Food Science of Animal Resources

Check for updates

Effect of Using Vegetable Powders as Nitrite/Nitrate Sources on the Physicochemical Characteristics of Cooked Pork Products

Jong Youn Jeong[*], Su Min Bae, Jiye Yoon, Da Hun Jeong, and Seung Hwa Gwak

School of Food Biotechnology & Nutrition, Kyungsung University, Busan 48434, Korea

OPEN ACCESS

*Corresponding author : Jong Youn Jeong
School of Food Biotechnology
& Nutrition, Kyungsung University,
Busan 48434, Korea
Tel: +82-51-663-4711
Fax: +82-51-622-4986
E-mail: jeongjy@ks.ac.kr

*ORCID
Jong Youn Jeong
https://orcid.org/0000-0001-5284-4510
Su Min Bae
https://orcid.org/0000-0002-9367-4594
Jiye Yoon
https://orcid.org/0000-0003-4781-6552
Da Hun Jeong
https://orcid.org/0000-0002-4831-2164
Seung Hwa Gwak
https://orcid.org/0000-0003-4975-1641

Abstract   This study investigated the potential for using vegetable powders as a natural replacement for sodium nitrite and their effects on the physicochemical characteristics of alternatively cured pork products. We analyzed pork products subjected to four treatments: control (0.015% sodium nitrite), Chinese cabbabe powder (CCP) treatment (0.4% Chinese cabbage powder), radish powder (RP) treatment (0.4% radish powder), and spinach powder (SP) treatment (0.4% spinach powder). Among the vegetable powders prepared in this study, SP had the highest ($p<0.05$) nitrate content, while CCP had the lowest ($p<0.05$). The cooking yields from these treatments were not significantly different from each other. However, the products with vegetable powders had higher ($p<0.05$) pH and thiobarbituric acid reactive substances values than the control. Pork products with vegetable powders also showed lower CIE L* values and higher CIE b* values than the nitrite-added control. RP treatment had similar ($p>0.05$) CIE a* values to the control, while SP treatment had the lowest ($p<0.05$) CIE a* values. The residual nitrite content was lower ($p<0.05$) in the vegetable powder added pork products than in the control, although nitrosyl hemochrome and total pigment contents in the CCP and RP treatments were similar ($p>0.05$) to those in the control. The control, CCP, and RP treatments showed curing efficiencies greater than 80%, indicating that CCP and RP would be promising potential replacements for sodium nitrite. The results of this study suggest that RP may be a suitable natural replacement for sodium nitrite to produce alternatively cured meat products, compared to other leafy vegetable powders.

Keywords   alternatively cured meat, sodium nitrite, Chinese cabbage powder, radish powder, spinach powder

Introduction

Meat curing is an ancient process developed to preserve highly perishable food products (Sebranek, 2009). In this process, salt, nitrite and/or nitrate, and other ingredients like sugar, phosphates, and spices are typically added to the food product (Parthasarathy and Bryan, 2012; Sebranek and Bacus, 2007). The use of nitrate and nitrite as curing agents is responsible for the distinctive properties that characterize

cured meat products and contributes to the development of cured color and flavor, prevents lipid oxidation, and provides antimicrobial protection (Alahakoon et al., 2015; Sebranek, 2009; Sebranek and Bacus, 2007; Sindelar and Milkowski, 2012). Despite the presence of multifunctional ingredients in cured meat, consumers have a negative perception of chemical sources of nitrate and nitrite in processed meat, mainly due to health concerns associated with the potential for the formation of carcinogenic nitrosamine (Bedale et al., 2016; Cropp et al., 2020). Therefore, consumer demand for organic or natural meat products has continued to rise. In response, the meat industry has been increasingly focusing on the development of nitrite alternatives (Alahakoon et al., 2015).

Alternatively cured meat products are produced using natural sources of nitrite and/or nitrate derived from vegetable-based ingredients (Sebranek and Bacus, 2007) and are perceived in a more positive light by consumers (Cropp et al., 2020). These natural ingredients are rich in nitrate, which can be reduced to nitrite using specific bacterial cultures during the processing or prior to adding to the fresh meat in the pre-converted form (Bedale et al., 2016). Celery juice or powder is the most commonly used commercially available natural source of nitrate, and it has a nitrate content of ~3% (Sebranek and Bacus, 2007; Sindelar and Houser, 2009). The concentration of celery juice/powder used to cure the meat products has to be limited to 0.2%–0.4% of the total formulation weight because larger amounts of it may negatively influence certain sensory properties such as a potential vegetable flavor and aroma in the final product (Sindelar et al., 2007a; Sindelar et al., 2007b). Another disadvantage with the use of celery is the prevalence of allergic reactions to celery among consumers (Ballmer-Weber et al., 2002). Researchers have previously attempted to develop alternatively cured meat products using beetroot (Sucu and Turp, 2018), spinach (Gabaza et al., 2013; Kim et al., 2019a), Swiss chard (Shin et al., 2017), and parsley (Riel et al., 2017) as nitrate sources. Sucu and Turp (2018) reported that the addition of beetroot powder increased the redness of Turkish fermented beef sausages and resulted in sensory properties comparable to those of control treated with 150 ppm sodium nitrite during storage. Shin et al. (2017) found that pork patties with pre-converted nitrite from Swiss chard had higher levels of nitrosoheme pigment and redness than control with sodium nitrite. However, standardizing nitrite/nitrate concentrations in vegetable powder before meat processing may be important for industrial applications, but this control has not been considered in most of the aforementioned studies.

