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7 **Investigating the Effects of Chinese Cabbage Powder as an Alternative Nitrate Source on**  
8 **Cured Color Development of Ground Pork Sausages**

10 **Abstract**

11 This study investigated the effects of Chinese cabbage powder as a natural replacement for  
12 sodium nitrite on the qualities of alternatively cured pork products. Chinese cabbages grown in  
13 Korea were collected and used for preparing hot air dried powder. Different levels of Chinese  
14 cabbage powder were added to pork products and evaluated by comparing these products to  
15 those with sodium nitrite or a commercially available celery juice powder. The experimental  
16 groups included control (100 ppm sodium nitrite added), treatment 1 (0.15% Chinese cabbage  
17 powder added), treatment 2 (0.25% Chinese cabbage powder added), treatment 3 (0.35%  
18 Chinese cabbage powder added), and treatment 4 (0.4% celery juice powder added). The  
19 cooking yields and pH values of treatments 1 to 3 were significantly lower ( $p < 0.05$ ) than the  
20 control. However, all of the alternatively cured products were redder (higher CIE  $a^*$  values;  
21  $p < 0.05$ ) than the control and this result was supported from higher nitrosyl hemochrome, total  
22 pigment, and curing efficiency. Furthermore, the inclusion of vegetable powders to these  
23 products resulted in considerably less residual nitrite content. However, Chinese cabbage  
24 powder (0.25% and 0.35%) was effective in producing alternatively cured meat products with  
25 a higher curing efficiency comparable to those of the traditionally cured control or the products  
26 with celery juice powder. Therefore, Chinese cabbage powder exhibited the efficacy for use as  
27 a natural replacer for alternatively cured meat products.

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29 **Keywords:** alternatively cured meat, Chinese cabbage powder, sodium nitrite, color, cured

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## Introduction

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Nitrite, which is essentially used for curing meat, is a typical synthetic additive to replace in processed meat products termed naturally cured meat products. Nitrite is a multifunctional ingredient in cured meat and contributes to the cured meat color and unique flavor, while effectively controlling rancidity by retarding lipid oxidation, and inhibiting the growth of microorganisms, particularly against *Clostridium botulinum* (Alahakoon et al., 2015; Sebranek, 2009; Sindelar and Houser, 2009; Sindelar and Milkowsk, 2012). Therefore, it is difficult to replace nitrite, and a complete replacement for nitrite has not yet been discovered that can serve similarly as a multifunctional ingredient (Alahakoon et al., 2015; Sindelar and Houser, 2009). In order to address this, the meat processing industry seeks to replace synthetic additives with natural sources (Alahakoon et al., 2015). Novel food technology ideas designed to replace synthetic nitrite have focused on a method for producing naturally cured meat products by adding a vegetable powder with high nitrate content and a starter culture, such as *Staphylococcus carnosus*, *Staphylococcus vitulinus*, and *Staphylococcus xylosus*, possessing nitrate-reducing activity (Sebranek and Bacus, 2007a; Terns et al., 2011). Celery juice powder is the most widely used as a natural nitrate source for alternatively cured meat products. However, there is a limit to the amount of celery powder that can be used in product formulation as it may adversely affect the sensory attributes (Sindelar et al., 2007b). Therefore, in order to overcome this problem, some researchers have trialed other types of vegetables with high nitrate content such as white kimchi, red beet, parsley, spinach, and Swiss chard as potential natural sources for nitrite to produce naturally cured meat sausages (Choi et a., 2020; Riel et al., 2017; Riyad et al., 2018; Sucu and Turp, 2018). Riyad et al. (2018)

54 reported that the addition of red beet powder increased the redness of cooked sausages and  
55 demonstrated similar lipid oxidation to the nitrite-added control after 4 weeks of storage.  
56 However, Riel et al. (2017) found that compared with the traditionally nitrite-cured control,  
57 alternatively cured sausages with 0.49% parsley extract powder showed a reduced residual  
58 nitrite content (40%) and similar CIE a\* values until 21 d of storage. Most of these studies  
59 appear to have achieved a successful outcome to replace nitrite because this indirect curing  
60 method is used mainly in comminuted meat products rather than the whole muscle products,  
61 including hams and jerkies. Since the naturally occurring nitrate is soluble and the starter culture  
62 is water insoluble, the results were ineffective in the whole muscle products (Sebranek and  
63 Bacus, 2007a; Sindelar et al., 2010).

