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Effect of the types of starter on microbiological and physicochemical properties of dry-cured ham

ABSTRACT

This study analyzed the microbiological (*Lactobacillus spp.*, *Staphylococcus spp.*, mold, yeast, aerobic bacteria) and physicochemical properties (pH, salinity, water activity, volatile basic nitrogen (VBN), and thiobarbituric acid reactive substances (TBARS)). The starters were used by mixing *Debaryomyces hansenii* separated from Korean *Doenjang* (D) and fermented sausage (S). The starter was inoculated with dry-cured ham and aged for 6 weeks at 20℃ and 25℃, respectively. The aerobic bacteria, *Lactobacillus* spp., and *Staphylococcus* spp. of D, S, and DS treatment showed significantly higher values at 25℃ than at 20℃. Among them, S25 treatment showed a high tendency. At week 6, the mold of the S25 treatment was significantly higher than the S20 treatment, and the yeast was 25℃ higher than 20℃ ($p < 0.05$). The pH of all treatment groups increased with the aging period. Compared with that at 25℃, the pH was significantly higher at 20℃ ($p < 0.05$). The water activity showed a significant decrease as the aging period increased, and the treatment of D25, S20, and DS20 showed a significantly higher value at week 6 ($p < 0.05$). Compared with that at 20℃, the VBN content was higher at 25℃. At week 6, the VBN contents of the C20, S25, and DS25 groups were higher than those of the other treatment groups. Therefore, inoculation of *D. hansenii* separated from fermented sausage produced in Korea starter at 25℃ is expected to improve the safety of harmful microorganisms and physiochemical properties in dry-cured ham.

Key words: dry-cured ham, *Debaryomyces hansenii*, *Penicillium nalgiovense*, starter culture

Introduction

Dry-cured ham products, for instance, Jamón (Spain), Xuanwei (China), Parma (Italy), and Country Ham (U.S.) are produced and consumed worldwide (Simonella et al., 2006). The process of making dry-cured ham involves curing, ripening, and smoking (Yim et al., 2015). During the curing process, the absorption and dispersion of salt can reduce the water activity of dry-cured ham and consequently inhibit the growth of harmful microorganisms and enhance storage stability (Kim and Yim, 2016). However, the values of VBN and TBARS increase due to the growth of harmful microorganisms during the aging period (Yu et al., 2018; Stadnik et al., 2022). Also, Since dry-cured ham is not produced using heat, various studies on the safety of microorganisms are investigated (Lee et al., 2004; Kim et al., 2015). Therefore, starters have been applied during the production of dry-cured ham to increase microbial safety and enhance the physiochemical properties (Chen et al., 2021).

Different microorganisms in dry-cured ham are detected depending on the temperature. These microorganisms differentially influence the quality attributes of the dry-cured ham (Marcel et al., 1992). *Penicillium nalgiovense* and *Debaryomyces hansenii*, which grow mainly in dry-cured ham and are used as starters, inhibit the growth of harmful microorganisms and enhance flavor (Lacumin et al., 2020; Zhou et al., 2022). Microorganisms such as *D. hansenii*, *Lactobacillus plantarum*, *Lactobacillus sakei*, and *Bacillus subtilis* can grow in fermented foods produced in Korea (Kim et al., 2021; Dharaneedharan and Heo, 2016). Penicillin produced by *P. nalgiovense*, separated from fermented meat products, is less toxic (Papagianni et al., 2007), and *D. hansenii*, which is mostly detected in fermented foods can inhibit the growth of toxin-producing microorganisms and enhance the food flavor (Andrade et al., 2014). Additionally, *D. hansenii* is also a microorganism suitable for dry-cured ham pro-

duction because it is highly resistant to NaCl and can survive under pH 3-10 and low water activity conditions (Cape Angela, and Patricia Romano, 2009).

Research on the characterization of microorganisms separated from *Doenjang* and fermented sausage were widely investigated (Seon et al., 2021; You et al., 2014). However, studies on the microbiological and physicochemical properties of dry-cured ham according to temperature by using separated and identified microorganisms in Korean fermented foods as a starter is lacking. Thus, in this study, the effect of a starter mixed with *D. hansenii* strain separated from *Doenjang* and fermented sausage and *P. nalgiovense* strain separated from fermented sausage on microbial safety and physiochemical properties during dry-cured ham aging was investigated.

Materials and Methods

Starter culture and bacterial strain

Debaryomyces hansenii of SMFM2021-D1 (separated from *Doenjang* produced in Korea; D*)* and SMFM2021-S8 (separated from fermented sausage produced in Korea; S), and *Penicillium nalgiovense* of SMFM2021-S6 (separated from fermented sausage) were supplied form Yoon Bio Tech (Seoul, Korea). The *Penicillium nalgiovense* Saterkulturen Edelchimmel of commercial strains was supplied by the National Institute of Animal Science (Wanju, Korea). All yeasts and molds were used at a concentration of 8 log colony forming unit (CFU) per gram (Log CFU/g).

Preparation of dry-cured ham

The raw pork used in this study was supplied and used by *Gluteus medius* (Ihomemeat, Seoul, Korea) 24 hours after slaughter, and each starter treatment used 10 *Gluteus medius* to make a total of 80 dry-cured ham. Connective tissues and excessive fat were eliminated from *Gluteus medius* and used for experiments. After the application of salt and spices (0.5% of black pepper, 0.15% of juniper berry, 0.2% of sugar, 0.1% of garlic powder, 0.15% of cilantro seed powder, 1% of nitrite pickling salt, 2.5% of salt, and 0.05% of bay leaf) to the surface of the *Gluteus medius*, salting was performed for 7 days under vacuum conditions. During the saline process, it was carried out in a vacuum state to increase the bonding property of salt to dry-cured ham and to uniformize the conditions. Then, the meat was turned over once every 24 h during the saltation period, followed by rinsing with cold water for 2 min. The description of the treatment and starter culture is shown in Table 1. The dry-cured ham was then dried at room temperature (20 ± 5℃) for 2 days and turned over once every 24 h. Dry-cured hams were ripened under the following conditions: Relative humidity, 70%; Temperature, 20℃ and 25℃ (Jenog et al., 2023). The aging temperature and humidity gradually decreased. Finally, dry-cured ham at 20℃ was aged at 16℃ and 65% relative humidity. Meanwhile, ham dry-cured at 25℃ was aged at 21℃ and 65% relative humidity. Referring to Maruicí et al. (2014), it was aged based on 0.8 moisture activity of all treatments and finally aged for 6 weeks. Samples were randomly collected from three dry-cured hams for each week and used in the experiment. The microbial, pH, salinity, and water activity were experimented with on the day of sampling. The volatile basic nitrogen and thiobarbituric acid reactive substance were experimented on after being stored in a deep freezer (TSE320GPD, Waltham, USA) at -80℃.

