Effects of Additions of Monascus and Laccaic acid on the Color and Quality Properties of Nitrite-Free Emulsion Sausage during Refrigerated Storage

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Abstract
This effect of Monascus and Laccaic acid on the chemical composition, physical, texture and sensory properties of sausage were investigated during storage. Eight treatments (T) of sausage such as T1 (12 ppm sodium nitrite), while T2, T3, T4, T5, T6 and T7 were formulated with different ratios of Monascus/Laccaic acid: 63/7.0, 108/12, 135/15, 59.5/10.5, 102/18 and 127.5/22.5 ppm, respectively. The batch formulated without nitrite or Monascus and laccaic acid was served as control (C). The control sausages had higher pH values compared to the treated ones at 3, 10 and 28 d storage (p<0.05). After 10 d storage, the pH values decreased in treated sausage samples (p<0.05). The T1 and T4 presented the lowest yellowness and lightness values, respectively over the storage period. The redness values were increased as increasing Monascus and Laccaic acid amounts (T2-T4, T5-T7). The addition of Monascus and Laccaic acid had significantly higher hardness and springiness values (p<0.05) compared with the control in 3, 19 or 28 d storage. The results indicated that the addition of Monascus and Laccaic acid could improve the redness of the products.

Keywords Monascus, Laccaic acid, sausage, color

Introduction
Sodium nitrite and sodium nitrate are used as curing agents in meat and they are very important not only as preservatives, but also for color and flavor formation (Honikel, 2008; Marriott et al., 1981; Sebranek and Bacus, 2007). For many years, nitrite salts, mainly sodium nitrite and potassium nitrite have been used in the preparation of cured meats due to their antioxidant and antimicrobial effects (Gill and Holley, 2003). Practically, nitrite can also be applied to preserve desirable meaty flavor (Hedrick et al., 1994), and the maximum allowable level of sodium nitrite in food products is 156 ppm (Oh et al., 2004). Although, the added levels of nitrites in cured meat products can be diminished during the processing and storage however a significant amount is still residual. The nitrite can react with amines and amides to produce N-nitroso compounds which related to an increasing risk of gastric, esophageal, nasopharyngeal and bladder cancers. During the 1970s the safety of cured meats was strongly debated. At issue was the question if preformed nitrosamines were present at all or at levels of concern and if the known levels of residual nitrite represented a risk to human health. The potential problem was recognized and dealt with by the meat processing industry. The use of nitrate...
was essentially eliminated, the levels of nitrite used were lowered and much tighter control of manufacturing processes was instituted (Cassens, 1997).

Monascus produces monacolin K, which lowers cholesterol synthesis (Chen et al., 2008). This genus also exhibits several medicinal properties such as antimicrobial, antihypertensive, antioxidant, anticarcinogenic, and anticancer properties (Patakova, 2013). The mold Monascus has been widely used in the Orient for red wine brewing, red soybean cheese processing, and food coloring (Onoe and Katayama, 1977). Since the finding of carcinogens in coal tar dyes in the 1960s, a series of synthetic food colors was successively banned, and Monascus pigment has been considered as a natural pigment to replace the synthetics (Lin and Iizuka, 1981). Many metabolic derivatives, such as ethanol, monascus pigments (red in color), c-aminobutyric, and monacolins K, can be produced by Monascus spp. (Ma et al., 2000).

Lac, a natural resin of insect origin, is used extensively for natural food additives, cosmetics and as a colorant for silk and cotton dyeing. The secretion exuded by these insects is stick or rubber lacquer that gives scarlet components of this secretion has been separated into four species. Lac dye, which is the soluble part of stick lac, is composed mainly of two major anthraquinone-based components: laccic acid A and B (Chairat et al., 2004).