64 Vegetable concentrates, or powders are advantageous as they can provide high concentrations  
65 of nitrate to produce naturally cured products. However, high levels of vegetable products may  
66 affect the sensory attributes, and therefore the specific vegetable used should not negatively  
67 influence the characteristic flavors and pigments of the cured meats or act as an allergen  
68 (Sebranek and Bacus, 2007a; Sebranek et al., 2012). Chinese cabbage is a green leafy vegetable  
69 and considered a good source for biologically active metabolites such as carotenoids, phenolic  
70 acids, flavonoids, and vitamins, which can act as antioxidants (Lee et al., 2015; Seong et al.,  
71 2016). Moreover, Chinese cabbage contains high concentrations of naturally occurring nitrate,  
72 which makes it a potential candidate for use as a natural alternative for meat products.  
73 Reportedly, the average nitrate content in fresh Chinese cabbage was 1,438.5 mg/kg (Kang et  
74 al., 2016) or 1,740 mg/kg (Chung et al., 2003) depending on the study. Thus, the nitrate  
75 concentration can increase following the drying process and a sufficient amount of nitrate will  
76 be supplied to the cured products in powder form (Choi et al., 2020; Sindelar, 2006). Therefore,

77 Chinese cabbage powder was expected to show potential as a new natural alternative for  
78 synthetic nitrite as it is not associated with allergens. However, studies focusing on the  
79 compatibility of this ingredient in naturally cured meat sausages have not yet been elucidated.  
80 Therefore, this study investigated the effects of the addition levels of Chinese cabbage powder  
81 on the quality attributes of alternatively cured pork sausages and evaluated their effects by  
82 comparing them with products containing synthetic sodium nitrite or those cured with  
83 commercially available celery powder.

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## Materials and Methods

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### The preparation of raw materials

88 Chinese cabbages grown in five provinces of South Korea (Gyeonggi-do, Gyeongsang-do,  
89 Jeolla-do, Chungcheong-do, and Gangwon-do) were purchased, randomly selected  
90 (approximately 10 kg/region), and used for manufacturing Chinese cabbage powder in this  
91 study. Following removal of the non-edible portion of the cabbages such as the outside leaves  
92 and roots, the cabbages were cut (3 × 5 cm), rinsed in distilled, deionized water, and then drained  
93 for 1 h in a strainer. The excess water was further removed by (pressing 10 pumps) using a  
94 spinner (OXO Good Grips Salad Spinner, OXO International, Chambersburg, PA, USA). The  
95 rinsed Chinese cabbages were homogenized for 4 min using a food cutter (C6 VV, Sirman,  
96 Marsango, Italy). The sides of the food cutter were scraped periodically and the sample  
97 homogenized for an additional 2 min. This process was repeated until the sample was  
98 homogenized for a total of 10 min (Nuñez de González et al., 2015). The homogenized samples  
99

100 were vacuum-packed in nylon/polyethylene bags (~500 g each) and then stored in a freezer  
101 (C110AHB, LG Electronics, Changwon, Korea) at  $-18^{\circ}\text{C}$  prior to hot air drying. Then the  
102 frozen samples were removed from the bags and dried at  $60^{\circ}\text{C}$  in a dryer (EN-FO-3925, Enex  
103 Science, Goyang, Korea) for 12 h. The dried samples were pulverized with a blender (51BL30,  
104 Waring Commercial, Torrington, CT, USA) for 3 min and screened using a 30 mesh sieve (Test  
105 sieve BS0600, Chunggye Sieve, Gunpo, Korea). The Chinese cabbage powder was vacuum-  
106 packed in nylon/polyethylene bags and stored in a freezer at  $-18^{\circ}\text{C}$  until further use. The  
107 prepared Chinese cabbage powder had a nitrate ion content of 36,863 ppm (equivalent to 50,479  
108 ppm sodium nitrate), a nitrite content of 0.2 ppm, a pH of 5.44, and a moisture content of 6.27%.  
109 Celery powder (VegStable 502, Florida Food Products, Inc., Eustis, FL, USA) with a nitrate ion  
110 content of 23,375 ppm (equivalent to 32,020 ppm sodium nitrate) and a starter culture (CS 299,  
111 CHR Hansen, Milwaukee, WI, USA) consisting of *Staphylococcus carnosus*, sodium nitrite  
112 (S2252, Sigma-Aldrich, St. Louis, MO, USA), and sodium ascorbate (#35268, Acros Organics,  
113 Geel, Belgium) were obtained from commercial suppliers.

114

### 115 **The manufacturing process of ground pork sausages**

116 The pork ham (*M. biceps femoris*, *M. semitendinosus*, and *M. semimembranosus*) muscles  
117 and back fat used in this study were purchased from a local meat processor (Pukyung Pig  
118 Farmers Livestock Co., Kimhae, Korea) at 24–48 h postmortem and. The intermuscular fat and  
119 visible connective tissues were trimmed from the fresh ham. The lean pork ham was cut into  
120 squares of approximately 4–5 cm, vacuum-packaged in nylon/polyethylene film bags, and then  
121 stored in a  $-18^{\circ}\text{C}$  freezer until processing within 1 mon. The pork back fat was also prepared  
122 similarly. In order to manufacture the ground pork products (total batches of 30 kg per trial),



