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Combined Effects of Sodium Substitution and Addition of Cellulose or Chitosan on Quality Properties of Pork Sausages

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Abstract The aim of this study was to assess the impacts of cellulose/chitosan addition in combination with sodium substitution, including KCl and MgCl₂, on the quality and sensory properties of sausages. Sausages (control, 100% NaCl; T1, 60% NaCl, and 40% KCl; T2, 50% NaCl, 40% KCl, and 10% MgCl₂) were formulated with cellulose/chitosan at concentrations of 3% and compared to control. T1 and T2 decreased the pH values ($p < 0.05$), while the use of cellulose increased these values. Biopolymer addition reduced lipid oxidation ($p < 0.05$). In sausages containing cellulose, volatile basic nitrogen (VBN) in T1 was lower than that in T2 ($p < 0.05$). The use of cellulose increased L*, a*, and W color values in T1 ($p < 0.05$). Furthermore, cellulose addition was associated with lower hardness ($p < 0.05$). Cellulose addition contributed to better overall acceptability ($p < 0.05$). Consequently, a combined mixture containing T1 and cellulose appears to be the best combination, indicating a possible synergistic effect.

Keywords sodium substitution, cellulose, chitosan, meat quality

Introduction

Sodium chloride (NaCl) is responsible for the physicochemical properties of processed meat products because it enhances the overall flavor and water-binding capacity, and prevents microbial growth by lowering water activity (Choi et al., 2014). However, excess sodium consumption is linked to health risks such as increased risks of hypertension and coronary heart diseases, including blood pressure (Wu et al., 2014). From a health point of view, decrease in NaCl level or substitution with salt alternatives in sausages is beneficial to reduce the risk of cardiovascular disorders (Choi et al., 2014). Some studies suggested reducing the amount of salt during meat processing by replacing NaCl with partial substitutes such as potassium chloride (KCl),

magnesium chloride (MgCl_2), with similar features to NaCl (Campagnol et al., 2011; Wu et al., 2014). However, the addition of KCl negatively affects the flavor and texture of sausages by increasing bitterness and hardness (Wu et al., 2014). Based on the results of our previous study (unpublished), we selected different concentrations of KCl and MgCl_2 , to evaluate the impact of partial substitution of sodium salts with these non-sodium salts.

Normally, when the sausages presented a reduction in the sodium levels over 25%, other additives should be added to increase textural properties and to compensate for the reduction in water binding (Jo et al., 2001). Cellulose, a natural linear carbohydrate biopolymer chain constituted of d-glucopyranose units combined by β -1,4-glycosidic linkages, is the richest natural biopolymer and can be discovered in sources such as cotton and wood (Khan et al., 2012). Almeida et al. (2014) described the amorphous cellulose as a replacement in low-fat sausages, showing that the addition of cellulose lowered the fat level without detrimentally affecting sensory traits. However, the result was inferior in nutritional quality because the substitute of fat with cellulose lowered the protein level (Almeida et al., 2014).

Chitosan, mainly found in the crustacean shells of crab, shrimp, and crayfish, is the second most rich polysaccharide after cellulose and the deacetylated form of chitin (Rinaudo, 2006). This substance not only exhibited antimicrobial and antioxidant characteristics, but also had texturizer, lipid and water binding capacity as a versatile biopolymer (Yen et al., 2008). Although chitin/chitosan derivatives have powerful bioactive substances, their high molecular weights and viscosity can limit the utilizations (Seo et al., 2000). Additionally, since majority of animal intestines, especially human, have no breakdown enzymes such as chitinase and chitosanase, which directly decompose β -glucosidic linkage in chitin and chitosan and, thereafter, affects absorption in the intestine (Fukamizo and Brzezinski, 1997). The antimicrobial activities of chitosan against a range of foodborne pathogenic organisms in sausages have been previously studied (Roller et al., 2002; Soutos et al., 2008). Little harmful impacts on the textural and sensory traits of sausages by the inclusion of chitosan were detected (Jo et al., 2001). As a result, chitosan became a potential natural antimicrobial additive in meat products (Roller et al., 2002). Few researches have been carried out to elucidate the suitability of cellulose or chitosan addition in sausage production (Faria et al., 2015). Based on the results of our preliminary study (unpublished), 3% of chitosan or cellulose had higher sodium absorption inhibitory effects both *in vitro* and in a mouse model, compared to pectin and dextrin. However, to date, no studies have been carried out exploring the combined use of KCl/ MgCl_2 for sodium substitution and cellulose/chitosan as additives in sausages. The aim of the present study was to investigate the effect of cellulose or chitosan in combination with sodium substitution on the physico-chemical and sensory characteristics of cooked sausages.

