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ARTICLE

Effect of Sodium Nitrite, Sodium Chloride and Concentrated Seawater on Physicochemical Properties of Meat Emulsion System

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Abstract The objective of this study was to compare the effects of various salts on the physicochemical properties of pork emulsion sausages. Pork sausages were prepared using two different salts, sodium nitrite (SN) and sodium chloride (SC), and concentrated seawater (CSW). The CIE L*, CIE a*, and CIE b*, and chroma values of cooked and uncooked sausages with added CSW were significantly higher than those of the sausages with added SC ($p<0.05$). However, uncooked and cooked sausages with added SN and CSW had similar CIE a* values ($p>0.05$). The residual NO_2^- content of sausages with added CSW was significantly lower than that of sausages with added SN. Addition of CSW to sausages resulted in a higher cooking yield compared to the other treatments ($p<0.05$). Addition of SC resulted in significantly higher volatile basic nitrogen (VBN) and thiobarbituric acid (TBA) values compared to the other treatments. Furthermore, addition of CSW enhanced important physicochemical properties, including CIE a*, CIE b*, residual nitrite content, cooking yield, VBN, TBA, textural properties, and cross-sectional area.

Keywords sausage, seawater, NO_2^-, replacement, physicochemical properties

Introduction

The World Health Organization reported in 2015 that processed meat products contained more than the acceptable maximum daily level of nitrite, drawing widespread public attention (Hur et al., 2019). The International Cancer Institute reported that meat products were defined as first-class carcinogens, and the incidence of colorectal cancer increased by 18% when consumed over 50 g per day (IARC, 2015), leading to a sharp decline in the consumption of processed meat products in Korea. Since then, the amount of residual nitrite in processed meat has been strictly regulated and limited to 71 mg/kg in Korea (KFSRI, 2020a), however, consumers remain reluctant to purchase these products because of concerns about nitrite.

Synthetic nitrite is used as a coloring agent in processed meats, including ham, sausages, and bacon, in order to give the meat a reddish color and make it appear more appetizing. It is also used to decrease rancid odors, enhance flavor, and delay microbial growth (Jung et al., 2002). However, ingestion of excessive amounts of synthetic nitrite has various side effects, including blood vessel dilation and a decreased ability of blood to transport enzymes (Gray and Randall, 1979). Owing to the carcinogenic potential of synthetic nitrite (WCRF/AICR, 2007), ongoing research aims to find natural salts and other substitutes to replace it.

The main natural salt substitutes that have been studied for potential use in processed meat products include kelp, bamboo salt baked in bamboo stems, sea salt from naturally evaporated seawater, and brine from processed seawater (Kim et al., 2010a; Kim et al., 2010b; Lee et al., 2020). There are three main types of brine: brine from the evaporation of sea salt; brine from the long-term storage of sea salt; and brine from machine salt, which is washed with water to remove impurities and the unique bitterness of seawater (Ha and Park, 1998). The brine used in this experiment was concentrated-seawater (CSW), that is, it is obtained after evaporating the sea salt.

CSW refers to deep SW removed natural salt. It contains a wide range of minerals and useful trace elements such as Cl, Na, Mg, S, Ca, K, Br, C, and N (Hardie and Eugster, 1980). Many patents have been obtained for fermented drink products produced using CSW (Kim and Oh, 2009). There have also been reports of effective treatment with SW of patients with atopic dermatitis and mice with obesity or high levels of cholesterol, demonstrating its suitability for use in drink products (Hsu et al., 2011; Kimata et al., 2002). Furthermore, SW is used in Korea for making tofu (Ko et al., 2013) and for fermenting soy sauce, in order to add flavor and nutrients (Ham et al., 2008). This study investigated the effects of the addition of different amounts of CSW on the color and quality of emulsion sausages. Also it is thought to be replaced NPS and replaced sodium nitrite using natural materials.

Materials and Methods

Preparation of sausage samples

CSW was supplied by Yeorumul (Incheon, Korea) and used as received. Pork meat (Jungwoo Food, Pocheon, Korea) and pork fat were cut and ground using a grinder (PA-82, Mainca, Barcelona, Spain) equipped with 3 mm plates. Pork ground meat (60%), ground fat (20%), and ice (20%) were placed in a bowl-cutter (K-30, Talsa, Valencia, Spain), with sugar (1%) as the subsidiary material. Three treatments were prepared: 1.2% sodium nitrite (SN) (nitrite: 0.006%), 1.2% sodium chloride (SC), and CSW (20% NaCl, 0.007% zinc, 3.3% sodium, 1.8% potassium, and 0.0004% arsenic). The pork meat mixtures with the respective treatments were stuffed into natural hog casings (Woosing Foodtec, Seoul, Korea) using a stuffer (EM-12, Mainca, Barcelona, Spain) and heated in a chamber (10.10 ESI/SK, Alto Shaham, Menomonee Falls, WI, USA) at 80℃ until the internal temperature of the samples reached 75℃ (Table 1). The treated samples were then left at room temperature (20℃) for 30 min before being stored at 5℃ until analysis. The above method was repeated 3 times and used in the experiment.

