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ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title	Effect of Modified Atmosphere Packaging (MAP) Varying in CO_2 and N_2 Composition on Quality Characteristics of Dry Fermented Sausage During Refrigeration Storage
Running Title (within 10 words)	Effect of MAP on Quality Characteristics of DFS
Author	Ammara Ameer[1], Semeneh Seleshe[1], Suk Nam Kang[1]
Affiliation	Department of Animal Resource, Daegu University, Gyeongsan 38453, Korea
Special remarks – if authors have additional information to inform the editorial office	
ORCID (All authors must have ORCID) https://orcid.org	Ammara Ameer (https://orcid.org/0000-0002-8110-2433) Semeneh Seleshe (https://orcid.org/0000-0002-2599-393X) Suk Nam Kang (https://orcid.org/0000-0002-9230-3070)
Conflicts of interest List any present or potential conflict s of interest for all authors. (This field may be published.)	The authors declare no potential conflict of interest.
Acknowledgements State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available. (This field may be published.)	This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (2017R1A2B201277).
Author contributions (This field may be published.)	**Conceptualization:** Suk Nam Kang **Data curation:** Ammara Ameer, Semeneh Seleshe **Formal analysis**: Ammara Ameer, Semeneh Seleshe **Methodology**: Ammara Ameer, Semeneh Seleshe, Suk Nam Kang **Software:** Ammara Ameer, Semeneh Seleshe, Suk Nam Kang **Validation:** Suk Nam Kang **Investigation:** Suk Nam Kang **Writing-original draft**: Ammara Ameer, Semeneh Seleshe, Suk Nam Kang **Writing-review & editing:** Ammara Ameer, Semeneh Seleshe, Suk Nam Kang
Ethics approval (IRB/IACUC) (This field may be published.)	This article does not require IRB/IACUC approval because there are no human and animal participants.

CORRESPONDING AUTHOR CONTACT INFORMATION

For the <u>corresponding</u> author (responsible for correspondence, proofreading, and reprints)	**Fill in information in each box below**
First name, middle initial, last name	Suk Nam Kang
Email address – this is where your proofs will be sent	whitenightt@hanmail.net
Secondary Email address	sk-kang@daegu.ac.kr
Postal address	Department of Animal Resource, Daegu University, Gyeongsan 38453, Korea
Cell phone number	+82-010-3783-7718
Office phone number	+82-53-850-6726
Fax number	+82-53-850-6729

Effect of CO_2 and N_2 Composition in Modified Atmosphere Packaging (MAP) on Quality Characteristics of Dry Fermented Sausage During Refrigeration Storage

Running title: Effect of MAP on Quality Characteristics of DFS

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Abstract

The current study investigated the effects of the most suitable modified atmosphere packaging (MAP) on the physicochemical, microbiological, and sensory properties of fermented dry sausages during 45 days of refrigeration (4℃) storage period. Treatments were vacuum-packed (control), 25%CO_2/75%N_2 (MAP1), 50%CO_2/50%N_2 (MAP2), 70%CO_2/30%N_2 (MAP3) and 100% CO_2 (MAP4). All MAP samples regardless of their CO_2 composition significantly ($p<0.05$) decreased in pH, a_w, total plate count (TPC), and LAB count values as compared to the vacuum-package during storage. The *Enterobacteriaceae* count in all MAP packaging was significantly ($p<0.05$) lower than the vacuum-packed samples and counts in MAP3 and MAP4 samples were markedly ($p < 0.05$) lower than all other treatments in prolonged storage of 15 and 45 days. Based on the TBARS content at day 15 and 30 storage time, treatments are ranked as follows: vacuum-packed >MAP1 >MAP2 >MAP3>MAP4. The a* value of MAP4 was higher than all other treatments. In the final storage days, no variation was exhibited ($p>0.05$) among treatments in lactic acid aroma and sourness, and MAP2 samples had the lowest ($p<0.05$) overall acceptability. The use of MAPs with an increase in the CO_2 from MAP1 to MAP4 samples can help in better microbial inhibition than vacuum package, and 70%CO_2/30%N_2 (MAP3) and 100% CO_2 (MAP4) were effective to maintain several quality parameters (a_w, pH, microbial inhibition, stability against lipid oxidation, and instrumental color traits) and extend the shelf life of dry fermented sausage.

Keywords: modified atmosphere packaging, microbiological, physicochemical, and sensory properties, vacuum-packed.

Introduction

Meat and meat byproducts are considered as integral part of human diet due to their nutritional properties such as protein source, fatty acid profile, minerals, vitamins and other bioactive compounds and potential booster for growth and development. These products are frequently contaminated with spoilage, pathogenic bacteria and other micro-organisms (viruses and parasites) causing food borne illness/diseases by *Escherichia coli*, *Staphylococcus aureus, L. monocytogenes*, *Clostridium perfringens* and *Salmonella spp*. Thus, food industries have been developing alternative techniques of meat bio preservation (Aymerich et al., 2008).

Now days, refrigeration, vacuum packing (VP), and modified atmosphere packaging (MAP) are all being utilized more and more to increase the shelf life of meat products for distribution and retail sale (Kim et al., 2014; Stiles, 1991). Beyond traditional protection features, modern food packaging has several advantages (Han, 2005). Modified atmospheric packaging (MAP) is one of the preservation and packaging solutions being employed to meet customer demand for food that is safe, additive-free, and nutritious (Esturk and Ayhan, 2009). Cann (1984) and Gokoglu et al. (2010) defined modified atmosphere packaging (MAP) as replacing the air in a food package with a different mixture of gases, often a combination of nitrogen, carbon dioxide, and oxygen. One of the technological requirements for meeting customer demands is to extend the shelf life of meat products (Adab et al., 2020). As a result, MAP paired with cold storage can improve the quality and extend shelf life of minimally processed foods (Church and Parsons 1995; Farberet al., 2003). As marketing sliced ready-to-eat meat products have gained popularity in recent years, the use of MAP and chill storage for meat products such as salami may considerably preserve the quality and increase the shelf life (Esturk and Ayhan, 2009). Modified atmosphere packaging (MAP) utilizes

63 different combinations of gases to improve the shelf life of meat and meat products (Ö zogul et al.,
64 2004). Because of its antibacterial properties, carbon dioxide (CO_2) is the most key component of
65 the gas mixtures in MAP (Adab et al., 2020; Farber, 1991). Carbon dioxide-enriched atmospheres
66 prevent the growth of unwanted microbes, and nitrogen gas, while inert to meat products, is used
67 as a filler to reduce the concentrations of more active gases (Fernandez- Fernandez et al., 2002;
68 Kim et al., 2014; Rubio et al., 2008).

