Meat Science 108 (2015) 132–137

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

The use of atmospheric pressure plasma-treated water as a source of nitrite for emulsion-type sausage

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ARTICLE INFO

Article history:
Received 4 April 2015
Received in revised form 11 June 2015
Accepted 16 June 2015
Available online 18 June 2015

Keywords:
Atmospheric pressure plasma
Nitrite
Curing
Emulsion-type sausage

ABSTRACT

We investigated the possible use of atmospheric pressure plasma-treated water (PTW) as a nitrite source in curing process. Emulsion-type sausages were manufactured with PTW, celery powder containing nitrite, and synthetic sodium nitrite at a concentration of nitrite ion 70 mg kg^{-1}. In terms of sausage quality, there were no noticeable effects of PTW on the total aerobic bacterial counts, color, and peroxide values of sausages compared with those of celery powder and sodium nitrite throughout 28 days of storage at 4 °C. Sausage with added PTW had lower concentrations of residual nitrite compared to those of added celery powder and sodium nitrite during the storage period ($P < 0.05$). The sensory properties of PTW-treated and sodium nitrite-treated sausages were not different, whereas the sausage with added celery powder received the lowest scores in taste and acceptability. From the results, it is concluded that PTW can be used as a nitrite source equivalent to a natural curing agent.

1. Introduction

Nitrite (NO_2^-) has been used as a curing agent since it was discovered to play a role in the development of distinctive cured meat color and flavor, the inhibition of lipid oxidation, and the control of spoilage and pathogenic microorganisms, including *Clostridium botulinum* and its spores in cured meat products (Pegg & Shahidi, 2000). Today both conventional and natural curing processes are used for production of cured meat products. Conventional curing processes generally use sodium nitrite as a nitrite source (Parthasarathy & Bryan, 2012; Sebranek & Bacus, 2007a, 2007b; Sebranek, Jackson-Davis, Myers, & Lavieri, 2012). Natural curing processes use vegetable juice concentrate that contains nitrate with a starter culture of bacteria, which reduces nitrate (NO_3^-) to nitrite (NO_2^-), or pre-converted (nitrate to nitrite) vegetable concentrate (Parthasarathy & Bryan, 2012; Sebranek et al., 2012). Among vegetable juices that contain nitrate, celery juice is mainly used in natural curing because of its low flavor and minimal content of vegetable pigment (Sebranek & Bacus, 2007a, 2007b). Recently, many consumers have been found to prefer meat products cured by the natural process to those cured by the conventional one, because of concerns about synthetic curing agents, including sodium nitrite (Sebranek & Bacus, 2007a, 2007b). Therefore, the market for naturally cured meat products has grown rapidly (Sebranek et al., 2012). However, the use of vegetable concentrates, especially celery concentrate, in curing processes has disadvantages, which include added cost and increased incubation time compared with the use of a chemical nitrite source (sodium nitrite) (Horsch et al., 2014). In addition, the microbiological safety of naturally cured meat products is generally lower than that of a conventionally cured one (Wanless, 2010). The concentration of nitrite in commercially available pre-converted celery concentrates is 10,000–15,000 ppm, and these concentrates must be used at less than 0.4% (w/w) of the formulation because of its characteristic flavor (Sindelar, Cordray, Sebranek, Love, & Ahn, 2007). At this concentration of added concentrate, the amount of nitrite in the naturally cured meat products is less than 40–45 ppm. This concentration is enough for the development of cured color and flavor, however that is certainly lower than the requirement to assure microbiological safety of cured meat products (Jafari & Emam-Djomeh, 2007). Therefore, a new curing process or new source of nitrite, which can be accepted by both consumers and industry, is needed for the production of cured meat.

Plasma is an ionized gas, and atmospheric pressure plasma treatment is regarded as an emerging non-thermal sterilization technology (Jayasena et al., 2015; Yong et al., 2015). Recently, it has been reported that this technology can increase the biological activity of natural compounds by molecular transformation (Kim et al., 2014, Kim et al., 2015). For inactivation of microorganisms, the ultraviolet, reactive species, and charged particles contained in the plasma interact directly with microorganisms on the surface of food (Deng, Shi, & Kong, 2006; Yong et al., 2015). On the other hand, Oehmigen et al. (2010) reported

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http://dx.doi.org/10.1016/j.meatsci.2015.06.009
0309-1740/

that plasma does not interact directly with microorganisms in a liquid. Instead, they found that the plasma–liquid interaction resulted in acidification of the liquid combined with the generation of reactive oxygen and nitrogen species, including nitrate (NO_3^-) and nitrite (NO_2^-), which inactivated the microorganisms. Based on the previous study, we hypothesized that water containing nitrite formed by plasma treatment could be used as a nitrite source for cured meat products.