Osterlie and Lerfall (2005) recommended mixing minced meat with a lycopene-containing product to reduce nitrite levels. Jiménez-Colmenero et al. (2001) pointed out that combination of nitrite with several compounds which having color, flavor, antioxidant and antimicrobial effects could be considered as an effective way to reduce the added level of nitrite in meat products. Fink-Gremmels et al. (1992) indicated that Monascus extracts might be used as an alternative to nitrite in some meat products. The aim of this study was to determine the effect of Monascus and Laccic acid additions on the color and quality properties of nitrite-free sausages during refrigerated storage.

### Materials and Methods

**Materials**

Monascus and Laccic acid was obtained from the aon-ecaf (Korea). Fresh pork ham, pork back-fat was obtained from a local commercial processor (Korea) after 24 h postmortem.

**Formulation and processing of fermented sausage**

The formulations of sausages are summarized in Table 1. Particular treatments (T) were prepared as follows: T1 was added with 12 ppm sodium nitrite, while the T2, T3, 4, T5, T6 and T7 were formulated with different levels of Laccic acid and Monascus: 63 and 7 ppm (T2, 9:1), 108 and 12 ppm (T3, 9:1), 135 and 15 ppm (T4, 9:1), 59.5 and 10.5 ppm (T5, 8.5:1.5), 102 and 18 ppm (T6, 8.5:1.5), and 127.5 and 22.5 ppm (T7, 8.5:1.5), respectively. The control (C) batch was prepared with pork, fat and additives without nitrite or Laccic acid and Monascus. The meat was trimmed off of all connective tissue and visible fats and then chopped through a 3 mm plate using a silent chopper (Model 7548, Biro MFG. Co., USA). For each

### Table 1. Formulation of sausage

| Ingredients (%) | C     | T1    | T2    | T3    | T4    | T5    | T6    | T7    |
|-----------------|-------|-------|-------|-------|-------|-------|-------|-------|
| Pork            | 69    | 69    | 69    | 69    | 69    | 69    | 69    | 69    |
| Fat             | 20    | 20    | 20    | 20    | 20    | 20    | 20    | 20    |
| Ice             | 9     | 9     | 9     | 9     | 9     | 9     | 9     | 9     |
| Phosphate       | 0.5   | 0.5   | 0.5   | 0.5   | 0.5   | 0.5   | 0.5   | 0.5   |
| Salt            | 1.2   | 1.2   | 1.2   | 1.2   | 1.2   | 1.2   | 1.2   | 1.2   |
| Sodium nitrite  | 12    |       |       |       |       |       |       |       |
| Laccic acid (ppm) | 7    | 12    | 15    | 10.5  | 18    | 22.5  |       |       |
| Monascus (ppm)  |       | 63    | 108   | 135   | 59.5  | 102   | 127.5 |       |
| Garlic          | 0.15  | 0.144 | 0.147 | 0.144 | 0.143 | 0.147 | 0.144 | 0.143 |
| Onion           | 0.15  | 0.144 | 0.147 | 0.144 | 0.143 | 0.147 | 0.144 | 0.143 |
| Total           | 100   | 100   | 100   | 100   | 100   | 100   | 100   | 100   |

1) C, control; T1, Sausages added 12 ppm sodium nitrite; T2, Sausages added 7 ppm Laccic acid and 63 ppm Monascus; T3, Sausages added 12 ppm Laccic acid and 108 ppm Monascus; T4, Sausages added 15 ppm Laccic acid and 135 ppm Monascus; T5, Sausages added 10.5 ppm Laccic acid and 59.5 ppm Monascus; T6, Sausages added 18 ppm Laccic acid and 102 ppm Monascus; T7, Sausages added 22.5 ppm Laccic acid and 127.5 ppm Monascus.
treatment, the chopped lean meat was placed in a bowl cutter (CR-40, Spain), chopped for about 10 s at low speed, and then the mixture of ingredients and replacing materials were gradually added while chopping. The meat mixture was chopped for further 1 min at high speed and then about one-third of ice-water was added and the batter was continuously chopped for 2 min at high speed. After that, the pork back-fat was added and the rest of ice water was gradually added, the batter was then chopped at high speed for further 5 min. The temperature of batter was maintained below 10°C during preparation. After chopping, the meat batter was immediately stuffed into 28-mm diameter collagen casings (Naturin Viscofan Co., Spain) using a vacuum stuffer (Model VF610, Handtmann Co., Germany). Finally, the sausages were placed in a smokehouse and cooked until the core temperature reached at 70°C. After cooking, the cooked sausages were immediately soaked in cold water to cool and left to drain the water. Thereafter, the samples were placed in polyethylene/polyamide bags and finally assigned into 4 different storage periods; 3, 10, 19 and 28 d and kept at 4°C.