Microbial composition

Five grams of dry-cured ham sample was placed in sample bags (193OF, 3M, Saint Paul, MN, USA) with 50 mL of 0.1% buffer peptone water (BPW). The samples were stomached in a stomacher (WH4000-2751-9, 3M) for 1 min. Then, 1 mL of the sample was diluted in 0.1% BPW at a ratio of 1:9. The dilution was repeated as many times as necessary. Diluted samples were plated onto potato dextrose agar (PDA; detection of mold and yeast), de Man, Rogosa, and Sharpe (MRS; detection of *Lactobacillus* spp.) agar, mannitol salt agar (MSA; detection of *Staphylococcus* spp.; BD Difco, Franklin Lakes, NJ, USA), and aerobic bacteria plate count (AC) and yeast and mold (YM) count petrifilm (3M, Saint Paul, USA). The samples plated on MRS, MSA, and AC petrifilm were incubated at 37℃ in an incubator (WSC-2610, ATTO, Tokyo, Janpan) for 24 h. Meanwhile, the samples plated on PDA and YM were incubated at 25℃ in an incubator (WSC-2610, ATTO) for 48 h. The colonies grown on plate and film were recorded as Log CFU/g. Although not explicitly shown, *E. coli*, *L. monocytogenes*, *Salmonella* spp., *S. aureuswas* not detected in any of the control or treatment groups in this study (data not shown).

pH

Three grams of the dry-cured ham and 12 mL of distilled water were measured and mixed. The mixed sample was homogenized under a speed condition of 6,451 ×g for 1 min using an Ultra Turrax homogenizer (HMZ-20DN, Poonglim Tech, Seongnam, Korea). The pH was measured using a pH meter (Model S220, Mettler-Toledo, Schwerzenbach, Switzerland) calibrated with the pH buffer solutions (pH 4.01, pH 7.0, and pH 10.0; Suntex Instruments Co, Ltd, Taipei, Taiwan).

Salinity

Three grams of the dry-cured ham and 12 mL of distilled water were measured and mixed. The mixed sample was homogenized under a speed condition of 6,451 ×g for 1 min using an Ultra Turrax homogenizer (HMZ-20DN, Poonglim Tech). The salinity of the homogenized samples was measured using a salt meter (SB-2000PRO, HM DIGITAL, Seoul, Korea). The salinity value was reported as percent of sample.

Water activity

Ten grams of dry-cured ham was transferred to a measuring container and measured the water activity (a_w) using a moisture activity measuring instrument (LabMaster aw, Novasina, Lachen, Switzerland) set the initial temperature at 25℃.

Volatile basic nitrogen (VBN)

The VBN described by Park et al. (2022) was used with some modifications. Ten grams of dry-cured ham and 30 mL of distilled water were homogenized with a homogenizer (AM-5, NISSEI, Tokyo, Japan) at 5,614 ×g speed conditions for 1 min. The homogenized sample was mass-up with 100 mL of distilled water and filtered with filter paper (Whatman No. 1, GE Healthcare, Chicago, IL, USA). And filled each outer and inner chamber of the Conway unit with 1 mL of the filtrate sample and 1 mL of 0.01 M H_3BO_3. Then, additionally filled each outer and inner chamber of the Conway unit with 1 mL of 50% K_2CO_3 and 100 μL of Conway reagent. The Conway unit was incubated at 37℃ for 2 h and the amount of volatile basic nitrogen was titrated by 0.02N H_2SO_4. The VBN content was calculated using the following formula:

$$\text{VBN (mg\%)} = 0.14 \times \frac{(V_2 - V_1)}{\text{W}} \times \text{a} \times \text{b} \times 100$$

W: sample weight

V_1: volume of sulfuric acid consumed for the blank titration (μL)

V_2: volume of sulfuric acid consumed for the sample titration (μL)

a: titer value of 0.02 N sulfuric acid

b: dilution factor

Thiobarbituric acid reactive substance (TBARS)

Ten grams of dry-cured ham was homogenized with 25 mL of 10% principal components analysis solution and 0.2 mL of 0.3% butylated hydroxytoluene (BHT) with a homogenizer (AM-5, NISSEI) at 5,614 ×g speed conditions for 1 min. The homogenized sample was filtered with filter paper (Whatman No. 1, GE Healthcare), and 5 mL of the filtrate sample was mixed up with 5 mL of 0.02M $C_4H_4N_2O_2S$ solution and boiled in a 100℃ set constant-temperature water bath (JSWB-30T, JSR, Gongju, Korea) for 10 min. The reacted sample has measured the absorbance at 532 nm using a multi-mode microplate reader (SpectraMax iD3, Molecular Devices, San Jose, CA, USA). And calculated the amount of malondialdehyde (MDA) using the standard curve prepared from 1,1,3,3-tetra-ethoxypropane. The TBARS value was reported as mg MDA/kg of sample.

Statistical analysis

All data in this study were used for statistical analysis and presented as the mean values and SEM. Experimental results were assessed by repeating experiments at least three times. Statistical analyses were performed using SAS (version 9.4 for windows, SAS Institute, Cary, NC, USA). Means were compared using an one-way analysis of variance and Duncan's multiple range tests. Differences were considered significant at $p < 0.05$.

