Combined Effects of Mugwort Herb and Vitamin C on Shelf-Life of Vacuum-Packed Seasoned Pork

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Abstract

This study was performed to investigate the possibility of the addition of mugwort herb extract (MH) and vitamin C (VC) alone (0.05%) and in combination (0.05% each) on shelf-life of seasoned pork. The combination of VC+MH demonstrated a significant reduction in thiobarbituric acid reactive substances, and volatile basic nitrogen in seasoned pork. Also, the pH values, total lactic acid concentration, lactic acid bacteria count, and the sensory properties (discoloration, flavor, and overall acceptability) of seasoned pork were not significantly affected by adding MH and/or VC. All seasoned pork were rejected by sensory panel when LAB count reached levels of 5-6 Log CFU/g, TLA concentration has been above a level of 3.6-3.9 mg lactic acid/g, and pH values ranged from 5.31-5.51 (15 d). Therefore, the findings showed that spoilage of seasoned pork does not appear to be the result of lipid oxidation, but is caused by lactic acid producing bacteria which result in sour odor.

Keywords: mugwort, vitamin C, shelf-life, seasoned meat, lactic acid bacteria

Introduction

Quality deterioration of seasoned meat can be delayed using several techniques, various packaging methods, including vacuum packaging and modified atmosphere packaging (MAP), or low temperature, such as refrigerated storage condition. In meat processing industry, the application of highly effective antioxidants has become a popular and effective mean to extend the shelf-life of muscle foods (Seol et al., 2010). Low-cost synthetic antioxidants, such as tertbutylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) have shown excellent antioxidant effects. However, synthetic antioxidants pose the possibility of health risk, toxicity, and carcinogenic effects (Cadun et al., 2008; Kim et al., 2013). For these reasons, replacements with more effective and non-toxic antioxidants are desired.

Mugwort (Artemisia princeps Pampanini) is a natural herb plant used in tea, food, and traditional medicine in the East Asian countries such as Korea, Japan and China. In Korea, mugwort can be found in markets in various forms such as cakes, emulsified sausages, sauces, and noodles. Also, this plant contains bioactive compounds including phenolics, alkaloids, and vitamins A, B₁, B₂, and C as well as various minerals (Hwang et al., 2013).

Vitamin C (ascorbic acid; VC) is very effective not only for increasing shelf-life and stabilizing the color of meat and meat products but also for acting as a synergist when used in combination with other antioxidants by promoting their antioxidant effects. However, VC either promotes or inhibits lipid oxidation reactions in meat products depending on its concentration (Hwang et al., 2013).

To our knowledge, some studies have reported that the addition of functional ingredients, such as ginseng, pine needles, saxon, chitosan, propolis, medicinal plants, tomato powder, and kiwifruit increased shelf-life in seasoned meat products (Cho et al., 2002; Cho et al., 2007; Han et al., 2006; Kim and Kim, 2007; Kim et al., 2009; Lee et al., 2009; Youn et al., 2004). However, scientific literature on the shelf-life of seasoned pork meat added with antioxidant combination is still insufficient. Also,
the evaluation of their effect in a range of food systems is still needed on successful application in meat and meat products. Therefore, the objective of this study was to determine the effects of mugwort extracts (MH) and vitamin C (VC) alone or in combination on the physicochemical, microbiological and sensory acceptance of seasoned pork during 20 d of storage at 4°C.

Materials and Methods

Preparation of mugwort extracts (MH)

Commercial samples of dried mugwort were purchased from a local market on Ganghwa Island in Korea. After separating the leaves from the dried mugwort, they were ground using a blender (KA-2610, Jworld Tech, Korea) for 1 min. Ten grams of ground leaves were mixed with 200 mL of 50% ethanol overnight (24 h) in a shaker at room temperature. The extracts were filtered through filter paper No. 1 (Whatman International, UK) and then evaporated with a rotary evaporator (EYELA N-1000, Rikakikai, Japan) at <50°C. The concentrated product represented MH.