123 frozen pork meat and back fat were thawed at 2–3°C for 24–36 h prior to processing and then  
124 sequentially ground with a chopper (TC-22 Elegant plus, Tre Spade, Torino, Italy) equipped  
125 with an 8-mm plate and 3-mm plate. The ground pork meat and back fat were randomly  
126 separated into five batches (Table 1) as follows: control, 0.01% sodium nitrite; treatment 1, 0.15%  
127 Chinese cabbage powder and 0.015% starter culture; treatment 2, 0.25% Chinese cabbage  
128 powder and 0.025% starter culture; treatment 3, 0.35% Chinese cabbage powder and 0.035%  
129 starter culture; and treatment 4, 0.4% celery juice powder and 0.04% starter culture. In the  
130 control samples, the addition of 100 ppm sodium nitrite was considered according to the  
131 regulations of The Korea Food Code (Ministry of Food and Drug Safety, 2020) in processed  
132 meat (< 70 ppm residual nitrite ion). The utilization level of the celery juice powder and starter  
133 culture was based on the suppliers' recommendations (Choi et al., 2020; Sindelar et al., 2007b).  
134 From the results of the nitrate analysis for Chinese cabbage powder (50,497 ppm nitrate) and  
135 celery juice powder (32,020 ppm nitrate), the various addition levels of Chinese cabbage powder  
136 in treatments 1–3 were selected based on the target nitrate concentration in 0.4% celery juice  
137 powder. The ingoing nitrate concentrations from the vegetable powders were calculated as  
138 75.75 ppm, 126.24 ppm, 176.74 ppm, and 128.08 ppm for treatments 1, 2, 3, and 4, respectively.  
139 For processing of the control samples, ground pork meat, pork fat, NaCl, dextrose, sodium  
140 tripolyphosphate, sodium nitrite, and sodium ascorbate were added to a mixer (5K5SS,  
141 Whirlpool, St. Joseph, MI, USA) with the ice/water and then mixed for 10 min (Table 1). For  
142 alternatively cured products, Chinese cabbage powder or celery juice powder and starter culture  
143 were incorporated in the same ingredients and procedure of the control, but sodium nitrite was  
144 not included. Each batch was stuffed into conical tubes (approximately 50 g) using a stuffer  
145 (MOD.5/W Deluxe, Tre Spade, Torino, Italy). The stuffed tubes were then centrifuged at 2000

146 × g for 10 min (Combi R515, Hanil Science Industrial Co., Incheon, Korea) to remove any air  
147 pockets. All tubes were closed with caps and placed on racks in a refrigerator at 10°C for the  
148 control or incubated for 2 h at 40°C in an incubator (C-IB4, Changshin Science, Pocheon, Korea)  
149 for treatments 1 to 4. After incubation, the tubes from each batch were sequentially cooked to  
150 an internal temperature of 75°C in a 90°C water bath (MaXturdy 45, Daihan Scientific Co.,  
151 Wonju, Korea) for approximately 9–12 min depending on the samples. The temperature was  
152 monitored using a 4-channel digital thermometer (Tes-1384, Ketch Scientific Instrument,  
153 Kaohsiung, Taiwan). Once cooked, the samples were immediately cooled for 20 min on slurry  
154 ice and stored at 2–3°C in the dark prior to analysis. Experiments were performed in triplicate.

155

#### 156 **Moisture content determination**

157 The moisture content of the Chinese cabbage powder was determined using the drying method  
158 (AOAC, 2016).

159

#### 160 **The pH values determination**

161 Five grams of Chinese cabbage powder or cooked meat products were homogenized with 45  
162 mL of distilled water for 1 min in a homogenizer (DI 25 basic, IKA<sup>®</sup>-Werke GmbH & Co. KG,  
163 Staufen, Germany). The pH values of each sample were measured using a pH meter (Accumet  
164 AB150, Thermo Fisher Scientific, Inc., Singapore).

165

#### 166 **Cooking yield determination**

167 The weight of each meat product sample in the conical tube was measured prior to cooking  
168 and then again after cooking and cooling to determine the cooking yield using the following

169 equation:

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

170

### 171 **CIE color measurements**

172 Color measurements were taken using a colorimeter (Chroma Meter CR-400, illuminant C,  
173 2° standard observer; Konica Minolta Sensing Inc., Osaka, Japan) calibrated with a white plate  
174 (L\* 94.90, a\* -0.39, b\* 3.88). The Commission Internationale de l'Eclairage (CIE) L\*  
175 (lightness), a\* (redness), and b\* values (yellowness) system was used to determine the color of  
176 freshly cut surfaces of each cooked sample immediately after cutting.

177

### 178 **Residual nitrite analysis**

179 The nitrate ion ( $\text{NO}_3^-$ ) and nitrite ion ( $\text{NO}_2^-$ ) of the homogenized Chinese cabbage prior to  
180 drying and the powder following the drying process were determined by the zinc reduction  
181 method described by Merino (2009). Results were reported as ppm. The residual nitrite content  
182 in the cooked pork sausages was analyzed according to the procedure of the AOAC (2016). A  
183 calibration curve was prepared using sodium nitrite (S2252, Sigma-Aldrich, St. Louis, MO,  
184 USA), and the residual nitrite content was reported as ppm.