Materials and Methods

Sausage preparation

Three different sausages were prepared, and the compositions for formulation were listed in Table 1. Each group was treated with 100% NaCl (control), 60% NaCl plus 40% KCl (T1); and 50% NaCl, 40% KCl plus 10% MgCl_2 (T2). This formula was determined by preliminary experimental results with different NaCl replacement levels ranging from 10% to 60%. Sausage preparation was carried out in triplicate, in 12 kg independent batches for each treatment. The raw material (fresh pork ham and back fat) was ground to a particle size of 5 mm. The formulation of the batches included 72.44% lean pork, 11.2% back fat, 13.8% ice, 0.01% sodium nitrite, 0.2% sodium triphosphate, 0.5% sugar, 0.05% monosodium glutamate, and 0.4% spices. For biopolymer groups, 3% cellulose (Esfood, Co. Ltd, Seoul, Korea) or 3% chitosan (Shinyoung Chitosan, Co. Ltd, Seoul, Korea) was added to the treated products to compare with the non-biopolymer group.

Table 1. The formulation of cooked sausage batter

Ingredient	Treatments (%)		
	Control	T1	T2
Pork lean meat	72.44	72.44	72.44
Pork backfat	11.2	11.2	11.2
Water (ice)	13.8	13.8	13.8
NaNO ₂	0.01	0.01	0.01
Phosphate	0.2	0.2	0.2
Sugar	0.5	0.5	0.5
MSG	0.05	0.05	0.05
Spices	0.4	0.4	0.4
NaCl (Sodium chloride)	1.4	0.84	0.7
KCl (Potassium chloride)	-	0.56	0.56
MgCl ₂ (Magnesium chloride)	-	-	0.14
Cellulose	-	3	-
Chitosan	-	-	3

The whole ingredients were mixed using a mixer (Fujee Co., Seoul, Korea) at low speed for 15 min. Emulsification was performed at high speed (2,840 rpm) for approximately 8 min. The temperature of the mixture was maintained below 14°C. The batters were stuffed into fibrous casings (approximate diameter of 55 mm). The product was cooked in a smokehouse (Thematec Food Industry Co., Korea) until the internal temperature of the product ranged from 75°C to 78°C. The sausages were cooled at 4°C in a refrigerator.

Physico-chemical analysis

Sausage pH was estimated on 3 g of samples homogenized in distilled water (1:9) using a pH-meter (MP330, Mettler Toledo, Switzerland). The salinity of the sample was tested by the method described by Chen et al. (2005). After homogenizing a 3 g sample with 27 mL of distilled water, the solution was filtered by Whatman No. 1 filter paper. Thiobarbituric acid reactive substances (TBARS) values of sausages were estimated by the method described by Buege and Aust (1978). Six grams of sample was diluted with 54 mL distilled water and 50 µL butylated hydroxyanisole (BHA) and then blended for 1 min on maximum speed. The blended sample (1 mL) was transferred to a test tube followed by addition of 2 mL TBA solution (0.25 N HCl, 15% TCA, and 0.375% TBA reagent). Afterwards, the tube was transferred to a water bath at 90°C for 15 min. The sample was centrifuged at 3,000×g and 5°C for 10 min and readings were taken using an X-MA 4000 spectrophotometer (Human Ltd., Korea) at 531 nm.

$$\text{TBARS value (mg of malondialdehyde/kg of sample)} = \text{Absorbance (O.D)} \times 7.8$$