Preparation and processing of seasoned pork

Fresh pork sirloin (M. longissimus dorsi), at 48 h post-mortem, was purchased from a local market, and sliced into 0.4-cm thickness. An antioxidant combination of vitamin C (VC; Sewoo Inc, Korea) and mugwort extract (MH; pH, 6.14±0.04; L*-value, 28.71±0.04; a*-value, 1.79±0.36; b*-value, 0.86±0.13) was prepared according to the formulations: Control (no antioxidant added), VC (0.05% VC), MH (0.05% MH) and VC+MH (0.05% VC + 0.05% MH). Seasoned pork sauce was prepared with the ingredients listed in Table 1. Sliced pork sirloin was marinated separately in prepared sauce with antioxidant for 30 min using the tumbler (MKR-150C, Ruhle GmbH., Germany). Then, seasoned pork was divided into 24 samples (4 treatments × 6 storage) in smaller portion (about 200 g each) and placed in polyethylene bags, and vacuum packaged using vaccum packaging system (FJ-500XL, Fuji Tech, Korea). All samples were stored at 4°C and evaluated after 0, 3, 5, 10, 15, and 20 d of storage periods.

pH

The pH values were measured in a homogenate (Ultra-Turrax T25, Janke and Kunkel, Germany) prepared with 5 g of sample and distilled water (20 mL) using a pH meter (Model 340, Mettler-Toledo GmbH, Switzerland). The pH meter calibrated with standard 4.00, 7.02, and 10.05 pH buffers (VWR Scientific Products) at a temperature of 20±1°C. All determinations were performed in triplicate.

Total lactic acid (TLA)

TLA concentration was determined according to the Valli et al. (2006) using Megazyme kit (Megazyme International Ltd., Ireland). Briefly, each ground sample (5 g) was blended using homogenizer (AM-7, Nihonseiki, Kaisa Ltd., Japan) with 20 mL of 1 M perchloric acid for 5 min and then transferred to a beaker. The solution was added 40 mL of distilled water and adjusted the pH to approx. 10.0 with 2 M KOH, using pH meter (Model 340, Mettler-Toledo GmbH, Switzerland). Then, the solution was transferred quantitatively to a 100 mL volumetric flask and filled to the mark with distilled water. The solution was placed in a refrigerator for 20 min to allow separation of fat and precipitation of potassium perchlorate. The sample was filtered through filter paper No. 1 (Whatman International, UK). After filtration, concentrations of TLA in the supernatant were measured with the D-lactic acid and L-lactic acid kit and expressed as milligrams per gram (mg/g) of seasoned meat. The absorbance at 340 nm was read in a UV/VIS spectrophotometer (Optizen 2120 UV plus, Mecasys Co. Ltd., Korea).

Volatile basic nitrogen (VBN) content

VBN content was determined by the Conway microdiffusion method (Kohsaka, 1975), and was express as mg% of the sample using the following steps: 5 g of each sample was mixed with 10 mL of distilled water in a 100 mL beaker. Then, 5 mL of 1 N HCl was added and the mixture was refluxed for 2 h in an oil bath. After cooling, the solution was transferred quantitatively to a 100 mL volumetric flask and filled to the mark with distilled water. The solution was placed in a refrigerator for 20 min to allow separation of fat and precipitation of potassium perchlorate. The sample was filtered through filter paper No. 1 (Whatman International, UK). After filtration, concentrations of VBN in the supernatant were measured with the Kjeldahl method and expressed as mg% of the sample.

Table 1. Seasoned pork formulations with mugwort herb (MH) and vitamin C (VC)

| Ingredients (%) | Treatments<sup>(1)</sup> | Control | VC | MH | VC+MH |
|-----------------|--------------------------|---------|----|----|-------|
| Pork loin       | 96                       | 96      | 96 | 96 |
| Ice             | 4                        | 4       | 4  | 4  |
| Total           | 100                      | 100     | 100| 100|
| Soy sauce       | 6                        | 6       | 6  | 6  |
| Sugars          | 2.5                      | 2.5     | 2.5| 2.5|
| Green onion     | 2                        | 2       | 2  | 2  |
| Garlic          | 2                        | 2       | 2  | 2  |
| Onion juice     | 12                       | 12      | 12 | 12 |
| Black pepper    | 0.05                     | 0.05    | 0.05| 0.05|
| Sesame oil      | 0.5                      | 0.5     | 0.5| 0.5|
| Starch syrup    | 1.7                      | 1.7     | 1.7| 1.7|
| Sesame          | 0.3                      | 0.3     | 0.3| 0.3|
| Vitamin C (VC)  | -                        | 0.05    | -  | 0.05|
| Mugwort herb extract (MH) | - | 0.05 | - | 0.05 |

