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Evaluation of Sodium Lactate Combined with Chitosans of Various Molecular Weights and Lac Pigment for the Extension of Shelf-life and Color Development of Low-fat Sausages during Refrigerated Storage

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Abstract The objective of this study was to investigate the color development and shelf-life effect of low-fat sausages (LFS) during refrigerated storage according to the additions of sodium lactate (SL), chitosan, and lac pigment. The LFS samples had 73~76% moisture, 3~4% fat, and 13~16% protein with a pH range of 6.4-6.6. The addition of chitosan (MW = 30~40 kDa) to LFS increased most textural properties. Hunter a (redness) values were increased by the addition of 0.05% lac pigment. The microbial growth of *Listeria monocytogenes* increased with increasing storage time. The addition of 2% SL and 0.3% chitosan with MW higher than 30~40 kDa effectively inhibited the growth of *L. monocytogenes*. The microbial growth of *L. monocytogenes* was further reduced with increasing chitosan MW. These results indicated that the combination of SL with chitosans (MW > 30 kDa, 0.3%) and lac pigment (0.05%) improved shelf-life and color development in LFS during refrigerated storage.

Key words: low-fat sausages, sodium lactate, chitosan, lac pigment, shelf-life

Introduction

Since the positive relationship between the consumption of excess fat and coronary heart disease (CHD) was reported, consumers have tended to select low-fat, low-salt foods. Thus, due to consumer's concern for "healthier foods" with reduced calorie values, salt content, saturated fatty acids and cholesterol content, the demand for low-fat meat products (LFMP) in Korea has increased. However, because they often have higher moisture contents (%) than regular-fat counterparts, LFMP have had problems related to increased microbial growth during storage. Among the pathogens found in low-fat sausages (LFS), *Listeria monocytogenes* has been reported be a specific problem (1-4). Thus, preservatives which retard microbial growth during storage should be added to the meat products to prolong the shelf-life. Furthermore, due to the increasing consumer focus on "health-concern", consumers prefer natural ingredients rather than chemical preservatives. The effect of sodium lactate (SL) as an antilisterial agent in LFS during storage has been well documented. Murano and Rust (1) reported that an antimicrobial effect was found with the addition of SL, especially against psychrotrophic bacteria in low-fat frankfurters (5-7% fat). Bloukas *et al.* (2) observed the extension of shelf-life in low-fat frankfurters (9%) when using protective culture and 2% SL which has been reported to have better antimicrobial effects than potassium sorbate and trisodium phosphate in Chinese-style sausages (3). LFSs (< 2% fat) containing at least 3.3% SL solution (60%) and a fat replacer had a greater antilisterial effect than the low-fat control (4).

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Chitin, as well as its deacetylated form, chitosan, is a polysaccharide found in the shells of crabs and shrimp. Since their functional properties, such as antimicrobial activity, antioxidant activity, and antitumor activity, have been reported, they have received great attention as potential food additives. The antimicrobial activity of chitosan has been studied to extend shelf-life in various products (5). Youn *et al.* (6) reported that chitosan inhibited the formation of nitrosamine. One year later, Youn *et al.* (7) suggested that antibacterial activity was increased with increasing molecular weight (MW) of chitosan. Lin and Chao (8) reported that the addition of chitosan to reduced-fat, Chinese-style sausages resulted in no detrimental effect on textural properties. They also suggested that addition of chitosan with MWs ranging from 150 and 1250 kDa did not adversely affect the textural and sensory traits of reduced-fat, Chinese-style sausage. Natural lac pigment is obtained from the secretions of an Indian insect (*Coccus Lacca*), and the main component is laccaic acid (lac). Kook *et al.* (9) reported that the addition of lac pigment improved cured pink color in LFS without sodium nitrite and concluded that it could be used to replace sodium nitrite partially in water-boiled frankfurters. Thus, the objective of this study was to determine the synergistic effect of the combination of SL and chitosans of various MWs, in combination with lac pigment, on the physicochemical properties, textural properties, and microbiological changes of LFS during refrigerated storage.