**Proximate composition**

The moisture, protein, fat and collagen contents of sausages were analyzed using a Food Scan™ Lab 78810 (Foss Tecator Co., Ltd., DK), according to the method of the Association of Official Analytical Chemists (AOAC, 2000).

**Color measurement**

Color was determined at 4 defined areas on the cut surface of each sausage sample using a Minolta Chroma Meter CR-400 (Minolta Camera Co., Ltd., Japan) that was standardized with a white plate (Y = 86.3, X = 0.3165 and y = 0.3242). Color was expressed according to the Commission International de l’Eclairage (CIE, 1978) system and reported as CIE \( L^* \) (lightness), CIE \( a^* \) (redness), CIE \( b^* \) (yellowness).

**pH measurement**

The pH values of sausage samples were determined in triplicates using a pH meter (Model 340, Mettler-Toledo GmbH, Switzerland). The pH was measured after homogenizing 3 g of each sample with 27 mL of distilled water for 30 s using a homogenizer.

**Texture analysis**

The texture properties of the sausages were analyzed using a puncture probe (7 mm diameter) attached to a texture analyzer (Model 4465, Instron Corp., UK). For texture analysis, the sample from each treatment was cut into 2.5 cm long pieces; the cube was axially compressed twice until reaching each time 80% of its initial height. The speed of load cell was set at 120 mm/min and the following parameters were calculated: hardness (kg), springiness (mm) and cohesiveness (kg*mm), gumminess (kg) and chewiness (kg*mm).

**Sensory evaluation**

Sensory evaluation of sausages in different treatments at 1 d storage was performed using the method as described by Deda et al. (2007) with suitable modifications. Briefly, eight panelists consisted of 2 males and 6 females with an average age of 27-35 years selected from the members of Animal Products Processing Division of the National Institute of Animal Science, Wanju, Korea were used. Before the sensory evaluation, the panelists were trained using commercial sausages for several months (once per every two weeks) to familiarize them with the characteristics to be evaluated. Prior to evaluation, the sensory samples were warmed at room temperature (about 25°C) for 1 h and cut into 1.5 cm long pieces, coded with random numbers. The panelists were laid to seat in private seats under fluorescent lighting and were served with the sensory samples in a random manner. The sensorial characteristics including texture, flavor, taste and overall acceptability specifically selected for frankfurters evaluation (Choe et al., 2013; Ozvural and Vural, 2011) were used. The samples were evaluated for the aforementioned sensorial traits using a 7-point scale (1 point = extremely undesirable, 7 point = extremely desirable) as described by Meilgaard et al. (1991). The panelists were asked to refresh their mouth with the drinking distilled water and salt-free crackers between samples. All sensory sessions were carried out in the sensory panel booth room equipped with white lighting at a constant temperature (20°C).

**Statistic analysis**

The data were subjected to statistical analysis using the Statistic Analysis System (SAS) package (SAS Institute, USA, 2014). All data were analyzed by the General Linear Model procedure considering treatment and storage time as the main effects. Means were compared using Duncan's Multiple Range Test. Significant differences (\( p<0.05 \)) between mean values of eight samples were determined for the proximate composition, color, pH and texture had five
replications. Significant differences (p<0.05) between sensory evaluations were determined (N=8).