Besides these vegetable sources, some leafy and root vegetables such as Chinese cabbage, radish, and spinach, which are mainly consumed in Korea (Korea Health Industry Development Institute, 2018), also have high concentrations of naturally occurring nitrates (Santamaria et al., 1999). Although the nitrate content in these vegetables vary based on many factors, such as cultivated area, environment, and harvest time, the average reported nitrate contents are 993–1,740 mg/kg in cabbage, 1,309–2,136 mg/kg in radish, and 748–4,259 mg/kg in spinach (Santamaria, 2006; Tamme et al., 2010). Moreover, vegetables are rich sources of vitamins such as ascorbic acid and antioxidant compounds, including carotenoids and polyphenols, which may be capable of accelerating the curing reaction or inhibiting lipid oxidation and the formation of N-nitroso compounds (Ranasinghe and Marapana, 2018; Sebranek et al., 2012; Septembre-Malaterre et al., 2018; Steinmetz and Potter, 1991). Furthermore, in European countries and some other countries, celery is considered a food allergen and it is required by law to show "celery-containing food" on food labels, while cabbage, radish, and spinach are not considered food allergens (Pawankar et al., 2013). Therefore, these vegetables can act as alternative natural sources of nitrate for producing alternatively cured meat products without any concern for allergens. However, there is very little information regarding the use of Chinese cabbage, radish, and spinach powder in directly cured meat products. To improve industrial practices, standardization procedures are needed for nitrate concentrations in vegetables added with starter culture.

Therefore, in this study, vegetable powders from cabbage, radish, and spinach cultivated in Korea were prepared and their

nitrate concentrations were standardized. Next, the potential of these powders for application as natural replacements for sodium nitrite in the curing of pork products was investigated.

Materials and Methods

The preparation of raw materials and processing of ground pork products

Chinese cabbages, radish, and spinach grown in the Jeolla-do region of Korea were purchased from local markets, randomly selected, and processed into vegetable powders. The Chinese cabbage was cut into pieces of approximately 3 cm×5 cm after removing the outer leaves and roots. For processing the radish, we first cut and removed 2 cm from the top and the bottom, and then cut into pieces of approximately 2 cm×3 cm×3 cm. The spinach was cut into pieces of 3 cm×5 cm after removing 1 cm from the root. After removing the inedible portions, the cut vegetables were rinsed in distilled, deionized water, and then drained for 1 h in a strainer. Excess water was further removed by pressing 10 pumps using a spinner (OXO Good Grips Salad Spinner, OXO International, Chambersburg, PA, USA). After then, each vegetable was homogenized for 4 min using a food cutter (C6 VV, Sirman, Marsango, Italy). The sides of the food cutter were then scraped, and the vegetables were homogenized for 2 min more. This was repeated until the sample was homogenized for a total of 10 min. The homogenized samples were vacuum-packed in nylon/polyethylene bags (~500 g each) and stored in a freezer (C110AHB, LG Electronics, Changwon, Korea) at −18°C. The frozen samples were then removed from the bags and dried at 60°C in a dryer (EN-FO-3925, Enex Science, Goyang, Korea) for 12 h. The dried samples were pulverized using a blender (51BL30, Waring Commercial, Torrington, CT, USA) for 3 min and passed through a 30-mesh sieve (Test sieve BS0600, Chunggye Sieve, Gunpo, Korea). The vegetable powders were vacuum-packed and stored at −18°C until further use (Table 1). Before testing on meat products, the nitrate content in each of the vegetable powders was adjusted by mixing them with maltodextrin to achieve a target nitrate content of 30,000 ppm (Table 2). A starter culture (CS 299, CHR Hansen, Milwaukee, WI, USA) consisting of *Staphylococcus carnosus*, sodium nitrite (S2252, Sigma-Aldrich, St. Louis, MO, USA), and sodium ascorbate (#35268, Acros Organics, Geel, Belgium) were obtained from commercial suppliers.

The raw pork ham (*M. biceps femoris*, *M. semitendinosus*, and *M. semimembranosus*) muscles and backfat used in this study were purchased from a local meat processor (Pukyung Pig Farmers Livestock, Kimhae, Korea) at 24–48 h postmortem and prepared as described by Choi et al. (2020) prior to manufacturing the pork products. Ground pork meat and backfat (30 kg/trial) were randomly separated into four groups: traditionally cured control group and three treatment groups cured with vegetable powders (Table 3). All batches also contained some basic ingredients, including 1.5% NaCl, 1.0% dextrose, 0.3% sodium tripolyphosphate, and 0.05% sodium ascorbate, based on the ground meat mixture (75% pork, 15% fat, and 15%

Table 1. The pH, moisture content, and nitrate content in various vegetable powders prepared from Chinese cabbage, radish, and spinach

Vegetable sources	pH	Moisture content (%)	Nitrate content (ppm)		
			Mean±SE	Min	Max
CCP	6.35±0.003[A]	5.68±0.10[A]	44,077±621[C]	42,080	46,078
RP	5.14±0.005[C]	3.74±0.45[B]	66,178±1,143[B]	62,141	70,449
SP	6.10±0.003[B]	2.78±0.20[C]	120,315±1,505[A]	113,768	124,535

[A–C] Means within a column with different superscript letters are significantly different (p<0.05).
CCP, Chinese cabbage powder; RP, radish powder; SP, spinach powder.

Table 2. The pH, nitrate content, and CIE color in vegetable powders with standardized nitrate content for manufacturing alternatively cured meat products

Vegetable sources	pH	Nitrate content (ppm)	CIE L*	CIE a*	CIE b*
CCP	6.41±0.01^A	30,434±380.4^A	72.97±0.79^B	−0.99±0.11^B	25.57±0.31^A
RP	5.18±0.00^C	30,514±457.9^A	83.71±0.37^A	1.91±0.12^A	22.47±0.39^B
SP	6.11±0.01^B	30,407±262.2^A	59.32±0.34^C	−5.90±0.04^C	12.35±0.08^C

All values are means±SE.
$^{A–C}$ Means within a column with different superscript letters are significantly different ($p<0.05$).
CCP, Chinese cabbage powder; RP, radish powder; SP, spinach powder.