185

### 186 **Nitrosyl hemochrome, total pigment, and curing efficiency determination**

187 Nitrosyl hemochrome and total pigment were determined using a method by Hornsey (1956).  
188 For nitrosyl hemochrome determination, 10 g of each cooked sample was blended with 40 mL  
189 acetone and 3 mL distilled, deionized water using a homogenizer (DI 25 basic, IKA®-Werke

190 GmbH & Co. KG, Staufen, Germany). The samples were kept in the dark for 15 min and filtered  
191 through a Whatman No. 1 filter paper, and then, the absorbance of the filtrate at 540 nm ( $A_{540}$ )  
192 was determined using a spectrophotometer (UV-1800, Shimadzu Co., Kyoto, Japan). The  
193 nitrosyl hemochrome concentration (ppm) was calculated as  $A_{540} \times 290$ . For the total pigment  
194 measurement, 10 g of each cooked sample was blended with 40 mL acetone, 1 mL HCl, and 2  
195 mL distilled, deionized water, which was allowed to stand in the dark at 2–3°C for 1 h and then  
196 filtered through a Whatman No. 1 filter paper. Absorbance was measured at 640 nm ( $A_{640}$ ). The  
197 total pigment concentration (ppm) was calculated as  $A_{640} \times 680$ . Curing efficiency (%) was  
198 calculated using the following equation:

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

199

200

### 201 **Statistical analysis**

202 The experiment was replicated three times. Data were statistically analyzed as a randomized  
203 block design with five treatments (control and four nitrite-free treatments) using the Proc GLM  
204 (general linear model) procedure of the SAS program (SAS, 2012). If significance was  
205 determined ( $p < 0.05$ ) in the model, the significance of the means was further separated by  
206 Duncan's multiple range test.

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209

## 209 **Results and Discussion**

210

### 211 **Cooking yield, pH values, and CIE color values**

212 The alternatively cured sausages (treatments 1 to 4) produced lower cooking yields ( $p < 0.05$ )  
213 compared to the nitrite-added control (Table 2). However, there were no differences ( $p > 0.05$ )  
214 in cooking yields by Chinese cabbage powder level or the addition of celery juice powder. Kim  
215 et al. (2019a) and Krause et al. (2011), who reported that the meat products cured with a natural  
216 nitrite source from vegetable juice showed no significant differences in cooking loss compared  
217 to the conventionally cured control. However, the effects of vegetable powder on cooking yields  
218 in this study are in agreement with Choi et al. (2020), who found that the naturally cured  
219 products with white kimchi powder or celery powder exhibited significantly lower cooking  
220 yields than the nitrite-added control.

221 Naturally cured sausages (treatments 1 to 3) with the Chinese cabbage powder had lower pH  
222 values ( $p < 0.05$ ) than the control (Table 2). Moreover, the pH values decreased ( $p < 0.05$ ) further  
223 as level of Chinese cabbage powder increased. These decreases in pH might be related directly  
224 to the vegetable powder or caused by the lactic acid produced by the starter culture during  
225 incubation (Gøtterup et al., 2008; Sebranek and Bacus, 2007a; Terns et al., 2011). Since the  
226 Chinese cabbage powder used in this study had a pH of 5.44, the powder could lower the pH of  
227 treatments 1 to 3. However, the pH values of treatment 4 were similar ( $p > 0.05$ ) to that of the  
228 control. This result was consistent with the findings of Sindelar et al. (2007b), who found that  
229 the addition of celery juice powder at various levels to hams did not change the pH of the  
230 products compared to the nitrite-added control.

231 No differences ( $p > 0.05$ ) in CIE L\* values were observed between the control and the other  
232 treatments (Table 2). Krause et al. (2011) reported similar results for the CIE L\* values in sliced  
233 hams with vegetable juice powder and Riyad et al. (2018) also found no differences in CIE L\*

234 values between the sodium nitrite-added control and beef sausages cured with celery, parsley,  
235 or spinach powder. Furthermore, the alternatively cured sausages (treatments 1 to 4) exhibited  
236 higher CIE a\* values than the control; however, these differences were not for the significant  
237 ( $p>0.05$ ) for the CIE a\* values by any Chinese cabbage powder level used in treatments 1 to 3  
238 (Table 2). Similar results were reported by Kim et al. (2019a), who found that when fermented  
239 spinach juice was added into pork loin along with organic acids, CIE a\* values were higher than  
240 in the control marinated with nitrite and ascorbic acid. Similarly, Sucu and Turp (2018) reported  
241 that the inclusion of beetroot powder as a nitrite alternative resulted in an increase in redness  
242 (higher CIE a\* values) in fermented beef sausages. In this study, treatment 2 and treatment 4  
243 provided equivalent nitrates from vegetable powder, while treatment 1 had 40% lower nitrate  
244 than the two treatments. Interestingly, the CIE a\* values for treatment 1 showed similar values  
245 to treatments 2 to 4 and higher values than control. Since the most important parameter in color  
246 is redness (a\* value) for cured meat products (Feng et al. 2016), these results indicated that the  
247 Chinese cabbage powder was very effective at increasing redness in the naturally cured  
248 sausages and showed the potential of this powder for use as a replacement for nitrite. Treatments  
249 1 and 2 showed similar ( $p>0.05$ ) CIE b\* values to the control (Table 2). However, treatments 3  
250 and 4 exhibited higher ( $p<0.05$ ) CIE b\* values compared to the control and other treatments.  
251 These results were similar to those of Riel et al. (2017), who found that higher ingoing amounts  
252 of parsley extract powder resulted in higher b\* values compared to traditionally cured sausages.  
253 Horsch et al. (2014) also reported that celery concentrate treatments were significantly more  
254 yellow (higher b\* values) than the hams cured with nitrite and an increase of the concentration  
255 of celery concentrate lead to increased yellowness in the final ham products. Riel et al. (2017)  
256 and Horsch et al. (2014) indicated that the higher b\* values might be related to the plant

257 pigments in the vegetable concentrates. Therefore, our results suggested that when vegetable  
258 powder with increased nitrate contents was added to the meat products, the effect of the color  
259 of the vegetable powder could be minimized, and similar yellowness to that of the nitrite added  
260 products might be obtained in the alternatively cured sausages.