Therefore, the objective of this study is to compare the quality of emulsion-type sausages cured by sodium nitrite, pre-converted celery concentrate, and plasma-treated water (PTW) to evaluate the suitability of PTW as a nitrite source in cured meat product manufacturing.

2. Materials and methods

2.1. Production of PTW

2.1.1. Plasma treatment system

As schematically illustrated in Fig. 1(a), a plasma device consisting of the powered electrode, the ground electrode, and a 0.6 mm-thick alumina plate that was placed between the electrodes was used for generating surface dielectric barrier discharge (SDBD). Due to the high concentration of gas-phase oxidants produced at the discharge layer, metallic parts are easily oxidized and induce the change of discharge properties. For this reason, in order to prevent the oxidation of electrodes due to the presence of high oxidant species, ground electrode which directly contacts with the discharge was made of a nickel–chromium alloy. A bipolar square waveform with 15 kHz was applied to powered electrode. Total discharge area is 20 cm^2, while the average power is 3.14 W and the peak power is 200 W. Fig. 1(b) presents real images of the ground electrode involving rounded square patterns with 3 mm × 3 mm size. Air surface discharge was generated at the opened area in the face where the ground electrode was installed. To produce PTW, 100 mL distilled water containing 1% sodium pyrophosphate (w/v) was exposed remotely by SDBD in atmospheric air for 120 min. The 1% sodium pyrophosphate (w/v) was used for inhibiting the decrease of pH in PTW because the amount of nitrite ion was decreased in acidic PTW.

2.1.2. Quantitation of nitrite and nitrate concentration

The concentration of nitrite (NO_2^-) and nitrate (NO_3^-) dissolved in PTW were measured using an UV–visible absorption system consisting of the continuum light source (ISS-UV–VIS, Ocean optics Inc., Dunedin, FL., USA), quartz cuvette (CV-Q-10, Ocean optics Inc.) having 10 mm of pathlength, UV–visible optical fiber (QP400-2-SR, Ocean optics Inc.), and spectrometer (MAYA2000 Pro, Ocean optics Inc.). In order to obtain the absorption coefficient of nitrite in the UV range, the standard solutions of nitrite having 100–1000 ppm of the nitrite concentration was prepared by dissolving $NaNO_2$ (Sigma-Aldrich Co., St. Louis, MO., USA) in distilled water. The absorption spectra of nitrite and nitrate show two distinct regions, a strong band caused by $\pi \rightarrow \pi^*$ transition near 200 nm and a weak band by $n \rightarrow \pi^*$ transition at 270–400 nm (Krishnan & Guha, 1934). The deconvolution of overlapping absorption bands of nitrite and nitrate at 200 nm is difficult to do, and the high absorption coefficients of both ions at 200 nm are not suitable in this experiment, whereas absorption bands at 270–400 nm are appropriate. Thus, the concentration of nitrite and nitrate were quantified by monitoring the absorption spectra in the wavelength range of 270–400 nm as seen in Fig. 1(c).

2.2. Manufacture and analysis of emulsion sausage

2.2.1. Manufacture of emulsion sausage

Emulsion-type sausages were manufactured using pork hind leg meat and back fat obtained from a commercial butcher (Seoul, Korea). Meat was trimmed free of visible fat and connective tissue and then ground using meat grinder with 6 mm plate. Ground meat was mixed with back fat, cooled water, and additives in bowl cutter depending on the each formula of four treatments (control, emulsion sausage cured with no nitrite source; PTW, emulsion sausage cured with PTW; Celery powder, emulsion sausage cured with pre-converted celery concentrate; Sodium nitrite, emulsion sausage cured with sodium nitrite, Table 1). The concentration of nitrite ion added to treatments was equal (70 mg kg^{-1}) except for control group. A total 5 kg of meat batter was prepared for each treatment per each trial in three trials. The meat batters were stored at 4 °C for 24 h prior to filling of meat batter. After storage, meat batter was filled with a respective treatment and trial in manufactured collagen casing (2.5 cm of diameter) using a continuous stuffer. Sausage (15 cm long in average) was cooked in water-bath at 80 °C for 30 min until internal temperature of the sausage reached 75 °C. Cooked sausage was vacuum-packaged in a low-density polyethylene/nylon vacuum bags (20 cm × 14 cm; oxygen permeability of 22.5 mL/m^2/24 h atm at 60% RH/25 °C; water vapor permeability of 4.7 g/m^2/24 h at 100% RH/25 °C) using a vacuum-packaging machine (FJ-600XL, Hankook Fujee Industries Co., Hwaseong, Korea) at −650 mm Hg. Packaged sausages were pasteurized in 85 °C hot water for 2 min and then cooled in 10 °C water. The properties except sensory parameters of three emulsion-type sausages in treatments per each trial were analyzed during storage at 4 °C for 28 days.

