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*Research Article*

# Quality of Sliced Cured Pork Loin with Spinach: Effect of Incubation Period with Starter Culture

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An increasing concern about the usage of chemical additives in meat products has resulted in the use of natural ingredients instead of chemical additives in meat products. Therefore, this study aimed to investigate the effect of incubation period on the physicochemical characteristics of meat products cured with spinach and starter culture containing *Staphylococcus carnosus*. The pH, color, TBARS lipid oxidation, volatile basic nitrogen, residual nitrite content, and microbial number in cured pork loin were determined by incubating it with spinach and starter culture for the following durations: 3, 6, 9, 12, 24, and 48 h. The pH and TBARS values of cured pork loin incubated with spinach and starter culture decreased in a time-dependent manner. An increase in the incubation time from 3 to 48 h resulted in a significant increase in the redness and volatile basic nitrogen content. The residual nitrite content was observed to be maximum in samples from the I48 group followed by the control (+), preconverted nitrite group, and I24 groups. Thus, we found that incubation with spinach and starter culture for 24 h yields a good-quality cooked sliced cured pork loin.

## 1. Introduction

Cured meat products are primarily manufactured by curing meat with salts, plant or plant extracts, and nitrites [1, 2]. The color of cured meat and meat products is the most important factor that determines the consumer's choice for purchasing meat products [3]. Sodium nitrite, an additive widely used for curing meat products, mainly in Europe, is implicated in several functions, including color formation [4]. Thus, sodium nitrite content in cured meat products should be adequately monitored.

Addition of synthetic nitrite has several key functions in the processing of meat and meat products [5]. It imparts a remarkable reddish pink color and a characteristic flavor to meat products. According to Lee et al. [6], nitrite inhibits the growth of pathogens and bacteria responsible for spoilage and lipid oxidation in the cured meat products. However, the use of synthetic nitrite in meat products is controversial owing to the carcinogenic potency of nitrosamines that are formed from nitrites and amines [7]. Although the currently permitted levels of nitrite in meat products are considered safe, many consumers prefer to reduce or eliminate the use of synthetic nitrites [8, 9]. Nitrate to nitrite conversion by a starter culture is being considered as an alternative to avoid the direct addition of synthetic nitrite to meat products. Among various microorganisms in starter culture, *Staphylococcus carnosus* is used widely because it could reduce nitrate to nitrite easily than any other microorganism without formation of lactic acid [10]. Hwang et al. [11] reported that current food culture trend for natural food materials has a profound effect on the meat industry, and that addition of synthetic nitrite during processing of meat and meat products is a major health concern. Previous studies have reported that alternative natural sources of nitrite are considered healthy by the consumers and they have recently been studied for their use in the meat-processing industries [12]. However, these studies were just focused on a minced meat or fermented sausage.

It is well known that vegetables contain high levels of nitrate and nitrite [13, 14]. Krause et al. [15] indicated that nitrite derived from natural sources is currently being used on a large scale to develop the reddish pink color of meat products. It has been reported that nitrate present in vegetables can be reduced to nitrite by adding starter cultures during the curing process of meat products [16]. According to Choi et al. [14], nitrate is converted to nitrite by the nitrogen cycle of microorganisms for rapidly being cured meat products before the formulation of meat products through the pregeneration process.

Spinach (*Spinacea oleracea*) is consumed mainly for its nutritional content such as carotenes, vitamin C, calcium, and iron [17]. Spinach has been shown to contain nitrate levels as high as 2,470 ppm [16, 18]. Thus, nitrate present in spinach can be used as a good source of nitrite. Preconverted nitrite from spinach can be used as a good source of nitrite with starter culture [9]. However, there are no studies on the effect of incubation and its period with spinach powder on whole muscle cured pork loin.

Thus, the aim of this study was to compare the effect of incubation period on the physicochemical properties of pork loins cured with an alternative natural source of sodium nitrite and synthetic nitrite.

## 2. Materials and Methods

*2.1. Chemical and Materials.* In this study, spinach and pork loin were obtained from a local market. Starter culture (S-B-61 Bactoferm™) containing *Staphylococcus carnosus* was purchased from Chr. Hansen Inc. (Gainesville, FL, USA). 2-Thiobarbituric acid, bromocresol green, methyl red, hydrochloric acid, boric acid, ethanol, acetic acid, sulfuric acid, sodium nitrite of analytical reagent grade, and all other chemicals were purchased from Sigma Chemical Co. (St. Louis MO, USA) and Junsei Chemical Co., Ltd. (Tokyo, Japan). Antifoaming agent (KMK-73) was obtained from Shin-Etsu Silicone Co., Ltd. (Seoul, Korea). Plate count agar was obtained from Difco (Sparks, MD, USA). Petrifilms were supplied by 3M™ Petrifilm™ (Seoul, Korea).