69 The packaging methods of aerobic, vacuum, and modified atmosphere affected the color, lipid
70 oxidation, pH, microbial counts, and texture profiles of dry-cured meat products differently (Aksu
71 et al., 2005; Cilla et al., 2006; Kim et al., 2014). Oxygen, carbon dioxide and nitrogen are used in
72 different combinations and many studies related to their composition in MAP have been done by
73 meat scientists to extend the shelf-life of meat. However, for the prolonged shelf-life of MAP, the
74 findings are inconsistent (Samelis and Georgiadou, 2000; Pexara et al., 2002; Santos et al., 2005).
75 Therefore, according to Møller et al. (2000), optimizing the gas composition is critical for product
76 quality and safety. The determination of the shelf life and its validation are very important for the
77 microbiological safety of dry fermented sausages. Moreover, maintaining the quality associated
78 physiochemical attributes and sensory characteristics of the product is important to address the
79 consumer demands. The objective of this study was to determine the quality changes and shelf life
80 of fermented dry sausages packed under varying modified atmospheres. The current study
81 investigated the effects of the most suitable modified atmosphere packaging (MAP) on the
82 important quality characteristics: microbiological, physiochemical, and sensory properties of
83 fermented dry sausages during 45 days of refrigeration (4°C) storage period.

84

Martials and methods

Dry Fermented Sausages Manufacture

The prototype meat processing centre at Daegu University's Animal Resources Department produced low-temperature dry fermented sausages. Fresh pork lion was purchased from the commercial market of Geyongsan, Korea which were vacuum packaged. The back fat was thawed for 24 h at 4℃. The lean meat was preserved in the refrigerator for later use after cutting the connective tissues and extra fat. With the use of a 3- 4 mm plate, chilled pork and pig fat were cut into small cubes and minced twice in a meat mincer (M-12S, Hankook fujee Industries Co., Ltd., Suwon, Korea). Ground pork (65%), pig fat (21.5%), ice water (10%), NPS (97:3, a blend of sodium chloride and nitrite) (0.34%), NaCl (1.70%), sugar (0.45%), glucose (0.45%), sodium ascorbate (0.20%), and sausage seasonings (0.36%) are all included in the basic sausage formulation. For the start of the fermentation, one ml/kg (v/w) mixed starter cultures of *Lactobacillus sakei* and *Staphylococcus xylosus* were added and properly blended using a rotary slice cutter (SF-2002, Samwoo Industry Co., Korea).Each starter culture had approximately 6 Log CFU/g, and the intended suspension put into the sausage batter was one mL/kg (v/w). With a vacuum filling machine (RVF 327, Düker-REX Fleischereimaschinen gmbh, Germany), the batter was filled into collagen casings (IKJIN Co. Ltd., Seoul, Korea), 2.4 cm diameter and 15 cm length. The sausages were fermented and ripened in a digital chamber system with a temperature and RH control unit (SMK-2000SL, Metatek, Korea). The temperature was kept at 23℃ for the first seven days of fermentation, and the relative humidity (RH) was regulated between 90 to 95 %. Following that, the ripening process was conducted for 28 days (following the fermentation process) at 15℃ (the temperature was gradually reduced from 23℃), with RH varying between 70 to 75%.

Modified atmosphere packaging (MAP) and Sampling

After the completion of the ripening process, sausages were packed in their respective treatments as follows. Nylon/PE bags (80 µm thick: 15 µm for Nylon and 65 µm for PE) (Gasung Pak Co., Ltd., Gyeonggi, Korea) with O_2 permeability of 9.5 ml $O_2/m^2/24$ h at 0℃, 0.98 g/cm^3 density was used in the current experiment. Five lots of sausage/bag and 8 packages for each treatment were used. The intended gases mixtures were purchased from a local gas supplier (Deokyang Co., Ltd. Ulsan, South Korea) in cylinders having an injection pipe and a gauge for the gas control system. One of the treatments was solely packed in a vacuum (Model 19/S, Röscherwerke gmbh, Hanover, Germany) and used as a control treatment, and the other four treatments were sealed after flushing with the following gas mixtures: MAP1, 25% CO_2/75% N_2; MAP2, 50% CO_2/50% N_2; MAP3, 70% CO_2/30% N_2; MAP4, 100% CO_2. Residual gases were initially removed with vacuum and the sausages to gas volume ratio in the MAP samples were 1/1 (Gokoglu et al., 2010). Storage study was conducted for a total of 45 days and sampling was done at 1, 15, 30, and 45 days of storage time for physiochemical analyses [a_w, pH, color, VBN and TBARS contents, and texture profile analysis (TPA)], microbial quality, and sensory characteristics of sausages samples. For each analysis time and batch, two packages of sausage were withdrawn, and each analysis was performed in triplicates.

Physiochemical analysis

After homogenizing three grams of a sample with 30 ml of distilled water in a homogenizer (Model Polytron® PT 2500 E Stand Dispersion Device, Kinematica AG, Switzerland), the pH values of the samples were determined. A digital pH meter (Mettler Toledo, Columbus, Ohio,

129 USA) was used for reading the values. After slicing the core of the samples into 4 mm cubes, water
130 activity (a_w) was assessed using a_w measurement apparatus (Lab master aw, Novasina AG,
131 Switzerland). Determination of volatile basic nitrogen (VBN) contents was performed according
132 to the Conway micro diffusion method (Conway, 1950), and total VBN values were expressed in
133 mg%. Analysis of the **2**-thiobarbituric acid reactive substances (TBARS), was conducted using
134 the method indicated by Pikul et al. (1989), and the content was calculated as mg malonaldehyde
135 equivalent per kg (mg MA/kg) of sample.

136 Instrumental color analysis was performed from the inner surface of the sliced sausages using
137 a portable chromameter (CR-400, Konica Minolta, NJ, USA). Prior to the analysis, the device was
138 calibrated using a standard calibration plate (Y=92.80, x=0.3136, and y=0.3194) and five readings
139 per sample were taken for L* (lightness), a*(redness), b*(yellowness). The viewing/illuminating
140 apertures were 11 mm/8 mm (8 mm) and 3 mm/3 mm, (3 mm) respectively. Average values were
141 calculated from five readings and expressed as L*, a*, and b* based on the CIE color system (CIE,
142 1976).