Results and Discussion

Counts of aerobic bacteria, *Lactobacillus*, and *Staphylococcus*.

Table 2 shows the counts of aerobic bacteria, *Lactobacillus*, and *Staphylococcus* according to the aging period of dry-cured ham under different temperatures and starter conditions. The aerobic bacterial counts of dry-cured ham increased in all treatment groups with the aging period. Compared with those at week 4, the aerobic bacterial counts were significantly higher at week 6 in the C20, C25, S20, S25, and DS20 groups ($p < 0.05$). All treatment groups showed increased *Lactobacillus* counts. Compared with those at week 4, the *Lactobacillus* counts were significantly higher at week 6 in the C25 and DS20 groups ($p < 0.05$). In the S20 group, the *Lactobacillus* counts significantly increased from week 2 to week 6 ($p < 0.05$). The *Staphylococcus* counts increased in all treatment groups, except for the C20 group. The *Staphylococcus* counts significantly increased from week 2 to week 6 in the S20 and DS20 groups ($p < 0.05$). Meanwhile, the *Staphylococcus* counts were not significantly different between weeks 2 and 4 in the C25 and D20 groups ($p > 0.05$) but significantly increased at week 6 ($p < 0.05$). *Lactobacillus* is used as a starter in fermented food and it is a beneficial bacterial strain that can grow even under approximately 6.5% salinity and pH 8 conditions

(Topçu et al., 2020; Hassan et al., 2020). *Lactobacillus* can exhibit the effect of reducing the risk of biogenic amines as well as reducing the incidence of diseases such as listeriosis (Sirini et al., 2021). Ryu et al. (2018) examined the types of meat-related microbial strains and their growth abilities in dry-aged beef. The authors demonstrated that the total bacterial and *Lactobacillus* counts increased with the aging period, which was consistent with the findings of this study. Therefore, it is considered that C20, D25, S25, and DS25, which showed faster growth ability of *Lactobacillus* than other treatments, will have a positive effect on the quality improvement of dry-cured ham. Among the *Staphylococcus* strains found in dry-cured ham, approximately 32% are *S. xylosus*, which is reported to be the main microbial strain in dry-cured ham (Vilar et al., 2000). The *S. xylosus* strain can be used as a starter in the production of fermented meat products as it contributes to flavor by degrading proteins and lipids and showing growth under high NaCl conditions (up to 15%) (Müller et al., 2016). The increase in *Staphylococcus* counts with the aging period may affect enhancing the flavor. Therefore, it is considered that treatment of D25, S25, and DS25, which showed higher growth capacity than other treatments, will have a positive effect on flavor formation.

Next, the effect of different temperatures on the counts of aerobic bacteria composition was evaluated. The aerobic bacterial counts in the 25℃ treatment groups were higher than those in the 20℃ treatment groups from week 2 to week 6 ($p < 0.05$). The aerobic bacterial counts in the D20 group were significantly lower than those in the other groups at week 6 ($p < 0.05$). The aerobic bacterial counts in the S25 group were significantly higher than those in the other groups at week 6 ($p < 0.05$). The *Lactobacillus* counts in the 20℃ treatment groups were significantly lower than those in the 25℃ treatment groups ($p < 0.05$). However, the *Lactobacillus* counts in the C25 group were significantly higher than those in the C20 group at week 6 ($p < 0.05$). From week 2 to week 6, the *Lactobacillus* counts in the 20℃ treatment groups

were significantly higher than those in the 25℃ treatment groups in the D20, D25, S20, S25, DS20, and DS25 groups ($p < 0.05$). At week 6, the *Lactobacillus* counts in the D25 and S25 groups were significantly higher than those in the C20, C25, D20, S20, and DS20 groups ($p < 0.05$). The *Staphylococcus* counts in the 20℃ treatment groups were significantly lower than those in the 25℃ treatment groups ($p < 0.05$), except for the C20 and C25 groups. The *Staphylococcus* counts in the C20, D20, and DS20 groups were significantly lower than those in the other treatment groups at week 6 ($p < 0.05$). Meanwhile, the *Staphylococcus* counts in the S25 group were significantly higher than those in the other groups at week 6 ($p < 0.05$). During the aging period, microorganisms affect the accumulation of amino acids and fatty acids through enzymatic protein degradation, lipid degradation, and oxidation, and the flavor is enhanced via Maillard reaction and Strecker degradation (Wang et al., 2021). Hence, microbial growth increases microbial metabolic activities to exert a positive effect on flavor (De Filippis et al., 2016). Kristjansson and Stetter (2021) reported a linear increase in the growth of aerobic bacteria with an increase in temperature from 15℃ to 25℃, which is consistent with the significantly increased aerobic bacterial counts at 25℃ (relative to 20℃) in this study ($p < 0.05$). Matejčeková et al. (2016) demonstrated that *Lactobacillus* grows at 8℃-40℃ and the growth of *Lactobacillus* at 25℃ was higher than that at 21℃. Also, similar results were showed in this study. The *Lactobacillus* counts at 25℃ were significantly higher than those at 20℃ ($p < 0.05$). The optimum growth temperature for *Staphylococcus* is 37℃. The *Staphylococcus* counts at 25℃ were higher than those at 20℃. This may be because 25℃ is close to the optimum growth temperature (Di Ciccio et al., 2015). Although not explicitly shown, S. aureus was not detected in any of the control or treatment groups in this study (data not shown). Therefore, harmful microbiological risks can be slightly reduced by *Staphylococcus* and *Lactobacillus* in aging at 25℃ than at 20℃. Among the treatment, Staphylococcus and lactobacillus tend to show high values of the S25 treatment and is con-

sidered a suitable starter for improving the microbiological safety and quality of dry-cured ham compared to other treatments (Zhou et al., 2022).