<sup>(1)</sup>Control: no antioxidant, VC: vitamin C 0.05%, MH 0.2: mugwort herb extract 0.05%, VC+MH: vitamin C 0.05% and mugwort herb extract 0.05%.
sample was mixed with 15 mL of distilled water for 1 min, and distilled water was added to bring the volume up to the final 50 mL followed by filtering through Whatman paper (No. 1). One milliliter of filtrate was placed in outer space of Conway unit, and 1 mL of 0.01 N \( \text{H}_2\text{BO}_3 \) and 200 \( \mu \text{L} \) of Conway reagent (0.066% methyl red in ethanol: 0.066% bromocresol green in ethanol = 1:1) were added in inner space. The Conway unit was sealed immediately after adding 1 mL of 50% \( \text{K}_2\text{CO}_3 \) to the outer space. The sealed Conway unit was shaken slowly and incubated at 37°C for 2 h. Then, 0.02 N \( \text{H}_2\text{SO}_4 \) was added to inner space for titration. The \text{VBN} content was calculated using the following equation.

\[
\text{VBN mg}\% = \frac{(a-b) \times f \times 28.014 \times 100}{S}
\]

Where, \( S \) = the sample weight in gram, \( a \) = the volume (mL) of added \( \text{H}_2\text{SO}_4 \) in sample, \( b \) = the volume (mL) of added \( \text{H}_2\text{SO}_4 \) in blank, \( f \) = the standard factor of \( \text{H}_2\text{SO}_4 \).

**Thiobarbituric acid reactive substances (TBARS) value**

Lipid oxidation was assessed in triplicate using the TBARS method of Tarladgis et al. (1960) with minor modifications and was expressed as milligrams of malondialdehyde (MD) per kilogram of seasoned pork. A 10-g sample was blended using homogenizer (AM-7, Nihonseiki, Kaisha Ltd., Japan) with 50 mL distilled water for 2 min and then transferred to a distillation tube. The cup used for blending was washed with an additional 47.5 mL of distilled water and added to the same distillation flask with 2.5 mL of 4 N \( \text{HCl} \) and a few drops of antifoaming agent (KMK-73, Shin-Etsu Silicone Co., Ltd., Korea). The mixture was distilled, and 50 mL of the distillate was collected. Five milliliter of 0.02 M TBA in 90% acetic acid (TBA reagent) was added to each test tubes containing 5 mL of the distillate and mixed well. The tubes were capped and heated in a boiling water bath for 30 min to develop the chromogen and cooled to room temperature. Absorbance was measured at 538 nm against a blank prepared with 5 mL distilled water and 5 mL TBA reagent using a UV/VIS spectrophotometer (Optizen 2120 UV plus, Mecasys Co. Ltd., Korea).

**Microbial analysis**

For all microbial counts, 25 g of samples were weighed and transferred into a sterile stomacher bag containing 225 mL of 0.1% peptone water followed by pummeling samples in a stomacher (Masticater-Paddle-Blender, IUL Instrument, Spain) for 2 min. From the prepared dilutions, total viable count (TVC), and lactic acid bacteria (LAB) count, total coliform bacteria count (TCC) and total Enterobacteriaceae count (TEC) were carried out. Plate Count Agar (PCA; Difco, USA) was used for TVC with an incubation period of 37°C for 2 d. Lactobacilli MRS agar (Difco, USA) was used for LAB with an incubation period of 35°C for 2 d. Escherichia coli/colicform count plate petrifilm and Enterobacteriaceae count plate petrifilm (3M Healthcare, USA) were used for TCC and TEC, respectively, with an incubation period of 35°C for 1 d under the same aerobic conditions.