*2.2. Preparation of Fermented Spinach Extracts.* The preconversion of nitrite from natural extracts was carried out using the method described by Kim et al. [9] who optimized the amount of fermented spinach juice. Spinach was freeze-dried, powdered, vacuum-sealed, and stored at 0°C until further use. Fermented spinach extracts containing nitrite were prepared as follows: 10% (w/v) spinach powder was incubated with 0.025% active nitrate-reducing culture containing *Staphylococcus carnosus* at 30°C for 24 h. Following incubation, the fermented spinach powder juice was filtered, and the filtrate containing fermented natural extracts was stored in amber flasks at 4°C and used within 24 h. The 10% (w/v) nonfermented spinach extracts were not incubated.

*2.3. Preparation of Cured Pork Loins.* The center portion of eighteen pork loins (castrated boars, Landrace × Yorkshire × Duroc; each weighing approximately 110 kg) from nine different pigs 48 h postmortem was cut into 5 equal portions of one inch thickness each and divided into nine groups randomly. Pork loins cured without nitrite were used as negative control (−) and those cured with synthetic nitrite served as positive control (+). Pork loins were cured with preconverted (PC) nitrite from spinach and in the remaining six groups pork loins were cured with 10% spinach extract and 0.02% starter culture (*S. carnosus*) for 3 (I3), 6 (I6), 9 (I9), 12 (I12), 24 (I24), and 48 (I48) h in a refrigerated room maintained at 4°C. Pork loins were pickled in 40% brine in proportion to the total pork loin weight. Pork loins were then mixed in a tumbler (MKR-150C, Ruhle GmbH., Stuttgart, Germany) at 4°C for 60 min followed by incubation at 15°C in a water bath (MC-31, Jeio tech Co., Ltd, Seoul, Korea). After this, cured pork loins were heated for 30 min at 75°C in a chamber (MAXi3501, Kerres, Sulzbach, Germany) and cooled at 25°C for 1 h. This procedure was performed in triplicate for all the assays.

*2.4. pH.* Five grams of cured pork loin was homogenized in 20 mL distilled water and pH was measured on a pH meter (Model 340, Mettler-Toledo GmbH, Schwerzenbach, Switzerland), which was calibrated with pH 4.0 and 7.0 buffers. The measurements were performed in triplicate.

*2.5. Color Instrument.* Changes in color of the cured pork loin samples were monitored using a colorimeter (Chroma meter CR-400, Minolta, Japan) (light source $D_{65}$, 2° standard observer), which was calibrated with a white plate ($L^* = +94.62$, $a^* = -0.36$, $b^* = +3.33$) composed of a 8 mm diameter measuring area and a 50 mm diameter illumination area. Color was measured by determining $L^*$ (100 = white, 0 = black), $a^*$ (positive = redness, negative = greenness), and $b^*$ (positive = yellowness, negative = blueness) values. Color readings were measured on ten randomly chosen spots on the cured pork loins and used as an estimate of discoloration. The hue angle ($H^\circ$), color difference ($\Delta E^*$), and chroma difference ($\Delta C^*$) were calculated as follows: $H^\circ = \arctan(b^*/a^*)$, $\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$, $\Delta C^* = (\Delta a^{*2} + \Delta b^{*2})^{1/2}$ [19].

*2.6. Thiobarbituric Acid Reactive Substances (TBARS) Values.* Malondialdehydes (MD) were measured using a TBARS method [20] with minor modifications to determine the extent of lipid oxidation and expressed as mg MD per kilogram of sample. Ten gram of cooked pork loin was homogenized in 50 mL distilled water at 10,000 rpm for 2 min in a homogenizer (AM-7, Nihon Seiki, Kaisha Ltd., Tokyo, Japan). Homogenate was then transferred to a distillation tube and washed with an additional 47.5 mL of distilled water. In the same distillation tube, 2.5 mL of 4 N hydrochloric acid (HCl) and 1 mL of antifoaming agent were mixed. After collecting 50 mL of distillate, 5 mL of distillate and 5 mL of thiobarbituric acid (TBA) reagent (0.02 M TBA in 90% acetic acid) were mixed thoroughly in a test tube. The tube was capped and heated in a boiling water bath for

35 min for color development and then cooled at room temperature. Absorbance was measured at 538 nm using a UV/VIS spectrophotometer (Optizen 2120 UV plus, Mecasys Co. Ltd., Seoul, South Korea) against blank sample containing 5 mL distilled water and 5 mL TBA reagent.