Volatile basic nitrogen (VBN) was determined as described by Conway (1950). Meat color of sausages from each formulation was measured with an appliance called Chroma Meter (CR-400, Minolta Co., Japan). Based on CIE, the L* (lightness), a* (redness), and b* (yellowness) values of sausages were read. Chroma meter [(a*² + b*²)] and hue-angle [tan⁻¹

(b^*/a^*) were calculated from a^* and b^* values. To evaluate texture properties, the sliced samples, with a 20-mm diameter and 10-mm thickness, were performed using a texture analyzer (TA-XT4, Stable Micro Systems, UK). Hardness, springiness, cohesiveness, gumminess, chewiness, and adhesiveness were obtained. The cross-head speed for measurement was 5 mm/s.

Statistical analysis

Calculations based on the general linear model were used using the SAS 8.3 software program (SAS Institute Inc., USA) and the results were reported as mean values with standard error of the means (SEM). Significant differences among the mean values were determined by the Duncan's multiple range test comparison test at a level of $p < 0.05$.

Results and Discussion

Table 2 presents the physicochemical traits of sodium-substituted sausage produced with cellulose or chitosan. Regardless of biopolymer addition, the pH values were the highest in the formulation with only NaCl added ($p < 0.05$). A similar trend was described by Gimeno et al. (2001). With regard to the effect of biopolymer, control samples containing cellulose exhibited the highest pH values, while control and T1 samples without containing biopolymer had the lowest values ($p < 0.05$). T1 samples containing cellulose or chitosan showed higher values compared to the samples without containing biopolymer ($p < 0.05$). A

Table 2. Quality characteristics of sodium-substituted sausages prepared with cellulose or chitosan

	Treatments ¹⁾	Polymer			SEM
		Non-polymer	Cellulose	Chitosan	
pH	C	6.34 ^{Ab}	6.57 ^{Aa}	6.46 ^{Aab}	0.036
	T1	6.18 ^{Bb}	6.29 ^{Ba}	6.32 ^{Ba}	0.022
	T2	6.17 ^B	6.23 ^B	6.27 ^B	0.019
	SEM ²⁾	0.036	0.025	0.025	
Salinity (%)	C	2.01 ^A	1.96	1.91	0.019
	T1	1.75 ^B	1.74	1.75	0.050
	T2	1.72 ^B	1.70	1.68	0.058
	SEM	0.048	0.051	0.046	
TBARS (mg malonaldehyde/kg)	C	1.24 ^{Ba}	0.35 ^b	0.35 ^b	0.058
	T1	1.18 ^{Ba}	0.36 ^b	0.36 ^b	0.054
	T2	1.42 ^{Aa}	0.36 ^b	0.35 ^b	0.069
	SEM	0.005	0.009	0.018	
VBN (mg%)	C	8.53 ^B	8.24 ^B	8.64	0.093
	T1	8.93 ^A	8.85 ^B	8.85	0.058
	T2	9.11 ^{Ab}	10.14 ^{Aa}	9.01 ^b	0.198
	SEM	0.219	0.079	0.073	

¹⁾ Treatments: C1, sausages with 1.4% sodium chloride; T1, sausages with 0.84% sodium chloride and 0.56% potassium chloride; T2, sausages with 0.7% sodium chloride, 0.56% potassium chloride and 0.14% magnesium chloride.

²⁾ Standard error of mean (n=16).

^{a,b} Figures with different letters within a same row differ significantly ($p < 0.05$).

^{A,B} Figures with different letters within a same column differ significantly ($p < 0.05$).

VBN, volatile basic nitrogen.

similar effect on pH values of sausages from the chitosan addition has also been noted by Georgantelis et al. (2007).