Table 3. The formulation for cooked pork products formulated using various vegetable powders

Ingredients (%)	Treatments			
	Control	CCP	RP	SP
Pork ham	70.00	70.00	70.00	70.00
Pork backfat	15.00	15.00	15.00	15.00
Ice/water	15.00	15.00	15.00	15.00
Sub total	100.00	100.00	100.00	100.00
NaCl	1.50	1.50	1.50	1.50
Dextrose	1.00	1.00	1.00	1.00
Sodium tripolyphosphate	0.30	0.30	0.30	0.30
Sodium nitrite	0.015	0	0	0
Sodium ascorbate	0.05	0.05	0.05	0.05
Chinese cabbage powder	0	0.40	0	0
Radish powder	0	0	0.40	0
Spinach powder	0	0	0	0.40
Starter culture	0	0.04	0.04	0.04
Total	102.865	103.290	103.290	103.290

Control, 150 ppm sodium nitrite added; CCP, 0.4% Chinese cabbage powder added; RP, 0.4% radish powder added; SP, 0.4% spinach powder added.

ice/water). In the control group, 0.015% sodium nitrite was added to the basic materials. The Chinese cabbage powder (CCP) group was treated with 0.4% CCP and 0.04% nitrate-reducing starter culture (*Staphylococcus carnosus*). The radish powder (RP) group was treated with 0.4% RP and 0.04% nitrate-reducing starter culture. The spinach powder (SP) group was treated with 0.4% SP and 0.04% nitrate-reducing starter culture. The calculated concentrations of ingoing nitrate from the vegetable powders were 122 ppm for CCP, RP, and SP treatments. For processing the control samples, ground pork meat, pork fat, NaCl, dextrose, sodium tripolyphosphate, sodium nitrite, and sodium ascorbate were added to a mixer (5K5SS, Whirlpool, St. Joseph, MI, USA) with half of the ice/water and then mixed for 5 min. The remaining ice/water was then added, followed by another 5 min of mixing. For the treatment groups, each vegetable powder and starter culture were added to the pork product with the same ingredients and using the same procedure as the control, with the exception of sodium nitrite. Each batch from the control and treatment groups was stuffed into conical tubes (approximately 50 g each) using a stuffer (MOD.5/W Deluxe, Tre Spade, Torino, Italy). The stuffed tubes were centrifuged at 2,000×g for 10 min (Combi R515, Hanil Science Industrial,

Incheon, Korea) to remove air pockets. All tubes were then closed with caps and stored in a refrigerator at 10°C for the control or held for 2 h at 40°C in an incubator (C-IB4, Changshin Science, Pocheon, Korea) for the treatment groups. The tubes from each batch were then cooked to an internal temperature of 75°C in a 90°C water bath (MaXturdy 45, Daihan Scientific, Wonju, Korea). The temperature was monitored using a thermometer (Tes-1384, Ketech Scientific Instrument, Kaohsiung, Taiwan). Once cooked, the samples were immediately cooled for 20 min on slurry ice and stored at 2°C–3°C in the dark prior to analysis. All experiments were performed in triplicate.

Moisture content determination

The moisture content of the hot air dried vegetable powders was determined using the drying method (AOAC, 2016).

pH determination

A pH meter (Accumet AB150, Thermo Fisher Scientific, Singapore) was used to measure pH on five grams of the vegetable powders or the cooked meat products homogenized in 45 mL of distilled water.

Cooking yield determination

Each meat sample in the conical tube was weighed before cooking, and then weighed again after cooking and cooling. The cooking yield was determined using the following equation:

$$\text{Cooking yield (\%)} = \frac{\text{Sample weight after cooking}}{\text{Sample weight before cooking}} \times 100$$

CIE color measurements

The Commission Internationale de l'Eclairage (CIE) L* (lightness), a* (redness), and b* (yellowness) values were measured on the freshly cut surfaces of each cooked sample using a colorimeter (Chroma Meter CR-400, illuminant C, 2° standard observer; Konica Minolta Sensing, Osaka, Japan) calibrated with a white plate (L* 94.90, a* –0.39, b* 3.88).

Nitrate and residual nitrite content analysis

The nitrate ion (NO_3^-) content in the prepared vegetable powders was determined using the zinc reduction method described by Merino (2009). The obtained results were converted in terms of the concentration of sodium nitrate and expressed as ppm. The residual nitrite content in the cooked meat products was analyzed using the procedures of the AOAC (2016) and reported as ppm.

Determination of nitrosyl hemochrome, total pigment, and curing efficiency

Nitrosyl hemochrome and total pigment content were determined on cooked cured products after extraction in 80% acetone and acidified acetone using a method established by Hornsey (1956). The nitrosyl hemochrome concentration (ppm) was calculated as $A_{540} \times 290$. The total pigment concentration (ppm) was calculated as $A_{640} \times 680$. Finally, curing efficiency (%) was calculated using the following equation:

$$\text{Curing efficiency (\%)} = \frac{\text{Nitrosyl hemochrome content}}{\text{Total pigment content}} \times 100$$

Determination of thiobarbituric acid reactive substances (TBARS)

Malondialdehyde (MDA) content was measured based on 2-thiobarbituric acid reactive substances (TBARS) values using a distillation method reported by Tarladgis et al. (1960). The TBARS values were reported as milligrams of MDA per kilogram of the cooked samples (mg MDA/kg).

