



## Effects of Gas Composition in the Modified Atmosphere Packaging on the Shelf-life of *Longissimus dorsi* of Korean Native Black Pigs-Duroc Crossbred during Refrigerated Storage

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**ABSTRACT:** This study was conducted to observe the effects of gas composition in modified atmosphere packaging (MAP) on the shelf-life of *Longissimus dorsi* of Korean Native Black Pigs-Duroc Crossbred (KNP×D) during refrigerated storage. Muscle sample was obtained from the left side of carcass of seven months old of KNP×D barrow. The sample was sliced into 1 cm in thickness, placed on trays (two slices/tray) and filled with different gas composition, i.e. 0:20:80/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub> (MAP1), 30:20:50/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub> (MAP2) and 70:20:10/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub> (MAP3). Other slices of sample were vacuum packed (VP) as a control. All packs were stored at 5±1°C. At 12 d of storage, pH value of MAP2 and MAP3 were higher (p<0.05) than that of MAP1 and pH value of MAP1 was higher (p<0.05) than that of VP. At 6 d of storage, redness (a\*) value of MAP2 and MAP3 were higher (p<0.05) than that of VP and MAP1 and, at 9 and 12 d of storage, redness value of MAP3 was higher (p<0.05) than that of VP, MAP1, and MAP2. At 3, 6, 9, and 12 d of storage, the 2-thiobarbituric acid reactive substances (TBARS) value of MAP3 was higher than that of MAP2 and TBARS value of MAP2 was higher than that of VP and MAP1. At 3, 6, 9, and 12 d of storage, volatile basic nitrogen values of MAP2 and MAP3 were higher (p<0.05) than those of VP and MAP1. At 3 d of storage, total aerobic plate counts of MAP2 and MAP3 were higher (p<0.05) than those of VP and MAP1 and, at 6 d of storage, total aerobic plate counts of MAP3 was higher (p<0.05) than that of MAP1 and MAP2. However, there was no significant different total aerobic plate count among MAP1, MAP2, and MAP3 at 9 and 12 d of storage. There was no significant different total anaerobic plate count among MAP1, MAP2, and MAP3 during storage. It is concluded that the MAP containing 30:20:50/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub> gas composition (MAP2) might be ideal for better meat quality for KNP×D meat. (**Key Words:** Modified Atmosphere Packaging (MAP), Korean Native Black Pigs Crossbred with Duroc, Storage Time, Meat Quality)

### INTRODUCTION

Korean native black pig (KNP) is typical of native pigs residing in the Korean peninsula which have particularly high intramuscular contents and high redness as compared to other commercial breeds, such as Landrace and Yorkshire (Jin et al., 2001; Kim et al., 2008). However, due to its slow

growth rate and light carcass (Hwang et al., 2004) it has been crossbred with commercial breeds, such as Duroc. Duroc is usually used as a sire breed due to its excellent growth rate (Suzuki et al., 2003) and intramuscular fat deposition and carcass traits (Latorre et al., 2003) and, therefore, KNP×Duroc crossbred (KNP×D) meat is regarded as having a high intramuscular fat and redness value.

Meat is usually offered to the consumers in a refrigerated showcase display. Appearance of meat during display in the showcase is the most important factor in attracting consumers to buy and the shelf-life of meat during display is important to maintain consumer's preference. Therefore, a study of packaging method to preserve the quality of KNP×D meat, particularly its red color, is needed.

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Modified atmosphere packaging (MAP) is commonly used in the food industry to maintain the quality and extend the shelf-life of meat. McMillin et al. (2008) stated that MAP is the removal and/or replacement of the atmosphere surrounding the product before sealing in vapor-barrier materials. There are three gases which are mainly used in MAP, i.e. oxygen (O<sub>2</sub>), carbon dioxide (CO<sub>2</sub>) and Nitrogen (N<sub>2</sub>). Oxygen is used for its ability to promote the reddish color of fresh meat and maintain it during storage (McMillin, 2008). Studies by meat scientists reported the effect of high oxygen MAP on preserving the bright red color of meat (Jayasingh et al., 2002; Seyfert et al., 2004; Mancini and Hunt, 2005). However, high oxygen MAP has limitations due to its oxidative activity (Grobbel et al., 2008; Zakrys et al., 2008) and caused premature browning during cooking (John et al., 2005). Moreover, oxygen generally stimulates aerobic bacterial growth and inhibits growth sensitive anaerobes (Church, 1994). Carbon dioxide performs antimicrobial effects but absorption of a large amount of carbon dioxide in meat tissue can cause a minor decrease in pH which might affect other chemical qualities and elevated carbon dioxide levels can cause pore formation in cooked meat (Jakobsen and Bertelsen, 2002). Nitrogen is used as a filler gas as well as to prevent pack collapse caused by high concentration of carbon dioxide (Phillips, 1996).