Materials and Methods

Processing of low-fat sausages Fresh pork hams and pork backfats were purchased from a retail meat market in Gwangju, Korea. The formulation of non-meat ingredients is shown in Table 1. The processing procedures of regular-

Table 1. Meat and non-meat ingredients incorporated into low-fat comminuted sausages

	Lean	NMI[1)]	AW[2)]	SL(60%)[3)] (%)	CH[4)]	LP[5)]	Total
Nitrite 0 ppm	55	10	35	0	0	0	100
Nitrite 150 ppm	55	10	35	0	0	0	100
SL, 2%	55	10	33.7	3.3	0	0	102
Chitosan-Low	55	10	33.7	3.3	0.3	0.05	102.35
Chitosan-Medium	55	10	33.7	3.3	0.3	0.05	102.35
Chitosan-High	55	10	33.7	3.3	0.3	0.05	102.35

[1)]NMI; non-meat ingredient, [2)]AW; added water, [3)]SL; sodium lactate (60% solution, Pursal L, Purac Inc., The Netherlands), [4)]CH; Chitosan, [5)]LP; Lac pigment

fat control (RFC) and LFS from the previous report by Chin *et al.* (10) were followed. Trimmed pork hams were chopped for 30 sec in a silent cutter (K15, Talsa, Xirivella, EU) to reduce the particle size. Salt, sodium nitrite, sodium erythorbate, SL (60%, Purac Inc., The Netherlands), and seasonings were added to the same volume of ice water. This mixture was then added to the chopped pork, and the meat batter was further chopped until the temperature reached 15-16°C. The meat batter was then vacuum-packaged to remove the air bubbles and stuffed into cellulose casing (26 mm diameter, Yujin Industry Co., Gunpo, Korea). The sausage batters were then smoked and cooked at an internal temperature of 71.7°C in a smoke chamber (ES-13, Nu-Vu Food System, Menominee, USA), according to the methods of the previous report by Chin *et al.* (10). During storage at 4°C (±1), microbiological changes were determined at 0, 1, 2, 4, 6, and 8 wks of storage. Chitosans with various MWs (1.5kDa~200 kDa) were added to the sausage mixture in order to obtain a final chitosan concentration of 0.3% in the final mixture. Low MW (1.5 kDa, purity 80%) chitosan, medium MW (MW=30~40 kDa) chitosan by lyase, and high MW chitosan (MW=200 kDa) were purchased from Kumho Whasung Co. (Kyungbook, Yoilzin, Korea), and the degree of deacetylation was 98%. To replace the sodium nitrite, lac pigment (Meatcol-L, Meattek Korea Co., Jincheon, Chungbook, Korea) was used at the level of 0.05%. In addition, 3.3% SL (60% solution) was also used to compare to the other treatments.

Inoculation of three pathogens in the sausage *Listeria monocytogenes* strain (ATCC, 43256), *Salmonella typhimurium* LT2 strain (ATCC 19585), and *E. coli* 0157:H7 were cultured on tryptic soy agar (TSA) and incubated at 37 °C for 48 h. The colonies on the TSA were harvested in 5 mL of tryptic soy broth and incubated in a shaking water bath at 37°C for about 19 h. After the cellulose casings were peeled off, the fully grown bacterial suspension (approximately 10^9 CFU/mL) was diluted with sterilized, double-distilled (dd)-water until a final concentration of 10^3 CFU/mL was reached. One milliliter of bacterial suspension was surface-inoculated and each was mixed with 25 g of sausage samples. Then, they were vacuum-packaged (TAEVAC 600MX, Yoiwang-city, Kyungki-do, Korea) into cryovac extruded film (7325B, Sealed Air Korea Inc., Seoul, Korea) at -20 mmHg and stored at 4°C until analyzed at 0, 1, 2, 4, 6, and 8 wks of storage, according to the methods of the previous report by Choi *et al.* (4).

pH and proximate analysis After a 10-g sausage sample was blended with 90 mL of dd-water, the pH was measured by a digital pH meter (Model 340, Mettler-Toledo, Schwarzenbach, Switzerland). Moisture, fat, and protein contents (%) were determined using the following AOAC (11) procedures. Moisture contents (%) were measured using the dry oven method, fat contents (%) using the Soxhlet extraction method, and crude protein contents using a Kjeltech protein system (Buchi B-322, Kjeldahl auto system, Switzerland).