2.2.2. Total aerobic bacterial counts

The meat batter or sausage (10 g) was blended with sterile saline (90 mL) for 2 min by using a stomacher (BagMixer® 400, Interscience Ind., St. Nom, France). A series of decimal dilutions was prepared using sterile saline. Each diluent (0.1 mL) was spread in triplicate on tryptic soy agar plates (Difco Laboratories, Detroit, MI, USA). The plates were incubated at 37 °C for 48 h, and the microbial counts were expressed as log CFU/g.

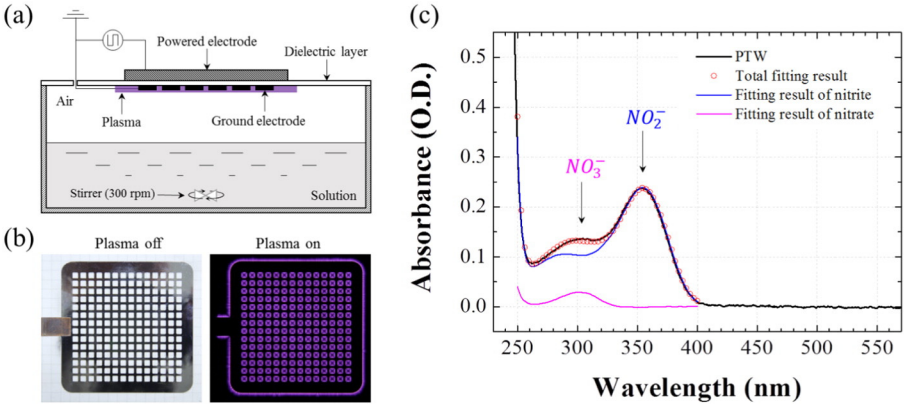

Fig. 1. Schematic drawing of plasma apparatus (a), real images of ground electrode (b), and ultra-violet absorption spectrum (c).

Table 1
Formulations (%) for manufacturing emulsion-type sausages.

Ingredients	Control	PTW	Celery powder	Sodium nitrite
Pork hind leg meat	60.0	60.0	60.0	60.0
Pork fat	20.0	20.0	20.0	20.0
Water	20.0	11.0	20.0	20.0
PAW	–	9.0	–	–
Total	100	100	100	100
Sodium chloride	1.5	1.5	1.5	1.5
Sodium pyrophosphate	0.2	0.11[a]	0.2	0.2
L-Ascorbic acid	0.04	0.04	0.04	0.04
Sugar	1.0	1.0	1.0	1.0
Black pepper powder	0.3	0.3	0.3	0.3
Wheat starch	5.0	5.0	5.0	5.0
Soy protein powder	2.0	2.0	2.0	2.0
Sodium nitrite	–	–	–	0.01
Celery powder	–	–	2.3	–

[a] PTW contained 1% sodium pyrophosphate.

2.2.3. Instrumental color measurements

The lightness (L^*), redness (a^*), and yellowness (b^*) of the sausage were measured using a spectrophotometer with the illuminant D_{65} (CM-3500d, Konica Minolta Inc., Tokyo, Japan). Measurements were taken perpendicularly to the cut surface of sausage with 8 mm diameter of illumination area at 2 different locations per sample.

2.2.4. Lipid oxidation

Lipid oxidation in sausage was measured using peroxide values. Lipid in sausage was extracted according to Folch's extraction method (Folch, Lees, & Sloane-Stanley, 1957). Sausage (10 g) was cut into small pieces in 50 mL of Folch solution (chloroform:methanol, 2:1) and it was shaken at room temperature for 24 h. The mixture was filtered with a filter paper and 15 mL NaCl (0.88%) was added. After shaking vigorously, 10 mL was collected from bottom layer and evaporated using nitrogen gas. The extracted lipids was treated with 35 mL of solvent mixture (acetic acid:chloroform, 3:2). The mixture was shaken thoroughly, and 0.5 mL of saturated potassium iodide solution was added. The mixture was kept in the dark for 5 min, 75 mL of distilled water was added, and the mixture was mixed. A 2.5 mL of starch solution (1%, w/v) was added as an indicator. The peroxide value was determined by titrating the iodine liberated from potassium iodide with standardized 0.005 N sodium thiosulfate solution. The peroxide value was calculated by following equation.