As shown in Table 2, partial substitution of NaCl by KCl or MgCl₂ reduced the salinity content of samples without containing biopolymer ($p < 0.05$). Similar results were shown by Campagnol et al. (2011). The TBARS value in T1 sausages was similar to the control ($p > 0.05$). This is also in accordance with the research by Stanley et al. (2017), that found no differences in TBARS values on partially replacing NaCl with KCl. Among the biopolymer treatments, samples containing cellulose or chitosan tended to have lower TBARS values compared to non-biopolymer groups ($p < 0.05$). This is also in accordance with the research by Georgantelis et al. (2007), that indicated that samples containing chitosan showed lower TBARS values compared to the controls. Zhao et al. (2018) also reported that TBARS values of fat-decreased emulsified sausages with the addition of cellulose tended to be lower compared to non-cellulose groups. Similarly, Jo et al. (2001) found that the TBARS values of pork sausages with the chitosan addition were lower than those of the control. Chitosan chelates free irons, which inhibit the lipid oxidation of food (Shahidi et al., 1999). TBARS values for cellulose or chitosan formulations did not differ. Groups in cellulose or chitosan formulations had TBARS values lower than 0.5 mg of MDA/kg which reflected the minimum rancidity level perceived by consumers (Greene and Cumuze, 1982). However, groups without containing cellulose or chitosan showed TBARS values higher than 0.5 mg of MDA/kg, regardless of sodium treatment. Thus, the addition of 3% cellulose or chitosan could retard lipid oxidation in sausages. Increased content of VBN, which is determined by protein decomposition by bacteria, can be an indicator for the freshness of meat (Chang et al., 2011). The VBN content in T1 sausages containing biopolymer was similar to that in the control. Among the biopolymer treatments, T2 samples containing cellulose had higher VBN content than the control and T1 groups ($p < 0.05$). Low VBN contents of sausages may be owing to a quick decrease in the microbial count and a reduced capacity of bacteria for oxidative deamination of nonprotein nitrogen compounds when inserting biopolymer to the sausages (Chang et al., 2011). Thus, T1 samples containing cellulose or chitosan had a positive influence on TBARS values and VBN contents in sausages.

Table 3 presents the color characteristics of sodium- substituted sausages produced with cellulose or chitosan. In sausages containing non-biopolymer, L* values were the highest in only NaCl formulation (control), while the values were the lowest in T2 ($p < 0.05$). With regard to the effect of biopolymer, the samples containing chitosan together with partial substitution of NaCl by the combination of KCl and MgCl₂ exhibited higher L* values compared to the non-biopolymer groups ($p < 0.05$). Similar effects were observed by chitosan addition to sausages (Jo et al., 2001). In addition, Giatrakou et al. (2010) reported that chitosan addition to ready-to-eat chicken resulted in higher L* value. In our study, the color of cellulose was bright white, which might have contributed to the increase in L* when cellulose was added to the sausages (Zhao et al., 2018). T1 and T2 samples containing polymer showed a higher a* value compared to the control ($p < 0.05$). A similar result was found in the data by Choi et al. (2014), in which a* values of samples with 60% NaCl and 40% KCl were higher than those of sausages with 100% NaCl. With regard to the effect of biopolymer, T1 samples containing cellulose exhibited higher a* values compared to the non-biopolymer and chitosan groups ($p < 0.05$). The sausages containing chitosan had lower a* values than or similar to the samples without biopolymer ($p < 0.05$). This may be owing to the antioxidant potential of chitosan (Wang et al., 2015). In addition, b* values were the highest in T1 samples containing NaCl 0.84% and KCl 0.56% ($p < 0.05$). The non-biopolymer groups showed a higher b* value compared to the biopolymer groups, while the non-biopolymer groups showed a lower W value ($p < 0.05$). Especially, b* values were lower in samples with chitosan than in samples without chitosan ($p < 0.05$). T1 samples showed a higher saturation index (C*) value compared to the control and T2 samples ($p < 0.05$). C* values were the highest in samples without biopolymer, and the lowest in samples containing chitosan ($p < 0.05$). Control samples showed the highest hue (h) value, while T2 samples showed the lowest value ($p < 0.05$). Among the biopolymer

Table 3. Color characteristics of sodium- substituted sausages prepared with cellulose or chitosan