Statistical analysis

The experimental design of this study was a randomized block design with four groups (control and three vegetable powder treatments). The obtained data were statistically analyzed using the Proc GLM (general linear model) procedure of the SAS program (SAS, 2012). If the model revealed a significant difference ($p < 0.05$), the significance of the means was further separated using Duncan's multiple range test.

Results and Discussion

The physicochemical properties of prepared vegetable powders

After hot air-drying, the pH of CCP, RP, and SP were 6.35, 5.14, and 6.10; the pH of CCP was significantly higher ($p < 0.05$) than those of the others (Table 1). The highest moisture content was observed ($p < 0.05$) in CCP (5.68%), followed by RP (3.74%), while SP had the lowest ($p < 0.05$) moisture content (2.78%). The mean nitrate contents in CCP, RP, and SP were 44,077 ppm, 66,178 ppm, and 120,315 ppm, respectively, with SP showing the highest ($p < 0.05$) nitrate content and CCP the lowest ($p < 0.05$). These results were consistent with the trends of previous studies on fresh Chinese cabbage, radish, and spinach (Chung et al., 2003; Suh et al., 2013). Suh et al. (2013) observed that the nitrate content in Chinese cabbage, radish, and spinach collected from retail markets in Korea averaged 1,059.9 mg/kg, 1,494.0 mg/kg, and 2,123.8 mg/kg, respectively. These results indicated that the nitrate concentration in vegetables was increased by the drying process in our study. Further, CCP, RP, and SP all had considerable higher nitrate contents than commercially available celery juice powders containing approximately 30,000 ppm of nitrate (Sindelar and Houser, 2009; Sebranek et al., 2012). This suggests that prepared vegetable powders may be promising natural alternative nitrate sources that could replace synthetic nitrite for the production of alternatively cured meat products.

Thus, in order to standardize the nitrate concentration in the vegetable powders used, we adjusted the nitrate content in each vegetable powder by adding sufficient maltodextrin to achieve a target nitrate content of 30,000 ppm, which corresponds to the nitrate content in commercially available celery powder (Table 2). The nitrate content in the standardized vegetable powders ranged from 30,407 ppm to 30,514 ppm, showing that the measured contents were very close to the target levels. Similarly, the pH of the standardized vegetable powders also followed the same trend as those of the hot air-dried powders prepared initially. As expected during the manufacturing of the vegetable powders, their colors varied based on their plant origins. RP had significantly higher ($p < 0.05$) CIE L* and CIE a* values than the powders from leafy vegetables, while SP had the lowest CIE L* and CIE a* ($p < 0.05$). CCP had the highest CIE b* values ($p < 0.05$), followed by RP, and SP had the lowest. Sebranek and Bacus (2007) described that vegetable products with low levels of vegetable pigment are ideal for use as natural sources of nitrate for meat processing. Besides, high levels of plant pigments could affect the color of the final meat product

(Lavieri et al., 2014; Horsch et al., 2014).

Physicochemical properties of cured pork products

The traditionally cured control and alternatively cured pork products did not have significantly different (p>0.05) cooking yields (Table 4), which was consistent with the results of Krause et al. (2011), who reported that the cooking yields of hams naturally cured with vegetable juice powder and those cured with nitrite were not significantly different. Kim et al. (2019a) and Kim et al. (2019b) also found that cooking loss was not significantly different between pork loins cured with nitrite and those treated with fermented spinach or Swiss chard, which is also consistent with our results. The addition of vegetable powders to cured meat products also affected the pH of the final products. The alternatively cured products (pH range 6.26–6.29) had higher pH (p<0.05) than the traditionally cured control (pH 6.20), although the difference of numerical value is small. However, there were no differences (p>0.05) in the pH of the products with the different vegetable powders, suggesting that the effects of the different vegetable powders on the pH of the cured meat products were hidden, although vegetable powders used had different pH values. This was probably due to the buffering capacity of the meat used or the added phosphates (Krause et al., 2011; Sebranek and Bacus, 2007).

The CIE L* values were highest (p<0.05) for the nitrite-added control and lowest (p<0.05) for the SP treatment (Table 4). Previous studies have shown that powders made from cabbages, Chinese cabbages, radishes, spinaches, and Swiss chard lowered the lightness of alternatively cured meat products (Kim et al., 2019a; Kim et al., 2019b; Ko et al., 2017). Further, our observation of the decrease in lightness after the addition of SP is consistent with the results of Kim et al. (2019a), who found that pork loin marinated with fermented spinach along with organic acid showed lower lightness than those with nitrite and ascorbic acid. In this study, the CIE a* values in alternatively cured products ranged from 8.17 to 10.74 (Table 4), higher than those found in cook pork sausages (0.3–4.8) with 3% fermented spinach, lettuce, celery, or red beet (Hwang et al., 2018). The RP treatment and the control did not show a difference (p>0.05) in their CIE a* values (Table 4), while CCP and SP treatments had lower CIE a* values than the control. Recently, similar results were reported by Ahn et al. (2019), who investigated the effects of dried radish leaves and roots on the quality of pork patties. They found that the combination of powders made from radish leaf and root did not change the redness of pork patties. Ko et al. (2017) reported that cooked sausages with pre-converted nitrite from radish were less red than sausages with sodium nitrite, but significantly redder than sausages treated with cabbage and Chinese cabbage powders, which was partially consistent with our findings. In this study, the lowest CIE a* values shown by the SP treatment (Table 4). According to a report from Bohn et al. (2004), leafy vegetables such as spinach and lettuce have a much higher chlorophyll content than other vegetables. Considered in light of

Table 4. Effects of various vegetable powders on cooking yield, pH, and CIE color of cooked meat products

