EFFECT OF SODIUM LACTATE USED ALONE OR IN COMBINATION WITH LYSOZYME ON THE PHYSICO-CHEMICAL AND MICROBIOLOGICAL PROPERTIES OF STEAMED SAUSAGE STORED UNDER THE REFRIGERATION

ADAM MALICKI, ANDRZEJ JARMOLUK and SZYMON BRUŻEWICZ

Department of Animal Products Hygiene, Veterinary Medicine Faculty, Agricultural University, 50-375 Wroclaw, Poland
e-mail: malicki@ozi.ar.wroc.pl

1Department of Animal Products Technology, Faculty of Food Science, Agricultural University, 50-375 Wroclaw, Poland
2Department of Hygiene, Wroclaw Medical University, 50-345 Wroclaw, Poland
e-mail: jar@ozi.ar.wroc.pl
e-mail: szybru@hyg.am.wroc.pl

Received for publication October 02, 2003.

Abstract

The purpose of the paper was the evaluation of 2% sodium lactate effects, alone or in combination with 200 ppm of lysozyme, on the microbial status, stability and physico-chemical properties of the steamed sausage. Lactate, used separately or with lysozyme, doubled the durability of the experimental sausages, when compared with the control samples. Both the substances worked synergististically against the lactic acid bacteria in the product. No synergism was, however, detected in case of the total plate count. The addition of sodium lactate and lysozyme did not affect the organoleptic and physico-chemical properties of the product. Consequently, the commercial application of both the substances as natural preservatives seems to be highly advisable.

Key words: sausages, sodium lactate, lysozyme, food preservation.

The safety and full satisfaction of the consumers require the extensive studies on biological preservatives, i.e. the natural components of human and animal tissues, effectively preventing the spoilage and the pathogen growth within the food.

The lactates – lactic acid salts formed during natural fermentation, seem to follow the aforementioned criterion. Their direct bactericidal effect results from the pH rise within the bacterial cell (3, 14, 21, 36, 39).

Sodium and potassium lactates are applied as the food preservatives. Their addition to animal origin products reflects in the significant increase in microbial stability if stored under the refrigeration (2, 3, 6, 7, 20, 24, 32, 37, 39). Considerably higher antibacterial efficacy was achieved if lactates were used in combination with the other factors, such as: acetates or diacetates (2, 19, 23, 34), nisin (24, 32), nitrites (23), bacteriocins (33), glucono-delta-lactone (30, 39), or storage under the carbon dioxide atmosphere (7).

Lysozyme, the polypeptide present in human and animal tissues and secretions, also seems to possess the biopreservative properties. It is potent to hydrolyse the β-glycoside linkages between N-acetylmuramic acid and N-acetylglucosamine of Gram-positive bacteria, destroying the cellular wall and consequently being bactericidal (15, 29, 38). Lysozyme is obtained commercially from hen’s egg white, where constitutes about 0.5% of the albumin fraction (29, 38). It is applied in the hydrochloride form for food use, mainly to preserve semidry cheeses, but also in fresh fruits and vegetables, tofu, potato salad, sea foods, meat and sausages (5, 8, 12, 29).

Although the highest antimicrobial activity of lysozyme was described in the plant-derived products, its high efficacy was also proved in those of animal origin, particularly against the Gram-negative bacteria. Antimicrobial spectrum of lysozyme is broaden when it is applied in combination with other preservatives, especially with nisin (4, 8, 9, 22, 25), but also with EDTA, nitrites and sodium chloride (5, 8, 9, 12). The use of lysozyme for food treatment, alone or with other substances, prevents the growth of numerous pathogens (8, 12, 25). Due to its thermostability, lysozyme exhibits antibacterial properties also after the thermal treatment of a product (5, 38).

According to Polish legislation (31) sodium, potassium and calcium lactates are admitted in meat products only as the acidulants of sausages and canned...
products. The lysozyme is registered as the preservative only for the fermented cheeses.

According to the relevant literature, either the lactates or the lysozyme were successfully tested antimicrobially in combination with different chemicals. The available references, however, lack the information on the associative application of the substances for food use. The studies on the problem seem to be highly appropriate, considering the increasing popularity of natural-based preservatives.

