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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Running title: Quality Characteristics of dry fermented sausages from different LAB starters

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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 plat 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 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.

Key words: dry fermented sausage, starter cultures, 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 (Swetwiwathana and Visessanguan, 2015). *Lactobacillus sakei*, *Lactobacillus curvatus* and *Lactobacillus plantarum* species were the most commonly identified in traditional fermented sausages (Talon et al., 2007; Hugas et al., 1993; Kittisakulnam et al., 2017); 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 US 2225783 A (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, 2000). 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

68 final product (Baka et al., 2011; Bonomo et al., 2011)

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

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

88 Under the conditions of fermentation and maturation of the sausages, the growth of the
89 LAB strains is decisive in order to be regarded as a possible starter. An important feature is the
90 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 *Pediococcus pentosaceus* (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* 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 (Ba et al., 2018). Similarly, *Pediococcus pentosaceus* 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, USA) was used for the enrichment of the starter cultures and incubated at 37 ℃ 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, Korea). The basic sausage formulation included lean pork meat (80%), pork fat (20%), water (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 divide in to 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 ~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 Co., Ltd., Taiwan) and stuffed into collagen casings (IKJIN Co. Ltd., Seoul, Korea), 24 mm diameter and 150 mm length, with vacuum filling machine (RVF 327, Düker-REX Fleischereimaschinen GmbH, Germany). Fermentation and ripening of sausages were done in digital chamber unit (SMK-2000SL, Metatek, Korea) equipped with temperature and RH control system. In the fermentation period of the first seven days, the temperature was maintained at 23℃ and relative humidity (RH) was alternated to 90–95%. In the ripening period, the next 21 days following the fermentation period, the temperature was maintained at 15℃ 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 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, 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 (Difco, USA) was used for total microbial counts and Lactobacillus MRS agar (Difco, USA) for LAB. Plates from different and appropriate dilutions were incubated in triplicate at 37℃

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, Ohio, 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)

Water activity (a_w) of sausages was analyzed using A_w measuring device (LabMaster-a_w, Novasina AG, Switzerland) at 25 ℃ 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 (CIE, 1976).

184

185 **Volatile basic nitrogen (VBN) analysis**

186 Measurements of the VBN content of samples were determined using the Conway micro
187 diffusion method (Conway, 1950). Two Conway's tools per each sample were used after
188 cleaning with a neutral detergent. A sealing agent (vaseline) was applied to the edge of the
189 outer ring of each unit. Three grams of the sample was homogenized with 30 mL of distilled
190 water at 1,000 rpm for 1 min using a homogenizer. The homogenate was filtered using
191 Whatman no. 1 filter paper (GE Healthcare Life Sci., Pittsburg, PA, USA). The filtrate (1 mL)
192 was pipetted to the outer chamber of a Conway micro diffusion unit, and 1 mL of 0.01 N boric
193 acid (H_3BO_3) and 100 μL of Conway indicator (0.066% bromocresol green:0.066% methyl red,
194 1:1) were pipetted to the inner chamber. Then, 1 mL of 50% potassium carbonate (K_2CO_3) was
195 added to the outer chamber of the Conway unit and sealed immediately. Incubation of the
196 materials was performed for 2 hours at 37°C. After the addition of 0.02 N sulfuric acid (H_2SO_4)
197 to the inner chamber of the Conway unit, the VBN contents were measured. Total volatile basic
198 nitrogen (VBN) values were expressed in mg%.

199

200 **Thiobarbituric acid reactive (TBARS) analysis**

201 Analysis of lipid oxidation was performed by analyzing the TBARS (Pikul et al., 1989).
202 Sausage sample (5 g) was homogenized with a 50ul of BHA (7.2% in ethanol) and 15mL of
203 distilled water and then centrifuged at 2,000 rpm for 15 min using a centrifuge (Hanil Science
204 Industrial, CO., Ltd., Incheon, Korea). The supernatant (2 mL) was mixed with 4 mL
205 thiobarbituric acid solution (20 mM TBA in 15% Trichloroacetic acid, TCA) followed by
206 heating in a water bath at 90° C for 30 min and then cooling to room temperature. Therefore,
207 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 water activity < 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 Duncans'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 total plate counts 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 total plate count, it speculates the growth characteristics of all groups of microorganisms using general-purpose media, plate count agar (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

280 is expected. In the present study, a significant positive correlation was exhibited between TPC
281 and LAB counts ($r=0.992$, $p<0.05$), (data not presented).