Mold and yeast

Table 3 shows the counts of mold and yeast according to the aging period of dry-cured ham under different temperature and starter conditions. The mold counts of dry-cured ham increased with the aging period in all treatment groups. The mold counts at week 6 were significantly higher than those at week 4 ($p < 0.05$). The D20 group showed a significant increase in mold counts with the aging period ($p < 0.05$). None of the groups with 25℃ treatment showed a significant difference in mold counts between weeks 2 and 6 ($p > 0.05$). The yeast counts increased in all treatment groups, except for the DS20 group (which did not show significant differences in yeast counts), between weeks 2 and 6. However, the yeast counts were not significantly different between weeks 4 and 6 ($p > 0.05$). During the production of dry-cured ham, the starter strains *D. hansenii* and *P. nalgiovense* suppress the growth of harmful microorganisms as well as the aging period (Garriga and Aymerich, 2014). Additionally, the decomposition of peroxides, proteins, and lipids results in increased flavor and aromatic compounds (Kołożyn-Krajewska and Dolatowski, 2012). *P. nalgiovense* and *D. hansenii* can grow under range of pH 3–10 and low water activity conditions and show strong resistance to NaCl (Díaz et al., 2002, Capece Angela and Patrizia Romano, 2009). Therefore, it is considered that the increase in yeast and mold according to the aging period may increase the safety of harmful microorganisms of dry-cured ham. In addition, it is considered that the treatment of C25, D20, D25, S25, and DS25 that showed a fast growth rate of yeast and mold among the used starters suitable.

Next, the effects of different temperatures on mold and yeast counts in dry-cured ham were examined. Mold counts did not show consistent variations across the treatment groups and aging weeks. At week 6, the mold counts in the C20 and C25 group were significantly lower than those in the D20, D25, DS20, and DS25 groups. The yeast counts in the 20℃ treatment groups were significantly lower than those in the 25℃ treatment groups at week 6 ($p < 0.05$). From weeks 2 to 6, the yeast counts in the C20 group were significantly lower than those in the other treatment groups ($p < 0.05$). Ludemann et al. (2004) isolated and identified *P. nalgiovense* in Argentine salami whose growth rate at 25℃ was higher than that at 14℃. The growth rate of this strain increased with an increased in temperature, which was consistent with the discovery of this study. Additionally, the secretion of proteases and lipases and the contents of free fatty acids (that determine the taste and flavor) at 25℃ were significantly higher than those at 14℃, suggesting that 25℃ is a suitable temperature for aging (Galvalisi et al., 2012). The temperature variation is a key influencing factor in the growth of *D. hansenii* (Masoud et al., 2021). Masoud and Jakobsen (2005) compared the growth of *D. hansenii* at 10℃ -25℃ and reported that its growth increases with the increase in temperature. The starter used in this research mixed yeast and mold, and it showed a tendency that yeast and mold values at 25℃ were higher than 20℃. Therefore, it is considered that aging at 25℃, which shows an appropriate growth temperature of the starter, will be effective. In addition, D25 and S25 of the 25℃ treatments showed high growth ability of yeast and mold. Therefore, it is believed that the use of starters D and S starter at 25℃ will reduce harmful microorganisms and improve flavor.

pH, salinity, and water activity

Table 4 shows the measurements of pH, salinity, and water activity according to the aging period of dry-cured ham under different temperatures and starter conditions. The pH of dry-cured ham increased with the aging period in all treatment groups. In the S20 group, the pH was not significantly different between weeks 2 and 4 ($p > 0.05$) but significantly increased at week 6 ($p < 0.05$). Compared with that at week 2, the pH was significantly higher at week 4 in the D25 and DS25 groups ($p < 0.05$) but was not significantly different between weeks 4 and 6 ($p > 0.05$). The pH was not significantly different between weeks 2 and 6 in the D20 group ($p > 0.05$). The C20, C25, S25, and DS20 groups showed a significant increase in pH with the aging period ($p < 0.05$). In aged products, the release of nitrogen and the formation of ammonia resulting from protein degradation and the activities of deaminase and deamidase increase the pH (Wang et al., 2022). As shown in Table 3, *D.hanenii* tends to increase as the aging period increases in this experiment, suggesting that the pH could have increased due to the release of nitrogen and the formation of ammonia owing to the degradation of proteins in dry-cured ham by *D. hansenii* (Kim et al., 2018). The pH of dry-cured ham with 20℃ treatment was significantly higher than that with 25℃ treatment across all starter groups ($p < 0.05$). In the final week, compared with that in other treatment groups, the pH was significantly higher in the S20 group ($p < 0.05$) and significantly lower in the C25 group ($p < 0.05$). This can be affected by the growth of *Lactobacillus* and the production of lactic acid and acetic acid as the main metabolites, leading to decreased pH with an increase in the aging period (Li et al., 2021). Silva et al. (2018) examined the growth ability of *Lactobacillus* at 4℃-30℃ and reported that the growth of *Lactobacillus* increases with an increase in temperature. As shown in Table 2 in this study, the *Lactobacillus* counts of dry-cured ham at 25℃ were higher than those at 20℃ with an effect on the pH. Microbial growth is facilitated at high pH val-

311 ues. Additionally, the strains used in this study (*D. hansenii* and *P. nalgiovense*) can grow at
312 low pH. The aging conditions of 25℃ and low pH are predicted to be effective for dry-cured
313 ham production (Odeyemi et al., 2020).