143

144 **Microbial quality analysis**

145 Microbiological quality characteristics were conducted by enumeration of total plate count
146 (TPC), lactic acid bacteria (LAB), *Enterobacteriaceae*, *E. Coli* O157:H7, and *Salmonella* spp.
147 Each dry fermented sausage sample was taken aseptically using a sterile spoon, combined with
148 225 mL of 0.1 % peptone water, and homogenized for 2 minutes in a Stomacher Lab Blender
149 (model 400 Circulator, Seward Laboratory, USA). Diluting one ml of the material in nine ml of
150 0.1% sterile peptone water yielded a series of 10-fold dilutions (10^1 to 10^7) After incubating

151 samples with their appropriate selective medium, enumerations of the developed colony of
152 microorganisms were undertaken. For total plate counts, LAB, *Enterobacteriaceae*, *E. Coli*
153 O157:H7, and *Salmonella* spp. counts, the media used were Plate Count Agar (mbcell, kisanbio
154 Co., Ltd, Seoul, Korea), Lactobacillus MRS agar (Difco, USA), Violet Red Bile Glucose Agar
155 (VRBGA) (Kisanbio Co., Ltd, Seoul, Korea), MacConkey Plates and Bismuth Sulfite Agar
156 (Kisanbio Co., Ltd, Seoul, Korea) respectively and appropriate dilutions were incubated for 48
157 hours in triplicate at 37℃ (Drosinos et al., 2005). The average number of colonies per countable
158 plate was determined, and the total number of colonies per gram (CFU/g) were calculated before
159 the data was presented in log CFU/g.

160 **Sensorial analysis**

161 Color, lactic acid aroma, sourness, and overall acceptability of dry fermented sausages were
162 all assessed using descriptive sensory analysis (scoring method). The sensory evaluation was
163 performed by seven experienced panelists who are researchers and students in Daegu university's
164 department of animal resources, meat science laboratory. Ahead of the actual evaluation session,
165 the panelists were trained on sensory characteristics of dry fermented sausages using five-point
166 scale. The intensity scale used to define the quality attributes ranged from 1 to 5 that corresponds
167 to the sensory attributes of samples as follows "extremely pale to very dark," for color, "very weak
168 fermented aroma to very strong fermented aroma," for aroma, and "light sour to strong sour" for
169 sourness. Three different types of commercial dry fermented sausage were used during training
170 session, and panel were given 3 slices (5 mm thickness) of samples on white plastic plates during
171 the judgment. To avoid carryover influences, all samples were individually labeled with three
172 digits and provided at random. The sample consisted of five series. Each series was made up five
173 batches manufacturing with the respective MAP gas mixture (0, 25,50,70 and 100% CO_2). Before

each sample was examined, the panel were provided cold water to rinse their mouths. The sensory analysis method was certified by the life management committee of Daegu University and given an IRB number (1040621-201905-HR-004-02).

Statistical analysis

Statistical data were analyzed by using Analysis Variance (ANOVA) for the three replicates. SAS software version 9.4 (SAS Institute, Cary, NC, USA) was used for the analysis, and a significance level of $p < 0.05$ was applied for all evaluations. Differences among the means were compared according to Duncans's Multiple Range Test.

Results and discussion

The effect of packaging conditions on pH and a_w characteristics

Effect of modified atmosphere packaging (MAP) on pH and a_w of dry fermented sausages during storage period is indicated in Table 1. Modified atmospheric packaging varying in gas composition had a significant ($p < 0.05$) effect on the pH value of samples during storage. Similarly, Gokoglu et al. (2010) reported a significant difference in pH among the modified atmospheres during the storage. In all storage time, the batches in MAP samples presented significantly lower pH values than the vacuum-packed, control samples. The finding agrees with Kim et al. (2014) who observed a significantly lower pH in the MAP samples than that in the VP of dry-cured pork neck products at the given storage time. During the storage study, the pH value of MAP packages decreased as CO_2 concentration increased from 25% to 100% CO_2 in MAP1-MAP4 samples. The current result

agrees with Cilla et al. (2006) and Martínez et al. (2005) reports that the increase in concentrations of CO_2 lowered pH in dry-cured meat products. The initial decrease of pH is due to CO_2 absorption. As possible reason, carbonic acid, H_2CO_3, may have been produced from absorbed carbon dioxide by meat (Dixon and Kell, 1989), increased corresponding to the increase in CO_2 concentration in the MAP treatment and became responsible for the pH variation. Gokoglu et al. (2010) assumed that the increase in LAB count caused for decrease in pH values. In the current investigation, the LAB, which are responsible for lactic acid generation, showed a progressive decline in the MAP treatments as CO2 concentration increased (Table 2), but the pH also fell, indicating that the pH value was unaffected by LAB. The storage time had a profound ($p < 0.05$) effect on the pH value of all MAP treatments and vacuum-packed batches. The pH value notably decreased in all treatments as the storage time extended. This may be associated with lower LAB activity. Muhlisin et al. (2014) documented the increase in pH values of all groups MAP varying in gas composition as storage time increased after studying the effect of MAP on the shelf-life of *Longissimus dorsi*. In the current study, the LAB was not completely inhibited in all treatments that lactic acid production at a steady rate and its accumulation as the storage time prolonged may be contributed for the decline in the pH. Houben and Van-Dijk (2001) for sliced hams, Pexara et al. (2002) for cured turkey fillets, Kim et al. (2014) for dry-cured pork neck products, and Muhlisin et al. (2014) for Longissimus dorsi of Korean native black pigs during storage all testified the decrease of pH values in MAP products with extended storage time.