261

### 262 **Residual nitrite, nitrosyl hemochrome, total pigments, and curing efficiency**

263 The residual nitrite contents of the cooked pork sausages was 37.32, 14.68, 16.58, 22.24, and  
264 16.35 ppm for the control, treatment 1, treatment 2, treatment 3, and treatment 4 (Table 3).  
265 Previous researchers have found this depletion of nitrite during product manufacturing. Xi et al.  
266 (2012) found that the amount of nitrite had reduced by about 75% of the initial concentration  
267 for frankfurters. Choi et al. (2020) also reported about 78% of the nitrite had depleted in ground  
268 pork products following the cooking and chilling process. In this study, the addition of a starter  
269 culture (*S. carnosus*) converted the nitrate from vegetable powder to nitrite in alternatively  
270 cured products although the added amount of starter culture varied depending on the treatments.  
271 Terns et al. (2011) indicated that the level of starter culture did not improve the cured meat  
272 properties in the indirectly cured, emulsified sausages. Nevertheless, all alternatively cured  
273 sausages had lower ( $p<0.05$ ) residual nitrite contents than the control. Sebranek and Bacus  
274 (2007a) found that the residual nitrite contents was lower in the naturally cured meat products  
275 than in the conventionally cured products. Similarly, Riel et al. (2017) reported that the residual  
276 nitrite contents of mortadella-type sausages produced with different levels of parsley extract  
277 powder were lower than the nitrite-added control. In this study, treatments 3 had higher ( $p<0.05$ )  
278 residual nitrite contents compared to other treatments. Higher residual nitrite contents in  
279 treatment 3 among the alternatively cured products were expected because the highest ingoing

280 amounts of nitrate (176 ppm) were from the Chinese cabbage powder in this study.

281 Nitrosyl hemochrome is a stable pink pigment formed by the reaction of nitric oxide  
282 converted from nitrite with myoglobin, resulting in a typical cured meat color after the cooking  
283 process (Cassens, 1997; Suman and Joseph, 2013). In this study, similar to the results of the  
284 CIE  $a^*$  values, the nitrosyl hemochrome contents were higher ( $p < 0.05$ ) for all of the  
285 alternatively cured sausages than the control (Table 3). However, treatment 1 showed lower  
286 ( $p < 0.05$ ) nitrosyl hemochrome contents than treatments 2 to 4, which exhibited similar results  
287 ( $p > 0.05$ ). This finding suggested that the increase in redness of the alternatively cured sausages  
288 might be associated with an increase in the nitrosyl hemochrome, and sufficient amounts of  
289 nitrite were facilitated for the curing reactions, which resulted in the reduction of residual nitrite  
290 as shown in this study. Similar to our results, Choi et al. (2020) found that the indirectly cured  
291 pork products with 0.2% or 0.4% white kimchi powder had a higher nitrosyl hemochrome  
292 contents than the conventionally cured control. Terns et al. (2011) also obtained similar results  
293 by adding celery juice powder and cherry powder to the indirectly cured, emulsified sausages.  
294 However, these results disagree with those of Sindelar et al. (2007b), who found no differences  
295 in the cured pigment contents between the nitrite-added control and the naturally cured hams  
296 treated with different concentrations of the celery juice powder and the incubation time. As a  
297 result, the results of the present study suggest that the addition of cabbage powder was very  
298 effective in the cured pigment formation in the alternatively cured products.

299 Total pigment contents of the products tested in this study ranged from 47.18 to 49.13 ppm  
300 and were lower than those found in previous research (Sindelar et al., 2007a) ranging from 58.1  
301 to 92.5 ppm. However, compared to the control, the addition of Chinese cabbage powder or  
302 celery juice powder to the products increased ( $p < 0.05$ ) the total pigment contents. However,



303 these increases were similar ( $p>0.05$ ) to each other. These results clearly followed the same  
304 trends as our CIE  $a^*$  values. The relationship between nitrosyl hemochrome and total pigment  
305 contents was supported by Ahn and Maurer (1989), who speculated that less nitrosyl  
306 hemochrome formation could be the primary cause of a low total pigment. This has been  
307 previously confirmed by Sullivan et al. (2012), who reported that naturally cured frankfurters  
308 collected from commercial brands had the greatest amount of cured pigment and the highest  
309 total pigment contents. Thus, our results suggested that nitrite derived from vegetable sources  
310 by the starter culture was rapidly converted to nitric oxide, resulting in more nitrosyl  
311 hemochrome and total pigment contents in final products. In addition, it might be possible that  
312 the curing reaction in the alternatively cured products could occur faster because the vegetables  
313 such as the Chinese cabbage powder and celery juice powder possess inherent ascorbic acid  
314 that functions as a curing accelerator facilitated the conversion of nitrite to nitric oxide  
315 enhancing color development (Li et al., 2018; Sebranek et al., 2012; Zhu et al., 2018).