Cooking yield

Raw samples were placed in natural casings and heated in a chamber (10.10 ESI/SK, Alto Shaham). Sample weights before and after heating were measured, and the following calculation was performed: Weight after heating/weight before heating×100.

Table 1. Formulation of pork emulsion sausage with various salts

Ingredients		SN (%)	SC (%)	CSW (%)
Main	Meat	60	60	60
	Fat	20	20	20
	Ice	20	20	5
	CSW	-	-	15
Total			100	
Additive	SN	1.2	-	-
	SC	-	1.2	-
	Sugar	1	1	1

SN, sodium nitrite; SC, sodium chloride; CSW, concentrated seawater.

Nitrite residue (NO)

The nitrite residue (NO) contents of the sausages were analyzed using the diazo method employed by the foodsaferykorea (MFDS, 2019). Ten g of each raw sample was added to 50 mL distilled water and homogenized (Ultra Turex, HMZ-20DN, Poolim Tech., Seoul, Korea). Then, 10 mL of 0.5 M NaOH and 10 mL 12% zinc sulfate solution were added to the turbidity solution, followed by shaking at 80°C. After cooling, 20 mL of ammonium acetate was added. The total volume was adjusted to 200 mL and the solution was mixed and turbidity was filtered. Next, 1 mL of sulfaminide was added to 20 mL of the filtrate solution, followed by 1 mL of naphthyl-ethylenediamin and an appropriate amount of distilled water to give a total volume of 25 mL. NO values were obtained using a spectrophotometer (SpectraMax iD3, Molecular Devices, San Jose, CA, USA) at 540 nm. A standard solution of nitrite was used to construct the calibration curve.

Color

CIE L*, a*, and b* values were measured for the inner surfaces of cooked and uncooked samples using a colorimeter (CR-10, Minolta, Tokyo, Japan). Hue angle values were calculated using the following formula: $\tan^{-1}(b^*/a^*)$. Chroma values were calculated using the following formula: $(a^{*2}+b^{*2})^{1/2}$.

Protein solubility

Protein solubility (total, myofibrillar, and sarcoplasmic proteins) was determined as described by Lee et al. (2020). To determine the total protein solubility content, 2 g of raw better was added to 20 mL of 1.1 M potassium iodide was added to a 0.1 M potassium phosphate (pH 7.4). The same procedure was used to prepare samples for sarcoplasmic protein solubility experiments, except that 0.025 M potassium phosphate (pH 7.4) was used. Samples were homogenized for 2 min and incubated overnight at 2°C, before being centrifuged at 4,032×g for 15 min at 4°C and filtered using Whatman No. 4 filter papers. Protein solubility values were obtained using a spectrophotometer (SpectraMax iD3, Molecular Devices) at 540 nm. Protein content was calculated using the following formula: optical density×upper diluent multiple×buffer dilution factor×a value (protein concentrate).

Cross-sectional area

Sausages were cut into 1cm slices and stored in a deep freezer (Thermo Fisher Scientific, Waltham, MA, USA) at −85°C.

The frozen samples were cut into 10 μm section at −25°C using a Cryostat Cryo-cut microtome (CM3050S, Leica, Nussloch, Germany). The sausage cross-sectional area was measured using a photo-activation imaging microscope (NiS-Element, Nikon, Tokyo, Japan).

Thiobarbituric acid and volatile basic nitrogen

The thiobarbituric acid (TBA) values of the pork sausages were determined according to the distillation method described by Choi et al. (2007). Volatile basic nitrogen (VBN) was measured by the Conway microdiffusion method. TBA values were expressed as mg malonaldehyde/kg sausage, and TBA values were expressed as mg%. TBA and VBN experiments were performed 10 days after manufacturing of the sausages. TBA values were calculated using the following formula: Absorbance of the sample×7.8, VBN values were calculated using the following formula: (Sample titration amount-Sample titration amount)×1×0.02×14.007×100/sample volume.

Statistical analysis

All data were analyzed based on a minimum of four independent experiments using one-way analysis of variance (ANOVA) in SAS (version 9.3). Results are expressed as mean values with standard error of the means. Significant differences ($p<0.05$) among the mean values were determined using ANOVA with Duncan's multiple range test ($p<0.05$).