Oxygen, carbon dioxide and nitrogen are used in different combinations and many studies related to their composition in MAP have been done by meat scientists to extend the shelf-life of meat. However, ambiguous results have been obtained with regard to the longer shelf-life of MAP (Samelis and Georgiadou, 2000; Pexara et al., 2002; Santos et al., 2005). Moreover, there is only a little information about packaging KNP×D meat. Therefore, this study was conducted to observe effects of the gas composition in the MAP on the shelf-Life of *Longissimus dorsi* of KNP×D during refrigerated storage.

## MATERIALS AND METHODS

### Sample preparation and experimental design

Seven months old KNP×D barrows were slaughtered in a commercial slaughtering house followed by overnight chilling. The *Longissimus dorsi* muscles were obtained from the left side of carcass. The muscles were vacuumed packaged and were transported to laboratory. The muscles were sliced into 1 cm in thickness and placed on trays (O<sub>2</sub> transmission rate = 0.1 cc/cm<sup>2</sup> at 23°C, 0% relative humidity (RH); water vapor transmission rate was 7.87 mg/24 h-cm<sup>2</sup> at 38°C, 100% RH, Cryovac Sealed Air Corp., Duncan, South Carolina, USA) for packaging. Every tray contained two slices of meat. The trays were filled with different gas compositions, i.e. 0:20:80/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub> (MAP1),

30:20:50/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub> (MAP2), and 70:20:10/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub> (MAP3). The gas filling was performed using MAP machine (Hypervac, Hwaseong, Korea) equipped with the gas mixture (MAP Mix 9001 ME, PBI Dansensor, Ringsted, Denmark). Gases were purchased from a local gas supplier (Baeklyung Specialty Gas Co., Chuncheon, Korea). Trays were sealed with O<sub>2</sub> barrier film (O<sub>2</sub> transmission rate = 0.39 mg/24 h-cm<sup>2</sup> at 4.4°C, 100% RH; Lid 1050, Cryovac Sealed Air Corp., USA). Other slices of sample were vacuum packed (VP) as a control. All packs were stored at 5±1°C.

### Shelf-life analysis

Shelf-life analysis included pH value, color changes, lipid oxidation, protein deterioration and microbial growth. The analyses were performed at 0, 3, 6, 9, and 12 d of storage. Two packages (two slices/samples each) were used at each time of storage.

Ten g of sample was homogenized with 100 mL of distilled water at 10,000 rpm for 60 s using a homogenizer (PH91, SMT Co. Ltd., Tokyo, Japan). The pH of meat slurry at room temperature was measured using a pH meter (SevenEasy pH, Mettler-Toledo GmbH, Greifensee, Switzerland).

Color changes of the surface of samples were monitored by measuring the Commission Internationale de l'Eclairage (CIE) color values using a color difference meter (CR-400, Konica Minolta Sensing Inc., Osaka, Japan) and an illuminant C. The color instrument was calibrated using white plate (Y = 93.6, x = 0.3134, and y = 0.3194). Color measurements were directly performed on the surface of samples immediately after the packs were opened.

Lipid oxidation was measured by analysis the 2-thiobarbituric acid reactive substances (TBARS) values according to Sinnhuber and Yu (1977). A half g of sample was mixed with 3 drops of antioxidant solution, 3 mL of TBA solution, and 17 mL of 25% (w/v) Trichloroacetic acid (TCA). The mixture was heated at 100°C for 30 min and centrifuged at 3,500 rpm for 30 min. Absorbance of supernatant was measured at 532 nm using a spectrophotometer (UVmini-1240, Shimadzu, Kyoto, Japan). The results were calculated as mg of malonaldehyde (MA) per kg of sample.