**Sensory evaluation**

The seasoned pork samples were evaluated by 60 panelists (32 females, 28 males; aged 20-30) representing graduate students and members of the Department of Food Sciences and Biotechnology of Animal Resources, Konkuk University. Panellists were informed according to methodology proposed by ISO regulations (KS Q ISO, 2003; KS Q ISO, 2009) with the attributes and the scale to be used. Seasoned pork were cooked using a convection oven (Convotherm, OES6.06, Germany) at about 170°C for 5 min. Each sample was served immediately after cooking. The attributes which have been studied are the following: discoloration, flavor, and overall acceptability using a 9-point hedonic rating scale (Lawless and Heymann, 1999). The scale includes the following ranking: 1=extremely unacceptable, 2=very much unacceptable, 3=moderately unacceptable, 4=slightly unacceptable, 5=between acceptable and unacceptable, 6=slightly acceptable, 7=moderately acceptable, 8=very much acceptable and 9=extremely acceptable. A mean score of 7 or above indicates an acceptable product. A mean score below 5.0 marks the end of seasoned pork shelf-life (Mexis et al., 2009). Water was provided to panelists to cleanse the palate between samples.

**Statistical analysis**

All data were subjected to the analysis of variance (ANOVA) using general linear model (GLM) procedure of SPSS 18.0 software (SPSS Inc., USA), with three replications, which was used as the storage periods (days 0, 3, 5, 10, 15, and 20) and antioxidant type (Control, VC, MH, VC+MH). When significant \((p<0.05)\) treatment effects were shown, Duncan’s multiple range test was used to compare the mean values. Mean values and standard error of the means (SEM) were reported. The relationship between the pH and the TLA concentration is essentially
linear and a standard linear regression model was used to describe it (Korkeala et al., 1990).

## Results and Discussion

### VBN and TBARS values

The VBN value is a good indicator of protein deterioration and decomposition in meat products freshness during storage periods (Choi et al., 2011). The influence of different antioxidant added on VBN content of seasoned pork is shown in Table 2. According to the results, MH or VC alone did not significantly affect the VBN content, whereas a significant effect from the storage time was noted \((p<0.05)\). Similar results were observed in seasoned pork formulated with yellow dock extracts (0, 1, 3%), where the VBN content was not significantly different \((p<0.05)\). However, VC+MH had a little effect on inhibiting the increase in VBN content in seasoned pork \((p<0.05)\) from day 5. The finding may be associated with synergistic effects MH and VC which inhibit proteolysis carried out by microorganisms and enzymes in meat products. SPISLP (2011) suggested that a VBN value of 20 mg% can be used as a threshold value to evaluate the degree of freshness of raw and packed meat. Kim et al. (2012) reported that many meat products do not decay, though their VBN content is 30 mg%. In this study, VBN contents in seasoned pork did not exceed this range during the 20-d storage period \((p<0.05)\).

The TBARS values represent the contents of secondary products as measured by MD contents and are illustrated in Table 2. As the storage period progressed, the TBARS values increased significantly \((p<0.05)\) in all samples. However, TBARS values were significantly lower at any day of storage in samples containing VC+MH, so TBARS formation was almost totally inhibited. This finding is in agreement with that of Turp and Serdaroglu (2004), who noted that chicken patty treated with an antioxidant mixture (vitamin C 500 ppm and \(\alpha\)-tocopherol 200 ppm) was the most resistant to lipid oxidation, as evidenced by the lowest TBARS values compared with the other treatments. The results showed that antioxidant combination would more effectively inhibit lipid oxidation than using them alone during storage periods. Furthermore, Sheard et al. (2000) recommended a TBARS value of 0.5 mg/kg as a threshold value for rancidity perception by consumers. The TBARS values of all seasoned pork samples did not exceed 0.5 mg/kg by the end of the storage period.

### pH values and total lactic acid contents

The pH is a dependable indicator of food stability and is associated with microbial and chemical reactions that cause food deterioration. The changes in pH value of sea-