$$\text{Peroxide value (meg/kg)} = \frac{(V1 - V0) \times F \times 0.01}{S} \times 1000$$

V1 : titration amount (mL) of 0.005 N $Na_2S_2O_3$ on the samples
V0 : titration amount (mL) of 0.005 N $Na_2S_2O_3$ on the blank
F : factor of 0.005 N $Na_2S_2O_3$
S : sample weight (g)

2.2.5. Residual nitrite content

Residual nitrite in sausage was measured according to AOAC method 973.31 (AOAC, 1990). The measurement of residual nitrite was duplicated for each sample, and the average value was used.

2.2.6. Sensory evaluation

A sensory evaluation of the emulsion-type sausages was conducted with an individual trial. Sausages were cut into a similar size pieces and served to the sensory panel. The semi-trained sensory panel consisted of ten panelists, who have had at least 2 years of experience in meat sensory analysis. The scoring of each sample was done on a single sheet using a 9-point hedonic scale (1 = extremely dislike, 9 = extremely like). The sensory parameters scored were color, flavor, taste, juiciness, springiness, off-odor, and acceptability.

2.2.7. Statistical analysis

Data were analyzed using the PROC GLM procedure of SAS software (version 9.3, SAS Institute Inc., Cary, NC, USA) in a randomized complete block design (trial as a block). The experimental unit was the packed sausage. The statistical model for total aerobic bacteria, instrumental color, peroxide value, residual nitrite, and sensory parameters were included the effect of nitrite source. The trials and panelists as random terms were statistically checked, but no effects were found. Thus they were excluded from the model. Specific comparisons were performed by Tukey's multiple range test when the main effect was significant. Results are reported as least square mean values and standard error of the least square means (SEM). Statistical significance was considered at $P < 0.05$.

3. Results and discussion

3.1. Chemical property of PTW

In addition to charged particles, many reactive neutral species are produced in the discharge layer near the ground electrode. Atmospheric nitrogen and oxygen molecules are dissociated by energetic electrons in the discharge layer mostly forming nitrogen oxides (e.g., NO_2, N_2O_3, and N_2O_5) in the gas phase through several reaction pathways (Sakiyama, Graves, Chang, Shimizu, & Morfill, 2012). Charged particles have a short lifetime (under 10^{-6} s) and are not able to diffuse significant distances in aqueous solution. On the other hand, neutral species such as ozone, nitrogen oxides, and nitric acid have greater lifetimes and are able to participate in chemical reactions at the gas–liquid interface. Dissolved NO_2, NO_3, N_2O_4, and N_2O_3 react irreversibly with H_2O to form nitric and nitrous acid via the following reactions; nitric oxide (NO) is sparingly soluble in water (Thomas & Vanderschuren, 1997 and 2000):

$$2NO_2 + H_2O \rightarrow HNO_2 + HNO_3 \quad (1)$$

$$N_2O_4 + H_2O \rightarrow HNO_2 + HNO_3 \quad (2)$$

$$N_2O_3 + H_2O \rightarrow 2HNO_2. \quad (3)$$

Because of non-negligible concentrations of nitric acid ($pK_a = -1.4$) and nitrous acid ($pK_a = 2.8 \sim 3.2$), protons are produced and cause the solution pH to drop from 7 to 2–3.

$$HNO_2 \rightleftharpoons H^+ + NO_2^- \quad (4)$$

$$HNO_3 \rightleftharpoons H^+ + NO_3^- \quad (5)$$

Even with the plasma turned off, the concentrations of reactive components in the aqueous solution vary continuously over several days. As a result, post-discharge reactions are thought to maintain the level of key chemical species, i.e. nitrite, in PTW. In acidic PTW, nitrous acid and nitrite coexist, with nitrous acid being continuously decomposed into nitrate and nitric oxide (Reaction 6) (Rayson, Mackie, Kenndy, & Dlugogorshi, 2012). Because nitric oxide is irreversibly oxidized to nitrogen dioxide by oxygen, the concentrations of nitrous acid and nitrite gradually decrease over time while that of nitrate increases. However, in an alkaline environment, nitrous acid is deprotonated via Reaction 4, and the decomposition of nitrous acid is prevented. In the presence of hydrogen peroxide, the concentrations of nitrate and nitrite in PTW are also affected by Reactions 7 and 8 (Thomas & Vanderschuren, 2000)