316 Curing efficiency of the pork products tested in this study ranged from 72.70% to 82.93%  
317 (Table 3). Cured pork sausages with Chinese cabbage powder or 0.4% celery juice powder had  
318 a higher ( $p<0.05$ ) curing efficiency than the control with sodium nitrite. Furthermore, no  
319 differences ( $p>0.05$ ) in curing efficiency were found between treatments 2 to 4. Kim et al.  
320 (2019b) reported that pork loins cured with fermented Swiss chard showed curing efficiency of  
321 62.9% to 90.19% with the concentrations of fermented Swiss chard solutions, which was lower  
322 or higher than that of the control cured with 120 ppm nitrite (86.03%). Choi et al. (2020) found  
323 that the curing efficiency of indirectly cured meat products with white kimchi powder ranged  
324 from 75.26% to 79.34%, resulting in higher curing efficiency than the nitrite control. However,  
325 the lower nitrosyl hemochrome contents in treatment 1 resulted in decreasing curing efficiency

326 and treatments 1 had less ( $p < 0.05$ ) curing efficiency than treatments 2 to 4. Therefore, these  
327 results suggested that the 0.15% addition (75 ppm ingoing nitrate) of Chinese cabbage powder  
328 did not show sufficient cured pigment and curing efficiency in the alternatively cured products  
329 even when it showed a higher curing efficiency than the control. Importantly, the addition of  
330 more than 0.25% Chinese cabbage powder (126 ppm ingoing nitrate) increased both the cured  
331 pigment and curing efficiency in the alternatively cured meat products.

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### **Conclusions**

335 Although cooking yield was slightly lower in the alternatively cured pork sausages with  
336 Chinese cabbage powder or celery juice powder, higher redness was found compared with the  
337 nitrite control. Moreover, less residual nitrite content were found in the alternatively cured meat  
338 products. In addition, the nitrite derived from the vegetable powder and starter culture with  
339 nitrate-reducing activity was more effectively involved in the curing reaction and more effective  
340 at the conversion of nitrite to nitric oxide, thereby resulting in higher cured pigment, total  
341 pigment, and curing efficiency. However, among the meat products with Chinese cabbage  
342 powder, 0.15% addition of Chinese cabbage powder (treatment 1) exhibited less cured pigment  
343 and curing efficiency, while the addition of more than 0.25% Chinese cabbage powder (126  
344 ppm ingoing nitrate) positively affect the cured meat sausages with an increase in the cured  
345 pigment and curing efficiency comparable to those of the traditionally cured control. Therefore,  
346 this study indicated that Chinese cabbage powder showed efficacy as a new natural ingredient  
347 to replace synthetic nitrite for alternatively cured meat sausages. Further research is needed to  
348 explore the effectiveness of Chinese cabbage powder on the sensory attributes and shelf life of

349 naturally cured meat products during storage for industrial practice.

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### **Conflicts of interest**

353 The authors declare no potential conflict of interest.

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### **References**

364 Ahn DU, Maurer AJ. 1989. Effects of added nitrite, sodium chloride, and phosphate on color,  
365 nitrosoheme pigment, total pigment, and residual nitrite in oven-roasted turkey breast.  
366 *Poult Sci* 68:100-106.

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

369 AOAC. 2016. Official methods of analysis of AOAC International. 20<sup>th</sup> ed. AOAC  
370 International. Rockville, MD, USA.

371 Cassens RG. 1997. Residual nitrite in cured meat. *Food Technol* 51:53-55.

372 Choi JH, Bae SM, Jeong JY. 2020. Effects of the addition levels of white kimchi powder and  
373 acerola juice powder on the qualities of indirectly cured meat products. Food Sci Anim  
374 Resour 40 (In press).

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

377 Feng X, Sebranek JG, Lee HY, Ahn DU. 2016. Effects of adding red wine on the  
378 physicochemical properties and sensory characteristics of uncured frankfurter-type  
379 sausage. Meat Sci 121:285-291.

380 Gøtterup J, Olsen K, Knöchel S, Tjener K, Stahnke LH, Møller JKS. 2008. Color formation in  
381 fermented sausages by meat-associated staphylococci with different nitrite- and nitrate-  
382 reductase activities. Meat Sci 78:492-501.

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

385 Horsch AM, Sebranek JG, Dickson JS, Niebuhr SE, Larson EM, Lavieri NA. 2014. The effect  
386 of pH and nitrite concentration on the antimicrobial impact of celery juice concentrate  
387 compared with conventional sodium nitrite on *Listeria monocytogenes*. Meat Sci 96:400-  
388 407.

389 Kang KH, Lee SJ, Ha ES, Sung NJ, Kim JG, Kim SH, Kim SH, Chung MJ. 2016. Effects of  
390 nitrite and nitrate contents of chinese cabbage on formation of n-nitrosodimethylamine  
391 during storage of kimchi. J Korean Soc Food Sci Nutr 45:117-125.