The chemical reactions and the survival of spoilage and pathogenic microorganisms rely on the water activity of the food products. Measurement of the water activity, therefore, increasingly important to determine the shelf-stability of meat products. Water activity (a_w) of treatments significantly varied on 1, 30 and 45 days of storage time. In the indicated days, all the MAP

218 treatments regardless of their CO_2 composition exhibited the lower a_w as compared to vacuum-
219 packed treatment. The current finding agrees with Kim et al. (2014) that the a_w values of the MAP
220 samples were significantly lower than those of the VP samples after 30, 60, and 90 days of
221 storage. At day 1 of storage, MAP4 had the lowest a_w than other MAP treatments. When the
222 storage time prolonged to 30 and 45 days, MAP3 and MAP4 presented similarly lowest a_w than
223 all the treatment. The decrease in a_w in MAP treatments was corresponding with the increase in
224 CO_2. After application of MAP to reduce the ripening time of dry-cured boneless hams, Wang
225 (2001) indicated that the stability of microbiological quality is attributed to low water activity in
226 dry-cured ham and bacteriostatic effect of modified atmospheres. Storage time had a significant
227 effect on the a_w of all MAP treatments regardless of their gas composition and the values noticeably
228 decreased as the storage time prolonged. In the vacuum-packed samples, a_w activity increased at
229 day 30 storage compared to earlier storage of days 1 and 15. However, it decreased again at 45
230 days of storage. Similarly, Rubio et al. (2006) observed a decrease in a_w values of sliced dry-
231 cured meat under both VP and MAP conditions when the storage time was extended.

232

233 **The effect of packaging conditions on microbiological characteristics**

234 Effect of modified atmosphere packaging (MAP) varying in CO_2 and N_2 composition
235 compared to the control, vacuum packaging, on microbial quality of dry fermented sausages during
236 storage (45 days) indicated in Table 2. All MAP batches showed a lower total count (TPC) than
237 vacuum-packed treatments throughout the storage period and the effect was significant ($p < 0.05$)
238 at day 1 and day 30 of the storage time. Microbial inhibition was effective as CO_2 composition
239 grew from 25% to 100% in MAP1-MAP4 treatments throughout these days, and the effect was

significantly (p 0.05) higher in MAP3 and MAP4 than other MAP treatments and the vacuum packaged control. Similarly, Gokoglu et al. (2010) reported the lowest count in samples packed under 100% CO_2 after studying the effect of modified atmosphere packaging on the quality and shelf life of frankfurter type-sausages. Kim et al. (2014) observed significantly lower total aerobic bacteria and LAB counts in MAP samples than those of the VP samples. It has been stated by Sorheim et al. (2004) that a concentration of 20–30% CO_2 was sufficient to prevent the growth of aerobic spoilage bacteria. In the current study, the effective inhibition could be achieved starting with the use of 25%, that is MAP1, and confirmed to the earlier finding; however, the effect gets pronounced as the concentration of CO_2 increased. The progressive reduction in water activity along with the increase in CO_2 of MAP treatments could be the possible reason for the decline in microbiological counts presented in the present study (Table 2). Storage time has a profound effect on the TPC of all MAP treatments and the control. TPC substantially increased ($p < 0.05$) as the duration time extended in all treatment batches.

Lactic acid bacteria (LAB) count showed a similar trend to TPC. There is a noticeable ($p < 0.05$) difference in LAB counts of MAP treatments varying in their gas mixture across the storage period (Table 2). In all the storage days, vacuum-packed samples had a significantly highest (LAB) count than all MAP packages regardless of their gas compositions. Similarly, Kim et al. (2014) documented that the LAB counts in MAP samples were significantly lower than those of the VP samples in dry-cured pork neck products. According to Aksu et al. (2005), MAP with CO_2 and N_2 greatly inhibited the growth of LAB. The prominent inhibitory effect for LAB starts in MAP1 packages, and the effect increased as the increment of CO_2, and a significant highest count is exhibited in MAP4 with 100% CO_2. It was recognized that the rate of carbon dioxide in the gas mixture affected the growth of lactic acid bacteria (Gokoglu et al., 2010). Previous investigations

of Blickstad and Molin (1984), Borch et al. (1996), and Metaxopoulos et al. (2002) have reached similar conclusions. In contrast, some researchers exhibited that modified atmosphere packaging did not have a growth rate hindering effect on the LAB compared to vacuum packaging (Samelis and Georgiadou, 2000; Pexara et al., 2002). Gokoglu et al. (2010) reported that a higher aerobic spoilage bacteria inhibition effect was observed when the gas mixture contained over 30% CO_2. The current finding, inhibition of lactic acid bacteria was achieved starting from 25% CO_2 composition which is comparable with the previous study. Storage time had a noticeable ($p < 0.05$) effect on LAB counts of all MAP treatments and the control, vacuum packaging. In all the batches, the count substantially decreased as storage time extended. However, dry fermented sausage is manufactured with deliberate addition of the LAB to achieve the important characteristics required for dry fermented sausages, further activity of the LAB is not required after products completed the ripening process to maintain the quality. In this regard, the decrease in LAB counts by the application of MAP and the storage time can be appreciated in keeping the quality of the products.

Evaluation of the microbiological quality and safety of food products are commonly carried out by determination of indicator microorganisms' levels and the one is *Enterobacteriaceae* (Moore et al., 2002; EFSA, 2010). The *Enterobacteriaceae* are a large family of facultative anaerobic, gram-negative bacilli that inhabit the intestines of many animal species. This family includes pathogenic *Escherichia*, *Salmonella serovars*, and *Klebsiella* species (Gwida et al., 2014; Ruby and Ingham, 2009). The high prevalence of *Enterobacteriaceae* could be attributed to inadequate sanitary conditions and poor general hygiene. MAP treatments varying in their gas mixture showed a significant ($p<0.05$) effect on *Enterobacteriaceae* counts at 1, 15 45 days of storage days (Table 2). On day 1, vacuum packing, MAP1, MAP2 had similar higher counts than MAP3 and MAP4 batches. In prolonged storage of 15 and 45 days, however, all MAP packaging

resulted in a significantly lower *Enterobacteriaceae* count than the vacuum-packed samples and counts in MAP3 and MAP4 samples were markedly ($p < 0.05$) lower than all other treatments during the stated period. According to Kim et al. (2014), MAP with a combination of CO_2 and N_2 inhibited the growth of LAB and *Enterobacteriaceae*. All MAP packaging presented a decrease in the counts at the final storage time of 45 days compared to day 1 storage time when the count increased for vacuum packaging samples.