Water holding capacity (WHC) Water holding capacity (WHC) was measured by a modified procedure of the centrifugal separator method (12). Each 1.5 g sausage sample was covered with filter paper (Whatman No.3). After centrifugation at 1000 x g for 20 min, expressible moisture (EM, %) was calculated as the weight difference between the total sample and the filter paper weight containing the moisture released from the sample during centrifugal action.

Hunter color values (L, a, b) Hunter L, a, b values were measured at three different locations of the sausage surface using a Color Reader (CR-10, Minolta Co., Ltd., Japan). Data presented are the means of three measurements of each sausage sample and are expressed as Hunter L (Lightness), a (redness), and b (yellowness) values.

Texture profile analysis (TPA) The texture profile analysis (TPA) of the sausage samples which had been cut

Table 2. Smokehouse schedule for the manufacture of comminuted sausages

	Time (min)	Temp (°C)[1)]	RH (%)	Smoking
Reddening	30	54	100	off
Drying	30	60	0	off
Smoking	30	60	0	on
Heating 1	30	77	60	off
Heating 2	30	88	80	off
Steam[2)]	30	88	100	off
Cold shower[3)]				

[1)]RH=(%); Percentage relative humidity
[2)]Steam: Comminuted sausages were heated until internal temperature (IT) reached 71.7°C
[3)]Cold shower: Approximately 20 min IT<40°C

into cylinder form (1.3 cm thick, 1 cm diameter) was performed with a texture meter (TA-XT2, Stable Micro System, Haslemere, England), which was set to compress 75% of the sample weight through a two-cycle compression, as described by Bourne (13). The sausage samples were laid on a flat plate equipped with 5-kg load cell and were measured at a rate of 120 mm/min. The following textural properties were determined: fracturability (g), hardness (g), springiness (cm), chewiness, gumminess, and cohesiveness.

Statistical analysis LFSs and regular-fat sausages were produced with the addition of 3.3% SL alone or in combination with 0.3% chitosans of various MWs, compared to LFSs with 150 ppm sodium nitrite. Physicochemical and textural properties were analyzed by one-way analysis of variance (ANOVA) to evaluate the treatment effects, whereas microbial data were analyzed by two-way ANOVA using the general linear models procedure of SAS (14). A 3 (MWs of chitosan) * 6 (storage time) factorial arrangement with three replicates was performed. The interaction between MWs of chitosans and storage time was also tested. If interactions between main effects were significant (P<0.05), data were separated by SL combination and storage time. Duncan's multiple range tests were performed for each treatment, if interactions were not observed between the two factors.

Results and Discussion

Proximate composition, pH, and expressible moisture (%) Moisture, fat, and protein content ranges (%) of LFSs containing various MWs of chitosans were 73.1-75.4, 2.9-3.4, and 13.4-16.4, respectively. These results were similar to those of our previous report (9), but due to the different cooking procedure, the moisture (%) was lower and fat (%) was higher than those with water-boiled sausage products (P<0.05). Thus, the proximate composition was not affected by the addition of SL, sodium nitrite, and various MWs of chitosans (Table 3). The addition of chitosans into LFS did not significantly affect (P>0.05) the pH values or EM %, regardless of chitosan MW. Jo *et al.* (15) reported that the pH value of pork sausages with 0.2 % chitosan oligomer (5kDa) was higher than that of the control in vacuum packaging. However, Lin and Chao (8) reported that the sausage samples containing chitosans (low=150 kDa, med=600 kDa, high=1250 kDa) had lower pH values than those without chitosans (p<0.05), due to their being dissolved in 1% lactic acid solution (pH 2.84). Since pH changes were not found with the addition of various chitosan MWs in our study, water-soluble chitosans appear to be more advantageous for the manufacturing of meat products. LFS containing low MW chitosan had lower EM values than those with medium MW chitosan in this study (P<0.05). Although the reason for the EM differences between low- and medium chitosan MW was not fully understood, it might be partially due to the differences of pH values among treatments, even though they were not different statistically. Our results were not in agreement with those of Lin and Chao (8), who reported that the water retaining index (WRI) of reduced-fat, Chinese-style sausages (22% fat) was not affected by the presence of a variety of chitosan levels. Since they used extremely high chitosan MWs (>150 kDa) in the formulation of reduced-fat, Chinese-style sausages, they might not be comparable to our results. However, Kim and Choi (16) reported that chitosans having MWs of 15-30 kDa reduced WHC in comminuted sausages, and that the addition of sodium nitrite might reduce the differences.