Results and Discussion

Proximate composition of pork sausages

Levels of moisture, protein, fat and collagen contents are presented in Table 2. As expected, the treatments significantly affected the levels of these contents. The moisture content was lower in the T2 than in the other treatments and control (62.14 vs. 62.52-63.11%, p<0.05). The protein contents in the C, T1 and T3 were lower compared to that in the other treatments (16.24-16.36 vs. 16.57-16.76%, p<0.05). For fat content, the T6 had the lowest level (18.77%). There was no difference (p>0.05) in collagen contents between the control, Monascus and Laccaic acid sausages. The reason why the addition of Monascus and Laccaic acid levels increased the fat and protein content of sausages has remained unknown.

Color and pH of pork sausages

The color and pH values of sausages in the control and treatments at different storage times are presented in Table 3. The pH values were significantly lower in the treatments in comparison to the control over the storage time (p<0.05). All the treated sausages decreased in pH values after 10 d refrigerated storage time. Banwart (1979) indicated that cured meat became sour, with lower pH values, because of the fermentation of carbohydrates by lactic acid bacteria. Similarly Liu et al. (2010) reported that the pH values of low-nitrite Chinese sausages made with anka rice decreased due to the increased lactic acid bacteria counts over storage time at 4℃.

The data indicated that sausages with added Monascus and Laccaic acid added tended to have higher CIE a* (redness) values, indicating more red color. Especially, T2, T3, T4, T6 and T7 samples had significantly higher CIE a* values than those of the C and T1 treatments (without Monascus and Laccaic acid added). Since the most dominant color of cured meat products is red, a difference in the CIE a* value may be considered to have the greatest impact on product color. In the present study, the addition of Monascus and Laccaic acid at all levels increased CIE a* values of sausages, but it decreased the CIE L* (lightness) values of sausages on all storage days examined. These pigments, produced by Monascus and Laccaic acid, might contribute to the increase of redness of the samples observed in this study. The sausages produced with 135 ppm and 15 ppm Monascus and Laccaic acid (T4) were generally redder compared with other treatments at all days examined. T4 was lowest lightness and highest redness in 3, 10 and 28 storage days. Regarding the CIE b* ( yellowness) values, all the samples produced with Monascus and Laccaic acid had higher CIE b* values than the sodium nitrite sausages in all storage days. The decline in the lightness and increase in redness probably result from the suppression of the product’s natural color owing to the addition Monascus and Laccaic acid. It is well known that nitrite contributes to the characteristic pink cured meat color (Honikel, 2008). In this study, level of nitrite residue in sausages with Monascus and Laccaic acid inoculation was lower compared to the control, whereas the redness was higher than that of the control. Probably, Monascus and Laccaic acid strain possesses nitrate reductase and heme-independent nitrite reductase activities, which directly involve in the mechanisms of nitrosomyoglobin formation, resulting in forming the typical pink color in meat products (Hammes et

| Treatments | Moisture | Fat | Protein | Collagen |
|------------|---------|-----|---------|----------|
| C          | 63.03±0.01<sup>A</sup> | 19.00±0.10<sup>A</sup> | 16.24±0.12<sup>A</sup> | 1.88±0.18 |
| T1         | 62.52±0.21<sup>B</sup> | 19.31±0.08<sup>A</sup> | 16.34±0.08<sup>B</sup> | 1.81±0.32 |
| T2         | 62.14±0.22<sup>C</sup> | 19.40±0.03<sup>A</sup> | 16.64±0.01<sup>A</sup> | 1.83±0.31 |
| T3         | 63.07±0.04<sup>A</sup> | 18.89±0.04<sup>C</sup> | 16.36±0.01<sup>B</sup> | 1.89±0.10 |
| T4         | 62.83±0.22<sup>AB</sup> | 19.09±0.05<sup>B</sup> | 16.64±0.02<sup>C</sup> | 1.68±0.24 |
| T5         | 62.76±0.14<sup>AB</sup> | 19.07±0.02<sup>B</sup> | 16.57±0.04<sup>AB</sup> | 1.94±0.01 |
| T6         | 63.11±0.06<sup>A</sup> | 18.77±0.01<sup>AB</sup> | 16.76±0.04<sup>A</sup> | 1.72±0.04 |
| T7         | 62.55±0.12<sup>B</sup> | 19.10±0.01<sup>AB</sup> | 16.70±0.17<sup>A</sup> | 1.69±0.13 |