Protein deterioration was evaluated by measuring the production of volatile basic nitrogen (VBN) values as described by Kohsaka (1975). Five grams of sample were mixed with 30 mL of 5% (w/v) TCA using a homogenizer (Ultra-Turrax T25 basic, IkaWerke GmbH & Co., Staufen, Germany) at 13,500 rpm for 2 min. The homogenate was made up with 5% (w/v) TCA to 50 mL of final volume and filtered using the Whatman filter paper No. 1. One mL of filtrate and 1 mL of borate buffer were placed in outer and inner of Conway dish, respectively. The Conway dishes

were incubated at 37°C for 100 min. Finally, the inner solution was titrated with 0.01 N HCl and the titration volume was recorded.

To determine the microbial growth, 10 g of sample was aseptically transferred into a stomacher bag. The sample then was mixed with 90 mL of sterile 0.1% peptone water using a stomacher (Lab blender 400Seward Laboratory, West Sussex, UK) for 2 min at room temperature. A serial of decimal dilutions was prepared by adding 1 mL of sample mixture with 9 mL of 0.1% peptone water. One mL inoculum of appropriate dilution was spread on a plate count agar (PCA; Difco, Sparks, Maryland, USA). Plates were incubated at 35°C for 48 h in atmospheric conditions for determination of total aerobic plate count and in anaerobic conditions for total anaerobic plate count. After incubation, plates with 30 to 300 colonies were counted. Microbiological data were transformed into logarithms of the number of colony forming units (Log CFU/g).

### Statistical analysis

The data were analyzed statistically using analysis of variance. Furthermore, Duncan's new multiple range test was used to observe the significant different between means. Analysis was performed using SPSS, version 19.0 (SPSS, 2010).

## RESULTS AND DISCUSSION

### Value of pH

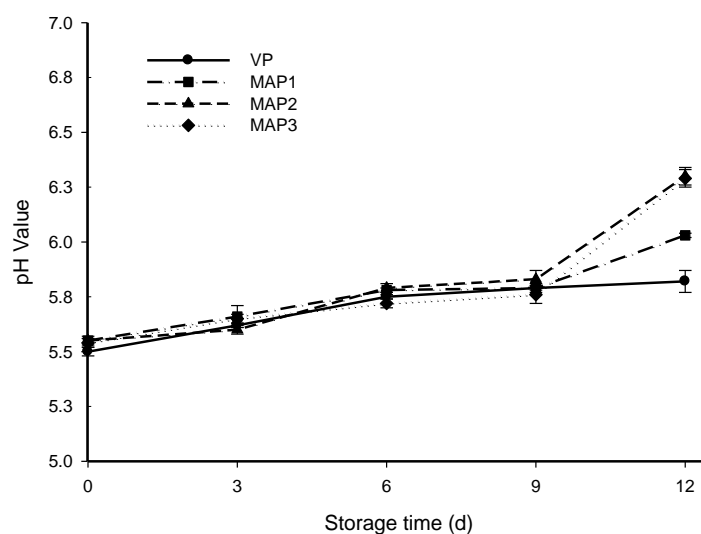
The pH values of all groups increased as storage time increased (Figure 1) and there was no significant difference in pH value among groups from 0 to 9 d of storage. At 12 d of storage, pH value of MAP2 and MAP3 were higher ( $p < 0.05$ ) than that of MAP1 and pH value of MAP1 was

higher ( $p < 0.05$ ) than that of VP. This showed that oxygen increased the pH during storage and nitrogen restrained the increasing of pH. However, the trend of pH regarding packaging type was not consistent. An inconsistent effect of packaging on pH of pork also was reported by Jeremiah et al. (1995). Different results were reported by Viana et al. (2005) in that MAP did not show strong effects on pH of fresh pork loin. According to Borch et al. (1996) and Brewer et al. (2001), pH stabilization in fresh meat is attributed to exhaustion of muscular glucose reserves to denaturation of glycolytic enzymes.