$$3HNO_2 \leftrightarrow H^+ + NO_3^- + 2NO + H_2O \quad (6)$$

$$2NO + 3H_2O_2 \rightarrow 2HNO_3 + 2H_2O \quad (7)$$

$$HNO_2 + H_2O_2 \rightarrow ONOOH + H_2O. \quad (8)$$

Therefore, alkalinization of the target solution prior to producing PTW improves the rate of formation of nitrite during plasma treatment and prevents the nitrite concentration from varying after plasma treatment (Choe et al., 2014). To utilize PTW as a nitrite source for emulsion-type sausage, the concentration of nitrite in PTW should satisfy the requirements of meat curing. In this work, sodium pyrophosphate, which is commonly used as a curing agent for meat products, was dissolved in distilled water to produce a 1% solution prior to plasma treatment to avoid the decomposition of nitrous acid. The pH of alkalized PTW before and after SDBD treatment for 120 min was 10.19 and 9.01, respectively. The absorption spectrum of PTW after plasma treatment for 120 min, shown in Fig. 1(c), corresponds to formation of 782 ppm of nitrite and 358 ppm of nitrate (data not shown). Although PTW contains NO_3^-, the latter was not considered when PTW was added as a nitrite source to emulsion-type sausage.

3.2. Total aerobic bacteria

The number of total aerobic bacteria in emulsion-type sausage was monitored during storage at 4 °C for up to 28 days (Table 2). Because of pasteurization (heating the packed sausages in hot water at 85 °C for 2 min), the number of total aerobic bacteria was lower than the detection limit of 10^1 CFU/g for all treatments at day 0 and was maintained at this level through day 14. The number of total aerobic bacteria increased to more than 2 log CFU/g after 21 days of storage. Addition of nitrite limited the growth of aerobic bacteria on days 21 and 28 compared to the control ($P < 0.05$). Among the different nitrite sources tested, there were no significant differences in the total aerobic bacteria count on day 21. On day 28, the PTW-treated sample had a similar number of total aerobic bacteria compared with the sodium nitrite-treated sample ($P > 0.05$) and had a lower number of total aerobic bacteria than the celery powder-treated sample ($P < 0.05$), although the difference between the bacteria counts of the PTW-treated and celery powder-treated samples was small. The sausages in this study were cured with the same concentration (70 mg kg^{-1}) of nitrite from different nitrite sources. Previous studies have reported that the antimicrobial effect of nitrite in meat products depends mainly on nitrite concentration rather than nitrite source (Horsch et al., 2014; Myers et al., 2013).

3.3. Instrumental color

The L^* values of sausage cured with nitrite were significantly lower than that of the control throughout the storage period (Table 3). This result agrees with a previous study, which found that curing fermented sausage with nitrite resulted in a decrease in L^* values (Tsoukalas, Katsanidis, Marantidou, & Bloukas, 2011). By comparison, the PTW-treated samples showed a significantly higher L^* value compared with the celery powder and sodium nitrite-treated samples immediately after manufacture (day 0, $P < 0.05$). However, the significant difference in L^* values between the PTW-treated and sodium nitrite-treated sausage was inconsistent with the increase of storage days while that was clear between PTW-treated and celery powder-treated sausage. Celery concentrate has its own pigment, and Horsch et al. (2014) found that ham cured by celery concentrate was dark regardless of nitrite concentration.

The addition of nitrite significantly increased the a^* values of sausage compared to the control (Table 3), and this difference was maintained throughout the storage period. The increase in the redness of a meat product cured by nitrite, regardless of nitrite sources, is well known (de Oliveira et al., 2012; Horsch et al., 2014; Tsoukalas et al., 2011). In the comparison of nitrite sources, the a^* values of sausages subjected to the PTW and celery powder treatments were significantly lower than the sausage treated with sodium nitrite throughout storage for 28 days ($P < 0.05$). A previous study found no difference between the a^* values of hams cured with sodium nitrite and celery concentrate at 100 mg kg^{-1} for 35 days and a lower a^* value for ham cured with celery concentrate than with sodium nitrite immediately after manufacture at 200 mg kg^{-1} of nitrite (Horsch et al., 2014). No significant differences in a^* values with storage time were found except for the control, in which there was no noticeable trend.

The b^* value of sausage cured with nitrite was significantly lower than that of the control throughout the storage period ($P < 0.05$). In comparing nitrite sources, there was no significant difference among the b^* values of the samples subjected to various treatments right

Table 2
The number (log CFU/g) of total aerobic bacteria in emulsion-type sausage.