282 The effect of different strains of LAB starter cultures on the pH, Aw, VBN, and TBARS
283 values of dry fermented sausage after the ripening period is presented in Table 2. Valyasevi et
284 al. (2001) described that LAB are the major producers of lactic acid responsible for the decrease
285 in pH and the increase in acidity during the fermentation. In the current study, a significant
286 variation ($p<0.05$) in pH values of treatments was exhibited due to the difference in
287 acidification capacity of different strains of LAB starter cultures. In pH values of treatments:
288 PP < LP2 < LP1 having 4.91, 5.05 and 5.23. As compered LP1 treatment, the lower pH values
289 exhibited in PP and LP2 inoculated lots may have resulted from better adaptation and fast
290 multiplication of the LAB starter cultures (Table 1) to the meat environment and the meat
291 processing conditions which may have mainly contributed to the acidification of the products.
292 The samples with the lower pH values corresponding with treatments those having higher LAB
293 counts at the end of the fermenting/ripening process. The rapid growth of LAB is important
294 because it leads to the carbohydrate breakdown and buildup of organic acids, primarily lactic
295 acids (Nie et al., 2014; Zhao et al., 2011). In the production of sausages, a lower pH value is
296 important because it helps to prevent the growth of undesirable microorganisms and enhance
297 the redder color of the products (Lorenzo et al., 2014a; Lorenzo et al., 2014b). The lower pH
298 plays a crucial role in the development of the distinctive flavor, color, and aroma, and
299 microbiological consistency of the fermented sausages (Hammes et al., 1990; Hugas and
300 Monfort, 1997).

301 It is well known that a food product's shelf-life stability is usually dependent on the water
302 activity (a_w); water activity at the end of ripening will improve the excellence of fermented
303 sausage and prolong its shelf life. Inoculation of different strain starter cultures resulted in a

304 significant variation ($p<0.05$) in the water activity value of ripened sausage samples (Table 2).
305 On the base of Aw values, treatments are ordered as follows: LP2 < LP1 < PP with the
306 corresponding values of 0.74, 0.78, 0.83, respectively. Our findings disagree with those
307 previous studies reports which found no significant difference in Aw value of treatments with
308 different starter cultures across the ripening period (Ba et al., 2018; Chen et al., 2020). At the
309 end of the ripening process, the a_w of all three batches was below 0.85. This result also disagrees
310 with Ba et al. (2017a) and Ba et al. (2017b) findings who reported higher a_w values (0.85-0.88)
311 for the same product type that could be attributed to elevated moisture contents of the samples
312 in their studies. The denaturation of sarcoplasmic proteins as result of the drop in pH during
313 fermentation and degradation of protein caused by microorganisms involved in fermentation
314 likely to decrease the water holding capacity; this occurrence in turn responsible in lowering
315 the water activity as the moisture vanished during drying. Despite all treatments in current
316 study exhibited pH values of ≤ 5.05 (Table 2), there was no significant correlation between a_w
317 and pH value of treatments ($r = -0.547$, $p>0.05$) (data not presented). Fermented sausages are
318 categorized in final products as "semi-dry" and "dry" based on a_w. "The sausages are
319 categorized as "semi-dry" with Aw between 0.90 and 0.95 and are labeled "dry" with a_w below
320 0.90 (Luecke 1998; Wang et al., 2015). The sausages fermented by three strain of LAB starter
321 cultures in the present study belonged to the dry fermented sausage according to these
322 classification criteria.

323 The content of VBN consists of NH_3, H_2S, and CH_3CH_2SH, etc., which are produced by
324 spoilage bacteria or endogenous enzymes from the decomposition/degradation of proteins
325 (Huang et al., 2014). These low molecular non-protein nitrogen compounds could possibly be
326 formed from protein degradation during fermentation (Ruiz-Capillas and Jiménez-Colmenero,
327 2005) that impart to the VBN content of the product. Consequently, VBN is commonly used

328 as an important indicator showing the shelf life and microbial consistency of processed meat
329 products (Ba et al., 2018). Our findings show that the quality of VBN varies significantly
330 ($p<0.05$) among treatments at the end of the ripening period, ranging from 11.63 mg% to 14.05
331 mg% for LP1 and LP2 treated samples, respectively (Table 2). VBN values ranging from 7
332 to18 mg % was reported by Lin and Lin (2002) in Chinese style dry-cured sausage during the
333 ripening period. In the current study, the VBN content was much lower compared to the levels
334 documented by Rai et al. (2010) for the same product type (20-25 mg percent). The findings
335 may be attributed to the inoculated bacteria's ability to neutralize the VBN content with their
336 organic acids (e.g. lactic acid) or bacteriocin production (Yin et al., 2002). Ruiz-capillas and
337 Jiménez-colmenero (2005) documented that low molecular non-protein nitrogen compounds
338 could be possibly resulted from protein degradation during fermentation. The present study
339 exhibited that VBN values to have a significant correlation with LAB counts ($r = 0.945$, $p<0.05$)
340 and a significant negative correlation with pH value ($r = -0.867$, $p>0.05$) of treatments (data
341 not presented).