Treatments	Cooking yield (%)	pH	CIE L*	CIE a*	CIE b*
Control	98.97±0.12[A]	6.20±0.00[B]	67.80±0.10[A]	10.72±0.07[A]	6.74±0.05[C]
CCP	98.71±0.16[A]	6.26±0.02[A]	67.21±0.29[B]	10.40±0.10[B]	7.01±0.05[AB]
RP	98.68±0.16[A]	6.28±0.01[A]	66.67±0.18[B]	10.74±0.09[A]	6.91±0.08[BC]
SP	98.59±0.16[A]	6.29±0.01[A]	65.27±0.18[C]	8.17±0.10[C]	7.12±0.05[A]

All values are means±SE.
[A–C] Means within a column with different superscript letters are significantly different (p<0.05).
Control, 150 ppm sodium nitrite added; CCP, 0.4% Chinese cabbage powder added; RP, 0.4% radish powder added; SP, 0.4% spinach powder added.

our results, this suggests that the inherent green color in the prepared SP may have affected the CIE a* values and contributed to the decreased redness in the alternatively cured meat products. This is supported by the results of Kim et al. (2019c), who reported that when pork loins were cured with 10% spinach extract and 0.02% starter culture, followed by incubation for 3–12 h, the CIE a* values of the cooked products were significantly lower than those of control products treated with sodium nitrite. In our study, CIE b* values of cooked pork products were increased (p<0.05) by CCP or SP addition (Table 4). However, the RP treatment and the control resulted in similar (p>0.05) CIE b* values, although the RP treatment did show a slight increase. This effect of vegetable-based sources of nitrite/nitrate on the CIE b* values of meat products has been previously reported for hams with celery juice concentrate (Horsch et al., 2014), mortadella-type sausages with parsley extract powder (Riel et al., 2017), and pork loins cured with fermented spinach (Kim et al., 2019a). In many of these previous studies, the authors have speculated that the characteristic color of plant-based ingredients may affect the yellowness of the naturally cured products. Our results suggest that the RP treatment was relatively less affected by the addition of vegetable powders, compared to other treatments with the leafy vegetable powders. RP would therefore be a promising natural replacement for synthetic nitrite for the production of alternatively cured meat products.

The residual nitrite contents of the final products were 75.98, 34.73, 40.08, and 43.46 ppm for the control, CCP, RP, and SP treatments, respectively (Table 5). Many studies have established the fact that the meat undergoes a depletion in nitrite content during thermal processing and chilling (Choi et al., 2020; Honikel, 2008; Xi et al., 2011). Li et al. (2013) reported that only 65% of the initially added nitrite was detected in the product immediately after the production of dry-cured sausages. Xi et al. (2011) found that nitrite was depleted by about 50% following heating, which was similar to the trend shown by the control group in our study. Moreover, the alternatively cured pork products had lower (p<0.05) residual nitrite content than control, while RP treatment were not different from CCP and SP treatments. Other studies have also reported similar trends, with lower residual nitrite content being reported in products cured with vegetable sources and nitrate-reducing starter cultures (Choi et al., 2020; Krause et al., 2011; Riel et al., 2017; Sebranek and Bacus, 2007; Shin et al., 2017). Shin et al. (2017) reported that the pork patties with Swiss chard or celery powder showed a lower residual nitrite content than those with sodium nitrite. In addition, the results obtained in this study suggest that the nitrite contents in the alternatively cured products could be lower than those in the control containing 150 ppm nitrite, because the initial nitrate levels (122 ppm) in the vegetable powders were relatively lower, even if the added nitrate was completely converted to nitrite after incubation.

Cured meats are typically formulated with sodium nitrite or nitrate, which are responsible for the pink cured meat pigment (nitrosyl hemochrome) during subsequent cooking (AMSA, 2012). In naturally cured meat products, nitrite generated from

Table 5. Effects of various vegetable powders on residual nitrite, nitrosyl hemochrome, total pigment, and curing efficiency of cooked pork products

Treatments	Residual nitrite (ppm)	Nitrosyl hemochrome (ppm)	Total pigment (ppm)	Curing efficiency (%)	TBARS (mg MDA/kg)
Control	75.98±1.56[A]	41.47±0.16[B]	49.75±0.14[B]	83.37±0.51[A]	0.065±0.002[B]
CCP	34.73±1.82[C]	41.04±0.33[B]	50.32±0.17[B]	81.54±0.49[B]	0.085±0.006[A]
RP	40.08±2.46[BC]	41.47±0.39[B]	49.92±0.21[B]	83.05±0.49[A]	0.094±0.005[A]
SP	43.46±1.56[B]	45.34±0.26[A]	62.11±0.23[A]	73.00±0.34[C]	0.083±0.002[A]

All values are means±SE.
[A–C] Means within a column with different superscript letters are significantly different (p<0.05).
TBARS, thiobarbituric acid reactive substances.
Control, 150 ppm sodium nitrite added; CCP, 0.4% Chinese cabbage powder added; RP, 0.4% radish powder added; SP, 0.4% spinach powder added.

the reduction of nitrate by the starter culture becomes available to participate in normal curing reactions, resulting in cured meat properties similar to nitrite-added meats (Terns et al., 2011). Interestingly, the SP treatment had the highest nitrosyl hemochrome contents ($p < 0.05$) although this treatment had the lowest CIE a* values. The CCP and RP treatments resulted in similar ($p > 0.05$) nitrosyl hemochrome contents to the conventionally cured control, even though products with CCP and RP had a lower residual nitrite content than the control. It is possible that nitrite converted from nitrate in vegetable sources by nitrate-reducing starter culture was already involved in the curing reactions in the reactive form during the meat processing (Sindelar et al., 2007b), which could explain the formation of the cured pigments in the products with vegetable powders.