Hunter color values Since it has been reported that sodium nitrite, a curing agent, could produce nitrosamine, a carcinogen, during high temperature frying, considerable research has been performed to reduce the level of sodium nitrite through procedural modifications (2, 6, 17). Although no differences were observed in the Hunter L values (P>0.05), the addition of lac pigment (0.05%) into the sausage mixtures improved the Hunter a and b values (Fig. 1). However, LFS containing 0.05% lac pigment still appeared to be less red than that with 150 ppm of sodium nitrite (P>0.05). These results indicated that although lac pigment was not completely replaced with 150 ppm of sodium nitrite, it was nevertheless able to improve the redness values compared to those of control (no addition of sodium nitrite). In our previous report, lac pigment

Table 3. Proximate composition, pH and expressible moisture (EM, %) of low-fat functional sausages manufactured with sodium lactate and chitosans of various molecular weights

Treatments	Proximate analysis (%)			pH	EM[1)]
	Moisture	Fat	Protein		
Nitrite(0 ppm)	75.3	3.4	15.0	6.43	29.1^{ab}
Nitrite(150 ppm)	75.4	3.2	16.4	6.39	29.1^{ab}
Sodium lactate (SL, 2%)	73.2	3.4	14.4	6.43	26.0^{ab}
SL+Chitosan (L)[2)]+LP[3)]	73.4	3.1	13.6	6.59	23.6^{b}
SL+Chitosan (M)+LP	73.1	3.3	14.2	6.47	31.6^{a}
SL+Chitosan (H)+LP	74.1	2.9	13.4	6.44	30.7^{ab}

[a,b]Means with same row having same superscript are not different (P>0.05).
[1)]EM= Expressible moisture (%)
[2)]Chitosan(L)=low(MW=1.5 kDa); (M)=medium(MW=30~40 kDa); (H)=high(MW=200 kDa)
[3)]LP=lac pigment

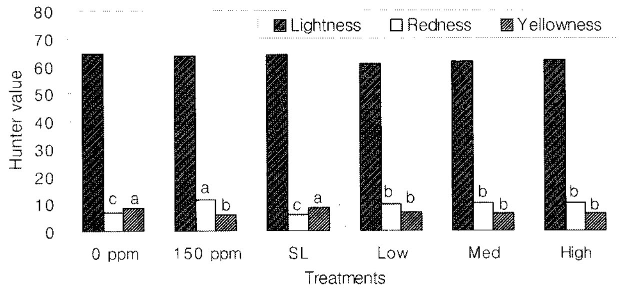

Fig. 1. Hunter color values of low-fat functional sausages made with sodium lactate and chitosans of various molecular weights. [a-c]Means with same row having same superscript are not different(P>0.05). Treatments : Sodium nitrite 0, 150 ppm; SL= 2 % sodium lactate; Low=low MW (1.5 kDa) chitosan + 2% SL+ 0.05% lac pigment (LP); Med=medium MW (30~40 kDa) chitosan + 2% SL+0.05% LP; High=high MW (200 kDa) chitosan + 2% SL+0.05% LP.