<sup>A</sup><sup>B</sup>Means with different superscript in the same column significantly differ at p<0.05.
<sup>C</sup>C, control; T1, Sausages added 12 ppm sodium nitrite; T2, Sausages added 7 ppm Laccaic acid and 63 ppm Monascus; T3, Sausages added 12 ppm Laccaic acid and 108 ppm Monascus; T4, Sausages added 15 ppm Laccaic acid and 135 ppm Monascus; T5, Sausages added 10.5 ppm Laccaic acid and 59.5 ppm Monascus; T6, Sausages added 18 ppm Laccaic acid and 102 ppm Monascus; T7, Sausages added 22.5 ppm Laccaic acid and 127.5 ppm Monascus.
Laccaic acid was detected in Monascus and its extracts (Chen and Johns, 1993). Similarly, Kim (2013) reported that addition of red yeast rice decreased lightness and increased redness of sausages.

### Texture profile of pork sausages

The texture characteristics of sausages are presented in Table 4. Hardness was higher in added Monascus and Laccaic acid sausages than control at 19 and 28 d (p<0.05). Springiness was higher in added Monascus and Laccaic acid sausages than control at 19 d (p<0.05). No significant differences were observed between control and treatments with respect to cohesiveness, gumminess and chewiness. These results suggest that the incorporation of Monascus and Laccaic acid did not cause a texture defect in the product. The sausages made with red yeast rice were lower hardness, more springiness and gumminess than the products made with no added red yeast rice (Kim, 2013).

Rhyu et al. (2003) also reported that the sausages with 2 g/100 g Monascus Koji significantly improved the color

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Table 3. Changes in the pH and color of added Monascus an Laccaic acid sausages during storages time at 4°C

| Treatments | 3 | 10 | 19 | 28 |
|------------|---|----|----|----|
| pH         |   |    |    |    |
| C          | 6.63±0.01<sup>a</sup>B | 6.73±0.01<sup>a</sup>B | 6.59±0.02<sup>a</sup>B | 6.65±0.04<sup>a</sup>B |
| T1         | 6.59±0.01<sup>b</sup>H | 6.67±0.00<sup>b</sup>H | 6.53±0.01<sup>b</sup>H | 6.44±0.01<sup>b</sup>H |
| T2         | 6.57±0.00<sup>c</sup>E | 6.57±0.01<sup>c</sup>E | 6.48±0.01<sup>c</sup>E | 6.39±0.01<sup>c</sup>E |
| T3         | 6.61±0.01<sup>c</sup>H | 6.67±0.01<sup>c</sup>H | 6.50±0.01<sup>c</sup>H | 6.43±0.01<sup>c</sup>H |
| T4         | 6.58±0.00<sup>f</sup>E | 6.58±0.01<sup>f</sup>E | 6.46±0.01<sup>f</sup>E | 6.40±0.01<sup>f</sup>E |
| T5         | 6.58±0.00<sup>g</sup>E | 6.62±0.00<sup>g</sup>E | 6.45±0.01<sup>g</sup>E | 6.39±0.00<sup>g</sup>E |
| T6         | 6.58±0.01<sup>c</sup>D | 6.64±0.01<sup>c</sup>D | 6.46±0.01<sup>c</sup>D | 6.38±0.01<sup>c</sup>D |
| T7         | 6.57±0.01<sup>e</sup>E | 6.59±0.01<sup>e</sup>E | 6.48±0.03<sup>e</sup>E | 6.41±0.01<sup>e</sup>E |

### Table 4. Hardness was higher in added Monascus and Laccaic acid sausages than control at 19 and 28 d (p<0.05).

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### References

al., 1990; Wolf et al., 1990). In addition to monascin, ankaflavin and rubropunctatina, a red pigment, monascusorubamine, was detected in M. purpureus extracts (Chen and Johns, 1993). Similarly, Kim (2013) reported that addition of red yeast rice decreased lightness and increased redness of sausages.
and texture.