	Treatments ¹⁾	Polymer			SEM
		Non-polymer	Cellulose	Chitosan	
L* ²⁾	C	79.14 ^{Ac}	80.64 ^b	81.25 ^a	0.141
	T1	78.90 ^{ABb}	80.89 ^a	81.20 ^a	0.154
	T2	78.70 ^{Bb}	81.06 ^a	81.36 ^a	0.179
	SEM ³⁾	0.082	0.075	0.059	
a*	C	6.03 ^{Ba}	5.77 ^{Cb}	5.44 ^{Bc}	0.053
	T1	6.17 ^{Bb}	6.53 ^{Aa}	6.15 ^{Ab}	0.054
	T2	6.57 ^{Aa}	6.22 ^{Bb}	6.17 ^{Ab}	0.048
	SEM	0.063	0.064	0.051	
b*	C	10.26 ^{Ba}	8.63 ^{Ab}	8.47 ^{Bc}	0.113
	T1	10.80 ^{Aa}	8.54 ^{Ab}	8.69 ^{Ab}	0.146
	T2	10.35 ^{Ba}	8.06 ^{Bb}	8.00 ^{Cb}	0.153
	SEM	0.043	0.047	0.050	
W	C	48.38 ^{Ac}	54.83 ^{Bb}	55.93 ^{Ba}	0.465
	T1	46.50 ^{Cb}	55.27 ^{Ba}	55.15 ^{Ca}	0.578
	T2	47.69 ^{Bc}	56.89 ^{Ab}	57.39 ^{Aa}	0.620
	SEM	0.164	0.153	0.153	
c	C	11.91 ^{Ca}	10.39 ^{Bb}	10.07 ^{Bc}	0.112
	T1	12.44 ^{Aa}	10.76 ^{Ab}	10.68 ^{Ab}	0.116
	T2	12.26 ^{Ba}	10.18 ^{Cb}	10.13 ^{Bb}	0.138
	SEM	0.041	0.044	0.040	
h	C	59.58 ^{Aa}	56.24 ^{Ac}	57.38 ^{Ab}	0.287
	T1	60.29 ^{Aa}	52.64 ^{Bc}	54.77 ^{Bb}	0.515
	T2	57.63 ^{Ba}	52.39 ^{Bb}	52.38 ^{Cb}	0.421
	SEM	0.353	0.375	0.270	

¹⁾ Treatments: C1, sausages with 1.4% sodium chloride; T1, sausages with 0.84% sodium chloride and 0.56% potassium chloride; T2, sausages with 0.7% sodium chloride, 0.56% potassium chloride and 0.14% Magnesium chloride.

²⁾ L*, lightness; a*, redness; b*, yellowness; c, chroma; h, hue value.

³⁾ Standard error of mean (n=16).

^{a-c} Figures with different letters within a same row differ significantly (p<0.05).

^{A-C} Figures with different letters within a same column differ significantly (p<0.05).

treatments, h values were the highest in the non-biopolymer groups (p<0.05).

Table 4 presents the texture profiles of sodium-substituted sausage produced with cellulose or chitosan. Regardless of biopolymer addition, control samples showed lower hardness values compared to the other samples, whereas T2 with partial substitution of NaCl by the combination of KCl and MgCl₂ showed higher values (p<0.05). However, the control showed higher cohesiveness values compared to the other samples in non-polymer and cellulose samples (p<0.05). T2 samples showed the highest shear force values, while control had the lowest (p<0.05). Among the biopolymer treatments, non-biopolymer samples had higher hardness values, whereas samples containing cellulose had lower hardness values (p<0.05). T2 samples produced with cellulose or chitosan showed the highest gumminess and chewiness values (p<0.05). Among the biopolymer treatments, non-biopolymer samples had higher gumminess, chewiness, and adhesiveness values, whereas samples containing cellulose had lower values (p<0.05). Control samples containing cellulose showed the lowest shear force

Table 4. Texture profile of sodium-substituted sausages prepared with cellulose or chitosan

