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Research Article

Effect of Sodium Lactate on the Microbiological Quality and Shelf Life of Sausages

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Abstract: This study was performed to investigate the effects of various levels of sodium lactate on the microbiological quality and shelf life of sausages, and determine whether it can be used as a nitrite alternative. For this purpose, 0%, 0.6%, 1.2%, and 1.8% NaL and 0.125% $NaNO_2$ were added to sausage samples. Samples were vacuum-packaged and stored at 4 °C for 60 days and periodically analyzed for their sensorial properties, microbial counts (aerobic plate count, lactic acid bacteria, yeast-mould, and sulphite reducing anaerobic bacteria), and pH values. Sensorial characteristics were not altered with NaL addition; the flavor of the samples was partially improved. In contrast to red-pinkish color in $NaNO_2$ containing samples, natural meat color was preserved in the lactate containing ones. NaL delayed microbial growth depending on the concentration used. There was a significant increase in the shelf life of the samples containing NaL compared to the control group. NaL caused no change in pH values during storage that would affect the microbial quality of the samples. This study indicated that the addition of NaL to sausages improved their microbiological quality depending on the concentration used, extended their shelf life, and exhibited a better antimicrobial effect than $NaNO_2$.

Key Words: Sodium lactate, sausages, microbiological quality, shelf life

Sodyum Laktatın Sosislerin Mikrobiyolojik Kalitesi ve Raf Ömrü Üzerine Etkisi

Özet: Bu çalışma değişik konsantrasyonlarda sodyum laktatın, sosislerin mikrobiyolojik kalitesi ve raf ömrü üzerine etkisinin araştırılması ve aynı zamanda nitrit ile karşılaştırılarak bir nitrit alternatifi olup olamayacağının belirlenmesi için gerçekleştirilmiştir. Bu amaçla hazırlanan sosis örneklerine % 0, % 0,6, % 1,2 ve % 1,8 NaL ile % 0,125 $NaNO_2$ ilave edilmiştir. Sosis örnekleri hazırlandıktan sonra vakum paketlenmiş ve 4 °C'de 60 gün süreyle muhafaza edilmiştir. Muhafaza sırasında örnekler periyodik olarak duyusal özellikleri, mikroorganizma sayıları (toplam aerob bakteri sayısı, laktik asit bakterileri, küf-maya ve sülfit redükte eden anaerobik bakteriler) ve pH değerleri yönünden analiz edilmiştir. NaL ilavesi ürünün duyusal özelliklerini değiştirmemiş, hatta tatda kısmen iyileşme sağlanmış; bununla birlikte nitritli ürünlerdeki kırmızı-pembe rengin yerine doğal et rengi muhafaza edilmiştir. NaL ilavesi kullanılan konsantrasyona bağlı olarak mikrobiyel üremeyi geciktirmiştir. NaL içeren örneklerin raf ömründe, kontrol grubuyla karşılaştırıldığında önemli bir artış kaydedilmiştir. NaL, muhafaza süresi boyunca deneysel sosis örneklerinin mikrobiyel kalitesi üzerine etki edecek bir pH değişikliğine sebep olmamıştır. Bu çalışma, sosislere NaL ilavesinin konsantrasyona bağlı olarak mikrobiyolojik kaliteyi iyileştirdiğini, raf ömrünü uzattığını, incelenen mikroorganizmalar bakımından antimikrobiyel ajan olarak $NaNO_2$'den daha iyi performans sergilediğini göstermiştir.

Anahtar Sözcükler: Sodyum laktat, sosis, mikrobiyolojik kalite, raf ömrü

Introduction

In recent years, there has been an increasing demand for cooked or ready-to-cook products. Products like sausages and salamis are among the mostly preferred ones due to their convenient consumption. However, sausages have limited shelf life even though they are subjected to high temperature during the production process. Many microorganisms causing undesired changes in the product can continue to survive after heat treatment and, by propagating during storage, pose a health risk for consumers (1-3).

The use of antimicrobial ingredients is one of the widely used methods to maintain microbiological safety and prolong the shelf life of food products. The nitrates

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and nitrites are preferred due to their efficiency on *Clostridium botulinum*, one of the most important food pathogens, and also due to their positive effect on the color of the product (4,5). However, alternative ingredients are needed as nitrites and nitrates have been reported to lead to the formation of nitrosamines, which are carcinogenic in nature.

In recent years, organic acids and their salts have been the preferred antimicrobial ingredients because they are natural (6). Lactic acids and their salts (lactates) are recommended as food additives because they are naturally present in meat and in many other fermented foods and are effective on many spoilage pathogen microorganisms; they do not pose any health risk for consumers, and they do not affect the sensory characteristics of the products (7,8).