**Sensory characteristics of pork sausages**

The sensory characteristics of sausages evaluated at 3 d storage are presented in Table 5. The scores of sensory quality attributes were highest in T1 compared to those of the added Monascus and Laccacic acid treatments and control. The sausages added with Monascus and Laccacic acid.
Table 5. Sensory evaluation of added Monascus and Laccaic acid sausages

| Treatments | Color | Flavor | Taste | Overall acceptability |
|------------|-------|--------|-------|-----------------------|
| C          | 4.00±1.10
t | 4.83±0.75 | 5.00±1.10 | 4.83±0.98 |
| T1         | 5.67±1.37
t | 5.17±0.75 | 5.50±0.84 | 6.00±1.10 |
| T2         | 4.83±1.74 | 4.67±0.82 | 5.00±1.26 | 4.83±1.33 |
| T3         | 5.00±1.10 | 5.00±0.89 | 5.00±1.10 | 5.17±0.98 |
| T4         | 4.83±0.98 | 5.33±0.82 | 5.50±0.84 | 5.17±0.75 |
| T5         | 4.50±1.05 | 4.67±1.03 | 5.00±1.26 | 4.83±1.17 |
| T6         | 5.17±0.75 | 5.00±0.00 | 5.50±0.55 | 5.33±0.52 |
| T7         | 5.00±0.63 | 5.00±0.63 | 5.17±0.75 | 4.83±0.98 |

A,BMeans with different superscript in the same column significantly differ at p<0.05.
1C, control; T1, Sausages added 12 ppm sodium nitrite; T2, Sausages added 7 ppm Laccaic acid and 63 ppm Monascus; T3, Sausages added 12 ppm Laccaic acid and 108 ppm Monascus; T4, Sausages added 15 ppm Laccaic acid and 135 ppm Monascus; T5, Sausages added 10.5 ppm Laccaic acid and 59.5 ppm Monascus; T6, Sausages added 18 ppm Laccaic acid and 102 ppm Monascus; T7, Sausages added 22.5 ppm Laccaic acid and 127.5 ppm Monascus. 2Scale: 1 = pale pink; 7 = dark red. 3Scale: 1 = very unacceptable; 7 = very acceptable. 4Scale: 1 = very unacceptable; 7 = very acceptable. 5Scale: 1 = very unacceptable; 7 = very acceptable.

acid added tended to have higher color scores than did the control samples, however, not significantly different. It was showed that the sausages made with Monascus and Laccaic acid had slight higher “a” values, indicating more red color (Table 3). Additionally, the color was also more enjoyed by panels, through a sensory color evaluation. There was no difference (p>0.05) in flavor, taste and overall acceptability score between the control and treatments. These did not significantly interfere with the results obtained from the sensory study showing that the samples added Monascus and Laccaic acid had no significant (p>0.05) effects on the flavor, taste and the overall acceptability of the sausages. Other studies (Kim, 2013; Liu et al., 2010; Rhyu et al., 2003) have reported that color is increased by additives with Monascus.

Conclusion

The Monascus and Laccaic acid can be used for improving the physicochemical and texture properties of pork sausages. Samples added with Monascus and Laccaic acid had darker red color, higher hardness and springiness. Based mainly on the results of overall acceptance, the amount of addition of Monascus and Laccaic acid increases redness is increased and lightness is decreased, there is a need for a suitable amount of adjustment.

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