Counts for *E. Coli* O157:H7 and *Salmonella* spp. was exhibited during day 1 in all MAP treatments and vacuum-packed batch. *E. Coli* O157:H7 count ranged from 1.43 log CFU/g in MAP4 to 2.71 log CFU/g in vacuum-packed samples, and the range for *Salmonella* spp. count was 2.55 log CFU/g in MAP3 to 2.87 log CFU/g in vacuum-packed batch. Gram-negative bacteria are generally more sensitive to CO_2 than Gram-positive bacteria (Church, 1994) because most Gram-positive bacteria are facultative or strict anaerobes (Gill and Tan, 1980). Despite, the variation was not significant, vacuum-packed samples resulted in higher *E. Coli* O157:H7 and *Salmonella* spp. counts than all MAP treatments at early 1-day storage time. Vacuum-packed samples were exhibited to have higher *Enterobacteriaceae* (Table 2) which includes *E. Coli* O157:H7 and *Salmonella* spp. at day one of the storage. As the storage time extended to 15, 30, and 45 days, both *E. Coli* O157:H7 and *Salmonella* spp. disappeared in all MAP and vacuum-packed treatments.

The effect of packaging conditions on TBARS and VBN contents

Table 3 displays the effect of modified atmosphere packaging (MAP) varying in CO_2 and N_2 composition compared to the control, vacuum packaging, on TBARS and VBN contents of dry fermented sausages during the storage period (45 days). Martinez et al. (2006) and Gokoglu et al.

(2010) documented those sausages are more sensitive to oxidation than intact muscle because grinding reduces particle size and disrupts membranes, allowing air and oxygen to enter the tissues. Treatments had a significant ($p < 0.05$) variation in TBARS content across the storage period. Vacuumed-packed samples presented the highest TBARS content on 1, 15 and 30 days of storage time and along with MAP_2, 50% CO_2 and 50% N_2, at the final storage time as compared to other treatments. The current finding is in agreement with Wanget al. (1995) who found less oxidation in modified-atmosphere packed samples than those in vacuum packed. In contrast, higher TBA values under modified atmosphere packaging than those packed under vacuum was reported by some researchers (Berruga et al., 2005; Gokoglu et al., 2010; Kerry et al., 2000; Martinez et al., 2006). On day 1 of storage, MAP4 showed a significantly ($p < 0.05$) lowest TBARS value than all treatments and other MAP treatments were the same in the content. The increase in concentration of CO_2 affected lipid oxidation. Based on the TBARS content at day 15 and 30 storage time, treatments are ranked as follows: vacuum-packed > MAP1 > MAP2 > MAP3 > MAP4 as the prevention of rancidity through an increase in CO_2 concentration. Jeremiah, (2001) documented that the occurrence of lipid oxidation can be prevented by anaerobic packaging that the present study could achieve the inhibition of rancidity by increasing the CO_2 concentration. During the storage period of 45-days, except for MAP2 treatment which had the same highest content as vacuum-packed, MAP treatments showed a decrease in TBARS content as the increase in CO_2 composition and MAP4 exhibited the lowest content throughout the storage study. Correspondingly, Gokoglu et al. (2010) reported the oxidation inhibition effect of carbon dioxide concentration based on TBARS analysis. The TBARS values significantly ($p < 0.05$) decreased in vacuum-packed, MAP3 and MAP4 samples, and increased in MAP3 treatment while the MAP1 batch was unaffected ($p > 0.05$) due to extended storage time. The increased TBARS value in

MAP2 samples indicates rancidity development when rancidity was inhibited in vacuum-packed, MAP3 and MAP4 samples during the storage.

The VBN content indicates protein degradation and the increase of VBN content in meat can be caused by either bacterial or enzymatic degradation of proteins (Egan et al., 1981; Kim et al., 2014). In the current study, the VBN values significantly ($p < 0.05$) varied among treatments up to 30 days of storage time and the variation disappeared and all the treatments at the final storage time (Table 3). Across the study period, the variation didn't show similar trend and the values of treatments were fluctuating inconsistently having no relation with the composition of gas used in MAP and the bacterial growth characteristics (Table 2) during storage. However, the MAP2 treatment with 50% CO_2 and 50% N_2 gas mixture had the highest VBN content throughout storage period. The highest VBN and TABRS content exhibited in MAP2 could be the reason for the lowest sensory attributes of color and overall acceptability of the samples as to the panel judgment in the current study (Table 5). Additionally, the change in storage time didn't show a clear trend in the VBN contents of the vacuum-packed samples and all the MAP treatments regardless of their gas composition. The VBN value noticeably ($p < 0.05$) increased from day 1 up to 30 days and then decreased at the final storage time for MAP1, MAP3, and MAP4 treatments. The VBN content for MAP2 increased on 15 and 30 days compared to initial storage time thereafter decreased significantly ($p < 0.05$) in the final storage time like other MAP treatments. The VBN content of the vacuum-packed samples increased at 30 days compared to the previous time but then declined substantially ($p < 0.05$) at the final storage time. All treatments tended to have a decrease in VBN at the final storage time (45 days).

353 **The effect of packaging conditions on color characteristics**

354 Color is an important qualitative factor that determines meat and meat products acceptability
355 of consumers (Glitsch, 2000; Gokoglu et al., 2010). The three primary (L*, a*, and b*) color
356 coordinates used in the Hunter system of color determination were performed in the current study
357 and the results are presented in Table 4. A significant variation in the L* color attribute of
358 treatments was observed during days 1 and 15 of the storage time and the variation disappeared
359 thereafter at 30 and 45 days of storage time. On days 1 and 15 storage time, all MAP treatments
360 showed a noticeable ($p < 0.05$) lower score in L* than the vacuum-packed samples. Similarly, Kim
361 et al. (2014) observed the highest L* values in VP samples than the MAP samples at all storage
362 times except at Day 45. Gokoglu et al. (2010) found lower L*values in samples packed under
363 30% CO_2/70% N_2 and 100% CO_2 atmospheres during the storage. In contrast, Rubio et al.
364 (2007) and García-Esteban et al. (2004) documented that the type of packaging system had little
365 influence on L* values, and a significant difference was not found between vacuum-packaged
366 and MAP treatments. Our results disagree also with Li et al. (2012) who reported higher L* values
367 of beef MAP than VP. As a result of metmyoglobin production, elevated CO_2 concentrations in
368 MAP cause a degree of discoloration. The redox chemistry of myoglobin can be altered by gases
369 in MAP, which affects color. In the current study, the variation among the MAP samples was not
370 substantial regardless of the difference in gas composition applied. The L* values of samples
371 packed with MAP tended to decrease as the storage time extended and the values were significantly
372 ($p < 0.05$) higher at day 1 storage than further storage period for all MAP samples varying in gas
373 composition. The vacuum-packed samples significantly ($p < 0.05$) decreased in L* value on 15
374 and 30 days of storage than at day 1. However, the value again increases at 45 days but still
375 maintained a lower value as compared to day one. The current finding disagrees with Garcia-

376 Esteban et al. (2003) who reported the increased L* value in vacuum packed samples and the
377 stability in the modified atmosphere packed samples during storage.