Treatment	Storage (days) 0	7	14	21	28	SEM
Addition of nitrite						
Control[1]	ND[c,4]	ND[c]	ND[c]	2.33[bx]	3.47[ax]	0.052
Nitrite[2]	ND[c]	ND[c]	ND[c]	2.13[by]	3.23[ay]	0.018
SEM	–	–	–	0.053	0.037	
Nitrite sources						
PTW	ND[c]	ND[c]	ND[c]	2.09[b]	3.18[ay]	0.016
Celery powder	ND[c]	ND[c]	ND[c]	2.12[b]	3.28[ax]	0.051
Sodium nitrite	ND[c]	ND[c]	ND[c]	2.19[b]	3.22[axy]	0.011
SEM[3]	–	–	–	0.067	0.021	

[a–c]Different letters within the same row differ significantly ($P < 0.05$).
[x,y]Different letters within the same column differ significantly ($P < 0.05$).
[1] Sausages cured with no nitrite.
[2] All sausages cured with PTW, celery powder, and sodium nitrite.
[3] Standard errors of least square mean.
[4] Viable cells were not detected with the detection limit of $<10^1$ CFU/g.

Table 3
Instrumental color (L^*, a^*, and b^*) of emulsion-type sausage.

Treatment	Storage (days) 0	7	14	21	28	SEM
L^*						
Addition of nitrite						
Control[1]	75.92[bx]	76.52[abx]	77.98[ax]	77.28[abx]	77.95[ax]	0.418
Nitrite[2]	74.05[y]	74.88[y]	74.63[y]	75.24[y]	75.26[y]	0.390
SEM	0.435	0.462	0.615	0.410	0.544	
Nitrite sources						
PTW	75.15[bx]	75.97[abx]	76.26[ax]	76.29[ax]	76.94[ax]	0.219
Celery powder	73.60[y]	74.03[y]	73.02[z]	74.16[y]	74.20[y]	0.311
Sodium nitrite	73.41[by]	74.64[abx]	74.61[aby]	75.26[ax]	74.63[aby]	0.347
SEM[3]	0.311	0.306	0.345	0.248	0.266	
a^*						
Addition of nitrite						
Control	1.07[aby]	0.95[by]	1.09[aby]	1.21[ay]	1.22[ay]	0.049
Nitrite	5.57[x]	5.62[x]	5.62[x]	5.47[x]	5.49[x]	0.097
SEM	0.091	0.137	0.133	0.111	0.120	
Nitrite sources						
PTW	5.48[y]	5.58[y]	5.49[y]	5.31[y]	5.35[y]	0.092
Celery powder	5.40[y]	5.29[y]	5.36[y]	5.33[y]	5.28[y]	0.094
Sodium nitrite	5.85[x]	6.00[x]	6.00[x]	5.77[x]	5.85[x]	0.051
SEM	0.051	0.081	0.097	0.090	0.081	
b^*						
Addition of nitrite						
Control	16.94[ax]	16.41[abx]	16.33[abx]	16.00[bx]	15.91[bx]	0.184
Nitrite	12.80[y]	12.81[y]	12.69[y]	12.46[y]	12.56[y]	0.187
SEM	0.402	0.234	0.295	0.234	0.215	
Nitrite sources						
PTW	12.41	12.31[y]	11.88[y]	11.98[y]	12.07[z]	0.167
Celery powder	13.09	13.41[x]	13.30[x]	13.11[x]	13.20[x]	0.199
Sodium nitrite	12.91	12.71[xy]	12.89[x]	12.29[y]	12.40[y]	0.150
SEM	0.160	0.200	0.230	0.161	0.070	

[a,b]Different letters within the same row differ significantly ($P < 0.05$).
[x–z]Different letters within the same column differ significantly ($P < 0.05$).
[1] Sausages cured with no nitrite
[2] All sausages cured with PTW, celery powder, and sodium nitrite
[3] Standard errors of least square mean

after manufacture (day 0, $P > 0.05$). After storage, the b^* values of the PTW-treated samples were significantly lower than those of the sodium nitrite-treated samples on days 14 and 28 and then those of the celery powder-treated samples from days 7 to 28 ($P < 0.05$). These results agree partially with previous studies. Horsch et al. (2014) found lower b^* values for ham cured with sodium nitrite compared with ham cured without nitrite. Moreover, high b^* values of meat cured with vegetable concentrate as a nitrite source compared with meat cured without nitrite were reported to be due to pigment in the vegetable concentrate (Horsch et al., 2014; Tsoukalas et al., 2011). However, the b^* values of the celery powder-treated samples were lower than those of the control throughout the storage period, although that were the highest among nitrite sources. All nitrite source treatments showed no significant differences in b^* values with increased storage, whereas the b^* values of the control decreased slightly with an increasing storage.