The purpose of the paper was the evaluation of 2% sodium lactate effect, alone or in combination with 200 ppm of lysozyme, on the microbial status, safety and physico-chemical properties of the steamed sausage.

**Material and Methods**

The experiment was performed on the high quality minced steamed sausage made of turkey breast at amount of about 160 kg. Three variants of the sausage were prepared: control (K), with 2% of sodium lactate (M), and with 2% of sodium lactate and 200 ppm of lysozyme (ML).

Experimental sausages were stored at 4°C for 28 d. Microbiological analyses, including the total plate count and the numbers of lactic acid bacteria, *Enterobacteriaceae*, proteolytic bacteria, anaerobic sporogenic bacilli, salmonellae, coagulase-positive staphylococci, yeast and moulds, were performed directly after production and on 3, 7, 14, 21, 28 d of the storage. The total plate count was measured with the plate method on the agar incubated at 30°C for 72 h, the lactic acid bacteria - on MRS medium (Oxoid, 30°C, 72 h), *Enterobacteriaceae* - on VRBG medium (Oxoid, 37°C, 24 h), proteolytic bacteria - on Smith-Goodner medium (30°C, 48 h), and yeast and moulds – on agar with yeast extract, glucose and chloramphenicol (25°C, 72 h). The numbers of staphylococci or the presence of salmonellae and anaerobic sporogenic bacilli were determined according to Polish Standards (26-28). The bacterial counts were transformed to logarithms and statistical calculations were carried out using Microsoft® Excel 2000 and Statistica 5. Version 97 software. Mean values were compared with the aid of Student’s t-test (P<0.01).

The contents of protein, fat and NaCl (1) in the experimental sausages were measured directly after production, as well as the water-binding capacity (10), the rate of hem pigment conversion (11), and pH. Organoleptic assessments were performed by means of multiple comparisons by 5-person commission (16) after production and on 3, 7, 14, 21, 28 d of the storage. Statistical calculations of the results were carried out using Statistica 5. Version 97 software. Multiple variance analyses were done and the significance of differences was determined on the basis of the least significant difference (LSD) value (P=0.05).

**Results**

The selected physico-chemical properties of the experimental sausages, measured directly after production are presented in Table 1. The contents of protein and fat in the experimental sausages, as well as their pH and water-binding capacity were not affected by the lactate and lysozyme addition. The NaCl contents in M and ML samples were about 0.5% lower than in the K sausages, but the difference did not alter the salty taste assessed at organoleptic evaluation. The rate of hem pigment conversion was about 6% higher in the sodium lactate added sausages, but the difference did not change the saturation and acceptability of colour tested organoleptically. The other organoleptic properties, i.e. smell, taste and consistency of the sausages were not affected by lactate and lysozyme addition. Mean organoleptic scores of the experimental sausages were not significantly different among the variants and amounted from about 4.75 directly after production to about 4.25 after 28 d of storage.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sausage variant</th>
<th>LSD</th>
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<tbody>
<tr>
<td></td>
<td>K</td>
<td>M</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>15.94a</td>
<td>16.18a</td>
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<tr>
<td>Fat (%)</td>
<td>10.90b</td>
<td>10.41a</td>
</tr>
<tr>
<td>NaCl (%)</td>
<td>2.53a</td>
<td>2.00a</td>
</tr>
<tr>
<td>Water-binding capacity (%)</td>
<td>35.23b</td>
<td>37.81a</td>
</tr>
<tr>
<td>pH (-)</td>
<td>6.35a</td>
<td>6.28a</td>
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<tr>
<td>Total haem pigments (ppm of haematin)</td>
<td>39.81a</td>
<td>40.58a</td>
</tr>
<tr>
<td>Nitric oxide pigments (ppm of haematin)</td>
<td>15.43a</td>
<td>17.90b</td>
</tr>
<tr>
<td>Rate of pigment conversion (%)</td>
<td>38.76a</td>
<td>44.11b</td>
</tr>
</tbody>
</table>

K – control, M – 2% of sodium lactate, ML – 2% of sodium lactate + 200 ppm of lysozyme, LSD – least significant difference, a, b – statistically significant differences (P=0.05).
Fig. 1. Total plate count in the experimental sausages stored under the refrigeration: K – control, M – 2% of sodium lactate, ML – 2% of sodium lactate + 200 ppm of lysozyme.