378 In meat and meat products, redness (a*) is considered a color stability indicator (Kim et al.,
379 2014). There was a significant difference in a* value among MAP, varying in CO_2 and N_2
380 composition, and the vacuum-packed batches on 1, 30, and 45 storage days, and vacuum-packed
381 samples exhibited a decrease in a* value than all MAPs treatments. Hur et al. (2013) stated that
382 CO_2 has a positive role in the reduction of lipid oxidation and negative effects in color
383 deterioration in meat packaging during storage. During the storage time, a* value of MAP4 was
384 higher than all other treatments, and other MAP packages presented similar values which were
385 higher than the vacuum-packed samples. The current finding is in agreement with Adab et al.
386 (2020) who observed an increase in a* values during the storage of sausages packaged under
387 modified atmospheres. Similarly, Jeremiah et al. (1995) reported that pork packaged with 100%
388 CO_2 had great color stability. On the contrary, Ruiz-Capillas and Jiménez-Colmenero (2010)
389 reported that a* values remain constant during the storage of meat products packaged under
390 modified atmosphere. Viana et al. (2005) reported that high CO_2 concentrations in meat MAP
391 application as the major disadvantage with a certain degree of darkening as a result of
392 metmyoglobin formation. Sørheim et al. (1997) reported that CO_2 did not effect on meat color.
393 According to Hur et al. (2013), a decreased redness is associated with rancidity. The decreased
394 TBARS values in the MAP samples of the current study (Table 3) can be related to the increase in
395 a* value which indicates the advantage of MAPs in color stability by inhibiting the oxidation of
396 lipids. Storage time had a profound ($p < 0.05$) effect in a* value of all the packages, MAP, and
397 vacuum-package, used in the current study. And a marked decline in redness was observed in all
398 samples at 30 and 45 days than earlier storage time. The present study is in agreement with Kim

et al. (2014) who reported a pronounced fading in the redness color of all packaging systems, both VP and MAP samples, at extended storage time. In contrast, Esturk and Ayhan (2009) reported a decrease in the redness of salami slices at all MAP applications with an increase in storage time.

The yellowness (b*) value of treatments significantly showed variation during day 1, 15 and 30 storage time and all MAPs samples exhibited lower b* values than vacuum-packed batch. All MAP samples regardless of the gas composition had similar value during the stated period. Then after, all the MAP and vacuum-packed samples presented a similar b* value on the final storage time of 45 days. The current finding disagrees with Kim et al. (2014) report who found a significantly higher b* values in the MAP samples than the VP samples at day 30, 60, and 90 of storage. Cilla et al. (2006) reported the increase in yellowness color in MAP samples is related to the increased pigment oxidation during storage. Martínez et al. (2005) has been demonstrated that myoglobin oxidation is favored as the concentrations of CO_2 increased. However, the TBARS analysis of the current study (Table 3) didn't support the stated hypothesis as MAP treatments showed a decrease in TBARS content as the increase in CO_2 composition having the lowest content in MAP4, 100% CO_2, throughout the storage study. Similarly, Wang et al. (1995) reported that TBA and peroxide values were lower in modified atmosphere than in vacuum conditions after analyzing the lipid oxidation in Chinese-style sausages both stored at 4 and 15 ℃ temperatures. All the MAP and vacuum-packed samples showed a decrease in b* value due to extended storage time and the values at day 45 storage was substantially lower than day 1 storage time. Similarly, Gokoglu et al. (2010) reported a decreased b* values of the samples packed under modified atmosphere and vacuum during storage.

The effect of packaging conditions on sensory characteristics

Effect of modified atmosphere packaging (MAP) varying in CO_2 and N_2 composition compared to vacuum packaging on sensory characteristics of color, lactic acid aroma, sourness, and overall acceptability attributes of dry fermented sausages during storage was investigated, and the results are presented in Table 5. Treatments showed a significant ($p < 0.05$) variation in color attribute of sensory characteristics on 1, 30, and 45 storage days, lactic acid aroma and sourness on 1st day of storage, and overall acceptability at the initial and final storage period. The current results disagree with those of Fernández-Fernández et al. (2002) who documented that packing methods did not affect any sensory property of dry sausages subjected to VP and MAP. The color was preferred in vacuum packed and MAP1 on day 1 and MAP3 joined the preferred group on day 30 and 45 days of storage. The color attribute in MAP2, 50% CO_2 and 50% N_2, samples were lower compared to all other treatments. The sensory color score significantly decreased in all MAPs due to extended storage and vacuum-packed samples didn't vary across the storage time. On day one, the lactic acid aroma in vacuum packed, MAP1 and MAP2 were noticeably ($p < 0.05$) higher than others, and sourness were preferred in all MAPs than vacuum-packed samples. Thereafter, variation was not detected ($p > 0.05$) among treatments in both sensory traits of the lactic acid aroma and sourness according to the panel's judgments. Both sensory traits of lactic acid aroma and sourness values were significantly ($p < 0.05$) decreased in all treatment samples, MAP sample and vacuum-package, due to prolonged storage, and the lowest scores were recorded in the final storage time of 45 days. The overall acceptability score in MAP2 samples was the lowest compared to all other treatments in the initial and final storage days and no variation was exhibited among other treatments during these days. The least score exhibited in color and overall acceptability of MAP2 samples can be related to the higher TBARS and VBN results (Table 3)

444 which is associated with lipid oxidation and protein degradations in the treatment. The overall
445 acceptability of MAP1, MAP3, and MAP4 samples didn't show changes due to storage time,
446 samples in vacuum-packed and MAP2 samples decreased significantly at final storage time than
447 the initial day 1 storage.