Results and Discussion

Cooking yield

Cooking yield has a significant influence on consumer preference as it affects the moisture content and tenderness of food (Ruusunen et al., 1999). Fig. 1 shows the cooking yields of emulsion pork sausages with the addition of SN, SC, and CSW. The CSW treatment group showed a significantly higher yield of 95.08% compared with the other treatment groups, with the

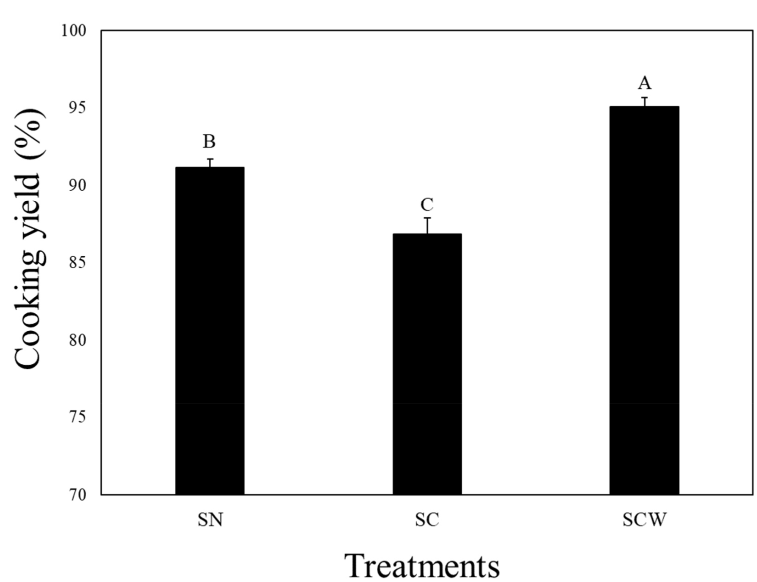

Fig. 1. Cooking yield of pork emulsion sausage formulated with various salts. A–C Mean in the same bars with different letters are significantly different ($p<0.05$). SN, sodium nitrite; SC, sodium chloride; CSW, concentrated seawater.

SC treatment group having the lowest value of 85.86%. The addition of CSW in solution is believed to maximize the extraction of salt-soluble proteins by dissociating into Na^+ and Cl^-. This facilitates the combination with myofibrillar molecules, in contrast to the SC and SN treatments, which are added in powder form (Kim et al., 2010a; Lee et al., 2020). Similarly, Lee et al. (2020) reported that the cooking yields of chicken breast sausages treated with 15% and 20% SW were higher than those of chicken breast sausages with added NPS.

Nitrite residue and color

Table 2 lists the NO content in emulsion pork sausages treated with SN, SC, and CSW. The SN treatment group had a significantly higher nitrite content (29.35 ppm) than the other treatment groups ($p<0.05$). Sebranek and Bacus (2007) reported that the nitrite content of sea salt varied depending on the region in which it was produced, and Froning et al. (1969) found that Mediterranean sea salt contained between 1.1 and 1.2 ppm of nitrite. The NO_2^- molecules of SN and CSW combine with myoglobin to form nitroso-myoglobin, resulting in a pink color. The remaining nitrite content of SW after the formation of the N-myoglobin complex was much lower, thus, CSW is considered to be appropriate for use as a coloring agent.

The colors of emulsion pork sausages treated with various amounts of SN, SC, and CSW are shown in Table 3. The lightness and yellowness values before and after heating were significantly higher in the CSW treatment group ($p<0.05$) than in the other groups, whereas the redness value showed no significant difference between the CSW and SN groups ($p>0.05$).

Table 2. Nitrite residue (NO) of pork emulsion sausage formulated with various salts

Traits	SN	SC	CSW	SEM[1)]
NO (ppm)	29.35[a]	3.77[b]	3.69[b]	12.64

All values are mean.
[1)] n=12.
[a,b] Mean in the same row with different letters are significantly different ($p<0.05$).
SN, sodium nitrite; SC, sodium chloride; CSW, concentrated seawater.