*2.7. Volatile Basic Nitrogen (VBN) Value.* VBN test was performed to determine the extent of deterioration of protein in the cooked pork loins. VBN was measured by the modified microdiffusion assay according to the method of Pearson [21].

$$\text{VBN (mg\%)} = \frac{(a-b)\times(f\times 0.02\times 14.007)}{S}\times 100, \quad (1)$$

where $a$ = titer for sample, $b$ = titer for blank, $f$ = standard factor of 0.02 $NH_2SO_4$, and $S$ = sample weight (g).

*2.8. Microbiological Analysis.* To determine the total viable count (TVC) of *Escherichia coli* and coliform bacteria in each cooked cured meat sample, 25 g of pork loin was homogenized in 225 mL of 0.1% peptone water in a sterile stomacher bag (Masticator Paddle Blender, IUL Instrument, Barcelona, Spain) for 3 min. The homogenate was diluted with 0.1% peptone water (1 mL), placed in a Petri dish, and a total of 20 mL of plate count agar (PCA) was poured on Petri dishes containing the diluted samples. Once the medium was solidified, plates were incubated at 37°C for 48 h and colonies formed on the plates were manually counted. To identify *E. coli* and coliform bacteria in the samples, Petrifilm (6414) was used. One milliliter of homogenized sample was placed on a Petrifilm and incubated at 37°C for 48 h. Each blue colony was recognized as an *E. coli* colony and purple and blue colonies were recognized as those of coliform bacteria.

*2.9. Residual Nitrite Contents.* The residual nitrite content was determined according to The Association of Official Analytical Chemists (AOAC) [22] and expressed as ppm of cured meat product. All the processes were performed in duplicate and processed at once to minimize time-induced variations. The residual nitrite content was calculated by a standard curve using nitrite solution.

*2.10. Sensory Properties.* A trained 12-member panel consisting of researchers from the Food Processing Research Center of Korea Food Research Institute (KFRI) in the Republic of Korea evaluated each treatment under fluorescent lighting. After each cured pork loin was cut ($1\times1\times1$ cm), 3 samples were served randomly to the panelists for evaluating sensory scores for color, flavor, chewiness, juiciness, and overall acceptability using the Hedonic test with three-digit code and the samples were evaluated triply (three different sessions) [23]. After heating at 75°C for 30 min in a chamber (MAXi3501, Kerres, Germany), the samples were cooled at 25°C for 1 h. Panelists were instructed to cleanse their plates between samples with water. The color, flavor, chewiness, and overall acceptability of the cooked samples were evaluated using a 9-point descriptive scale (1 = extremely undesirable, 9 = extremely desirable) and panelists, time, temperature, lighting, humidity, and place were fixed and other conditions were random.

*2.11. Statistical Analysis.* Experimental design was completely randomized at each time point and cured pork loins were also evaluated in a completely randomized manner. A total of 45 slices were used in each batch; the slices were divided randomly into nine different treatment groups containing five slices each and all tests were performed in triplicate. Source of nitrite and incubation time were considered as fixed effects and pork meat was considered as a random variable. All replicates were analyzed using the PROC MIXED procedure of SAS 9.4 software (SAS Institute Inc.) and results were expressed as mean ± standard error of the mean.

## 3. Results and Discussion

*3.1. Changes in pH Values and Color of Raw Cured Pork Loin.* Changes in the pH values of raw pork loins cured with spinach powder and starter culture at different time intervals are given in Table 1. Pork loins cured in absence of nitrite had the highest pH ($P < 0.05$), whereas those cured with spinach and starter culture showed a gradual decline in the pH as the incubation time increased. These observations are in accordance with a previous study [24], where a significant difference was observed in the pH of ham cured with vegetable juice powder. When *Staphylococcus carnosus* exist, pH of semidry fermented sausage was lower because of proteolysis activity of *S. carnosus* [25]. pH value and color of cured meats have a intimate connection because NO that combined with myoglobin can be reduced from nitrite more easily when the condition is acidic. Therefore, higher nitrite and myoglobin contents in acidic condition is better to form a NO-myoglobin and NO-porphyrin ring system that have a red color [4].

Table 1 depicts changes in the color values of the raw pork loins cured with spinach powder and starter culture at different time intervals. The lightness of raw cured pork loin was highest after 48 h of incubation (I48) ($P < 0.05$) and lowest after 3 h incubation (I3) ($P < 0.05$). The redness value of the pork loins increased significantly with an increase in the incubation time ($P < 0.05$). The yellowness of raw cured pork loin also increased with an increase in the incubation time ($P < 0.05$) with a maximum value for samples incubated for 48 hrs with spinach juice and starter culture (I48) ($P < 0.05$). A significant difference was observed in the hue angle index ($H^\circ$) and chroma difference ($\Delta C^*$) of raw cured pork loin samples following treatment with preconverted nitrite from spinach at different time intervals (Table 1). No significant variation was observed in the color difference ($\Delta E^*$) among different treatment groups ($P > 0.05$). These results are similar to those reported by Kim et al. [9] who demonstrated that the color values of cured meat treated with preconverted nitrite from spinach increased in a concentration-dependent manner. Sindelar et al. [26] reported the effect of vegetable juice powder on uncured ham