Lactates, salts of L(+) lactic acid, show a preservative effect by decreasing the water activity (a_w) of the products to which they are added, and by exhibiting a specific inhibitory effect (7,9,10). Antimicrobial effects of lactates have been investigated in meat and meat products, and their efficiency against many pathogens and spoilage microorganisms was established (10-13). In addition to their antimicrobial effects, lactates are also shown to improve sensory characteristics of the products such as color, texture, and flavor, and they are shown to exert an antioxidant effect (14-16).

This study was performed to investigate the effects of sodium lactate on the microbiological quality and shelf life of sausages.

Materials and Methods

Experimental sausage production: The main mixture, composed of chopped beef (85%) and fat (15%), was processed in a cutter. Ice (25%), sugar (0.25%), starch (4%), sodium caseinate (1.0%), sodium polyphosphate (0.4%), soybean protein (0.5%), salt (1.2%), and spice mix (3.0%) were added to the mixture. The sausage dough was subdivided into 5 equal groups, 10 kg each. The first group was kept as the control group. To the second group, 0.125% sodium nitrite ($NaNO_2$, BASF, Germany) was added. To the other groups, 0.6%, 1.2%, and 1.8% sodium lactate (60% NaL, Purac, Gorinchem, The Netherlands) were added, respectively. Each sausage dough was thoroughly mixed by regrinding in the cutter. Ascorbic acid (1.0%) was then added to each group. The dough was filled into an artificial casing and fastened at both ends (21 mm × 15 cm). The raw sausages were then subjected to drying for 10 min at 50 ºC, smoking for 10 min at 65 ºC, and cooking for 15 min at 80 ºC. The cooked sausages were then showered with cold water. The cooled sausages were vacuum-packaged (Multivac C100, Sepp Haggenmüller GmbH & Co., Wolfertschwenden, Germany) in portions of 5 and stored at 4 ºC for 60 days.

Laboratory analysis: The experimental sausage samples were analyzed for their microbiological (aerobic plate count (APC), sulphite-reducing anaerobic bacterial counts, lactic acid bacteria (LAB), mould, and yeast), physico-chemical (pH), and sensory properties after 1, 5, 10, 20, 30, 45, and 60 days of refrigerated storage.

Microbiological analysis: For microbiological analysis, 25 g of sausage sample from each package was transferred to a sterile stomacher bag with 225 ml of 0.1% peptone water (Oxoid CM 9, UK) and blended for 2 min in a stomacher (Labblender 400, Model BA 6021, Steward Lab., London, UK). Serial decimal dilutions were prepared using the same diluent. Duplicate 0.1 ml or 1 ml inoculum of appropriate dilutions were spread on pre-poured plates or pour plated, respectively, on the following media: aerobic plate counts on plate count agar (PCA – (Oxoid, CM 325, Hampshire, UK), spread plates incubated at 35 °C for 48 h), lactic acid bacteria (LAB) in de Man, Rogosa, Sharpe agar (MRS – (Oxoid, CM 361, Hampshire, UK), pour plates, with overlay, incubated at 35 °C for 48 h), sulphite-reducing anaerobic bacteria in Perfringens agar base (SPS – (Oxoid CM 543, Hampshire, UK) roll tube method used for incubation at 37 °C for 24 h), and mould and yeast on Yeast Extract Glucose Chloramphenicol Agar (YGC – (Merck 1.16000, UK), spread plates incubated at 25 °C for 3-5 days) (17,18).

Determination of pH: Sausage samples (10 g) from each package (n = 5/treatment) at each sampling day were separately blended with 100 ml of distilled-deionized water (ddH_2O) in a stomacher (Labblender 400, Model BA 6021, Steward Lab., London, UK). The pH of the homogenates was measured using a pH electrode (Hanna HI 1131) attached to a pH meter (HI 9321 Microprocessor pH meter, Hanna Instruments, Germany).

Sensorial test: Sausage samples belonging to different groups were visually examined for signs of spoilage after 1, 5, 10, 20, 30, 45 and 60 days of storage. The development of unpleasant odour, pale color, softening, and the formation of a sticky layer on the surface were evaluated as the indicators of spoilage.

Statistical analysis: The experimental sausage production was performed in triplicate and data were analysed by analysis of variance (ANOVA) in 5 (treatments) × 7 (storage times) factorial design with a control using the general linear model (GLM) procedure (19). Moreover, ANOVA for a 5 × 7 factorial design was performed to determine whether the interactions between treatment and storage times were significant ($P < 0.05$). If the significant interactions were observed ($P < 0.05$), means were separated using pooled means via Duncan's test.