The total plate count in ML, M and K sausages remained at the similar level (about 2.5 log CFU x g⁻¹) for three initial days of the storage under the refrigeration (Fig. 1). The number of aerobic bacteria increased gradually in all the variants studied since the 3rd d of the experiment, being 1 (14th d) to nearly 2.5 log CFU x g⁻¹ (28th d) higher in K samples, when compared to M and ML sausages. The limit of the bacterial spoilage of the K samples (total plate count >5 log CFU x g⁻¹) was exceeded already on the 14th d of the storage, whereas in M and ML sausages the number of the aerobic bacteria amounted 4.8 and 4.5 log CFU x g⁻¹, respectively, at the end of the experiment.

The total plate count differences between M and ML sausages were insignificant in course of the entire experiment and did not exceed 0.5 log CFU x g⁻¹.

Fig. 2. Lactic acid bacteria number in the experimental sausages stored under the refrigeration: K – control, M – 2% of sodium lactate, ML – 2% of sodium lactate + 200 ppm of lysozyme.
The lactic acid bacteria were not isolated from ML samples at any stage of the storage (Fig. 2), whereas the application of the lactate alone (M samples) reflected in the inhibition of the microorganism studied by 21st d of the experiment. The growth of the lactic acid producing bacteria was, however, observed since the 3rd d of control sample (K) storage.

The Enterobacteriaceae, proteolytic bacteria, anaerobic sporogenic bacilli, salmonellae, coagulase-positive staphylococci, yeast and moulds were not isolated either from ML and M, or from K samples, during the entire period investigated.

Discussion

Our results on total plate count revealed that the durability of sausage with added 2% of the sodium lactate, alone or in combination with 200 ppm of the lysozyme, was twice longer than that of control samples. It is consonant with the literature information on the effects of separately applied lactate on the stability of meat products. The lag-phase of the aerobes is prolonged due to the addition of 2-3% of the lactate (3, 39). Tyszkievicz (39) claims that the stability of cured meat products increases by 45-85% after the 3% lactate addition. According to different authors (6, 37) microbial stability of the meat products treated with 2-3% of lactate amounts 30 to 40 d.

The lactates, applied as the food preservatives, work synergistically with numerous substances (2, 7, 19, 23, 24, 30, 33, 34, 39). The studies on antibacterial cooperation of lactates and lysozyme were not performed as far. Considered as the criterion of microbial stability, the total plate count in the sausage with added 2% of the sodium lactate combined with 200 ppm of lysozyme, was not significantly different from the parameter achieved for the lactate alone. Consequently, the substances seemed not to work synergistically on aerobic inhibition.

The 2% sodium lactate addition to the material studied reflected in 3-week inhibition of the lactic acid bacteria. According to the relevant literature, 2-4% of the lactate caused 2-3 week inhibition of the germs discussed (20, 37).

The lack of the lactic acid bacteria in the material treated with 2% of sodium lactate and 200 ppm of lysozyme in course of the entire experiment (4 weeks) indicates the synergism of the substances added against the microorganisms. The phenomenon was not described in the literature as far, but it is not surprising, considering the data on lysozyme efficiency against the lactic acid producing bacteria (4).

The lack of Enterobacteriaceae, proteolytic bacteria, anaerobic sporogenic bacilli, salmonellae, coagulase-positive staphylococci, yeast and moulds in all the samples tested, proves for the adequate hygienic conditions during the processing and storage of the sausages. Otherwise, it is known that either the lactate or the lysozyme used separately for food use, prevent the growth of the most pathogens (3, 8, 12, 13, 17-19, 23, 25, 30, 32-35, 40).

Concluding, the 2% addition of sodium lactate, alone or in combination with 200 ppm of lysozyme, effectively prolonged the microbial stability of the steamed sausage stored under the refrigeration. Consequently, the commercial application of both the substances as the natural preservatives seems to be highly advisable.

References


35. Rozporządzenie Ministra Zdrowia z dnia 27 grudnia 2000 r. w sprawie wykazu dopuszczalnych ilości substancji dodatkowych i innych substancji obcych dodawanych do środków spożywczych lub używek, a także zanieczyszczeń, które mogą znajdować się w środkach spożywczych lub użytkach (Dz. U. Nr 9, poz. 72, 2001).