could be completely replaced with 75 ppm of sodium nitrite in water-boiled LFS (9). Although LFS containing chitosans seemed to be redder before cooking, redness values completely disappeared after cooking. Therefore, chitosan alone could not have improved the redness of LFS at all. These results were not in agreement with those of the previous study by Youn *et al.* (7), who suggested that chitosan alone improved the redness and could be replaced with 15 ppm of sodium nitrite. In addition, they suggested that the residual level of sodium nitrite in emulsified sausage reduced with increased chitosan level and increased chitosan MW. Kim and Choi (16) reported that meat batter containing chitosans was redder than that without chitosans. However, the redness values were reduced after cooking. These results were also supported by Lin and Choa (8), who reported that Chinese-style sausages containing chitosans had lower redness values than those of controls. Thus, natural lac pigment (0.05%) was used to aid the development of the cured LFS color in the replacement of sodium nitrite. Bloukas *et al.* (2) tested various natural colorants, such as curcumin, carminic acid beta carotene, and paprika extract, and reported that the combination of nitrite with the natural colorants improved the redness of frankfurters and increased the consumer satisfaction with the color. Byun *et al.* (17) reported that a dose of 5 kGy was as effective as the use of 200 ppm of sodium nitrite for maintaining a desirable color of the product for 30 days.

Texture profile analysis (TPA) Table 4 shows the properties of LFS as affected by the combination of SL and various chitosan MWs. The properties of LFSs containing low-MW (1.5 kDa) chitosan were not different from those without chitosans (P>0.05). Nevertheless, LFS containing medium MW (30~40 kDa) chitosans were harder, springier, and chewier than those with low-fat control (150 ppm, sodium nitrite) (Table 4, P<0.05). These results were supported by the previous study, in which water-boiled LFS containing medium MW chitosans were harder, springier, and chewier (9). Lin and Chao (8) reported that sausage springiness was lower in sausages with high MW (1,250 kDa) chitosans than in those with medium MW (600 kDa) chitosans. No textural differences were observed among the various tested sausages, regardless of the chitosan MWs. The discrepancy between the results of the two studies was partially due to the differences in the chitosan MWs. In other words, the high chitosan MW in our study was 200 kDa, compared to 1,250 kDa in their study. Thus, the high MW chitosans in our study were equivalent to the medium MW chitosans in their study. In another study, Chen and Tsaih (18) reported that the viscosity was reduced with increasing chitosan MW (19).

Table 4. Textural characteristics of low-fat functional sausages manufactured with sodium lactate and chitosans of various molecular weights

Parameters[1)]	Nitrite (ppm)		SL 2%	SL 2% + LP +Chitosan[2)]		
	0	150		Low	Med	High
FR (g)	4537^{ab}	4255^{b}	5769^{ab}	6515^{ab}	7313^{a}	5575^{ab}
HR (g)	6142^{ab}	6015^{b}	7659^{ab}	7544^{ab}	8229^{a}	6870^{ab}
SP (cm)	0.28^{b}	0.29^{b}	0.31^{b}	0.35^{ab}	0.39^{a}	0.32^{b}
GU	1178^{bc}	1030^{c}	1171^{bc}	1571^{ab}	1943^{a}	1227^{bc}
CH	2283^{bc}	264^{c}	355^{bc}	618^{ab}	756^{a}	388^{bc}
CO (ratio)	0.20	0.19	0.18	0.21	0.24	0.20

[a-c]Means with same row having same superscript are not different (P>0.05).
[1)]Parameters: FR=fracturability; HR=Hardness; SP=springiness; GU=gumminess; CH=chewiness; CO=cohesiveness.
[2)]SL = sodium lactate, LP=lac pigment (0.05%)

Microbial changes of low-fat sausages as affected by treatment and storage time LFSs were cooked by smoking and then cooled to temperature below 5°C in the refrigerator. *Listeria monocytogenes, E. coli* O157:H7, and *Salmonella typhimurium* were inoculated at a level of 10^3 cfu/g. After inoculation, the microbial changes of the sausages were measured during storage at 0, 1, 2, 4, 6, and 8 weeks. Microbial counts of *E. coli* O157:H7 and *Salmonella typhimurium* tended to plateau or decrease with increased storage time, regardless of treatment (data not shown). Since these microorganisms are mesophiles, they do not typically exhibit growth at such a low-temperature (4°C) storage (9). These results were supported by Mustapha *et al.* (20) who reported that the viable number of *E. coli* 0157:H7 in vacuum packaged raw beef slowly declined during refrigerated storage, regardless of treatment with or without polylactic acid, lactic acid and nisin. However, the microbial growth of *L. monocytogenes* increased with increased storage time (Fig. 2). This increased microbial growth was inhibited from 2 weeks of storage time by the addition of 2% SL to LFS. The addition of low MW chitosan to the 2% SL LFS increased microbial growth, whereas medium or high MW chitosan inhibited microbial growth when combined with SL. The inhibition rate was also increased with increased chitosan MW. These results indicated that microbial growth was synergistically inhibited by the combination of the chitosan