### Color changes

The change of  $L^*$ ,  $a^*$  and  $b^*$  values is shown in Table 1. The effect of gas composition of MAP on  $L^*$  value can be seen from 3 d of storage, in which MAP1 (0%  $O_2$ ) had a lower  $L^*$  value ( $p < 0.05$ ) than the other MAPs and VP. The  $L^*$  values of samples packed with MAPs tended to increase over storage, while vacuum packed samples were constant. Our results disagree with Li et al. (2012) who reported higher  $L^*$  values of beef MAP than VP. Garcia-Esteban et al. (2003) reported that optimized  $L^*$  value increased during storage in vacuum packed samples but was stable in MA packed samples. In general, MAP applications do not affect  $L^*$  values (Soldatou et al., 2009; Esmer et al., 2011).

There was no significant difference of redness ( $a^*$ ) value among groups at 0 and 3 d of storage. At 6 d of storage, redness values of MAP2 and MAP3 were higher ( $p < 0.05$ ) than that of VP and MAP1 and, at 9 and 12 d of storage, redness value of MAP3 was higher ( $p < 0.05$ ) than that of VP, MAP1, and MAP2. Kerry et al. (2006) stated that the major function of oxygen is to maintain the myoglobin in its oxymyoglobin state which is cherry cherry-red in color. Oxygen contained in MAP had a



**Figure 1.** Effect of gas composition in modified atmosphere packaging on pH value of *Longissimus dorsi* of Korean Native Black Pig crossbred with Duroc during refrigerated storage. VP, vacuum packaging; MAP, modified atmosphere packaging. MAP1 = 0:20:80/ $O_2$ : $CO_2$ : $N_2$ , MAP2 = 30:20:50/ $O_2$ : $CO_2$ : $N_2$ , MAP3 = 70:20:10/ $O_2$ : $CO_2$ : $N_2$ .

**Table 1.** Effect of gas composition in modified atmosphere packaging on pH and instrumental color of *Longissimus dorsi* of Korean Native Black Pig crossbred with Duroc during refrigerated storage

Parameters	Packaging <sup>1</sup>	Storage time (d)				
		0	3	6	9	12
L*	VP	54.76±3.16 <sup>A</sup>	53.02±1.89 <sup>aA</sup>	54.52±4.45 <sup>aA</sup>	54.50±3.05 <sup>abA</sup>	54.50±1.43 <sup>bA</sup>
	MAP1	53.63±5.01 <sup>A</sup>	51.45±2.01 <sup>bB</sup>	51.88±2.28 <sup>bB</sup>	53.19±3.02 <sup>bA</sup>	52.81±2.55 <sup>cAB</sup>
	MAP2	53.63±5.07 <sup>B</sup>	54.86±1.99 <sup>aAB</sup>	55.97±1.90 <sup>aAB</sup>	55.52±1.96 <sup>aAB</sup>	56.13±1.83 <sup>aA</sup>
	MAP3	54.76±3.16 <sup>AB</sup>	53.19±2.04 <sup>abB</sup>	53.17±1.27 <sup>abB</sup>	55.56±1.27 <sup>aA</sup>	55.94±2.49 <sup>abA</sup>
a*	VP	6.11±1.03 <sup>B</sup>	7.54±1.32 <sup>A</sup>	7.63±0.90 <sup>bA</sup>	5.79±1.70 <sup>bB</sup>	6.83±0.87 <sup>bB</sup>
	MAP1	6.66±1.03 <sup>B</sup>	8.42±1.15 <sup>A</sup>	6.45±1.04 <sup>bB</sup>	6.21±1.39 <sup>bB</sup>	6.87±1.06 <sup>bB</sup>
	MAP2	6.62±1.03 <sup>B</sup>	9.66±0.88 <sup>A</sup>	9.90±1.65 <sup>aA</sup>	6.01±0.74 <sup>bB</sup>	6.54±1.49 <sup>bB</sup>
	MAP3	6.11±1.39 <sup>C</sup>	9.73±1.20 <sup>A</sup>	9.75±1.67 <sup>aA</sup>	8.17±1.46 <sup>abB</sup>	7.98±1.08 <sup>abB</sup>
b*	VP	2.96±1.64 <sup>C</sup>	7.06±1.09 <sup>bA</sup>	5.35±0.94 <sup>bB</sup>	3.19±0.69 <sup>bC</sup>	5.30±1.07 <sup>bB</sup>
	MAP1	3.43±1.07 <sup>A</sup>	4.52±1.12 <sup>cA</sup>	4.55±1.12 <sup>bA</sup>	3.62±0.63 <sup>bA</sup>	4.12±0.83 <sup>cA</sup>
	MAP2	3.43±1.08 <sup>E</sup>	8.67±0.33 <sup>aA</sup>	7.02±0.90 <sup>aC</sup>	7.98±0.93 <sup>abB</sup>	6.69±1.21 <sup>aD</sup>
	MAP3	2.95±1.64 <sup>C</sup>	7.57±0.80 <sup>bAB</sup>	7.18±0.71 <sup>abB</sup>	7.98±0.93 <sup>aA</sup>	6.69±1.21 <sup>abB</sup>