Table 1: Changes of pH and color of raw cured pork loin with spinach juice and different incubating periods.

| Treatments[1] | pH | Color | | | | | |
|---|---|---|---|---|---|---|---|
| | | $L^*$-value | $a^*$-value | $b^*$-value | $H^{\circ}$[2] | $\Delta E^*$[3] | $\Delta C^*$[4] |
| Control (−) | $5.69 \pm 0.01^{a}$ | $48.75 \pm 0.22^{b}$ | $5.18 \pm 0.22^{e}$ | $2.90 \pm 0.28^{e}$ | $29.24 \pm 1.58^{e}$ | — | — |
| Control (+) | $5.66 \pm 0.02^{b}$ | $47.29 \pm 0.40^{bc}$ | $7.10 \pm 0.19^{a}$ | $5.08 \pm 0.22^{ab}$ | $35.58 \pm 0.60^{c}$ | $3.25 \pm 0.82$ | $2.90 \pm 0.49^{ab}$ |
| PC | $5.65 \pm 0.01^{bc}$ | $47.26 \pm 0.75^{bc}$ | $5.90 \pm 0.03^{cd}$ | $4.47 \pm 0.27^{bc}$ | $37.15 \pm 1.56^{a}$ | $2.28 \pm 0.42$ | $1.73 \pm 0.20^{c}$ |
| I3 | $5.66 \pm 0.00^{b}$ | $46.59 \pm 1.05^{c}$ | $5.38 \pm 0.18^{de}$ | $3.61 \pm 0.30^{de}$ | $33.86 \pm 1.75^{c}$ | $2.28 \pm 1.08$ | $0.74 \pm 0.18^{d}$ |
| I6 | $5.66 \pm 0.00^{b}$ | $47.87 \pm 0.42^{bc}$ | $5.42 \pm 0.11^{de}$ | $3.42 \pm 0.13^{e}$ | $32.25 \pm 0.70^{d}$ | $1.05 \pm 0.46$ | $0.57 \pm 0.22^{d}$ |
| I9 | $5.64 \pm 0.00^{bc}$ | $48.32 \pm 0.56^{bc}$ | $5.63 \pm 0.11^{cde}$ | $4.17 \pm 0.19^{cd}$ | $36.53 \pm 0.87^{b}$ | $1.41 \pm 0.62$ | $1.35 \pm 0.36^{cd}$ |
| I12 | $5.63 \pm 0.00^{bc}$ | $48.80 \pm 0.48^{b}$ | $6.18 \pm 0.11^{bc}$ | $4.30 \pm 0.28^{cd}$ | $34.83 \pm 1.22^{c}$ | $1.72 \pm 0.55$ | $1.72 \pm 0.23^{c}$ |
| I24 | $5.63 \pm 0.00^{c}$ | $49.09 \pm 0.40^{b}$ | $6.48 \pm 0.13^{b}$ | $4.44 \pm 0.12^{bc}$ | $34.42 \pm 1.75^{c}$ | $2.04 \pm 0.51$ | $2.02 \pm 0.29^{b}$ |
| I48 | $5.52 \pm 0.01^{d}$ | $51.62 \pm 0.53^{a}$ | $7.18 \pm 0.36^{a}$ | $5.74 \pm 0.31^{a}$ | $38.64 \pm 0.96^{a}$ | $4.51 \pm 0.85$ | $3.47 \pm 0.43^{a}$ |
| SEM | 0.01 | 0.53 | 0.16 | 0.23 | 1.22 | 0.59 | 0.27 |

All values are mean ± standard error of three replicates ($n = 3$). [a–e]Means within a column with different letters are significantly different ($P < 0.05$). [1]The difference between treatments was reported in the Materials and Methods section. [2]$H^{\circ}$; arctan ($b^*/a^*$). [3]$\Delta E^*$; $(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$. [4]$\Delta C^*$; $(\Delta a^{*2} + \Delta b^{*2})^{1/2}$.

at different time points and showed that the color value of the cured meat product decreased over time. Djeri and Williams [16] evaluated the effect of celery juice powder on turkey bologna and found that the hue angles were in the range of 64.08–78.49°, indicative of a red color that was included in the range of nitrosohemochrome cured meat color. Hunt et al. [27] reported that higher values of the hue angle index corresponded to a more brown color and higher values of chroma difference indicated an intense red color. Thus, color values of the raw pork loin cured with spinach powder and starter culture at different time points tended to increase over time. The lightness value of raw cured pork loin after 24 h incubation (I24) was similar to that of the control (+) group treated with nitrite.