448 In conclusion, the use of MAPs showed a better microbial inhibition than vacuum package
449 with an increase in the CO_2 from 25% to 100% in MAP1- MAP4 samples. Modified atmospheric
450 packaging with 70%CO_2/30%N_2 (MAP3) and 100% CO_2 (MAP4) are found to be more effective
451 to maintain several quality parameters (a_w, pH, microbial inhibition, stability against lipid
452 oxidation, and instrumental color traits) of dry fermented sausage and extend the shelf life without
453 any effect on sensory quality characteristics during storage.

454 **Conflict of interests**

455 The authors have no conflict of interest.

456 **Acknowledgments**

457 This research was supported by Basic Science Research Program through the National Research
458 Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (2017R1A2B201277).

459

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593 modified atmospheres. Meat Sci. 59:15–22.

594 Table 1. Effect of MAP varying in gas composition on pH and water activity a_w of dry fermented
595 sausages during storage

Parameter	Days	Treatments[1]					SEM
		Control	MAP1	MAP2	MAP3	MAP4	
pH	1	6.01^{aA}	5.87^{bA}	5.81^{cA}	5.74^{dA}	5.62^{eA}	0.03
	15	5.83^{aB}	5.78^{abB}	5.76^{bB}	5.73^{cB}	5.58^{dB}	0.00
	30	5.75^{aC}	5.71^{bC}	5.69^{bC}	5.63^{cC}	5.56^{dC}	0.01
	45	5.61^{aD}	5.59^{bD}	5.56^{cD}	5.51^{dD}	5.40^{eD}	0.00
	SEM	0.03	0.00	0.01	0.01	0.00	
a_w	1	0.73^{bB}	0.74^{aA}	0.74^{aA}	0.74^{aA}	0.73^{bA}	0.03
	15	0.73^{B}	0.73^{B}	0.74^{A}	0.74^{A}	0.73^{A}	0.00
	30	0.75^{aA}	0.73^{bB}	0.73^{bB}	0.72^{cB}	0.72^{cB}	0.01
	45	0.73^{aB}	0.72^{bC}	0.72^{bC}	0.71^{cC}	0.71^{cC}	0.00
	SEM	0.00	0.00	0.00	0.00	0.00	

596 [1)] Treatments are control (vacuum packaging); MAP1, 25% CO_2/75% N_2; MAP2, 50% CO_2/50%

597 N_2; MAP3, 70%CO_2/30%N_2; MAP4, 100% CO_2

598 [2)] SEM: standard error of mean. (n=3)

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

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

601

602 Table 2. Effect of MAP varying in gas composition on microbial quality characteristics of dry
603 fermented sausages during storage

Parameter	Days	Treatments [1]					SEM [2]
		Control	MAP1	MAP2	MAP3	MAP4	
TPC (log CFU/g)	1	8.96^{aA}	8.53^{bA}	8.41^{bcA}	8.24^{cA}	8.28^{cA}	0.10
	15	8.56^{B}	8.32^{A}	8.40^{A}	8.24^{A}	7.90^{AB}	0.30
	30	8.32^{aC}	8.28^{abA}	8.06^{bA}	7.61^{cB}	7.52^{cB}	0.28
	45	7.69^{D}	7.47^{B}	7.36^{B}	7.64^{B}	7.35^{B}	0.34
	SEM	0.22	0.21	0.29	0.27	0.34	
LAB (log CFU/g)	1	8.57^{aA}	8.43^{abA}	8.26^{abA}	8.32^{abA}	8.17^{cA}	0.19
	15	8.42^{aA}	8.33^{aA}	8.16^{abB}	8.05^{abAB}	7.85^{bB}	0.20
	30	8.49^{aA}	8.09^{bAB}	7.59^{cC}	7.44^{cB}	7.58^{cC}	0.15
	45	7.37^{aB}	7.33^{abC}	7.31^{abD}	7.24^{abC}	7.15^{bD}	0.12
	SEM	0.22	0.17	0.21	0.11	0.10	
Enterobacteriaceae (log CFU/g)	1	3.89^{aB}	3.96^{aA}	3.82^{aA}	3.05^{bA}	3.20^{b}	0.06
	15	3.89^{aB}	3.42^{bC}	3.03^{cB}	2.82^{cB}	2.86^{c}	0.10
	30	2.59^{C}	2.46^{C}	2.48^{C}	2.67^{B}	2.61	0.18
	45	4.11^{aA}	3.32^{bB}	3.20^{bB}	2.66^{cB}	2.44^{c}	0.10
	SEM	0.03	0.11	0.11	0.12	0.17	
	1	2.71	2.53	2.01	1.36	1.43	ND
	15	-	-	-	-	-	ND

E. coli (log CFU/g)	30	-	-	-	-	-	ND
	45	-	-	-	-	-	ND
	SEM	ND	ND	ND	ND	ND	
Salmonella spp. (log CFU/g)	1	2.87	2.80	2.61	2.55	2.57	ND
	15	-	-	-	-	-	ND
	30	-	-	-	-	-	ND
	45	-	-	-	-	-	ND
	SEM	ND	ND	ND	ND	ND	

[1)] Treatments are control (vacuum packaging); MAP1, 25% CO_2/75% N_2; MAP2, 50% CO_2/50% N_2; MAP3, 70%CO_2/30%N_2; MAP4, 100% CO_2

[2] SEM: standard error of mean, (n=3)

[a-c] Means with different letters within a row are significantly different ($p < 0.05$)

[A-D] means with different letters within a column of significantly different ($p < 0.05$)

ND: Not determined

611 Table 3. Effect of MAP varying in gas composition on TBARS and VBN of dry fermented sausages

612 during storage

Parameter	Days	Treatments [1]					SEM [2]
		Control	MAP1	MAP2	MAP3	MAP4	
TBARS (mg MA/kg)	1	1.01^{aA}	0.87^{b}	0.78^{bB}	0.77^{bA}	0.58^{cA}	0.00
	15	0.95^{aB}	0.73^{b}	0.84^{bB}	0.73^{cA}	0.54^{dA}	0.00
	30	0.91^{aB}	0.77^{b}	0.80^{bB}	0.70^{cA}	0.50^{dB}	0.01
	45	0.85^{aC}	0.79^{b}	0.94^{aA}	0.67^{cB}	0.43^{dC}	0.00
	SEM	0.01	0.00	0.00	0.00	0.00	
VBN (mg%)	1	8.87^{cB}	7.47^{dC}	10.36^{aB}	6.62^{eD}	9.52^{bC}	0.86
	15	8.96^{cB}	8.78^{cB}	13.54^{aA}	13.10^{abB}	12.16^{bB}	1.78
	30	10.92^{cA}	11.86^{cA}	14.47^{aA}	13.62^{bA}	12.42^{cA}	1.61
	45	8.68^{B}	7.36^{C}	8.73^{D}	8.49^{C}	8.70^{D}	1.38
	SEM	1.65	1.27	1.44	0.99	1.77	