Mean values±standard deviation.

VP, vacuum packaging; MAP, modified atmosphere packaging.

<sup>1</sup> MAP1 = 0:20:80/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub>, MAP2 = 30:20:50/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub>, MAP3 = 70:20:10/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub>.

<sup>a-d</sup> Means in the same column followed by different superscript upper cases are significantly different (p<0.05).

<sup>A-E</sup> Means in the same row followed by different superscript upper cases are significantly different (p<0.05).

significant effect on a\* values of samples (p<0.05), the higher percentage of oxygen showed a greater effect. MAP3 which contained 70% oxygen increased the initial redness of sample from 6.11 to 9.73 on 3 d of storage and maintained the highest a\* values among other packaging methods until the end of study. Similar effect of oxygen also was observed from MAP2, in which 30% oxygen increased the redness on 3 d. These findings agree with Jakobsen and Bartelsen (2000), Jayasingh et al. (2002) and Martinez et al. (2006). Oxygen is required by myoglobin to remain in an oxygenated form, which gives the bright cherry color in meat (Mancini and Hunt, 2005). However, the effect of oxygen was seen only until 6 d. Commencing from 9 d, a\* values of MAP2 decreased. a\* values of MAP1 and VP were not different (p>0.05) over storage and remained steady. Martinez et al. (2006) reported that fresh pork sausages stored under vacuum kept a steady acceptable a\* value.

Oxygen contained in MAP played role in the increase of b\* values (Table 1). Oxygen concentration of 30% and 70% on MAP2 and MAP3 respectively significantly increased the b\* values (p<0.05) from 3 to 12 d of storage. Aida et al. (2013) found an increase of b\* values of samples packed under 15:30:50/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub> which is also agree with the report of Rubio et al. (2008). The b\* value of VP was increased on 3 d then decreased for the rest of storage time. The lowest b\* values were found in MAP1 samples.

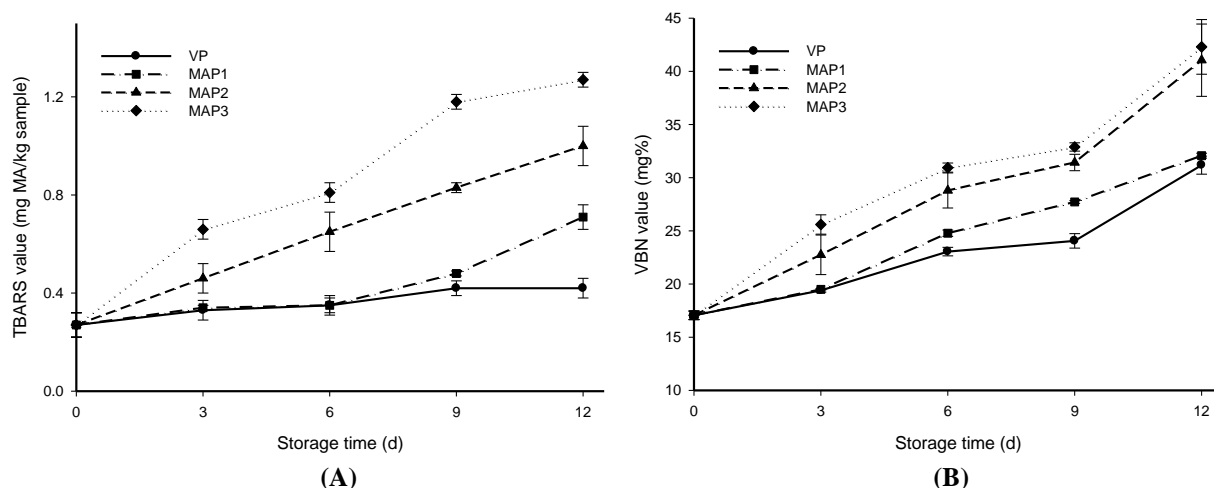
### Lipid oxidation

TBARS value of all groups increased during storage (Figure 2A). At 3, 6, 9, and 12 d of storage, TBARS value of MAP3 was higher than that of MAP2 and TBARS value