Table 3. Color of pork emulsion sausage formulated with various salts

Traits			SN	SC	CSW	SEM[1)]
Color	Uncooked	CIE L*	66.10[b]	64.45[c]	69.48[a]	2.30
		CIE a*	11.95[a]	7.33[b]	12.18[a]	2.35
		CIE b*	18.70[b]	18.68[b]	19.80[a]	0.65
		Hue angle (H°)	57.42[b]	68.58[a]	58.41[b]	0.09
		Chroma (C)	22.19[b]	20.06[c]	23.24[a]	3.03
	Cooked	CIE L*	70.35[b]	70.00[b]	71.53[a]	0.77
		CIE a*	5.95[a]	5.00[b]	5.83[a]	0.47
		CIE b*	16.63[b]	16.68[b]	17.60[a]	0.50
		Hue angle (H°)	70.31[c]	73.31[a]	71.69[b]	0.02
		Chroma (C)	17.66[b]	17.41[b]	18.54[a]	9.73

All values are mean.
[1)] n=12.
[a–c] Mean in the same row with different letters are significantly different ($p<0.05$).
Hue angle calculated as $Tan^{-1}(b/a)$: 90°=yellow, 180°=green and 0°=red Chroma calculated as $(a^2+b^2)^{1/2}$.
SN, sodium nitrite; SC, sodium chloride; CSW, concentrated seawater.

The preservation of redness in meat products is an important factor that promotes consumers' desire to buy (Sánchez-Escalante et al., 2003). According to Resurreccion (2004), natural additives are preferred over synthetic additives owing to consumers' increasing interest in health. CSW can be used to achieve the nitroso-myoglobin coloration effect without the addition of synthetic nitrite. The hue angle value before and after heating was significantly increased by the addition of SC ($p<0.05$); that is, the redness value of the SC group was lower than those of the SN and CSW groups, as the hue angle is inversely related to redness. The chroma values before and after heating were much higher in the CSW group; this can be attributed to the presence of minerals in the SW (Hataguchi et al., 2005). All these results indicate that synthetic nitrite could be replaced by CSW.

Protein solubility and cross-sectional area

Table 4 lists the protein solubility content in emulsion pork sausages treated with SN, SC, and CSW. Total protein solubility content was no significant difference was observed among the treatments ($p>0.05$). The sarcoplasmic protein solubility was significantly higher in the CSW treatment group. The samples in the CSW group showed a significantly higher myosibillar protein solubility content than those in the SC and SN ($p<0.05$). Myofibillar protein solubility is an important factor in processed meat products, as it affects emulsion, physical properties, viscosity, and customer preference (Doyle and Glass, 2010). The CSW group had the highest myofibrillar protein solubility at 530 mg/mL, which increased its emulsifying capacity and resulted in the highest cooking yield.

Fig. 2 displays cross-sections of emulsion pork sausages with the addition of SN, SC and CSW observed under electron microscopy. As shown in the figure, the fat molecules in part CSW were more uniformly distributed than those in parts SN and SC. This visually confirmed that the sausages treated with CSW were better able to form stable emulsions than those treated with SN and SC. Youssef and Barbut (2010) reported that salt-soluble proteins are extracted during the production of sausages to concentrate and build protein matxices in the O/W interface. Conversely, proteins are pulverized during the formation of emulsions, which destroys components including muscle fibers and filaments and continuously decreases the size of fat tissues (Intarasirisawat et al., 2014). These findings, together with the observation of uniform particles in the electron microscopy images, suggest that the samples in the SW treatment group had the highest emulsifying capacity.

VBN and TBA

Fig. 3 depicts the VBN and TBA values for emulsion pork sausages with the addition of SC, SN, and CSW. The VBN of the SC treatment group was 15.87±0.93 mg%, significantly higher than those of the SN treatment group (11.91±0.70 mg%) and the 15% CSW group (11.21±1.14 mg%) ($p<0.05$). This suggests that safe production is possible without exceeding the

Table 4. Protein solubility of pork emulsion sausage formulated with various salts

Traits (mg/mL)	SN	SC	CSW	SEM[1)]
Total protein solubility	743.83	689.27	785.16	35.53
Sarcoplasmic protein solubility	254.15^{b}	251.59^{b}	269.19^{a}	0.04
Myofibrillar protein solubility	494.68^{b}	427.68^{c}	530.98^{a}	14.65

All values are mean.
[1)] n=12.
$^{a-c}$ Mean in the same row with different letters are significantly different ($p<0.05$).
SN, sodium nitrite; SC, sodium chloride; CSW, concentrated seawater.