342 Analysis of TBARS is used as an important indicator for the development of secondary
343 lipid oxidation products, primarily malondialdehyde, which can lead to oxidized fat off-flavor.
344 Lipid oxidation in meat products may change their nutritive values, colors, and flavors (Kim et
345 al., 2015), and associated with health risks (Grun et al., 2006). At the end of the ripening
346 process, treatments inoculation with different strains of LAB starter cultures had a significant
347 difference ($p< 0.05$) in the TBARS content (Table 2), in the following order: LP1 > PP > LP2.
348 This result is concordant with that of Bingol et al. (2014), who found a difference in TBARS
349 values and contemplated the variation in the result as it could be from lactic activities of starter
350 cultures that importantly decrease the pH value. The current finding disagrees with Yim et al.
351 (2017) who demonstrated no significant difference in TBARS value between the fermented

352 sausages treated with the commercial cultures mix and the commercial cultures mix +
353 *Lactobacillus plantarum* at the end of the ripening process. TBARS values of fermented
354 sausages in the range of 0.6−2.8 mg MDA / kg are acceptable (Marco et al., 2006). In this study,
355 TBARS values of all treatments did not exceeded the stated range.

356 Customers pay a considerable attention to its color when determining meat products,
357 which, like visual perception, is primarily caused by the existence of pigments but also depends
358 on the tissue composition and meat structure. The color of meat products is, therefore, one of
359 the mainly important quality parameters that govern the response and decision at the retail
360 outlet of the product. Color depends on a variety of factors in the case of dry-fermented sausage,
361 such as the composition of the sausage, the fat-lean ratio, the quantity and kind of spices and
362 the additives and technical operations applied (Perez-Alvarez and Fernandez-Lopez, 2011). In
363 most instances, however, sausages become red (cured color) during the fermentation stage due
364 to the development of nitrosomyoglobin resulting from the combination of nitric oxide (NO),
365 produced by the bacterial conversion of nitrate to nitrite, and myoglobin (Cavalheiro et al.,
366 2013). The color traits lightness, redness, and yellowness of fermented sausages at the end of
367 the ripening process are presented in Table 3. Treatments added with PP and LP2 exhibited a
368 higher value of L* (Lightness), whereas the lowest L^* values of sausages were in batches
369 inoculated with LP1 starter cultures (p<0.05). Samples inoculated with LP2 had elevated b*
370 (Yellowness) value than other strains treated samples. Redness (a*) is the most important trait
371 for determining the degree of products oxidation, and the lower redness value in meat is
372 contemplated as a sign of oxidation (Ergezer et al., 2018). In the present study, the addition of
373 different strains of LAB starter cultures did not have a substantial difference (p>0.05) in the
374 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 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, 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 spices), starter cultures, processing circumstances

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

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

413

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418

419 **CONFLICT OF INTEREST**

The authors have no conflict of interest.

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571

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

Parameter	Treatments [1)]			SEM [2)]
	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

574 [1)] Treatments are different strain of LAB starter culture used in the present study: PP,
575 *Pediococcus pentosaceus* (KCTC-13100); LP1, *Lactobacillus plantarum* (KCTC-21004)
576 and LP2, *Lactobacillus plantarum* (KCTC-13093). n=3. [2)] SEM: standard error of mean. [a-b]
577 Means with different superscript are significantly different ($p < 0.05$).

578

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

Attributes	Treatments [1)]			SEM [2)]
	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 MA/kg)	0.83^{b}	0.90^{a}	0.74^{c}	0.01

581 [1)] Treatments are as described in the Table1. n=3. [2)] SEM: standard error of mean. [a-b] Means
582 with different superscript are significantly different ($p < 0.05$).

583

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

Parameter	Treatments [1)]			SEM [2)]
	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

586 [1)] Treatments are as described in the Table1. n=3. [2)] SEM: standard error of mean. [a-b] Means
587 with different superscript are significantly different ($p < 0.05$).

588

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

Attributes	Treatments [1)]			SEM [2)]
	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

591 [1)] Treatments are as described in the Table 1. n=3. [2)] SEM: standard error of mean. [a-c] Means
592 with different superscript are significantly different ($p < 0.05$).

593

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

Attributes	Treatments [1)]			SEM [2)]
	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. [2)] SEM: standard error of mean. [a-c] Means with different superscript are significantly different ($p < 0.05$).