of MAP2 was higher than that of VP and MAP1. This was in accordance with the findings of (Borch et al., 1996; Cayuela et al., 2004; John et al., 2005; Santos et al., 2005; McMillin, 2008). A meat system whereby oxygen is readily available is more oxidatively labile (Smiddy et al., 2002). The occurrence of lipid oxidation can be prevented by anaerobic packaging (Jeremiah, 2001), whereas a greater degree of lipid oxidation is observed in meat stored under high level of oxygen (Jakobsen and Bartelsen, 2000). The TBARS value of samples packed with VP, MAP1, MAP2, and MAP3 increased from initial value of 0.27 mg MA/kg sample on 0 d to 0.42, 0.71, 1.00, and 1.27 mg MA/kg sample respectively, after 12 d of storage. Taking into account that TBARS values up to 0.6 mg MA/kg of fresh meat are considered as fresh (Tarladgis et al., 1960), samples packed with VP, MAP1, MAP2, and MAP3 were categorized as fresh meat up to 12 d, 9 d, 3 d and less than 3 d of storage respectively. However, TBARS values of sample from all packaging methods were below 2 mg MA/kg sample. A TBARS value of 2 was considered the limiting threshold for oxidized meat acceptability (Campo et al., 2006). The TBARS values increased throughout storage in all packaging methods, and the increasing rate of TBARS was higher as the oxygen content in packaging increased. The increasing rate of TBARS throughout storage can be attributed to the increased of oxidation of unsaturated fatty acid (Mendes et al., 2008) which is accelerated by the presence of oxygen (McMillin, 2008).

### Protein deterioration

VBN value of all groups increased during storage (Figure 2B). At 3, 6, 9, and 12 d of storage, VBN values of



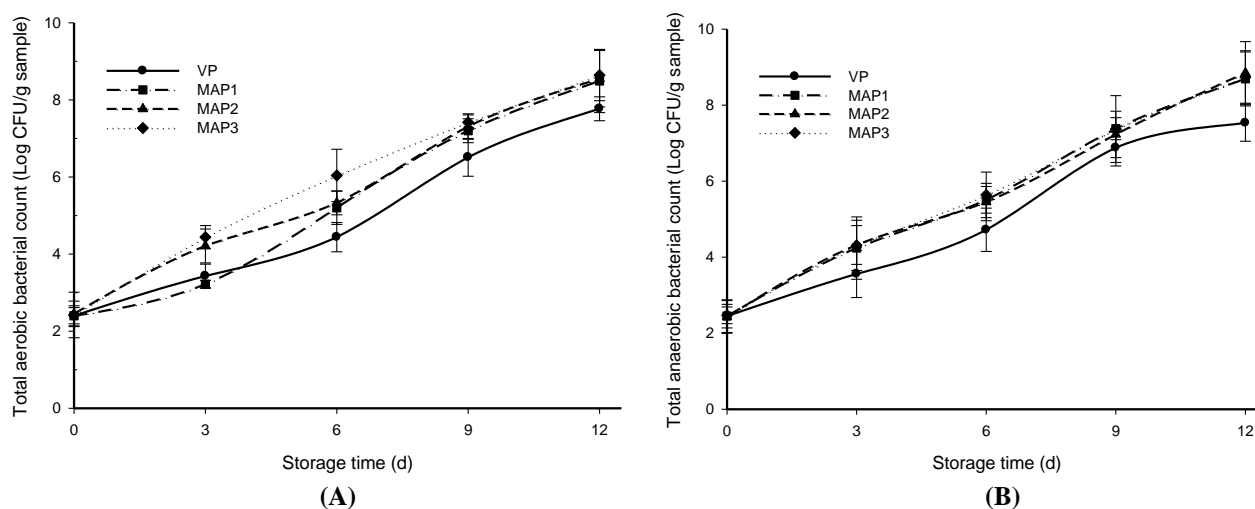
**Figure 2.** Effect of gas composition in modified atmosphere packaging on TBARS (A) and VBN (B) of *Longissimus dorsi* of Korean Native Black Pig crossbred with Duroc during refrigerated storage. VP, vacuum packaging; MAP, modified atmosphere packaging; TBARS, thiobarbituric acid reactive substances; VBN, volatile basic nitrogen. MAP1 = 0:20:80/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub>, MAP2 = 30:20:50/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub>, MAP3 = 70:20:10/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub>.

