:188-194.
- Ravyts F, Vuyst LD, Leroy F. 2012. Bacterial diversity and functionalities in food fermentations. *Eng Life Sci* 12:356-367.
- Ruiz-Capillas C, Jiménez-Colmenero F. 2005. Biogenic amines in meat and meat products. *Crit Rev Food Sci Nutr* 44:489-599.
- Swetwathana A, Visessanguan W. 2015. Potential of bacteriocin-producing lactic acid bacteria for safety improvements of traditional Thai fermented meat and human health. *Meat Sci* 109:101-105.
- Tabanelli G, Coloretti F, Chiavari C, Grazia L, Lanciotti R, Gardini F. 2012. Effects of starter cultures and fermentation climate on the properties of two types of typical Italian dry fermented sausages produced under industrial conditions. *Food Control* 26:416-426.
- Talon R, Leroy S, Lebert I. 2007. Microbial ecosystems of traditional fermented meat products: The importance of indigenous starters. *Meat Sci* 77:55-62.
- Toldrá F. 2006. Meat fermentation. In *Handbook of food science, technology, and engineering*. vol 4. Hui YH (ed), CRC Press, Boca Raton, FL, USA.
- Valyasevi R, Jungsiriwat P, Smitinont T, Praphilong W, Chowalitnitithum C. 2001. Improvement of starter culture for Nham fermentation. Final report submitted to National Center for the Genetic Engineering and Biotechnology. National Science and Technology Development Agency.
- Van Ba H, Seo HW, Cho SH, Kim YS, Kim JH, Ham JS, Park BY, Seong PN. 2017a. Effects of extraction methods of shiitake by-products on their antioxidant and antimicrobial activities in fermented sausages during storage. *Food Control* 79:109-118.
- Van Ba H, Seo HW, Cho SH, Kim YS, Kim JH, Park BY, Kim HW, Ham JS, Seong PN. 2017b. Utilisation possibility of *Enterococcus faecalis* isolates from neonate's faeces for production of fermented sausages as starter cultures. *Int J Food Sci Technol* 52:1660-1669.
- Van Ba H, Seo HW, Seong PN, Kang SM, Kim YS, Cho SH, Park BY, Ham JS, Kim JH. 2018. *Lactobacillus plantarum* (KACC 92189) as a potential probiotic starter culture for quality improvement of fermented sausages. *Food Sci Anim Resour* 38:189-202.

- Wang X, Ren H, Wang W, Zhang Y, Bai T, Li J, Zhu W. 2015. Effects of inoculation of commercial starter cultures on the quality and histamine accumulation in fermented sausages. *J Food Sci* 80:M377-M384.
- Yim DG, Chung YH, Nam KC. 2017. Effects of starter cultures on physicochemical properties of fermented sausages. *Korean J Food Nutr* 30:1105-1112.
- Yin LJ, Pan CL, Jiang ST. 2002. Effect of lactic acid bacterial fermentation on the characteristics of minced mackerel. *J Food Sci* 67:786-792.
- Zagorec M, Champomier-Vergès MC. 2017. *Lactobacillus sakei*: A starter for sausage fermentation, a protective culture for meat products. *Microorganisms* 5:56.
- Zhao L, Jin Y, Ma C, Song H, Li H, Wang Z, Xiao S. 2011. Physico-chemical characteristics and free fatty acid composition of dry fermented mutton sausages as affected by the use of various combinations of starter cultures and spices. *Meat Sci* 88:761-766.