314 The salinity in all treatment groups increased with the aging period. Compared with that at
315 week 2, salinity in the S25 and DS20 groups was significantly higher at week 4 ($p < 0.05$) but
316 was not significantly different between weeks 4 and 6 ($p > 0.05$). From week 2 to week 6,
317 salinity significantly increased in the C20, C25, D20, D25, S20, and DS25 groups ($p < 0.05$).
318 This is due to the dehydration of the surface of dry-cured ham over the aging period. During
319 the aging period, water evaporates from the surface, reducing the water content (Chengliang
320 et al., 2020) and the osmotic pressure during curing that causes the absorption of salt and re-
321 lease of water (Martuscelli et al., 2017). Thus, salinity may have decreased due to the reduced
322 water content with an increase in the aging period (Bou et al., 2022). The salinity of dry-
323 cured ham in the 20℃ treatment groups was lower than that in the 25℃ treatment groups. In
324 the C20 and C25 groups, the salinity with 25℃ treatment was significantly higher at week 2
325 ($p < 0.05$) and significantly lower at week 4 ($p < 0.05$) when compared with that with 20℃
326 treatment. The D20, D25, DS20 and DS25 groups showed significantly increased salinity
327 with 25℃ treatment from week 2 to week 6 ($p < 0.05$). In the S20 and S25 group, the salinity
328 with 25℃ treatment was significantly higher than that with 20℃ treatment at weeks 2 and 4
329 ($p < 0.05$). However, the salinity did not vary significantly between the two temperatures at
330 week 6 ($p > 0.05$). The dehydration rate at high temperatures is higher than that at low tem-
331 peratures. Thus, high temperatures increase the release of water (Liu et al., 2019). The low
332 salinity at 20℃, a temperature lower than 25℃, may be due to the relatively decreased re-
333 lease of water during the same aging period. Efforts are ongoing to improve the effects of the
334 salt content in dry-cured ham to enhance nutritional and health-promoting effects (Pinna et al.,

2020). Thus, 20℃ treatment with decreased salinity may be optimal for dry-cured ham production. Among the 20℃ treatment groups with decreased salinity, D20, D25, S20, and S25 treatments may be effective for dry-cured ham production.

The water activity in all treatment groups significantly decreased from week 2 to week 6 over the aging period ($p < 0.05$). During the aging of dry-cured ham, the water content and water activity generally decrease (Toldrá, 2006) due to the effect of dehydration during which the water on the surface evaporates to reduce the moisture and disperse the salinity, resulting in decreased water activity (Vestergaard et al., 2000). In this study, the evaporation of water and the consequent increase in salinity over the aging period. may have decreased the water activity. Next, the effects of different temperatures on the water activity of dry-cured ham were examined. In the D20 and D25 groups, the water activity with 20℃ treatment was significantly higher than that with 25℃ treatment from week 2 to week 4 ($p < 0.05$). However, the water activity increased with 25℃ treatment at week 6 ($p < 0.05$). In the C20, C25, S20, S25, DS20 and DS25 groups, the water activity significantly increased with 20℃ treatment at week 6 ($p < 0.05$). Compared with that in the other groups, the water activity was significantly higher in the D25, S20, and DS20 groups ($p < 0.05$) and significantly lower in the S25 group at week 6 ($p < 0.05$). This may be due to the increased mobility of water molecules at higher temperatures, which decrease the level of bound water (Puri and Khamrui, 2016), and the increased level of NaCl during curing that converted free water to bound water in dry-cured ham (Betiol et al., 2020). The salinity of dry-cured ham at 25℃ was higher than that at 20℃. Temperature and salt content may have decreased the water activity at 25℃. As decreased water activity prevents the growth of harmful bacteria and the release of toxin in food products, 25℃ treatment with low water activity may be optimal for dry-cured ham production (Erkmen and Faruk Bozoglu, 2016). Among the 25℃ treatment groups, the water activi-

ty in the S treatment group was significantly lower than that in the other groups. Thus, S treatment may be effective for dry-aged ham production.

VBN and TBARS

Table 5 shows the measurements of VBN and TBARS according to the aging period of dry-cured ham under different temperature and starter conditions. The VBN content of dry-cured ham significantly and time-dependently increased with the aging period (from week 2 to week 6) across all treatment groups ($p < 0.05$). This may be due to the release of amino acids through protein degradation during the aging period by *D. hansenii* applied to dry-cured ham (Fröhlich-Wyder et al., 2019). As shown in Table 3, the yeast counts increased with the aging period. The growth of *D. hansenii* during the aging period may have increased protein degradation, which results in increased VBN content (Yim et al., 2016). The VBN content of dry-cured ham with 20℃ treatment was lower than that with 25℃ treatment. In the C20, C25, S20 and S25 groups, the VBN content with 25℃ treatment was significantly higher than that with 20℃ treatment ($p < 0.05$) from week 2 to week 6. However, the VBN content did not vary between 20℃ and 25℃ treatments in the D20 and D25 groups ($p < 0.05$). The VBN content in the DS20 and DS25 groups did not vary significantly between 20℃ and 25℃ treatments at week 2 ($p > 0.05$) but significantly increased with 25℃ treatment at weeks 4 and 6 ($p < 0.05$). At week 6, the VBN content in the C20, S25, and DS25 groups was significantly higher than that in the other treatment groups ($p < 0.05$) but was significantly down-regulated in the C20 group ($p < 0.05$). The decreased VBN content of dry-cured ham at lower temperatures can be affected by the decreased growth of microorganisms, which consequently reduced the rate of protein degradation (Kang et al., 2022). The growth rate of yeast at 25℃ was higher than that at 20℃ in this study, which may affect the VBN content. As pro-

383 tein degradation indicates the production of amino acids and volatile compounds with effects
384 on the taste and flavor, the S and DS starter treatments at 25℃ with high VBN and no signif-
385 icant difference from the control group, are predicted to be suitable for dry-cured ham pro-
386 duction (Pérez-Santaescolástica et al., 2018).