3.4. Peroxide value

Sausage peroxide values, which are an indicator of lipid oxidation, are shown in Table 4. Peroxide values of both control and nitrite-treated sausage increased significantly with increased storage from day 7 to day 21 ($P < 0.05$). This means that the lipid oxidation in sausage increases during storage. However, peroxide values of both the control and nitrite-treated samples at day 28 were significantly lower than those at day 21. This observation may be the result of the depletion of peroxides, which are primary products of lipid oxidation, and the formation of secondary oxidation products upon further lipid oxidation (Abd El-Alim, Lugasi, Hovari, & Dworschak, 1999). The peroxide values resulting from nitrite treatment were significantly lower than those of the control throughout the storage period apart from day 0 ($P < 0.05$). Inhibition of lipid oxidation in meat products by nitrite addition has been reported in various studies (Al-Shuibi & Al-Abdullah, 2002; de Oliveira et al., 2012; Tsoukalas et al., 2011). Generally, myoglobin in meat is denatured by cooking and processing, which results in the release of iron from heme that catalyzes lipid oxidation (Jung, Nam, Ahn, Kim, & Jo, 2013). However, nitrite can form nitrosyl-myoglobin, which is a stable compound in meat products that diminishes the availability of iron and its action as an accelerator of lipid oxidation (Honikel, 2008). In comparing nitrite sources in Table 4, there were no significant differences in peroxide values among the PTW-treated, celery powder-treated, and sodium nitrite-treated samples through the entire period of storage ($P > 0.05$).

3.5. Residual nitrite

The residual nitrite concentration in sausages treated with nitrite was predictably higher than that of the control throughout the storage period (Table 5). Among the different nitrite sources, the PTW-treated sample had a lower concentration of residual nitrite than the ones treated with celery powder and sodium nitrite throughout the storage period ($P < 0.05$). In the curing process of meat, the nitric oxide that is generated immediately after nitrite addition participates in the development of color and flavor and suppresses the growth of microbial entities (Pegg & Shahidi, 2000). It seems that either the reduction of nitrite to nitric oxide is rapid or the time required for nitrite to react with ascorbic acid, which is a reductant, is short when added nitrite is dissolved in water (PTW) rather than in the solid state (celery powder or sodium nitrite). The low concentration of residual nitrite in sausage when PTW is used as a nitrite source can be viewed in two ways. Fernandez-Lopez, Sendra, Sayas-Barbera, Navarro, and Perez-Alvarez (2008) have reported that residual nitrite is important for maintaining the quality of cured meat products during storage. However, it also has been reported that nitrite in cured meat is a potential human health hazard because of the formation of nitrosamines (Pegg & Shahidi, 2000). Therefore, a low concentration of residual nitrite in meat products may have both risks and benefits. In the present study, the concentration of residual nitrite declined gradually with increasing storage for all the three types of treatments investigated. A decrease in residual nitrite levels in meat products during storage also has been reported in various studies (Horsch et al., 2014; Myers et al., 2013).

3.6. Sensory evaluation

As expected, the scores of color, flavor, taste, and acceptability for sausage cured with nitrite were significantly higher than those of the control (Table 6). In the comparison of nitrite sources, the PTW-treated sample did not differ from the sodium nitrite-treated sample in terms of its sensory properties ($P > 0.05$). However, the scores of taste and acceptability of the celery powder-treated sample were significantly lower than those of the PTW-treated and sodium nitrite-treated samples ($P < 0.05$). In this study, 23 g kg^{-1} of celery powder was added to the sausage to produce a nitrite concentration of 70 mg kg^{-1}. Therefore, such a high concentration of celery powder may have generated an undesirable taste in the sausage. This is consistent with a previous study that reported an increase in undesirable sensory properties in ham cured with increasing quantities of vegetable concentrate (Sindelar et al., 2007).

4. Conclusion

The PTW produced in this study contained enough nitrite (782 ppm) to be used as a nitrite source for curing emulsion-type sausage. When emulsion-type sausage was manufactured with addition of PTW, properties such as the number of total aerobic bacteria, color, peroxide

Table 4
Peroxide value (meq kg^{-1}) of emulsion-type sausage.