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Table 2. Changes in TBARS values (mg MD/kg) and VBN (mg%) of seasoned pork containing different antioxidant during refrigerated storage for 20 d

| Traits| Storage time (d) | Control | VC | MH | VC+MH | SEM |
|-------|-----------------|---------|----|----|-------|-----|
|       |                 |         |    |    |       |     |
|       | 0               | 0.18<sup>a</sup> | 0.19<sup>b</sup> | 0.16<sup>a</sup> | 0.15<sup>a</sup> | 0.003 |
|       | 3               | 0.22<sup>b</sup> | 0.22<sup>b</sup> | 0.19<sup>b</sup> | 0.17<sup>b</sup> | 0.004 |
|       | 5               | 0.30<sup>c</sup> | 0.27<sup>c</sup> | 0.23<sup>b</sup> | 0.20<sup>c</sup> | 0.006 |
|       | 10              | 0.36<sup>d</sup> | 0.32<sup>c</sup> | 0.28<sup>d</sup> | 0.24<sup>d</sup> | 0.006 |
|       | 15              | 0.40<sup>e</sup> | 0.37<sup>c</sup> | 0.33<sup>b</sup> | 0.28<sup>e</sup> | 0.006 |
|       | 20              | 0.44<sup>f</sup> | 0.43<sup>e</sup> | 0.40<sup>e</sup> | 0.35<sup>f</sup> | 0.005 |
| SEM  |                 | 0.009   | 0.008 | 0.008 | 0.007 |     |
| VBN  | 0               | 13.17<sup>a</sup> | 13.38<sup>a</sup> | 13.45<sup>a</sup> | 13.10<sup>a</sup> | 0.087 |
|       | 3               | 13.94<sup>b</sup> | 13.80<sup>b</sup> | 13.69<sup>a</sup> | 13.38<sup>a</sup> | 0.095 |
|       | 5               | 13.96<sup>b</sup> | 13.82<sup>b</sup> | 13.83<sup>a</sup> | 13.40<sup>a</sup> | 0.075 |
|       | 10              | 14.55<sup>b</sup> | 14.42<sup>b</sup> | 14.54<sup>b</sup> | 14.02<sup>b</sup> | 0.076 |
|       | 15              | 14.56<sup>b</sup> | 14.56<sup>b</sup> | 14.84<sup>b</sup> | 14.12<sup>b</sup> | 0.081 |
|       | 20              | 15.32<sup>b</sup> | 15.36<sup>b</sup> | 15.55<sup>b</sup> | 14.79<sup>b</sup> | 0.080 |
| SEM  |                 | 0.108   | 0.109 | 0.123 | 0.102 |     |

1) Traits: TBARS, thiobarbituric acid reactive substances, VBN: volatile basic nitrogen.
2) Control: no antioxidant, VC: vitamin C 0.05%, MH 0.2: mugwort herb extract 0.05%, VC+MH: vitamin C 0.05% and mugwort herb extract 0.05%.
3) SEM: standard error of the means \((n=12)\).

Means with different superscripts are significantly different \((p<0.05)\).

A-F antioxidant effects.

a-f storage day effects.
Effect of Antioxidant on Shelf-Life of Seasoned Pork

Table 3. Changes in pH values, lactic acid concentration (mg/g), and microbial analysis of seasoned pork containing different antioxidant during refrigerated storage for 20 d