387 The TBARS values of dry-cured ham in all treatment groups increased with the aging period.
388 Compared with those at week 2, the TBARS values in the S20 and DS20 groups were signifi-
389 cantly higher at week 4 ($p < 0.05$). However, the TBARS values were not significantly dif-
390 ferent between weeks 4 and 6 ($p > 0.05$). The TBARS values in the D20 and DS25 groups
391 were not significantly different between weeks 2 and 4 ($p > 0.05$). However, the TBARS val-
392 ues at week 6 were significantly higher than those at week 4 ($p < 0.05$). From week 2 to week
393 6, the TBARS values significantly increased in the C20, C25, D25, and S25 groups ($p < 0.05$),
394 which may be due to the production of malondialdehyde (MDA) during the oxidation of pol-
395 yunsaturated fatty acids in meat with an increase in the aging period (Harkouss et al., 2015).
396 Cano-García et al. (2014) applied *D. hansenii* to fermented sausage. The TBARS values of
397 these fermented sausage with *D. hansenii* were lower than those of the control, indicating the
398 inhibitory effect on lipid oxidation. In this study, the TBARS value in the control group was
399 higher than that in the treatment groups. The TBARS value of dry-cured ham with 20℃
400 treatment was lower than that with 25℃ treatment in all groups. From week 2 to week 6, in
401 the C20, C25, DS20 and DS25 groups, the TBARS values with 25℃ treatment were signifi-
402 cantly higher than those with 20℃ treatment ($p < 0.05$). In the D20 and D25 groups, the
403 TBARS values with 25℃ treatment were significantly higher than those with 20℃ treatment
404 at week 2 ($p < 0.05$) but were not significantly different at weeks 4 and 6 ($p > 0.05$). The
405 TBARS values in the S20 and S25 groups were not significantly different between weeks 2
406 and 4 ($p > 0.05$). However, the TBARS value with 25℃ treatment was significantly higher

than that with 20℃ treatment at week 6 ($p < 0.05$). At week 6, the TBARS value in the C25 group was significantly higher than that in the other treatment groups ($p < 0.05$). In contrast, the TBARS values in the S20 and DS20 groups were significantly lower than those in the other treatment groups ($p < 0.05$). This can be attributed to the rate of MDA formation during lipid oxidation, which increased with the increase in aging temperature. Thus, the TBARS values at 25℃ were higher than those at 20℃ (Kang et al., 2022). The TBARS values are reported to be positively correlated with the aldehyde content (Domínguez et al., 2014). The contents of aldehydes (the compounds mainly responsible for the bitterness) in the ham are approximately 50%, which can potentially affect the flavor (Huan et al., 2005). TBARS is used as an indicator of storage stability with the criteria of 2.0 mg MDA/kg. Based on this criterion, 20℃ treatment with decreased TBARS is suitable for dry-cured ham production (Wereńska et al., 2022). Among the 20℃ treatment groups, DS starter treatment, which was associated with significantly decreased TBARS values relative to other treatments, is predicted to be effective for dry-cured ham production.

Conclusions

This study aimed to investigate the microbial composition and physicochemical properties of dry-cured ham after treatment with starters containing *Penicillium nalgiovense* and *Debaryomyces hansenii* separated from Korean fermented foods. The microbial content, which was enumerated on the AC, MRS, MSA, PDA, and YM plates, increased with the aging period. The level of aging at 25℃ was higher than that at 20℃. Among the treatment groups, the microbial counts in the S25 group were higher than those in the other groups, which suggested that dry aging at 25℃ using the *Debaryomyces hansenii* separated from fermented sausage produced in Korea will yield the highest growth rates of *D. hansenii* and *P. nalgiovense* and the highest inhibitory effect on the growth of harmful microorganisms. The water activity significantly decreased with the aging period ($p < 0.05$). Compared with that in the other groups, the water activity was significantly lower in the S25 group ($p < 0.05$). The pH, salinity, VBN, and TBARS values increased with the aging period. Compared with that at 20℃, the pH was lower at 25℃. This may be due to the high counts of *D. hansenii* with protein degradation and deaminase and deamidase activities, promoting the release of nitrogen and the formation of ammonia and consequently lowering the pH. The VBN and TBARS values at 25℃ were higher than those at 20℃. Thus, as the starter strains grow, low pH and low water activity can inhibit the growth of harmful microorganisms. Additionally, high VBN and low TBARS exert a positive effect on the flavor. Thus, S25 treatment is predicted to be the most suitable condition for the production of dry-cured ham.

Conflict of interest

The authors declare no potential conflicts of interest.

Acknowledgments

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Author contributions

Conceptualization: Kim HY. Data curation: Kim SG. Formal analysis: Kim SG. Methodology: Kim SG. Software: Kim SG. Validation: Kim SG, Kim HY. Investigation: Kim HY. Writing - original draft: Kim SG. Writing - review & editing: Kim SG, Kim HY.

Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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622 Table 1. Experimental design for dry-cured ham with starter cultures separated from Korean fermented food

Temperature (°C)	Treatments	Starter culture			
		Commercial starter	*D. hansenii* SMFM2021-D1 (D)	*D. hansenii* SMFM2021-S8 (S)	*P. nalgiovense* SMFM2021-S6
20 25	C (Control)	Inoculated			
20 25	D		Inoculated		Inoculated
20 25	S			Inoculated	Inoculated
20 25	DS		Inoculated	Inoculated	Inoculated

623 Table 2. Change in the counts of aerobic bacteria, *Lactobacillus*, and *Staphylococcus* of dry-
624 cured ham with various starters and temperatures during the aging period.