*3.2. pH and Color of Cooked Cured Pork Loin.* Table 2 shows the pH and color values of cooked cured pork loin incubated with spinach juice and starter culture at different time intervals. pH value was the highest for the control (+) ($P < 0.05$) samples; pH of the cooked cured pork loin decreased with an increase in the incubation time ($P < 0.05$). These findings are in accordance with those reported by Kim et al. [9] who demonstrated that pH of cured meat treated with fermented spinach extract was lower than the meat treated with sodium nitrite. Low pH of the fermented spinach may have resulted in the low pH of the cured meat. Choi et al. [28] observed a decrease in the pH of cooked meat emulsions treated with fermented red beet extract with a concomitant increase in the concentration of the extract; the pH of cooked meat emulsions showed a trend similar to that of raw meat emulsions. Sindelar et al. [26] reported a similar trend in the pH of cured hams treated with vegetable juice powders.

The color values of cooked cured pork loin incubated with spinach powder and starter culture for different time intervals are summarized in Table 2. The lightness value of the cooked cured pork loin was lowest following 3 h of incubation (I3) ($P < 0.05$) and increased with an increase in the incubation time up to 24 h. The lightness value decreased after 24 h incubation time. Redness value of the cooked cured pork loin with spinach significantly increased with an increase in the incubation time ($P < 0.05$). The yellowness value, on the other hand, decreased as the incubation time increased ($P < 0.05$). The hue angle index and color difference of cooked cured pork loin were maximum after 3 h of incubation (I3) ($P < 0.05$), while chroma difference was the highest in pork loins incubated for 48 h (I48) ($P < 0.05$). Terns et al. [29] reported that the storage time had a significant effect on the color values of raw and cooked sausages cured with cherry powder and starter culture. Hwang et al. [11] reported that the color values of frankfurter were affected by fermented red beet and starter culture since frankfurter cured with fermented red beet had lower lightness and yellowness values and a higher redness value. Furthermore, the lightness and yellowness values of frankfurter with fermented red beet decreased and the redness value increased with an increase in the duration of storage under refrigerated conditions. Sindelar et al. [26] reported that the redness value of cooked sausages cured with vegetable juice powder and starter culture increased with an increase in the incubation time. Furthermore, NO-porphyrin ring system (red colorant) can exist until it is heated to 120°C. It is dependent on NO generation in cured meat [4]. Therefore, samples that had lower pH had a higher redness than samples that had a higher pH except for control (−) which was nitrite-free. The color difference of meat products can provide a measure for the overall color variation of meat with time [30]. Higher the color difference of cured meat products, poorer is the color stability. Thus, color development of cured meat products can be maintained by incubating samples with spinach powder and starter culture. Thus, spinach and starter culture may replace synthetic nitrite for color development in cooked cured meat products.

*3.3. VBN and TBARS Content in Cured Pork Loin.* Table 3 shows VBN values of the cured pork loins treated with spinach powder and starter culture at different time intervals. Highest VBN values were observed in pork loins incubated for 24 h (I24) and 48 h (I48). A significant increase in VBN value was observed with an increase in the incubation period ($P < 0.05$). Kohsaka [31] showed that VBN value is an important indicator of deterioration of freshness of meat products during storage. Kim et al. [32] reported that meat products are affected by the activity of microbial

Table 2: Changes of pH and color of cooked cured pork loin with spinach juice and different incubating periods.