| Traits | Storage time (d) | Control | VC | MH | VC+MH | SEM (1) |
|--------|------------------|---------|----|----|-------|---------|
| pH values | 0 | 5.52a | 5.51c | 5.53c | 5.52c | 0.004 |
| | 3 | 5.52a | 5.49c | 5.51c | 5.51c | 0.004 |
| | 5 | 5.45d | 5.42d | 5.45d | 5.43d | 0.005 |
| | 10 | 5.35c | 5.37a | 5.36a | 5.37c | 0.004 |
| | 15 | 5.32b | 5.30b | 5.31b | 5.30b | 0.004 |
| | 20 | 5.20a | 5.19a | 5.21a | 5.18a | 0.003 |
| SEM (1) | 0.012 | 0.121 | 0.012 | 0.012 | 0.012 |
| TLA (mg/g) | 0 | 2.6a | 2.6a | 2.6a | 2.6a | 0.013 |
| | 3 | 2.7a | 2.7a | 2.7a | 2.7a | 0.018 |
| | 5 | 3.0a | 3.0a | 3.0a | 2.8b | 0.028 |
| | 10 | 3.1a | 3.1a | 3.1a | 3.0b | 0.033 |
| | 15 | 3.1a | 3.0a | 3.0a | 3.0a | 0.027 |
| | 20 | 3.1a | 3.1a | 3.1a | 4.8b | 0.052 |
| SEM (1) | 0.148 | 0.145 | 0.129 | 0.119 | 0.119 |
| LAB (CFU/g) | 0 | 3.4a | 3.5c | 3.5c | 3.4a | 0.057 |
| | 3 | 3.7a | 3.8a | 3.6a | 3.7a | 0.075 |
| | 5 | 4.8b | 4.9b | 4.8b | 4.7b | 0.061 |
| | 10 | 5.2b | 5.2b | 5.0b | 4.8b | 0.125 |
| | 15 | 5.8b | 6.1c | 5.9c | 5.8c | 0.082 |
| | 20 | 7.2d | 7.2d | 7.1d | 7.0d | 0.101 |
| SEM (1) | 0.228 | 0.225 | 0.215 | 0.225 | 0.225 |
| TVC (CFU/g) | 0 | 4.4a | 4.4a | 4.4a | 4.3a | 0.123 |
| | 3 | 4.5a | 4.5a | 4.5a | 4.5a | 0.093 |
| | 5 | 5.1b | 5.2b | 5.1b | 5.1b | 0.043 |
| | 10 | 5.5b | 5.4b | 5.5b | 5.4b | 0.057 |
| | 15 | 6.0b | 6.0b | 6.0b | 5.9b | 0.048 |
| | 20 | 7.7d | 7.7d | 7.6d | 7.4a | 0.064 |
| SEM (1) | 0.199 | 0.196 | 0.192 | 0.184 | 0.184 |

1) Traits: TLA, total lactic acid concentration, LAB: lactic acid bacteria, TVC: total viable count.
2) Control: no antioxidant, VC: vitamin C 0.05%, MH 0.2: mugwort herb extract 0.05%, VC+MH: vitamin C 0.05% and mugwort herb extract 0.05%.
3) SEM: standard error of the means (n=9).
4) SEM: standard error of the means (n=6).
5) SEM: standard error of the means (n=9).

Means with different superscripts are significantly different ( p < 0.05).
A-D antioxidant effects.
a-d storage day effects.

Seasoned pork were not affected by the antioxidants (MH and/or VC), whereas storage time had a significant effect (Table 3). As storage time progressed from 0 to 20 d, the pH values of all treatments decreased gradually from 5.51 to 5.18. Aksu and Kaya (2005) observed that the pH values were not significantly influenced by antioxidant addition. Also, Korkeala et al. (1990) noted that decrease in pH during storage was due to lactic acid generation by microorganisms.

The TLA of control and all the treated raw samples ranged from 2.6 to 2.7 mg/g with counts increasing from 4.6 to 5.2 mg/g after 20 d (Table 3). Korkeala et al. (1990) recommended that lactic acid could be used as an indicator of spoilage of meat products. If the TLA concentration is over 4 mg/g or pH is below 5.8 the samples can be deemed spoiled. In addition, Nassos et al. (1983) suggested that ground beef containing TLA level greater than 7.3 mg/g of meat would likely be found unacceptable by odor evaluation.

The scatter diagram between TLA concentration and the pH values of seasoned pork is shown in Fig. 1. The equation was pH = 5.853 – 0.137 × TLA (1 mg of TLA/g lead to a drop of 0.14 pH value). Included in the figure are 95% confidence limits for both predicted means and individual observation. These results are supported by the good correlation coefficients between pH and lactic acid
concentration values. In this study, above a level of 3.6 mg lactic acid/g and pH values below 5.3, all samples were considered unacceptable as sensory panel noticed sour odor (15 d).

**Microbial analysis**

The effect of the addition of different antioxidant on microbial contents (LAB, TVC, TCC, TEC) during refrigerated storage of seasoned pork is presented in Table 3, and Fig. 2, respectively.

The initial LAB of seasoned pork ranged from 3.4-3.5 Log CFU/g and it increased to 7.0-7.2 Log CFU/g after 20 d. Similar trends in LAB were observed in studies by Fernandez-Lopez et al. (2005) who indicated that the growth of LAB was similar for control and treatments with rosemary oil and water extract during 12 d at 8°C. According to Vermeiren et al. (2004), the sensory panel considered the rejected samples unfit for human consumption after LAB count reached levels of 7-8 Log CFU/g. In this research, the first evidence of sensory defects was an acidic odor, which occurred at a LAB of about 5-6 Log CFU/g (15 d). Vermeiren et al. (2004) suggest that when stored under vacuum-packaging and refrigeration conditions, LAB will dominate the spoilage process. Because the metabolic activity of LAB results in spoilage appearing as sour, off-flavors, off-odors, slime production, swelling of the package through gas production, and discoloration such as greening.