MAP2 and MAP3 were higher ( $p < 0.05$ ) than those of VP and MAP1. Similar findings also were reported by Lund et al. (2007) and Zakrys-Waliwander et al. (2012). Oxygen promoted the protein deterioration of meat represented by the higher VBN values. The higher VBN values of samples packed with higher composition of oxygen might be correlated with the higher counts of bacteria found in those packs (Figure 3A and 3B). Volatile basic nitrogen is a product of bacterial spoilage and endogenous enzyme action (Mendez et al., 2008). Fraser and Sumar (1998) indicated that bacterial catabolism of amino acids results in the accumulation of ammonia and other volatile bases.

### Microbial growth

Total aerobic plate count of all groups increased during

storage (Figure 3A). At 3 d of storage, total aerobic plate counts of MAP2 and MAP3 were higher ( $p < 0.05$ ) than those of VP and MAP1, and at 6 d of storage, total aerobic counts of MAP3 was higher ( $p < 0.05$ ) than that of MAP1 and MAP2. However, at 9 and 12 d of storage, there was no significant different total aerobic plate count among MAP1, MAP2 and MAP3. At 3, 6 and 9 d of storage, total aerobic plate count of VP was lower ( $p < 0.05$ ) than that of MAP1, MAP2 and MAP3. Esmer et al. (2011) showed higher viable counts of microbial groups in aerobic packaging (contained O<sub>2</sub>) than those of other packaging methods and this finding agrees with Ercolini et al. (2006). In the lack of oxygen, aerobic microbial grew during refrigerated storage and the presence of oxygen boosted their growth. Church (1994) stated that O<sub>2</sub> generally stimulates aerobic bacterial



**Figure 3.** Effect of gas composition in modified atmosphere packaging on total aerobic (A) and anaerobic bacteria (B) of *Longissimus dorsi* of Korean Native Black Pig crossbred with Duroc during refrigerated storage. VP, vacuum packaging; MAP, modified atmosphere packaging. MAP1 = 0:20:80/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub>, MAP2 = 30:20:50/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub>, MAP3 = 70:20:10/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub>.

growth and inhibits growth sensitive anaerobes. Martinez et al. (2006) reported that samples stored without O<sub>2</sub> (vacuum or the presence an O<sub>2</sub> scavenger) showed the lowest value of aerobic bacterial counts. In addition, meat packaged under vacuum had slower aerobic bacterial growth than that of MAP with O<sub>2</sub> (Sheridan et al., 1997). Total anaerobic plate count of all groups increased during storage (Figure 3B). At 3, 6, 9, and 12 d of storage, total anaerobic plate count of VP was lower (p<0.05) than that of MAP1, MAP2, and MAP3. There was no significant different total anaerobic plate count among MAP1, MAP2, and MAP3. This might due to the percent of CO<sub>2</sub> contained in MAP, where all of three MAP contained 20% CO<sub>2</sub> (McMillin, 2008).

## CONCLUSION

From the present study, it is concluded that MAP with higher oxygen content maintained a higher a\* value of sample during 12 d storage and higher L\* value at the end of storage. However, the higher rate of lipid oxidation was found in oxygen containing MAP than in non-oxygen MAP and vacuum packaging. Vacuum packaging maintained the lower growth of both aerobic and anaerobic bacteria than MAP throughout the storage. The lower counts of bacteria on VP contributed to the lower protein deterioration of VP than MAP. It is concluded that the MAP containing 30:20:50/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub> gas composition (MAP2) might be ideal for better meat quality for KNP×D meat.

## ACKNOWLEDGMENTS

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