613 [1)] Treatments are control (vacuum packaging); MAP1, 25% CO_2/75% N_2; MAP2, 50% CO_2/50%

614 N_2; MAP3, 70%CO_2/30%N_2; MAP4, 100% CO_2

615 [2] SEM: standard error of mean, (n=3)

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

617 [A-D] Means with different letters within a column of significantly different ($p < 0.05$)

618

619 Table 4. Effect of MAP varying in gas composition on instrumental color characteristics of dry
620 fermented sausages during storage

Parameter	Days	Treatments [1]					SEM [2]
		Control	MAP1	MAP2	MAP3	MAP4	
L* (lightness)	1	51.91^{aA}	47.29^{bA}	46.40^{bA}	46.28^{bA}	44.58^{bA}	2.01
	15	45.64^{aC}	44.92^{bB}	44.73^{bA}	43.18^{abA}	41.18^{bB}	2.22
	30	41.15^{C}	40.79^{B}	40.96^{B}	40.42^{B}	40.03^{B}	2.21
	45	40.64^{B}	40.74^{B}	39.76^{B}	39.14^{C}	39.08^{C}	2.41
	SEM	2.25	2.25	2.45	2.28	1.82	
a* (redness)	1	8.90^{bA}	9.63^{abA}	9.69^{abA}	10.01^{aA}	10.23^{aA}	1.09
	15	8.82^{A}	9.28^{A}	9.33^{A}	9.52^{B}	9.98^{B}	1.19
	30	7.96^{cB}	8.10^{bB}	8.35^{bB}	8.87^{bC}	9.65^{aC}	0.79
	45	6.56^{cC}	7.23^{bC}	7.30^{bC}	7.99^{bD}	8.87^{aD}	0.67
	SEM	0.61	0.99	0.96	0.94	1.19	
b* (yellowness)	1	10.74^{aA}	9.89^{bA}	9.65^{bA}	9.32^{bA}	9.15^{bA}	0.96
	5	10.15^{aA}	9.80^{abA}	9.51^{bA}	8.40^{bB}	8.11^{bB}	1.48
	30	9.88^{aA}	8.56^{abB}	8.32^{abB}	7.96^{bC}	7.82^{bC}	1.84
	45	8.17^{B}	8.09^{B}	8.00^{B}	7.50^{C}	7.43^{C}	1.04
	SEM	1.77	1.05	1.69	1.29	0.83	

621 [1)] Treatments are control (vacuum packaging); MAP1, 25% CO_2/75% N_2; MAP2, 50% CO_2/50%
622 N_2; MAP3, 70%CO_2/30%N_2; MAP4, 100% CO_2

623 [2] SEM: standard error of mean, (n=3)

624 [a-c] Means with different letters within a row are significantly different ($p < 0.05$)

625 [A-D] means with different letters within a column are significantly different ($p < 0.05$)

626

627 Table 5. Effect of MAP varying in gas composition on sensory attributes of dry fermented sausages
628 during storage

Parameter	Days	Treatments [1]					SEM [2]
		Control	MAP1	MAP2	MAP3	MAP4	
Color	1	4.50^{a}	4.08^{aA}	3.76^{bA}	3.50^{bA}	2.78^{bB}	0.63
	15	3.99	3.76^{B}	3.64^{B}	3.48^{A}	3.20^{A}	0.67
	30	3.73^{a}	3.64^{aB}	2.42^{cC}	3.41^{aB}	2.28^{cB}	0.44
	45	2.59^{a}	3.02^{aC}	2.00^{cD}	2.46^{abC}	2.24^{bB}	1.00
	SEM	0.83	0.66	0.63	0.63	0.63	
Lactic acid aroma	1	5.00^{aA}	4.54^{aA}	4.00^{abA}	3.76^{bA}	3.76^{bA}	0.12
	15	4.02^{B}	4.00^{B}	3.42^{A}	3.34^{A}	3.25^{A}	0.64
	30	3.50^{B}	3.38^{BC}	3.22^{A}	3.16^{B}	3.10^{AB}	0.31
	45	3.56^{B}	3.18^{C}	3.09^{B}	3.02^{C}	2.99^{B}	0.86
	SEM	0.61	0.67	0.62	0.29	0.53	
Sourness	1	3.60^{bA}	4.40^{aA}	4.58^{aA}	4.18^{aA}	4.05^{aA}	0.50
	15	3.52^{A}	3.30^{B}	3.04^{B}	3.15^{B}	3.00^{B}	1.03
	30	3.36^{AB}	3.12^{B}	3.19^{B}	3.12^{BC}	3.10^{B}	0.45
	45	2.90^{B}	2.86^{C}	3.37^{B}	3.00^{C}	2.46^{C}	1.02
	SEM	0.79	0.72	0.85	0.58	0.99	
	1	4.42^{aA}	3.72^{a}	3.09^{bA}	3.79^{a}	3.69^{a}	0.68
	15	3.68^{B}	3.36	3.00^{A}	3.75	3.56	0.82
	30	3.46^{B}	3.40	2.74^{B}	3.70	3.21	0.31

	45	3.25^{aB}	3.19^{a}	2.20^{bC}	3.56^{a}	3.01^{a}	1.17
	SEM	0.78	1.03	0.63	0.82	0.70	
Overall acceptability							

[1)] Treatments are control (vacuum packaging); MAP1, 25% CO_2/75% N_2; MAP2, 50% CO_2/50% N_2; MAP3, 70%CO_2/30%N_2; MAP4, 100% CO_2

[2] SEM: standard error of mean. (n=3)

[a-c] Means with different letters within a row are significantly different ($p < 0.05$)

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

0= extremely pale to 5= very dark (color),

0=very weak fermented aroma to 5=very strong fermented aroma (Lactic acid aroma)

0=light sour to 5= strong sour (sourness).