Traits	Treatment	Aging period (Weeks)				SEM
		0	2	4	6	
Aerobic bacteria (log CFU/g)	C20	6.86^{c}	7.57^{CDb}	7.71^{Eb}	8.25^{Da}	0.17
	C25	6.86^{c}	8.63^{Bb}	8.63^{Cb}	9.43^{Ba}	0.26
	D20	6.86^{c}	7.22^{Db}	7.62^{Ea}	7.74^{Ea}	0.09
	D25	6.86^{b}	9.69^{Aa}	9.71^{ABa}	9.63^{Ba}	0.35
	S20	6.86^{c}	7.42^{CDb}	7.48^{Eb}	8.27^{Da}	0.16
	S25	6.86^{b}	9.50^{Aa}	9.83^{Aa}	9.93^{Aa}	0.26
	DS20	6.86^{d}	7.75^{Cc}	8.23^{Db}	8.69^{Ca}	0.20
	DS25	6.86^{c}	9.46^{Ab}	9.50^{Bb}	9.62^{Ba}	0.32
SEM			0.20	0.18	0.12	
Lactobacillus spp. (log CFU/g)	C20	7.77^{c}	8.33^{Bb}	8.83^{Ca}	8.98^{Ca}	0.14
	C25	7.77^{c}	8.36^{Bb}	8.64^{Cb}	9.37^{Ba}	0.16
	D20	7.77^{a}	6.21^{Cc}	6.57^{Ebc}	7.14^{Eb}	0.18
	D25	7.77^{c}	9.00^{ABb}	9.79^{Aa}	9.72^{Aa}	0.22
	S20	7.77^{a}	6.41^{Cd}	7.10^{Dc}	7.56^{Db}	0.11
	S25	7.77^{c}	9.43^{Ab}	9.66^{Aab}	9.96^{Aa}	0.22
	DS20	7.77^{a}	6.67^{Cc}	6.50^{Ec}	7.36^{EDb}	0.13
	DS25	7.77^{b}	9.13^{Ba}	9.22^{Ba}	9.64^{ABa}	0.16
SEM			0.22	0.24	0.18	
Staphylococcus spp. (log CFU/g)	C20	2.26^{d}	8.60^{Bb}	9.19^{Ba}	7.80^{Dc}	0.78
	C25	2.26^{c}	8.37^{Bb}	8.36^{Cb}	9.45^{Ba}	0.68
	D20	2.26^{c}	5.96^{Cb}	6.11^{Eb}	7.69^{Da}	0.60
	D25	2.26^{b}	9.53^{Aa}	9.63^{Aa}	9.74^{ABa}	0.96
	S20	2.26^{d}	6.53^{Cc}	7.40^{Db}	8.26^{Ca}	0.70
	S25	2.26^{b}	9.59^{Aa}	9.67^{Aa}	9.85^{Aa}	0.71
	DS20	2.26^{d}	6.53^{Cc}	7.14^{Db}	7.76^{Da}	0.61
	DS25	2.26^{b}	9.48^{Aa}	9.59^{Aa}	9.59^{ABa}	0.78
SEM			0.26	0.24	0.17	

625 $^{a-d}$ Means in the same row with different letters are significantly different ($p < 0.05$).
626 $^{A-E}$ Means in the same column with different letters are significantly different ($p < 0.05$).

C: commercial starter culture; D, *Debaryomyces hansenii* of *Doenjang* produced in Korea and *Penicillium nalgiovense* of fermented sausage; S: *D. hansenii* of fermented sausage and *P. nalgiovense* of fermented sausage; DS: D + S; SEM, standard error of mean (n=80); CFU, colony forming units.

Table 3. Change in the counts of mold and yeast of dry-cured ham with various starters and temperatures during the aging period.

Traits	Treatment	Aging period (Weeks)				SEM
		0	2	4	6	
Mold (log CFU/g)	C20	5.79^{c}	6.98^{Db}	7.12^{Eb}	7.45^{Da}	0.20
	C25	5.79^{b}	7.65^{ABCDa}	7.72^{CDa}	7.71^{Ca}	0.22
	D20	5.79^{d}	7.54^{BCDc}	7.80^{CDb}	8.11^{ABa}	0.21
	D25	5.79^{b}	8.10^{ABa}	8.17^{Aa}	8.19^{ABa}	0.34
	S20	5.79^{c}	7.25^{CDb}	7.68^{Db}	7.69^{Ca}	0.22
	S25	5.79^{b}	7.96^{Aa}	8.04^{ABa}	8.28^{Aa}	0.31
	DS20	5.79^{c}	7.75^{ABCDb}	7.88^{BCDb}	8.17^{ABa}	0.29
	DS25	5.79^{b}	7.88^{ABCa}	7.92^{BCa}	8.02^{Ba}	0.29
	SEM		0.11	0.06	0.06	
Yeast (log CFU/g)	C20	3.41^{c}	6.56^{Bb}	6.97^{Da}	7.25^{Ea}	0.34
	C25	3.41^{c}	7.36^{Ab}	8.00^{ABa}	7.83^{Cab}	0.47
	D20	3.41^{c}	7.34^{Ab}	7.81^{BCa}	7.88^{Ca}	0.41
	D25	3.41^{c}	7.16^{ABb}	8.18^{Aa}	8.23^{Aa}	0.54
	S20	3.41^{c}	6.56^{Bb}	7.81^{BCa}	7.41^{Da}	0.46
	S25	3.41^{c}	7.00^{ABb}	7.95^{ABCa}	8.12^{ABa}	0.45
	DS20	3.41^{b}	7.60^{Aa}	7.66^{Ca}	7.76^{Ca}	0.52
	DS25	3.41^{c}	6.97^{ABb}	8.10^{ABa}	8.07^{Ba}	0.54
	SEM		0.08	0.08	0.06	

$^{a-d}$ Means in the same row with different letters are significantly different ($p < 0.05$).
$^{A-E}$ Means in the same column with different letters are significantly different ($p < 0.05$).
C: commercial starter culture; D, *Debaryomyces hansenii* of *Doenjang* produced in Korea and *Penicillium nalgiovense* of fermented sausage; S: *D. hansenii* of fermented sausage and *P. nalgiovense* of fermented sausage; DS: D + S; SEM, standard error of mean (n=80); CFU, colony forming units.

641 Table. 4. Change in pH, salinity, and water activity of dry-cured ham with various starters
642 and temperatures during the aging period.