| Treatments[1] | pH | Color | | | | | |
|---|---|---|---|---|---|---|---|
| | | $L^*$-value | $a^*$-value | $b^*$-value | H°[2] | $\Delta E^*$[3] | $\Delta C^*$[4] |
| Control (−) | $5.95 \pm 0.01^{b}$ | $75.45 \pm 0.13^{a}$ | $6.00 \pm 0.15^{f}$ | $7.13 \pm 0.25^{b}$ | $49.92 \pm 0.69^{b}$ | — | — |
| Control (+) | $6.01 \pm 0.00^{a}$ | $72.45 \pm 0.11^{b}$ | $8.36 \pm 0.08^{c}$ | $5.19 \pm 0.04^{e}$ | $31.83 \pm 0.38^{e}$ | $4.28 \pm 0.14^{d}$ | $3.06 \pm 0.10^{c}$ |
| PC | $5.79 \pm 0.00^{de}$ | $73.02 \pm 0.04^{b}$ | $9.95 \pm 0.03^{a}$ | $6.98 \pm 0.04^{b}$ | $35.05 \pm 0.46^{d}$ | $4.64 \pm 0.31^{d}$ | $3.95 \pm 0.17^{b}$ |
| I3 | $5.93 \pm 0.02^{b}$ | $59.71 \pm 0.55^{e}$ | $3.99 \pm 0.18^{g}$ | $8.71 \pm 0.06^{a}$ | $65.39 \pm 1.28^{a}$ | $15.95 \pm 0.35^{a}$ | $2.56 \pm 0.22^{d}$ |
| I6 | $5.92 \pm 0.02^{b}$ | $69.78 \pm 0.31^{c}$ | $7.19 \pm 0.16^{e}$ | $6.48 \pm 0.15^{c}$ | $42.03 \pm 1.07^{b}$ | $5.83 \pm 0.35^{c}$ | $1.36 \pm 0.20^{f}$ |
| I9 | $5.86 \pm 0.02^{c}$ | $72.37 \pm 0.32^{b}$ | $7.86 \pm 0.09^{d}$ | $6.45 \pm 0.12^{c}$ | $39.40 \pm 0.58^{c}$ | $3.66 \pm 0.14^{e}$ | $1.98 \pm 0.10^{e}$ |
| I12 | $5.82 \pm 0.02^{d}$ | $74.63 \pm 0.15^{a}$ | $8.00 \pm 0.08^{d}$ | $6.24 \pm 0.29^{cd}$ | $37.95 \pm 1.60^{d}$ | $2.34 \pm 0.26^{f}$ | $2.19 \pm 0.18^{d}$ |
| I24 | $5.78 \pm 0.00^{e}$ | $75.01 \pm 0.12^{a}$ | $9.54 \pm 0.04^{b}$ | $5.86 \pm 0.06^{d}$ | $31.56 \pm 0.26^{e}$ | $3.79 \pm 0.32^{e}$ | $3.76 \pm 0.06^{b}$ |
| I48 | $5.76 \pm 0.00^{f}$ | $68.48 \pm 0.16^{d}$ | $10.10 \pm 0.06^{a}$ | $4.25 \pm 0.02^{f}$ | $22.82 \pm 0.12^{f}$ | $8.58 \pm 0.21^{b}$ | $5.01 \pm 0.08^{a}$ |
| SEM | 0.01 | 0.21 | 0.10 | 0.12 | 0.72 | 0.23 | 0.12 |

All values are mean ± standard error of three replicates ($n = 3$). [a–g]Means within a column with different letters are significantly different ($P < 0.05$). [1]The difference between treatments was reported in the Materials and Methods section. [2]H°; arctan ($b^*/a^*$). [3]$\Delta E^*$; $(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$. [4]$\Delta C^*$; $(\Delta a^{*2} + \Delta b^{*2})^{1/2}$.

Table 3: Changes of VBN and TBARS of cooked cured pork loin with spinach juice and different incubating periods.

| Treatments[1] | VBN (mg/100 g) | TBARS (mg MD/kg) |
|---|---|---|
| Control (−) | $12.27 \pm 0.55^{c}$ | $0.68 \pm 0.01^{a}$ |
| Control (+) | $12.24 \pm 0.52^{c}$ | $0.11 \pm 0.01^{fg}$ |
| PC | $15.83 \pm 0.32^{b}$ | $0.09 \pm 0.01^{g}$ |
| I3 | $14.95 \pm 0.38^{b}$ | $0.28 \pm 0.01^{b}$ |
| I6 | $15.09 \pm 0.32^{b}$ | $0.26 \pm 0.01^{c}$ |
| I9 | $15.38 \pm 0.37^{b}$ | $0.24 \pm 0.01^{d}$ |
| I12 | $15.28 \pm 0.32^{b}$ | $0.20 \pm 0.01^{e}$ |
| I24 | $17.18 \pm 0.63^{a}$ | $0.11 \pm 0.01^{f}$ |
| I48 | $17.54 \pm 0.36^{a}$ | $0.06 \pm 0.01^{h}$ |
| SEM | 0.42 | 0.01 |

All values are mean ± standard error of three replicates ($n = 3$). [a–h]Means within a column with different letters are significantly different ($P < 0.05$). [1]The difference between treatments was reported in the Materials and Methods section.

enzymes, such as amino acid decarboxylase. Microbial growth is associated with an increase in the VBN value of meat products, and nitrite in the meat samples reduces the formation of volatile compounds [33]. In this study, there no specific difference between control (−) and control (+) ($P > 0.05$) and an increase in the incubation period of the cured pork loins with spinach powder and starter culture may have resulted in the increased production of volatile basic nitrogen, as evident by their high VBN values.