Total pigment contents in cooked pork products ranged from 49.75 to 62.11 ppm (Table 5). The total pigment contents in the control, CCP, and RP treatments were lower ($p < 0.05$) than those in SP treatment, but were similar ($p > 0.05$) to each other. The general trends in total pigment contents were similar to those observed for the nitrosyl hemochrome contents, suggesting that nitrosyl hemochrome formation may be positively correlated with the total pigment content in meat products. Consistent with our results, Shin et al. (2017) reported that higher nitrosoheme pigment in pork patties with pre-converted Swiss chard powder resulted in higher total pigment content. Sullivan et al. (2012) also found that commercial meat products having the highest cured pigment content had the highest total pigment content.

Curing efficiency is the percentage of total pigment converted to nitroso pigments; the parameter also indicates the degree of cured color fading (AMSA, 2012). In this study, the curing efficiency of the cured pork products ranged from 73.00% to 83.37% (Table 5). The curing efficiency of the RP treatment (83.05%) was not different ($p > 0.05$) from that of the control, and both were higher ($p < 0.05$) than those of the CCP (81.54%) and SP (73.00%) treatments, which were much higher than those reported by Sullivan et al. (2012) in commercially available naturally cured frankfurters (31.3%–46.2%), hams (26.5%–37.3%), and bacons (22.8%–45.6%). According to Pearson and Tauber (1984), the percentage conversion of nitrosoheme to total heme in well-cured meats typically exceeds 80%. In this study, the nitrite-added control, CCP, and RP treatments showed curing efficiencies of over 80%. Shin et al. (2017) obtained similar results upon treating pork patties with 2% pre-converted Swiss chard powder or celery powder. Therefore, CCP and RP may be potentially suitable replacements for sodium nitrite as a natural source of nitrate for curing meat products, in terms of curing efficiency. Surprisingly, SP treatment resulted in the lowest ($p < 0.05$) curing efficiency in this study (Table 5), suggesting that lower levels of nitrosyl hemochrome were converted to total pigment. This may explain the low CIE a* values observed in the SP treatment, despite high nitrosyl hemochrome and total pigment content. Kim et al. (2019b) reported similar results for redness and curing efficiency in cured pork loins treated with fermented Swiss chard.

Nitrite is a highly effective antioxidant that can inhibit lipid oxidation in cooked meat products. As little as 50 ppm nitrite could reduce rancidity in cured meat products (Morrissey and Techivangana, 1985; Sebranek, 2009). The nitrite-added control had lower TBARS values ($p < 0.05$) than the alternatively cured pork products (Table 5). Apparently, the higher TBARS values obtained upon treatments with vegetable powders could have resulted from lower residual nitrite contents obtained in the present study. The alternatively cured products were not expected to have higher TBARS values than the control because vegetables contain natural antioxidants that can reduce lipid oxidation (Septembre-Malaterre et al., 2018; Steinmetz and Potter, 1991). It could be assumed that the addition of 0.4% vegetable powders was not sufficient to compensate for the low residual nitrite in the final products as a result of the loss of natural antioxidants during powdering by the drying process (Kamiloglu et al., 2016; Raikos et al., 2015).

However, among the alternatively cured products, the TBARS values were not different ($p > 0.05$). Tarladgis et al. (1960) suggested 0.5−1.0 mg MDA/kg as a threshold range for detecting off-odor in cooked pork. Thus, our measured TBARS levels

(0.083−0.094 mg MDA/kg) in the alternatively cured products were considerably lower than those indicated by Tarladgis et al. (1960). Other studies on naturally cured meat products have shown varying effects of vegetable sources on lipid oxidation, depending on types and added quantity of vegetables (Djeri and Williams, 2014; Kim et al., 2019b; Sindelar et al., 2007b; Sucu and Turp, 2018). Djeri and Williams (2014) reported that turkey bologna with cherry powder and/or celery powder containing pre-generated nitrite had similar TBARS values to the nitrite-added control. However, Kim et al. (2019b) found low TBARS value in the nitrite-added control, compared to pork treated with fermented spinach; they also reported that TBARS values decreased with increasing amounts of fermented spinach extracts. Further, Sindelar et al. (2007b) reported that the nitrite-added control had a significantly lower TBARS value than hams cured with either 0.2% or 0.35% celery juice powder when incubated for 120 min, which was consistent with our results.

Conclusion

In this study, vegetable powders from Chinese cabbage, radish, and spinach were tested for their potential as natural sources of nitrate for replacing sodium nitrite in the curing of meat products. We investigated the effects of the three vegetable powders on the physicochemical characteristics of alternatively cured pork products. Treatment with SP negatively affected the color properties as well as curing efficiency of the meat products. The addition of CCP to pork products resulted in similar nitrosyl hemochrome and total pigment contents to the conventionally cured control, but slightly decreased the redness of final products. However, RP treatment resulted in meat products with redness, cured pigment, total pigment, and curing efficiency comparable to the traditionally cured control. Our results indicate that RP may be a more promising and suitable natural nitrate source for replacing synthetic nitrite for curing meat products, compared to powders from leafy vegetables.

Conflicts of Interest

The authors declare no potential conflict of interest.

Acknowledgements

Author Contributions

Conceptualization: Jeong JY. Data curation: Bae SM. Formal Analysis: Bae SM, Yoon J, Jeong DH, Gwak SH. Methodology: Bae SM, Yoon J, Jeong DH, Gwak SH. Software: Bae SM. Validation: Jeong JY. Investigation: Jeong JY, Bae SM, Yoon J, Jeong DH, Gwak SH. Writing - original draft: Jeong JY, Bae SM. Writing - review & editing: Jeong JY, Bae SM, Yoon J, Jeong DH, Gwak SH.

Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

References

Ahn SJ, Kim HJ, Lee N, Lee CH. 2019. Characterization of pork patties containing dry radish (*Raphanus sativus*) leaf and roots. Asian-Australas J Anim Sci 32:413-420.

Alahakoon AU, Jayasena DD, Ramachandra S, Jo C. 2015. Alternatives to nitrite in processed meat: Up to date. Trends Food Sci Technol 45:37-49.

AMSA. 2012. Meat color measurement guidelines. American Meat Science Association, Champaign, IL, USA. pp 80-83.

AOAC. 2016. Official methods of analysis of AOAC International. 20th ed. AOAC International. Rockville, MD, USA.

Ballmer-Weber BK, Hoffmann A, Wüthrich B, Lüttkopf D, Pompei C, Wangorsch A, Kästner M, Vieths S. 2002. Influence of food processing on the allergenicity of celery: DBPCFC with celery spice and cooked celery in patients with celery allergy. Allergy 57:228-235.

Bedale W, Sindelar JJ, Milkowski AL. 2016. Dietary nitrate and nitrite: Benefits, risks, and evolving perceptions. Meat Sci 120:85-92.

Bohn T, Walczyk T, Leisibach S, Hurrell RF. 2004. Chlorophyll-bound magnesium in commonly consumed vegetables and fruits: Relevance to magnesium nutrition. J Food Sci 69:S347-S350.

Choi JH, Bae SM, Jeong JY. 2020. Effects of the addition levels of white kimchi powder and acerola juice powder on the qualities of indirectly cured meat products. Food Sci Anim Resour 40:636-648.

Chung SY, Kim JS, Kim M, Hong MK, Lee JO, Kim CM, Song IS. 2003. Survey of nitrate and nitrite contents of vegetables grown in Korea. Food Addit Contam 20:621-628.

Cropp MS, Dickson JS, Tarté R, Sebranek JG. 2020. Use of nitrite-embedded packaging film for color stability of alternatively-cured, fully cooked bologna. Meat Muscle Biol 4:1-17.

Djeri N, Williams SK. 2014. Celery juice powder used as nitrite substitute in sliced vacuum-packaged turkey bologna stored at 4C for 10 weeks under retail display light. J Food Qual 37:361-370.

Gabaza M, Claeys E, De Smet S, Raes K. 2013. Potential of fermented spinach extracts as a nitrite source for meat curing. Proceedings of the 59th International Congress of Meat Science and Technology (ICOMST), Izmir, Turkey. pp 1-5.

Honikel KO. 2008. The use and control of nitrate and nitrite for the processing of meat products. Meat Sci 78:68-76.

Hornsey HC. 1956. The colour of cooked cured pork. I. Estimation of the nitric oxide-haem pigments. J Sci Food Agric 7:534-540.

Horsch AM, Sebranek JG, Dickson JS, Niebuhr SE, Larson EM, Lavieri NA, Ruther BL, Wilson LA. 2014. The effect of pH and nitrite concentration on the antimicrobial impact of celery juice concentrate compared with conventional sodium nitrite on *Listeria monocytogenes*. Meat Sci 96:400-407.

Hwang KE, Kim TK, Kim HW, Seo DH, Kim YB, Jeon KH, Choi YS. 2018. Effect of natural pre-converted nitrite sources on color development in raw and cooked pork sausage. Asian-Australas J Anim Sci 31:1358-1365.

Kamiloglu S, Toydemir G, Boyacioglu D, Beekwilder J, Hall RD, Capanoglu E. 2016. A review on the effect of drying on antioxidant potential of fruits and vegetables. Crit Rev Food Sci Nutr 56:S110-S129.

Kim TK, Hwang KE, Lee MA, Paik HD, Kim YB, Choi YS. 2019a. Quality characteristics of pork loin cured with green nitrite source and some organic acids. Meat Sci 152:141-145.

Kim TK, Hwang KE, Song DH, Ham YK, Kim YB, Paik HD, Choi YS. 2019b. Effects of natural nitrite source from Swiss chard on quality characteristics of cured pork loin. Asian-Australas J Anim Sci 32:1933-1941.

Kim TK, Yong HI, Jang HW, Lee H, Kim YB, Jeon KH, Choi YS. 2019c. Quality of sliced cured pork loin with spinach: Effect of incubation period with starter culture. J Food Qual 2019:6373671.

Ko YM, Park JH, Yoon KS. 2017. Nitrite formation from vegetable sources and its use as a preservative in cooked sausage. J Sci Food Agric 97:1774-1783.

Korea Health Industry Development Institute [KHIDI]. National nutrition statistics 2018. Available from: https://www.khidi.or.kr/kps/dhraStat/result2?menuId=MENU01653&gubun=&year=2018. Accessed at May 4, 2020.

Krause BL, Sebranek JG, Rust RE, Mendonca A. 2011. Incubation of curing brines for the production of ready-to-eat, uncured, no-nitrite-or-nitrate-added, ground, cooked and sliced ham. Meat Sci 89:507-513.

Lavieri NA, Sebranek JG, Brehm-Stecher BF, Cordray JC, Dickson JS, Horsch AM, Jung S, Larson, EM, Manu DK, Mendonca AF. 2014. Investigating the control of *Listeria monocytogenes* on alternatively-cured frankfurters using natural antimicrobial ingredients or post-lethality interventions. Meat Sci 97:568-574.

Li L, Shao J, Zhu X, Zhou G, Xu X. 2013. Effect of plant polyphenols and ascorbic acid on lipid oxidation, residual nitrite and N-nitrosamines formation in dry-cured sausage. Int J Food Sci Technol 48:1157-1164.

Merino L. 2009. Development and validation of a method for determination of residual nitrite/nitrate in foodstuffs and water after zinc reduction. Food Anal Methods 2:212-220.