Treatment	Storage (days) 0	7	14	21	28	SEM
Addition of nitrite						
Control[1]	1.37^{d}	2.34^{cx}	4.36^{abx}	4.94^{ax}	4.18^{bx}	0.136
Nitrite[2]	1.33^{c}	1.47^{cy}	3.53^{by}	4.15^{ay}	3.60^{by}	0.100
SEM	0.105	0.228	0.137	0.144	0.150	
Nitrite sources						
PTW	1.33^{b}	1.57^{b}	3.68^{a}	4.20^{a}	3.67^{a}	0.167
Celery powder	1.34^{b}	1.45^{b}	3.43^{a}	4.12^{a}	3.57^{a}	0.194
Sodium nitrite	1.32^{b}	1.40^{b}	3.47^{a}	4.12^{a}	3.56^{a}	0.220
SEM[3]	0.160	0.088	0.210	0.231	0.243	

[a–d]Different letters within the same row differ significantly ($P < 0.05$).
[x,y]Different letters within the same column differ significantly ($P < 0.05$).
[1] Sausages cured with no nitrite.
[2] All sausages cured with PTW, celery powder, and sodium nitrite.
[3] Standard errors of least square mean.

Table 5
Residual nitrite concentration (mg kg^{-1}) of emulsion-type sausage.

Treatment	Storage (days) 0	7	14	21	28	SEM
Addition of nitrite						
Control[1]	3.20^{ay}	2.09^{aby}	2.41^{aby}	0.83^{by}	0.67^{by}	0.459
Nitrite[2]	32.38^{ax}	28.68^{abx}	24.57^{bcx}	20.56^{cdx}	18.77^{dx}	1.062
SEM	1.312	1.404	1.493	1.139	1.150	
Nitrite sources						
PTW	28.37^{ay}	24.25^{by}	19.82^{cy}	16.97^{dy}	15.39^{dz}	0.579
Celery powder	33.91^{ax}	30.74^{bx}	27.26^{cx}	22.04^{dx}	19.35^{ey}	0.401
Sodium nitrite	34.86^{ax}	31.06^{bx}	26.63^{cx}	22.67^{dx}	21.56^{dx}	0.557
SEM[3]	0.533	0.585	0.492	0.466	0.509	

[a–d]Different letters within the same row differ significantly ($P < 0.05$).
[x–z]Different letters within the same column differ significantly ($P < 0.05$).
[1] Sausages cured with no nitrite.
[2] All sausages cured with PTW, celery powder, and sodium nitrite.
[3] Standard errors of least square mean.

Table 6
Sensory evaluation of emulsion-type sausage.

	Addition of nitrite			Nitrite sources			
	Control[1]	Nitrite[2]	SEM[1]	PTW	Celery powder	Sodium nitrite	SEM[3]
Color	3.78[b]	6.28[a]	0.268	6.33	6.17	6.33	0.326
Flavor	4.56[b]	5.81[a]	0.244	5.89	5.39	6.17	0.278
Taste	4.56[b]	5.65[a]	0.333	6.22[a]	4.56[b]	6.17[a]	0.352
Juiciness	4.61	5.06	0.209	5.00	4.72	5.44	0.238
Springiness	4.94	5.28	0.302	5.56	4.94	5.33	0.407
Off-odor	2.17	2.07	0.271	1.72	2.67	1.83	0.349
Acceptability	4.61[b]	5.67[a]	0.315	6.22[a]	4.72[b]	6.06[a]	0.299

[a,b]Different letters within the same row differ significantly ($P < 0.05$).
[1] Sausages cured with no nitrite.
[2] All sausages cured with PTW, celery powder, and sodium nitrite.
[3] Standard errors of least square mean.

value, and sensory qualities were similar to those of sausage cured with sodium nitrite.

In the meat product industry, the use of both chemical (sodium nitrite) and natural (i.e., celery concentrate) nitrite sources has generated concerns such as low consumer acceptance of the chemical form and the disadvantages of limited availability, high cost, and less pleasing flavor of the natural form. At this time, it is difficult to provide a distinctive classification of PTW, because it is neither a chemical reagent nor a natural nitrite source. Recently, Foster, Sommers, Gucker, Blankson, and Adamovsky (2012) suggested that plasma treatment is a water purification method that can remove harmful contaminants in water. In this context, PTW can be classified as purified water containing nitrite. Therefore, we conclude that PTW can be used as a nitrite source without concerns associated with the limited availability and unfavorable sensory properties of natural curing agents.

Acknowledgment

This work was carried out with the support of Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ011617), Rural Development Administration, Institute of Green Bio Science and Technology, Seoul National University, and R&D Program of Plasma Advanced Technology for Agriculture and Food (Plasma Farming; Project No. EN1425-1) through the National Fusion Research Institute of Korea (NFRI) funded by Government funds.

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