The TVC of all samples were about 4.3-4.4 Log CFU/g, and significantly increased as storage progressed (Table 3). These data agree with the findings of Han et al. (2006), who noted that the microbial population on oriental medicinal seasoned pork added with propolis as being 3-4 Log CFU/g at day 0. Also, several authors have mentio-
ned that the addition of functional ingredients to seasoned meat inhibited the growth of TVC during refrigerated storage (Han et al., 2006; Kim et al., 2009; Youn et al., 2004). However, in this study, there was no significant difference between treatment containing control or MH (either alone with vitamin C) throughout the storage time ($p<0.05$). As storage time progressed, increase in TVC and number of LAB showed a similar pattern. According to Mantis et al. (2007), the TVC and LAB count were in similar number when sensory defects were first detected in Greek sausage. They recommended that various sensory defects were associated with the up to TVC of about 8 Log CFU/g.

The TCC and TEC of seasoned pork formulated with different antioxidant are given in Fig. 2. Among all treatments, VC+MH effectively delayed both TCC and TEC growth during the whole periods of storage ($p<0.05$). These results agree with that of Zhang et al. (2009), who obtained similar results when total coliform bacteria decreased significantly after adding mixed spice extracts (rosemary and liquorice) compared with control in emulsified sausage. This trend of controlling TCC and TEC may be due to synergistic effect of the mugwort in combination with vitamin C on the inhibitory effect of growth. Karabegović et al. (2011) noted that extracts of various mugworts possess antimicrobial activity and the differences in antimicrobial activities of various mugworts can result in differences in their qualitative and quantitative components, which may be attributed to different growth environment and extraction conditions. TCC and TEC of all samples increased steadily during the first 15 days of storage and decreased thereafter. The decrease population supports the view that the formation of organic acid by bacterial metabolism caused decrease in pH, which may inhibit the coliform growth (Lee et al., 2009). These authors noted that the population of total coliform bacteria increased in seasoned pork treated with medicinal plants significantly on 15 d, followed by a decrease thereafter. Signorini et al. (2006) found that lactic acid was the most efficient compound for controlling spoilage population such as *enterobacteriaceae* species, *Pseudomonas* spp., and *B. thermosphacta* of meat stored at refrigeration condition.

**Sensory evaluation**

The sensory values for seasoned pork containing differ-

### Table 4. Changes in sensory evaluation of seasoned pork containing different antioxidant during refrigerated storage for 20 d

| Traits $^1$ | Storage time (d) | Control | VC | MH | VC+MH | SEM $^3$ |
|-------------|------------------|---------|----|----|-------|----------|
| **Discoloration** | 0 | 8.14$^a$ | 8.19$^a$ | 8.39$^a$ | 8.25$^a$ | 0.057 |
| | 3 | 7.94$^a$ | 8.00$^a$ | 8.08$^d$ | 8.31$^d$ | 0.071 |
| | 5 | 7.42$^a$ | 7.42$^a$ | 7.69$^a$ | 7.58$^c$ | 0.064 |
| | 10 | 6.92$^b$ | 6.83$^b$ | 6.86$^b$ | 6.81$^b$ | 0.061 |
| | 15 | 5.00$^d$ | 5.11$^d$ | 5.25$^d$ | 5.14$^d$ | 0.064 |
| SEM $^3$ | 0.101 | 0.098 | 0.105 | 0.103 | |
| **Flavor** | 0 | 8.17$^a$ | 8.25$^a$ | 8.31$^a$ | 8.36$^a$ | 0.061 |
| | 3 | 8.09$^a$ | 8.03$^a$ | 7.78$^a$ | 7.83$^a$ | 0.053 |
| | 5 | 7.72$^a$ | 7.89$^a$ | 7.86$^a$ | 7.94$^c$ | 0.049 |
| | 10 | 6.91$^b$ | 6.99$^b$ | 7.03$^b$ | 7.08$^b$ | 0.054 |
| | 15 | 5.11$^d$ | 5.11$^d$ | 5.22$^d$ | 5.36$^d$ | 0.062 |
| SEM $^3$ | 0.096 | 0.098 | 0.103 | 0.097 | |
| **Overall acceptability** | 0 | 8.57$^a$ | 8.65$^a$ | 8.61$^a$ | 8.69$^a$ | 0.054 |
| | 3 | 8.22$^a$ | 8.25$^a$ | 8.44$^a$ | 8.53$^a$ | 0.046 |
| | 5 | 7.98$^a$ | 8.08$^a$ | 8.11$^b$ | 8.11$^b$ | 0.059 |
| | 10 | 6.55$^c$ | 6.49$^c$ | 6.53$^c$ | 6.72$^a$ | 0.048 |
| | 15 | 4.96$^d$ | 4.96$^d$ | 5.19$^d$ | 5.23$^a$ | 0.054 |
| SEM $^3$ | 0.113 | 0.115 | 0.108 | 0.106 | |