Results

The sensorial characteristics of the product did not change with the addition of NaL, but a partial improvement in flavor was achieved. Furthermore, instead of the red-pinkish color present in sodium nitrite containing samples, the natural color of the dough was preserved after the cooking process in NaL containing samples.

Addition of NaL delayed the microbial growth depending on the concentration used. Shelf life of the samples containing NaL was extended significantly compared to the control group. The control group was spoiled on day 30, whereas signs of spoilage were observed in 0.6% and 1.2% NaL containing samples on days 45 and 60, respectively. Moreover, in samples containing 1.8% NaL, no spoilage was detected even after 60 days of storage.

The initial pH in the control group was 5.95; it was 5.93 in the group containing $NaNO_2$, and 5.90, 5.87, and 5.85 in the groups containing 0.6%, 1.2%, and 1.8% NaL, respectively. The pH of sausages, with or without NaL, remained unchanged (Table 1).

In the microbiological analysis of the sausage samples, it was observed that during the storage period there was no statistically significant difference between the groups for total aerobic plate count. However, it was determined that the addition of lactate decreased the initial microbial count partially and slowed down the development during the storage period parallel to the increasing concentration (Table 2). It was also observed that an increase in the NaL concentration led to a

Table 1. pH values of the sausages during storage.

GROUP	Storage period (4 °C)						
	Day 1	Day 5	Day 10	Day 20	Day 30	Day 45	Day 60
Control	5.95^{a*}	5.88^{a}	5.86^{a}	5.79^{a}	5.83^{a}	5.72^{a}	5.70^{a}
$NaNO_2$	5.93^{a}	5.91^{a}	5.86^{a}	5.77^{a}	5.68^{a}	5.57^{a}	5.53^{a}
0.6% NaL	5.90^{a}	5.88^{a}	5.84^{a}	5.80^{a}	5.78^{a}	5.66^{a}	5.69^{a}
1.2% NaL	5.87^{a}	5.88^{a}	5.83^{a}	5.79^{a}	5.66^{a}	5.64^{a}	5.65^{a}
1.8% NaL	5.86^{a}	5.87^{a}	5.82^{a}	5.77^{a}	5.65^{a}	5.63^{a}	5.64^{a}

*means in the same column given with different letters are significantly different from the other $P < 0.05$

Table 2. Aerobic plate counts of the sausages during storage (log_{10} cfu/g).

GROUP	Storage period (4 °C)						
	Day 1	Day 5	Day 10	Day 20	Day 30	Day 45	Day 60
Control	3.23^{a*}	4.01^{a}	5.43^{a}	6.35^{a}	6.51^{a}	5.58^{a}	5.13^{a}
$NaNO_2$	3.47^{a}	4.41^{a}	4.98^{a}	5.58^{a}	6.67^{a}	5.64^{a}	5.47^{a}
0.6% NaL	3.40^{a}	4.03^{a}	5.06^{a}	5.73^{a}	6.56^{a}	5.61^{a}	5.47^{a}
1.2% NaL	3.31^{a}	3.59^{a}	4.72^{a}	5.87^{a}	6.54^{a}	5.70^{a}	5.43^{a}
1.8% NaL	2.84^{a}	3.40^{a}	4.20^{a}	5.50^{a}	6.02^{a}	6.35^{a}	6.27^{a}

*means in the same column given with different letters are significantly different from the other $P < 0.05$

decrease in the number of sulphite reducing anaerobic bacteria. The results of this study show that as NaL concentration increases the reduction rate in the anaerobic bacteria also increases. Even though the initial levels were low, there was a reduction in the number of anaerobic bacteria including the control group. This reduction was even faster in samples containing 1.2% and 1.8% NaL; no growth of anaerobic bacteria was observed in the analysis after 5 days of storage (Table 3). The number of lactic acid bacteria was 10^3 cfu/g at the beginning in all groups. However, there was a continuous increase during the storage period. The increase in the lactic acid bacteria number was comparatively slow in NaL containing samples. All groups showed a rapid increase between days 10 and 20; following day 20, the increase in the number of microorganisms stayed at the level of 10^6 cfu/g until the last day of storage (Table 4). The effect of NaL on mould and yeast was observed only after day 30 (Table 5).