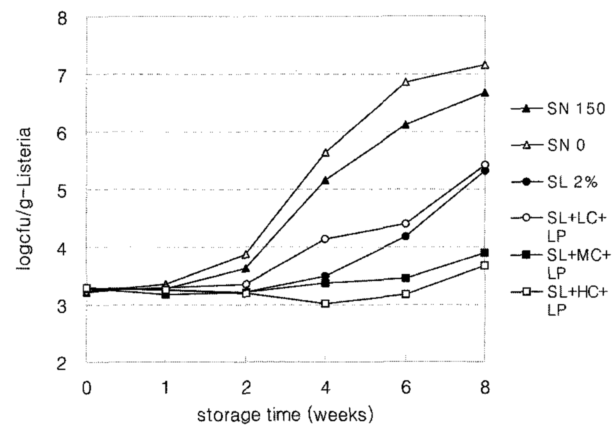

Fig 2. Changes of bacterial counts (log cfu/g) for *Listeria monocytogenes* of low-fat functional sausages made with sodium lactate and chitosans of various molecular weights and cooked in a smoke chamber during storage at 4°C. Ed- on the right axis of the figure above, I suggest that you put the control, SN 0, above SN 150. Treatments : Sodium nitrite(SN) 0, 150 ppm; SL=sodium lactate; LC=low MW (1.5 kDa) chitosan + 2% SL + 0.05% lac pigment (LP); MC=medium MW (30~40 kDa) chitosan + 2% SL + 0.05% LP; HC=high MW (200 kDa) chitosan + 2% SL + 0.05% LP.

MW and SL, compared to the addition of SL alone.

Jo *et al.* (15) reported that the addition of chitooligomer (MW=5000) to the emulsion-type sausage did not reduce the number of microorganisms. Lin and Lin (3) reported that, due to the lower pH values for chitosan-containing treatments, the reduced-fat, Chinese style sausages containing chitosan with MW of 150 kDa had the lowest total plate counts. However, Youn *et al.* (7) reported that 0.2% chitosan (MW=120 kDa), which was dissolved in 1% lactic acid solution, reduced microbial growth and could provide protection for the sausages. Our study was different from these previous studies because we incorporated the additives into the sausage mixture directly, rather than dissolving in 1% lactic acid solution. Youn *et al.* (7) reported that chitosan with a MW of 30 kDa had an antibacterial effect of more than 70% if the level of chitosan was more than 0.35%. Chitosan with a MW of 120 kDa, however, had an antibacterial effect of more than 70% if the level was more than 0.2%. Roller *et al.* (21) reported that 0.6% chitosan combined with low sulphite level (170 ppm) retarded the growth of spoilage microorganisms more effectively than that with high sulphite level (340 ppm) at 4°C for up to 24 days. In our study, 1-2 log cycle reduction were observed when the medium and high chitosan MWs were combined with 2% SL, thereby delaying the lag phase for approximately 2-4 wks. Helander *et al.* (22) reported that the antibacterial effect of chitosans against gram negative bacteria was partially due to the attachment of chitosans in the cell wall and the consequent loss of the possibility of nutrient transportation on the outside of the cell wall. The microbial counts of *L. monocytogenes* in water-boiled products tended to be rapidly increased compared to the smoked cooking counterparts performed in this study (9). Our previous study reported that the water boiled, low-fat control took approximately 4 wks to reach a microbial count of 10^8 cfu/g, whereas the SL and chitosan-treated sausage groups, either SL alone or in combination with various chitosan MWs, took 6-wks of refrigerated storage. Compared to the current study, smoking could therefore be recommended to increase the shelf-life of LFSs, compared to the water-boiled counterparts.

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