Traits	Treatment	Aging period (Weeks)				SEM
		0	2	4	6	
pH	C20	5.73^{d}	6.06^{Db}	6.02^{Fc}	6.29^{Da}	0.04
	C25	5.73^{d}	5.83^{Fc}	5.92^{Gb}	6.01^{Ha}	0.04
	D20	5.73^{b}	6.45^{Aa}	6.49^{Ba}	6.47^{Ca}	0.10
	D25	5.73^{c}	6.14^{Cb}	6.18^{Da}	6.20^{Fa}	0.04
	S20	5.73^{c}	6.22^{Bb}	6.22^{Db}	6.72^{Aa}	0.07
	S25	5.73^{d}	5.89^{Ec}	6.05^{Eb}	6.11^{Ga}	0.03
	DS20	5.73^{d}	6.53^{Ac}	6.60^{Ab}	6.61^{Ba}	0.09
	DS25	5.73^{c}	5.85^{Fb}	6.22^{Ca}	6.22^{Ea}	0.02
SEM			0.04	0.03	0.04	
Salinity (%)	C20	0.60^{d}	3.97^{Bc}	5.44^{Bb}	5.76^{ABa}	0.41
	C25	0.60^{d}	4.64^{Ac}	5.36^{Cb}	5.85^{Aa}	0.49
	D20	0.60^{d}	2.75^{CDc}	4.65^{Db}	5.11^{Da}	0.37
	D25	0.60^{d}	4.03^{Ac}	5.13^{Bb}	5.98^{Aa}	0.41
	S20	0.60^{d}	2.49^{Dc}	4.25^{Eb}	5.25^{CDa}	0.36
	S25	0.60^{c}	4.11^{Bb}	5.73^{Ca}	5.17^{Da}	0.40
	DS20	0.60^{c}	2.82^{Cb}	5.43^{Ba}	5.51^{BCa}	0.42
	DS25	0.60^{d}	4.75^{Bc}	5.05^{Ab}	5.94^{Aa}	0.53
SEM			0.13	0.08	0.06	
Water activity (a_w)	C20	0.97^{a}	0.89^{Cb}	0.84^{Cc}	0.74^{Cd}	0.02
	C25	0.97^{a}	0.87^{Eb}	0.77^{Gc}	0.72^{Dd}	0.03
	D20	0.97^{a}	0.92^{Bb}	0.82^{Dc}	0.76^{Bd}	0.02
	D25	0.97^{a}	0.88^{Db}	0.81^{Ec}	0.78^{Ad}	0.02
	S20	0.97^{a}	0.92^{Ab}	0.84^{Bc}	0.78^{Ad}	0.02
	S25	0.97^{a}	0.86^{Eb}	0.79^{Fc}	0.71^{Ed}	0.03
	DS20	0.97^{a}	0.92^{ABb}	0.85^{Ac}	0.78^{Ad}	0.02
	DS25	0.97^{a}	0.89^{CDb}	0.78^{Fc}	0.75^{Cd}	0.03
SEM			0.01	0.01	0.01	

643 $^{a-d}$ Means in the same row with different letters are significantly different ($p < 0.05$).
644 $^{A-H}$ Means in the same column with different letters are significantly different ($p < 0.05$).

645 C: commercial starter culture; D, *Debaryomyces hansenii* of *Doenjang* produced in Korea
646 and *Penicillium nalgiovense* of fermented sausage; S: *D. hansenii* of fermented sausage and
647 *P. nalgiovense* of fermented sausage; DS: D + S; SEM, standard error of mean (n=80).

Table 5. Change in volatile basic nitrogen (VBN) and thiobarbituric acid reactive substances (TBARS) of dry-cured ham with various starters and temperatures during the aging period

Traits	Treatment	Aging period (Weeks)				SEM
		0	2	4	6	
VBN (mg/%)	C20	3.02^{d}	9.65^{Dc}	11.29^{Fb}	14.37^{Da}	1.27
	C25	3.02^{d}	15.40^{Ac}	17.66^{Ab}	19.30^{Aa}	1.96
	D20	3.02^{d}	8.62^{Dc}	14.37^{CDb}	17.66^{Ba}	1.67
	D25	3.02^{d}	11.50^{CDc}	13.76^{DEb}	17.25^{Ba}	1.59
	S20	3.02^{d}	11.70^{CDc}	13.14^{Eb}	16.02^{Ca}	1.48
	S25	3.02^{d}	12.73^{Bc}	14.99^{Cb}	19.51^{Aa}	1.83
	DS20	3.02^{d}	11.09^{Bc}	16.17^{Bb}	17.56^{Ba}	1.48
	DS25	3.02^{d}	12.73^{Bc}	17.66^{Ab}	19.10^{Aa}	1.91
SEM			0.41	0.42	0.34	
TBARS (mg MDA/kg)	C20	0.22^{d}	0.29^{Cc}	0.36^{BCb}	0.40^{CDEa}	0.02
	C25	0.22^{d}	0.39^{Ac}	0.57^{Ab}	0.96^{Aa}	0.07
	D20	0.22^{c}	0.34^{Bb}	0.34^{CDb}	0.39^{DEa}	0.02
	D25	0.22^{d}	0.26^{Dc}	0.35^{CDb}	0.46^{CDa}	0.02
	S20	0.22^{c}	0.28^{CDb}	0.32^{Da}	0.34^{Fa}	0.01
	S25	0.22^{d}	0.29^{Cc}	0.34^{CDb}	0.49^{Ca}	0.03
	DS20	0.22^{c}	0.26^{CDb}	0.28^{Ea}	0.29^{Fa}	0.01
	DS25	0.22^{c}	0.35^{Bb}	0.38^{Bb}	0.77^{Ba}	0.05
SEM			0.01	0.02	0.04	

$^{a-d}$ Means in the same row with different letters are significantly different ($p < 0.05$).
$^{A-F}$ Means in the same column with different letters are significantly different ($p < 0.05$).
C: commercial starter culture; D, *Debaryomyces hansenii* of *Doenjang* produced in Korea and *Penicillium nalgiovense* of fermented sausage; S: *D. hansenii* of fermented sausage and *P. nalgiovense* of fermented sausage; DS: D + S; SEM, standard error of mean (n=80); MDA, malondialdehyde.