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

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

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

400 Lee DS, Jeon DS, Park SG, Arasu MV, Al-Dhabi NA, Kim SC, Kim SJ. 2015. Effect of cold  
401 storage on the contents of glucosinolates in Chinese cabbage (*Brassica rapa* L. ssp.  
402 *pekinensis*). South Indian J Biol Sci 1:38-42.

403 Li MY, Hou XL, Wang F, Tan GF, Xu ZS, Xiong AS. 2018. Advances in the research of celery,  
404 an important Apiaceae vegetable crop. Crit Rev Biotechnol 38:172-183.

405 Merino L. 2009. Development and validation of a method for determination of residual  
406 nitrite/nitrate in foodstuffs and water after zinc reduction. Food Anal Methods 2:202-220.

407 Ministry of Food and Drug Safety. 2020. The Korea Food Code. Available from:  
408 [http://www.foodsafetykorea.go.kr/foodcode/01\\_03.jsp?idx=37](http://www.foodsafetykorea.go.kr/foodcode/01_03.jsp?idx=37). Accessed at May 4,  
409 2020.

410 Nuñez de González MT, Osburn WN, Hardin MD, Longnecker M, Garg HK, Bryan NS, Keeton  
411 JT. 2015. A survey of nitrate and nitrite concentrations in conventional and organic-  
412 labeled raw vegetables at retail. J Food Sci 80:C942-C949.

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

416 Riyad YM, Ismail IMM, Abdel-Aziz ME. 2018. Effect of vegetable powders as nitrite sources  
417 on the quality characteristics of cooked sausages. *Biosci Res* 15:2693-2701.

418 SAS. 2012. SAS/STAT<sup>®</sup> software for PC. Release 9.4, SAS Institute Inc., Cary, NC, USA.

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

421 Sebranek JG, Bacus JN. 2007b. Natural and organic meat products: Regulatory, manufacturing,  
422 marketing, quality and safety issues. American Meat Science Association White Paper  
423 Series No. 1, American Meat Science Association, Champaign, IL, USA. pp 1-15.

424 Sebranek JG, Jackson-Davis AL, Myers KL, Lavieri NA. 2012. Beyond celery and starter  
425 culture: Advances in natural/organic curing processes in the United States. *Meat Sci*  
426 92:267-273.

427 Sebranek JG. 2009. Basic curing ingredients. In *Ingredients in meat products: Properties,*  
428 *functionality and applications.* Tarté R (ed). Springer Science, New York, NY, USA. pp  
429 1-24.

430 Seong GU, Hwang IW, Chung SK. 2016. Antioxidant capacities and polyphenolics of Chinese  
431 cabbage (*Brassica rapa* L. ssp. *Pekinensis*) leaves. *Food Chem* 199:612-618.

432 Sindelar JJ. 2006. Investigating uncured no-nitrate-or-nitrite-added processed meat products.  
433 Ph. D. thesis, Iowa State Univ., Ames, IA, USA.

434 Sindelar JJ, Cordray JC, Olson DG, Sebranek JG, Love JA. 2007a. Investigating quality  
435 attributes and consumer acceptance of uncured, no-nitrate/nitrite-added commercial  
436 hams, bacons, and frankfurters. *J Food Sci* 72:S511-S559.

437 Sindelar JJ, Cordray JC, Sebranek JG, Love JA, Ahn DU. 2007b. Effects of varying levels of  
438 vegetable juice powder and incubation time on color, residual nitrate and nitrite, pigment,

439 pH, and trained sensory attributes of ready-to-eat uncured ham. *Journal of Food Science* 72:  
440 S388-S395.

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

444 Sindelar JJ, Terns MJ, Meyn E, Boles JA. 2010. Development of a method to manufacture  
445 uncured, no-nitrate/nitrite-added whole muscle jerky. *Meat Science* 86:298-303.

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

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

450 Sullivan GA, Jackson-Davis AL, Schrader KD, Xi Y, Kulchaiyawat C, Sebranek JG, Dicson J.  
451 2012. Survey of naturally and conventionally cured commercial frankfurters, ham, and  
452 bacon for physico-chemical characteristics that affect bacterial growth. *Meat Science* 92:808-  
453 815.

454 Suman SP, Joseph P. 2013. Myoglobin chemistry and meat color. *Annual Review of Food Science and Technology*  
455 4:79-99.

456 Terns MJ, Milkowski AL, Rankin SA, Sindelar JJ. 2011. Determining the impact of varying  
457 levels of cherry powder and starter culture on quality and sensory attributes of indirectly  
458 cured, emulsified cooked sausages. *Meat Science* 88:311-318.

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

- 462 Xi Y, Sullivan GA, Jackson AL, Zhou GH, Sebranek JG. 2012. Effects of natural antimicrobials  
463 on inhibition of *Listeria monocytogenes* and on chemical, physical and sensory attributes  
464 of naturally-cured frankfurters. Meat Sci 90:130-138.
- 465 Zhu Z, Wu X, Geng Y, Sun DW, Chen H, Zhao Y, Zhou W, Li X, Pan H. 2018. Effects of  
466 modified atmosphere vacuum cooling (MAVC) on the quality of three different leafy  
467 cabbages. LWT-Food Sci Technol 94:190-197.