The effects of spinach powder and starter culture on concentration of TBARS in pork loins cured for different incubation periods are summarized in Table 3. The highest TBARS value was observed for the control (−) ($P < 0.05$) group. TBARS value of the cooked pork loins cured with spinach powder and starter culture decreased significantly with an increase in the incubation time ($P < 0.05$). Among various roles of nitrite, antioxidant is a major property [4]. As incubation time went on, nitrite generated gradually [15]. Therefore, TBARS value was decreased over time. These results are in agreement with those reported by Choi et al. [28] who demonstrated that the TBARS value of samples treated with fermented red beet and starter culture was lower than that of the untreated samples. On the contrary, Krause et al. [15] reported no significant reduction in lipid oxidation following treatment with fermented vegetable powder compared to treatment with sodium nitrite. Djeri and Williams [16] also reported no significant change in TBARS value of turkey bologna cured with celery juice powder or sodium nitrite upon storage at 4°C for 10 weeks. This suggested that nitrite from celery juice showed an antioxidant effect similar to that of the synthetic nitrite. Lipid oxidation leads to rancidity of meat products [34], which in turn changes their nutritive value, flavor, and color [35]. TBARS value between 0.5 and 1.0 mg MD/kg of meat weight is considered as the lower threshold for observable oxidized odor and flavor of meat [20]. In the present study, the TBARS values of pork loins from different treatment groups were less than 0.5 mg MD/kg of meat weight except for the pork loins from the control (−) group. Thus, curing of meat products with spinach powder and starter culture inhibited the production of MD.

*3.4. Total Viable Count (TVC) and the Number of Escherichia coli and Coliform Bacteria in Cured Pork Loin.* TVC, *E. coli*, and coliform bacteria analysis was performed to suggest basic microbial information and evaluate of quality and safety of cooked cured meat [25]. TVC was observed in cured pork loins from the I48 ($1.68 \pm 0.52$ CFU/g) group but remained undetected in other treatment groups (data not shown). *E. coli* and coliform bacteria were not observed in pork loins from different treatment groups. Djeri and Williams [16] reported a low count of anaerobic bacteria in turkey bologna cured with cherry juice and starter culture upon storage due to the availability of nitrite from cherry juice. These results are in agreement with Jackson et al. [36] who reported that microbial growth was inhibited in frankfurters cured with nitrite from natural sources, indicating the usefulness of natural preconverted nitrite as an antimicrobial agent. Choi et al. [28] showed the highest TVC for meat emulsions cured in absence of nitrite and reported the absence of *E. coli* and coliform bacteria in meat emulsions cured with red beet extract and ascorbic acid.

*3.5. Residual Nitrite Content of Cured Pork Loin.* Figure 1 shows the residual nitrite level of pork loins cured with

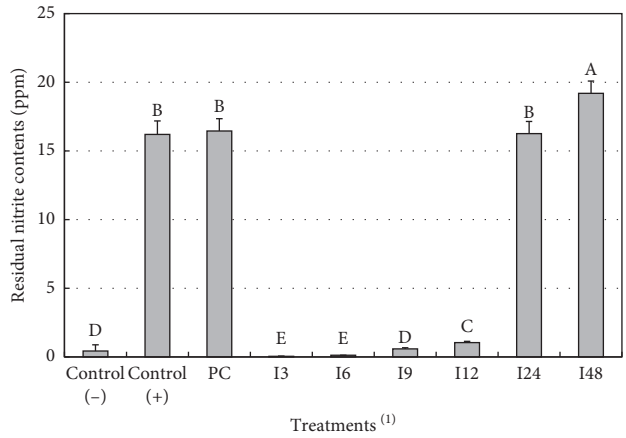


Figure 1: Changes of residual nitrite content of cooked cured pork loin with spinach juice and different incubating time. [A–E]Means with different letters are significantly different ($P < 0.05$). [(1)]Control (–), curing meat with nitrite free; control (+), curing meat with 120 ppm nitrite; PC, curing meat with preconverted nitrite source from spinach; I3, incubating for 3 h curing meat with spinach and starter culture; I6, incubating for 6 h curing meat with spinach and starter culture; I9, incubating for 9 h curing meat with spinach and starter culture; I12, incubating for 12 h curing meat with spinach and starter culture; I24, incubating for 24 h curing meat with spinach and starter culture; I48, incubating for 48 h curing meat with spinach and starter culture. [(1)]The difference between treatments was reported in the Materials and Methods section.