	Treatments ¹⁾	Polymer			SEM
		Non-polymer	Cellulose	Chitosan	
Hardness (kg)	C	0.24 ^{Ba}	0.17 ^{Bc}	0.20 ^{Bb}	0.002
	T1	0.24 ^{Ba}	0.19 ^{Ac}	0.21 ^{Bb}	0.004
	T2	0.26 ^{Aa}	0.20 ^{Ac}	0.23 ^{Ab}	0.004
	SEM ²⁾	0.003	0.002	0.003	0.004
Cohesiveness (%)	C	0.60 ^A	0.60 ^A	0.58	0.002
	T1	0.57 ^B	0.57 ^B	0.57	0.005
	T2	0.57 ^{Bb}	0.59 ^{ABa}	0.56 ^b	0.004
	SEM	0.004	0.004	0.005	0.003
Springness (mm)	C	1.02	1.00	1.01	0.001
	T1	1.00	1.00	1.00	0.004
	T2	1.00	1.00	1.00	0.001
	SEM	0.001	0.001	0.004	0.001
Gumminess (kg)	C	0.14 ^a	0.11 ^{Bc}	0.12 ^{Bb}	0.001
	T1	0.14 ^a	0.11 ^{ABb}	0.12 ^{Bb}	0.003
	T2	0.15 ^a	0.12 ^{Ac}	0.13 ^{Ab}	0.002
	SEM	0.002	0.002	0.002	0.002
Chewiness (kg,mm)	C	0.15 ^a	0.11 ^{Bc}	0.12 ^{Bb}	0.001
	T1	0.14 ^a	0.11 ^{ABb}	0.12 ^{Bb}	0.003
	T2	0.15 ^a	0.12 ^{Ac}	0.13 ^{Ab}	0.002
	SEM	0.002	0.002	0.002	0.002
Adhesiveness (kgf)	C	0.13 ^{Aa}	0.11 ^b	0.11 ^b	0.001
	T1	0.12 ^{Ba}	0.11 ^b	0.11 ^b	0.002
	T2	0.14 ^{Aa}	0.10 ^c	0.12 ^b	0.002
	SEM	0.002	0.001	0.001	0.002
Shear force (kg/cm ²)	C	1.18 ^{Ba}	0.96 ^{Bb}	1.08 ^{Ba}	0.024
	T1	1.24 ^B	1.27 ^A	1.19 ^{AB}	0.019
	T2	1.46 ^A	1.31 ^A	1.33 ^A	0.040
	SEM	0.028	0.031	0.037	

¹⁾ Treatments: C1, sausages with 1.4% sodium chloride; T1, sausages with 0.84% sodium chloride and 0.56% potassium chloride; T2, sausages with 0.7% sodium chloride, 0.56% potassium chloride and 0.14% magnesium chloride.

²⁾ Standard error of mean (n=16).

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^{A,B} Figures with different letters within a same column differ significantly (p<0.05).

values (p<0.05). Laranjo et al. (2015) found that high sodium content contributed to a better binding of the meat mixture, improving cohesiveness. The higher hardness and chewiness values found for T2 samples might be caused by the reduction in the NaCl content (Fieira et al., 2015).

Conclusions

Consequently, it was demonstrated that cellulose or chitosan addition was efficacious in maintaining the physicochemical properties of sodium-substituted sausages. Especially, experiments also demonstrated that the use of cellulose at a concentration

of 3% improved the technological quality of sodium-substituted sausages and there was no adverse impact on quality. Also, the decrease in NaCl by 40% and its substitution with KCl in sausage manufacture can reduce the sodium content without detrimentally affecting physicochemical traits. Therefore, the combined use of KCl, at the expense of sodium in meat batters and the inclusion of cellulose at a concentration of 3%, are valuable to improve meat quality and produce healthier and safer acceptable sausages. Further study is needed to investigate the combination of KCl and polymer in sausages as well as the use of different levels than those added in our study to optimize binding effects and microbiological stability.

Conflicts of Interest

The authors declare no potential conflict of interest.

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Author Contributions

Conceptualization: Jin SG. Formal analysis: Jin SG. Validation: Hur SJ. Writing - original draft: Yim DG. Writing - review & editing: Jin SG, Hur SJ, Yim DG.

Ethics Approval

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

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