Morrissey PA, Techivangana JZ. 1985. The antioxidant activities of nitrite and nitrosylmyoglobin in cooked meats. Meat Sci 14:175-190.

Parthasarathy DK, Bryan NS. 2012. Sodium nitrite: The "cure" for nitric oxide insufficiency. Meat Sci 92:274-279.

Pawankar R, Canonica GW, Holgate ST, Lockey RF, Blaiss MS. 2013. World allergy organization (WAO) White book on allergy: update 2013. World Allergy Organization, Available from: https://www.worldallergy.org/UserFiles/file/WhiteBook2-2013-v8.pdf. Accessed at Jul 20, 2020.

Pearson AM, Tauber FW. 1984. Analytical methods. In Processed meats. 2nd ed. The AVI, Westport, CT, USA. pp 351-388.

Raikos V, Neacsu M, Morrice P, Duthie G. 2015. Anti- and pro-oxidative effect of fresh and freeze-dried vegetables during storage of mayonnaise. J Food Sci Technol 52:7914-7923.

Ranasinghe RASN, Marapana RAUJ. 2018. Nitrate and nitrite content of vegetables: A review. J Pharmacogn Phytochem 7:322-328.

Riel G, Boulaaba A, Popp J, Klein G. 2017. Effects of parsley extract powder as an alternative for the direct addition of sodium nitrite in the production of mortadella-type sausage: Impact on microbiological, physicochemical and sensory aspects. Meat Sci 131:166-175.

Santamaria P, Elia A, Serio F, Todaro E. 1999. A survey of nitrate and oxalate content in fresh vegetables. J Sci Food Agric 79:1882-1888.

Santamaria P. 2006. Nitrate in vegetables: toxicity, content, intake and EC regulation. J Sci Food Agric 86:10-17.

SAS. 2012. SAS/STAT® software for PC. Release 9.4, SAS Institute, Cary, NC, USA.

Sebranek JG, Bacus JN. 2007. Cured meat products without direct addition of nitrate or nitrite: What are the issues? Meat Sci 77:136-147.

Sebranek JG, Jackson-Davis AL, Myers KL, Lavieri NA. 2012. Beyond celery and starter culture: Advances in natural/organic curing processes in the United States. Meat Sci 92:267-273.

Sebranek JG. 2009. Basic curing ingredients. In Ingredients in meat products: Properties, functionality and applications. Tarté R (ed). Springer Science, New York, NY, USA. pp 1-24.

Septembre-Malaterre A, Remize F, Poucheret P. 2018. Fruits and vegetables, as a source of nutritional compounds and phytochemicals: Changes in bioactive compounds during lactic fermentation. Food Res Int 104:86-99.

Shin DM, Hwang KE, Lee CW, Kim TK, Park YS, Han SG. 2017. Effect of Swiss chard (*Beta vulgaris* var. *cicla*) as nitrite replacement on color stability and shelf-life of cooked pork patties during refrigerated storage. Korean J Food Sci Anim Resour 37:418-428.

Sindelar JJ, Cordray JC, Sebranek JG, Love JA, Ahn DU. 2007a. Effects of vegetable juice powder concentration and storage time on some chemical and sensory quality attributes of uncured, emulsified cooked sausages. J Food Sci 72:S324-S332.

Sindelar JJ, Cordray JC, Sebranek JG, Love JA, Ahn DU. 2007b. Effects of varying levels of vegetable juice powder and incubation time on color, residual nitrate and nitrite, pigment, pH, and trained sensory attributes of ready-to-eat uncured ham. J Food Sci 72:S388-S395.

Sindelar JJ, Houser TA. 2009. Alternative curing systems. In Ingredients in meat products: Properties, functionality and applications. Tarté R (ed). Springer Science, New York, NY, USA. pp 379-405.

Sindelar JJ, Milkowski AJ. 2012. Human safety controversies surrounding nitrate and nitrite in the diet. Nitric Oxide 26:259-266.

Steinmetz KA, Potter JD. 1991. Vegetables, fruit, and cancer. II. Mechanisms. Cancer Causes Control 2:427-442.

Sucu C, Turp GY. 2018. The investigation of the use of beetroot powder in Turkish fermented beef sausage (sucuk) as nitrite alternative. Meat Sci 140:158-166.

Suh J, Paek OJ, Kang Y, Ahn JE, Jung JS, An YS, Park SH, Lee SJ, Lee KH. 2013. Risk assessment on nitrate and nitrite in vegetables available in Korean diet. J App Biol Chem 56:205-211.

Sullivan GA, Jackson-Davis AL, Schrader KD, Xi Y, Kulchaiyawat C, Sebranek JG, Dickson JS. 2012. Survey of naturally and conventionally cured commercial frankfurters, ham, and bacon for physico-chemical characteristics that affect bacterial growth. Meat Sci 92:808-815.

Tamme T, Reinik M, Roasto M. 2010. Nitrates and nitrites in vegetables: Occurrence and health risk. In Bioactive foods in promoting health: Fruits and vegetables. Watson RR, Preedy VR (ed). Academic Press, Salt Lake City, UT, USA. pp 307-321.

Tarladgis BG, Watts BM, Younathan MT, Dugan L. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J Am Oil Chem Soc 37:44-48.

Terns MJ, Milkowski AL, Claus JR, Sindelar JJ. 2011. Investigating the effect of incubation time and starter culture addition level on quality attributes of indirectly cured, emulsified cooked sausages. Meat Sci 88:454-461.

Xi Y, Sullivan GA, Jackson AL, Zhou GH, Sebranek JG. 2011. Use of natural antimicrobials to improve the control of *Listeria monocytogenes* in a cured cooked meat model system. Meat Sci 88:503-511.