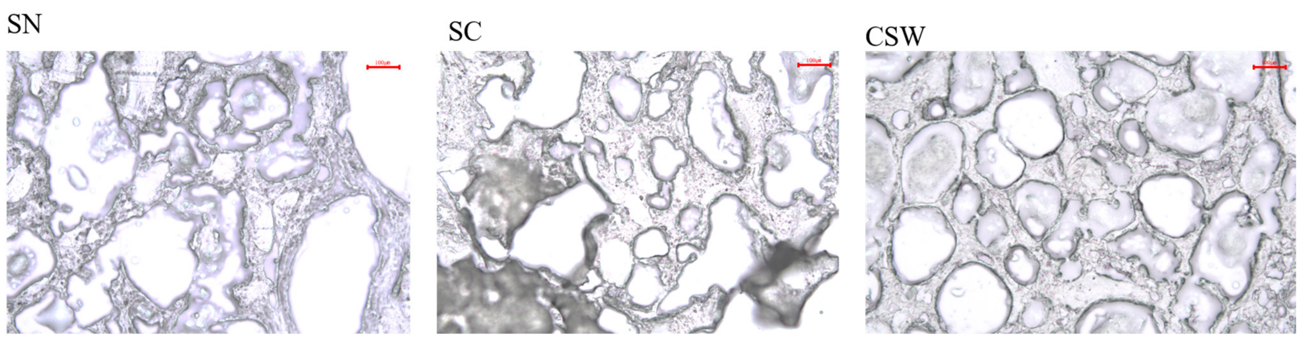

Fig. 2. Sausage cross section area of pork emulsion sausage formulated with various salts. SN, sodium nitrite; SC, sodium chloride; CSW, concentrated seawater.

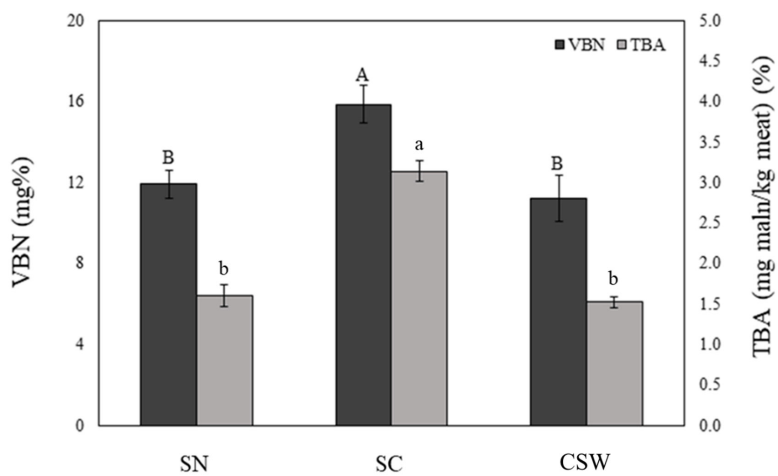

Fig. 3. VBN and TBA of pork emulsion sausage formulated with various salts. [A,B] Mean of VBN value in the same bars with different letters are significantly different ($p<0.05$). [a–c] Mean of TBA in the same row with different letters are significantly different ($p<0.05$). VBN, volatile basic nitrogen; TBA, thiobarbituric acid; SN, sodium nitrite; SC, sodium chloride; CSW, concentrated seawater.

maximum VBN content of 20 mg% stipulated in the food code (KFSRI, 2020b). Hwang et al. (2018) reported similar results, in which the group treated with SN had lower VBN values than the group treated with SC. Since the increase in VBN levels is due to chemical changes during storage and increased proteolytic microorganisms (Han et al., 2006), it is common to add NPS to sausages to prevent deterioration. Since CSW and SN showed similar values, it was considered that adding CSW produced excellent results.

De las Heras et al. (2003) identified TBA as a critical indicator for food storage. Previously, Tarladgis et al. (1960) had discussed the importance of extracting substances that react with TBA from food using a distillation method. In our work, the SC treatment group had significantly higher TBA values (3.12±0.01 mg/kg) than the other treatment groups ($p<0.05$). These results were consistent with those of Shahidi and Hong (1991), who found that SN had a greater ability than SC to suppress fat oxidation in the production of sausages. The similarity of both the VBN and TBA values between the SN and CSW treatment groups indicates that CSW is a suitable natural substitute for SN.

Conclusion

Addition of CSW resulted in a significantly higher cooking yield compared to addition of SN or SC, and similar redness to that achieved with addition of SN, despite CSW containing less nitrite than the SN. CSW treatment also resulted in significantly higher myofibillar protein content compared to the other two treatments, and similar VBN and TBA values to those obtained using SN. These results propose that CSW could be used to replace the synthetic additive SN in processed meat products.

Conflicts of Interest

The authors declare no potential conflicts of interest.

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Author Contributions

Conceptualization: Kim HY. Data curation: Lee SH, Kim HY. Formal analysis: Lee SH. Validation: Lee SH, Kim HY. Writing - original draft: Lee SH, Kim HY. Writing – review & editing: Lee SH, Kim HY.

Ethics Approval

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

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