Discussion

One of the essential properties of a food ingredient is not to cause any undesired sensorial changes in a food product. In this study, the addition of NaL did not cause any changes in the sensory characteristics of the product. Papadopoulos et al. (16) observed that the addition of NaL enhanced the typical meat flavor and minimized the flavor deterioration during storage. Palatability was improved by adding 1% NaL, but there was no improvement beyond 1% to 2%. Bloukas et al. (20) reported that 2% NaL used in frankfurters exhibited no negative effect on texture, sensory characteristics, and color. Brewer et al. (14) observed that 1% NaL maintained the red color of fresh pork sausage and enhanced the salty flavor of the meat. However, in the study the main observed difference between the NaL containing sausages and the sodium nitrite containing ones was in the color. Of the samples, the sodium nitrite containing sausages were red-pink, whereas the NaL

Table 3. Sulphite reducing anaerobic bacteria counts of the sausages during storage (log_{10} cfu/g).

GROUP	Storage period (4 °C)						
	Day 1	Day 5	Day 10	Day 20	Day 30	Day 45	Day 60
Control	1.79^{a*}	1.21^{a}	0.24^{a}	-	-	-	-
$NaNO_2$	1.70^{a}	0.92^{a}	-	-	-	-	-
0.6% NaL	1.20^{b}	0.33^{b}	-	-	-	-	-
1.2% NaL	1.08^{b}	-	-	-	-	-	-
1.8% NaL	0.63^{c}	-	-	-	-	-	-

*means in the same column given with different letters are significantly different from the other $P < 0.05$

Table 4. Lactic acid bacteria counts of the sausages during storage (log_{10} cfu/g).

GROUP	Storage period (4 °C)						
	Day 1	Day 5	Day 10	Day 20	Day 30	Day 45	Day 60
Control	3.30^{a*}	3.44^{a}	4.91^{a}	5.80^{a}	6.17^{a}	6.73^{a}	6.88^{a}
$NaNO_2$	3.16^{a}	3.50^{a}	4.81^{a}	5.28^{b}	5.97^{ab}	6.48^{ab}	6.56^{ab}
0.6% NaL	3.33^{a}	3.44^{a}	4.74^{a}	5.09^{bc}	5.70^{bc}	5.90^{bc}	5.97^{bc}
1.2% NaL	3.18^{a}	3.58^{a}	4.50^{a}	4.90^{bc}	5.30^{cd}	5.64^{c}	5.73^{c}
1.8% NaL	3.26^{a}	3.33^{a}	4.48^{a}	4.75^{c}	4.93^{d}	5.37^{c}	5.59^{c}

*means in the same column given with different letters are significantly different from the other $P < 0.05$

Table 5. Mould and Yeast counts of the sausages during storage (log_{10} cfu/g).

GROUP	Storage period (4 °C)						
	Day 1	Day 5	Day 10	Day 20	Day 30	Day 45	Day 60
Control	2.14[a*]	2.47[a]	2.73[a]	3.50[a]	3.77[a]	5.01[a]	4.50[a]
$NaNO_2$	2.32[a]	2.57[a]	2.86[a]	2.69[a]	2.61[b]	2.78[b]	2.87[c]
0.6% NaL	2.46[a]	2.64[a]	2.88[a]	3.26[a]	3.39[ab]	3.60[ab]	3.83[ab]
1.2% NaL	2.33[a]	2.43[a]	2.70[a]	2.84[a]	3.03[ab]	3.30[b]	3.43[bc]
1.8% NaL	2.51[a]	2.74[a]	2.91[a]	3.26[a]	3.37[ab]	3.49[ab]	3.27[bc]

*means in the same column given with different letters are significantly different from the other $P < 0.05$

containing ones were the color of natural cooked meat. This might be considered a negative aspect in terms of consumer preferences.

The most important effect of NaL on meat products is its ability to extend the shelf life. In this study, the addition of NaL produced a significant improvement in the shelf life of sausages. Samples containing 1.8% NaL exhibited no signs of spoilage after 60 days of refrigerated storage whereas the control group, in which no preservative was used, showed signs of spoilage after 30 days. In samples containing 0.6% and 1.2% NaL, there were changes in the texture and color after 45 and 60 days, respectively. Similar results were reported by previous researchers. The shelf life of meat and poultry products could be prolonged about 30% to 150% by NaL (9). Debevere (11) reported that NaL used in 1% and 2% concentrations in liver paste caused significant extensions in the shelf life. Lamkey et al. (21) determined that NaL used in fresh pork sausage was effective in maintaining low microbial count and extending the shelf life of chub packs by more than 2 weeks over control packs. De Wit and Rombouts (7) reported that NaL extended shelf life 1-2 weeks in meat products such as hams, sausages, and pâtés. Cegielska-Radziejewska and Pikul (22) reported that the addition of 1% or 2% NaL extended the shelf life of sliced poultry sausages (packaged in air atmosphere and stored at 5 to 7 ºC) by 3 or 4 times, respectively. Brewer et al. (23) reported that the addition of 2% and 3% NaL delayed the microbial deterioration of fresh pork sausages 7 days at 4 ºC storage. Buric and Koos (24) reported that when NaL was used in combination with NaCl there was an increase in shelf life, but NaL decreased the sharp salty flavor. The same authors found that 2.2% NaL increased the shelf life 2-fold in cooked salami.