$^1$Sensory score: 1=extremely unacceptable, 2=very much unacceptable, 3=moderately unacceptable, 4=slightly unacceptable, 5=between acceptable and unacceptable, 6=slightly acceptable, 7=moderately acceptable, 8=very much acceptable and 9=extremely acceptable. A mean score of 7 or above indicates an acceptable product. A mean score below 5.0 marks the end of seasoned pork shelf-life.

$^2$Control: no antioxidant, VC: vitamin C 0.05%, MH 0.2: mugwort herb extract 0.05%, VC+MH: vitamin C 0.05% and mugwort herb extract 0.05%.

$^3$SEM: standard error of the means (n=4). Means with different superscripts are significantly different ($p<0.05$). a-d/storage day effects.
ent antioxidant are displayed in Table 4. Addition of MH and/or VC in seasoned pork did not significantly affect the sensory characteristics, whereas a significant effect from the storage time was noted \( (p<0.05) \). These results are in accordance with that of Naveena et al. (2008), who reported that addition of pomegranate juice and rind powder in chicken patties did not impart a significant difference in sensory characters (off-odor, sweet-flavor, chicken flavor, or overall palatability score). As the storage time increased, sensory characters (discoloration, flavor, and overall acceptability) had lower scores than those of initial storage periods. In this study, sensory panel rejected the seasoned pork unfit for human consumption above a level of 3.6-3.9 mg lactic acid/g. LAB reached levels of 5-6 Log CFU/g, and pH values ranged from 5.31-5.51 (15 d). These results are in agree with that of Choi and Lee (2002), who reported that seasoned pork meat, which the LAB count increased with more than 7 Log CFU/g and pH values decreased with less than 5.5, had rancid odors and off-flavors. Several authors have found no correlation between total LAB counts and sensory deterioration (Leroi, 2010; Leroi & Joffraud, 2001). However, Paludan-Müller et al. (1998) succeeded in increasing the shelf-life of cold-smoked salmon by inhibiting LAB with nisin, suggesting a possible spoiling effect of this bacterial group. The sensory deterioration due to functions of LAB, the compounds responsible and their associated precursors have been summarized by Huss et al. (1995). The carbohydrate fermentation causes sour tastes and marinated off-odors and flavors due to organic acid production (Leroi, 2010). Therefore, producing bacterial have been shown to be responsible for the spoilage of vacuum packed seasoned pork meat; a fermented, sour odor and taste are described as the main sensory defects.

**Conclusion**

An antioxidant mixture of MH and VC was effective in inhibiting lipid oxidation (TBARS formation) and microbial growth (TEC and TCC) in seasoned pork during refrigerated storage at 4°C. Additionally, TLA concentration, LAB count, and the sensory properties (discoloration, flavor, and overall acceptability) of seasoned pork were not significantly affected by adding MH and/or VC. In this study, the increase in both LAB populations and TLA concentration, as well as decrease in the pH values, generally coincided with the sensory scores detected by the sensory panel. Further studies are needed to control LAB in vacuum packed seasoned meat, especially if combined with MH, which is capable of inhibiting the growth of lactic acid bacteria.

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