Table 4: Changes of sensory evaluation of cooked cured pork loin with spinach juice and different incubating time.

| Treatments[(1)] | Color | Flavor | Chewiness | Overall acceptability |
|---|---|---|---|---|
| Control (–) | $5.50 \pm 1.20^{c}$ | $6.38 \pm 0.92^{b}$ | $7.13 \pm 0.64$ | $6.38 \pm 0.92^{b}$ |
| Control (+) | $8.38 \pm 0.52^{a}$ | $7.38 \pm 0.52^{a}$ | $7.50 \pm 0.76$ | $7.38 \pm 0.52^{a}$ |
| PC | $7.50 \pm 1.20^{ab}$ | $6.00 \pm 1.07^{bc}$ | $6.88 \pm 0.35$ | $5.88 \pm 0.83^{bc}$ |
| I3 | $5.00 \pm 1.20^{c}$ | $5.50 \pm 0.93^{bc}$ | $6.88 \pm 0.35$ | $5.63 \pm 0.92^{bc}$ |
| I6 | $5.25 \pm 1.04^{c}$ | $5.63 \pm 1.19^{bc}$ | $6.87 \pm 0.83$ | $5.63 \pm 1.19^{bc}$ |
| I9 | $5.87 \pm 0.83^{c}$ | $5.88 \pm 0.64^{bc}$ | $6.88 \pm 0.35$ | $5.88 \pm 0.64^{bc}$ |
| I12 | $6.00 \pm 0.76^{c}$ | $6.38 \pm 0.74^{b}$ | $7.00 \pm 0.53$ | $5.38 \pm 0.74^{c}$ |
| I24 | $7.25 \pm 0.71^{b}$ | $6.13 \pm 0.64^{bc}$ | $7.00 \pm 0.76$ | $6.25 \pm 0.89^{b}$ |
| I48 | $7.75 \pm 1.04^{ab}$ | $3.87 \pm 0.99^{d}$ | $6.87 \pm 0.83$ | $4.00 \pm 1.07^{d}$ |
| SEM | 0.17 | 0.16 | 0.07 | 0.15 |

All values are mean ± standard deviation of three replicates ($n = 9$). [a–d]Means within a column with different letters are significantly different ($P < 0.05$). [(1)]The difference between treatments was reported in the Materials and Methods section.

spinach powder extract and starter culture for different incubation periods. Highest content of residual nitrite was found in pork loins from I48 group ($P < 0.05$). Control (+) samples containing 120 ppm of nitrite showed results similar to PC and I24 groups. This result is in agreement with Choi et al. [28] who demonstrated that meat emulsions cured with fermented red beet extract had lower residual nitrite content compared to the control samples. Kim et al. [9] reported that the residual nitrite content of cured pork loin increased with an increase in the concentration of fermented spinach extract, as the residual nitrite content is likely to be influenced by the presence of preconverted nitrite in the fermented spinach. In the present study, an increase in the incubation time resulted in gradual conversion of nitrate to nitrite by the starter culture. This increase in the nitrite level in turn affected the residual nitrite content of cured pork loins.

*3.6. Sensory Properties of Cooked Cured Pork Loin.* The sensory properties of the cured pork loin treated with spinach powder and starter culture for different incubation time points are shown in Table 4. The color score was the highest for the control (+) and PC ($P < 0.05$), but no significant difference was observed between PC, I24, and I48 groups ($P > 0.05$). Thus, preconverted nitrite from spinach appears to exhibit coloring effect similar to that of nitrite treatment after 24 h incubation. The flavor score of the cured pork loin was maximum for the (+) groups ($P < 0.05$) and treatments were lower than control (+) ($P < 0.05$) owing to the effect of the unique flavor of the fermented spinach [9]. The overall acceptability scores of the cured pork loin on control (+) with nitrite were the highest ($P < 0.05$), and a significant decrease in scores for overall acceptability was observed after 24 h incubation ($P < 0.05$). Choi et al. [28] reported an increase in the color score for meat with the increase in the amount of fermented red beet. Sindelar et al. [26] reported that the color of cured meat products was affected by incubation time and concentration of vegetable juice. Terns et al. [29] reported no significant difference in the overall acceptability between cured meat products treated with cherry powder and starter culture and their control (156 ppm sodium nitrite). Djeri and Williams [16] reported no significant difference in the overall acceptability score between bologna treated with celery juice as well as starter culture and nitrite. Although flavor of fermented spinach had negative effect on overall

acceptability, color scores were improved with incubation time.

## 4. Conclusion

The findings of this study reveal the potential application of natural vegetable extracts in the curing process. Synthetic nitrite can be substituted with nitrite from spinach and starter culture to maintain the color development of cured pork loin. Our results demonstrate that spinach with starter culture can serve as a good natural source of nitrite for developing the reddish pink color of cured pork loins. pH value and TBARS value were decreased and redness, residual nitrite, and VBN value of incubated sample were increased while incubation time went on and excessive incubation time (48 h) had a negative effect on quality characteristics of cooked cured meat. Therefore, 24 h incubation time is best to achieve cured pork loin of good quality.

## Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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