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468 **Table 1. The formulation for cooked ground pork products formulated with vegetable powders**

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Main materials (%)	Treatments				
	Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Pork ham	70.00	70.00	70.00	70.00	70.00
Pork back fat	15.00	15.00	15.00	15.00	15.00
Ice/water	15.00	15.00	15.00	15.00	15.00
<b>Ingredients (%)</b>					
NaCl	1.50	1.50	1.50	1.50	1.50
Dextrose	1.00	1.00	1.00	1.00	1.00
Sodium tripolyphosphate	0.30	0.30	0.30	0.30	0.30
Sodium nitrite	0.01	-	-	-	-
Sodium ascorbate	0.05	0.05	0.05	0.05	0.05
Chinese cabbage powder	-	0.15	0.25	0.35	-
Celery powder	-	-	-	-	0.40
Starter culture	-	0.015	0.025	0.035	0.04
<b>Total</b>	<b>101.860</b>	<b>103.015</b>	<b>103.125</b>	<b>103.235</b>	<b>103.29</b>

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471 **Table 2. Effects of Chinese cabbage powder on cooking yield, pH values, and CIE color of alternatively cured meat products**

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Treatments <sup>1</sup>	Dependent variables				
	Cooking yield (%)	pH	CIE L*	CIE a*	CIE b*
Control	99.64 ± 0.01 <sup>A</sup>	6.15 ± 0.009 <sup>A</sup>	68.79 ± 0.17	8.85 ± 0.14 <sup>B</sup>	6.98 ± 0.05 <sup>B</sup>
Treatment 1	99.24 ± 0.08 <sup>B</sup>	6.12 ± 0.003 <sup>B</sup>	68.41 ± 0.16	10.10 ± 0.10 <sup>A</sup>	6.97 ± 0.07 <sup>B</sup>
Treatment 2	99.24 ± 0.02 <sup>B</sup>	6.10 ± 0.002 <sup>C</sup>	68.28 ± 0.16	10.12 ± 0.09 <sup>A</sup>	7.04 ± 0.06 <sup>B</sup>
Treatment 3	99.19 ± 0.05 <sup>B</sup>	6.08 ± 0.003 <sup>D</sup>	68.44 ± 0.10	10.06 ± 0.07 <sup>A</sup>	7.39 ± 0.06 <sup>A</sup>
Treatment 4	99.20 ± 0.07 <sup>B</sup>	6.16 ± 0.005 <sup>A</sup>	68.29 ± 0.15	9.98 ± 0.08 <sup>A</sup>	7.52 ± 0.03 <sup>A</sup>

473 All values are means ± standard errors.

474 <sup>A-D</sup> Means within a column with different superscript letters are significantly different (p<0.05).

475 <sup>1</sup> Treatments: control, 100 ppm sodium nitrite; treatment 1, 0.15% Chinese cabbage powder; treatment 2, 0.25% Chinese cabbage powder;  
 476 treatment 3, 0.35% Chinese cabbage powder; treatment 4, 0.4% celery juice powder.

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479 **Table 3. Effects of Chinese cabbage powder on residual nitrite, nitrosyl hemochrome, total pigment, and curing efficiency of alternatively**  
 480 **cured meat products**

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Treatments <sup>1</sup>	Dependent variables			
	Residual nitrite (ppm)	Nitrosyl hemochrome (ppm)	Total pigment (ppm)	Curing efficiency (%)
Control	37.32 ± 0.53 <sup>A</sup>	34.29 ± 0.09 <sup>C</sup>	47.18 ± 0.18 <sup>B</sup>	72.70 ± 0.36 <sup>C</sup>
Treatment 1	14.68 ± 1.67 <sup>C</sup>	37.85 ± 0.24 <sup>B</sup>	48.79 ± 0.11 <sup>A</sup>	77.57 ± 0.50 <sup>B</sup>
Treatment 2	16.58 ± 1.62 <sup>C</sup>	39.73 ± 0.28 <sup>A</sup>	48.62 ± 0.22 <sup>A</sup>	81.73 ± 0.69 <sup>A</sup>
Treatment 3	22.24 ± 2.05 <sup>B</sup>	40.53 ± 0.09 <sup>A</sup>	49.13 ± 0.11 <sup>A</sup>	82.49 ± 0.09 <sup>A</sup>
Treatment 4	16.35 ± 2.13 <sup>C</sup>	40.60 ± 0.13 <sup>A</sup>	48.96 ± 0.18 <sup>A</sup>	82.93 ± 0.35 <sup>A</sup>

482 All values are means ± standard errors.

483 <sup>A-C</sup> Means within a column with different superscript letters are significantly different (p<0.05).

484 <sup>1</sup> Treatments: control, 100 ppm sodium nitrite; treatment 1, 0.15% Chinese cabbage powder; treatment 2, 0.25% Chinese cabbage powder;  
 485 treatment 3, 0.35% Chinese cabbage powder; treatment 4, 0.4% celery juice powder.

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