The extension of shelf life by NaL is a result of the improved microbial quality of the products it is added (3,9). In this study, as the NaL content increased, a more gradual growth was observed in the microbial count of the sausages during the storage period. Papadopoulos et al. (13) reported that in cooked, vacuum-packaged beef, increasing levels of NaL resulted in lower total bacteria counts and higher lactic acid concentrations of roasts. Lin and Lin (25) determined that adding 3% NaL decreased the total bacteria count in low-fat Chinese-style sausages. Bloukas et al. (20) reported that the total plate count of low-fat frankfurters produced with 2% NaL remained lower than the control group after 4 weeks of refrigerated storage. Meng and Genigeorgis (26) emphasized the antibotulinal effect of NaL on *Clostridium botulinum*, an important anaerobic sporadic microorganism. They reported that no toxin was formed up to 120 days in 1.2%, 2%, and 3% NaL added cooked turkey and chicken breast kept at 4 ºC. Even though there is no particular finding regarding *C. botulinum* in the present study, it was observed that NaL decreases the number of anaerobic sporadic bacteria in sausages during the storage period and this particular effect of NaL is stronger than that of nitrite. Maas et al. (27) determined that the antibotulinal effect of NaL was dependent on its concentration; the development of toxin was delayed in 2.0%, 2.5%, 3.0%, or 3.5% NaL containing vacuum-packaged turkey breast samples for 4-5, 4-6, 7, or 7-8 days, respectively. De Wit and Rombouts (7) reported that NaL was specifically used to extend the cold storage of a variety of ready-to-eat meat products such as hams, sausages, and turkey and that the main flora causing microbial spoilage in these products were composed of psychotrophic lactic acid bacteria. The researchers also

found that NaL inhibited the growth of these bacteria and thus the refrigerated storage of these products could be prolonged by 1-2 weeks. Similarly, Cegielska-Radziejewska and Pikul (22) reported that the addition of 2% NaL to sliced poultry sausage inhibited the growth of aerobic psychotrophic bacteria and lactic acid bacteria during refrigerated storage. Lemay et al. (12) reported that 3.3% NaL delayed the growth of *Lactobacillus alimentarius* and other lactic acid bacteria in cooked and acidified chicken meat for 14 days.

The yeast strains like *Debaryomyces hansenii, Candida* spp., and *Rhodotorula mucilaginosa* isolated from meat products are reported to resist high concentrations of NaL (700-1300 mM). Houtsma et al. (28) reported that yeasts isolated from meat products were found to resist NaL over 10% levels. In this study, it was also observed that NaL had no significant effect on yeast, and differences between the groups after 30 days of storage resulted from mould inhibition by NaL.

NaL was reported to have neutral pH and is used as a pH control agent. Brewer et al. (23) reported that the addition of 2% or 3% NaL to fresh pork sausage delayed pH decrease. Wang (29) reported that there was no change in the pH value of Chinese sausages containing 3% NaL and kept at 20 ºC for 30 days. On the other hand, Lin and Lin (25) reported that the addition of 3% NaL to low-fat Chinese-styled sausages kept the pH stable at 4 ºC for 12-week storage; the researchers concluded that these findings were probably due to the buffering capacity of NaCl. Choi and Chin (30) established that the addition of 3.3% NaL did not affect the pH values of regular-fat frankfurters. Bloukas et al. (20) also observed that in 2% NaL containing low-fat frankfurters produced with olive oil the pH was almost stable for 6 weeks of refrigerated storage. In our study, NaL causes no changes in pH, which would affect the microbial properties of the experimental sausage samples during the storage period.

The results of this study indicated that the addition of lactates to sausages slowed down microbial growth (compared to nitrites), showed a better performance as an antimicrobial agent on the studied microorganisms, caused no abnormal changes in sensory characteristics, and led to a partial improvement in flavor; and yet could negatively influence consumer preferences as no red-pinkish color, known as the typical sausage color of sodium nitrite-containing sausages, occurred.

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