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FOOD MICROBIOLOGY - SHORT COMMUNICATION

Application of nisin as biopreservative of pork meat by dipping and spraying methods

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Abstract

Comparison between dipping and spraying methods to inhibit bacterial growth on artificially contaminated pork meat showed greater effectiveness of the latter method during the whole low-temperature one-week storage of product. These results suggest that the spraying method could be successful in directly applying antimicrobials to food products.

Keywords Natural antimicrobial · Dipping method · Meat preservation · Spraying method

Bacteriocins are peptides ribosomally synthesized by either Gram-positive or Gram-negative bacteria [1, 2]. Among them, those produced by lactic acid bacteria (LAB) are of great interest for many sectors of the food industry [1] since LAB are considered as generally recognized as safe (GRAS) [3].

Nisin, which is the only bacteriocin commercially produced and authorized by the Food and Drug Administration (FDA), has been used in many countries [1] to preserve either plant or animal products [4]. Furthermore, it is the only one approved for use in meat, poultry, ready-to-eat meat products and sausage casing [5].

Nisin is a polycyclic, heat-stable peptide produced by *Lactococcus lactis* subs. *lactis* that inhibits the growth of most Gram-positive bacteria, but it is not effective against yeasts and molds [6]. It is commercialized by DuPont Danisco under the trade name Nisaplin®, which has in its formulation 2.5% nisin as the active compound, 77.5% NaCl (salt), and nonfat dry milk comprising 12.0% proteins and 6.0% carbohydrates [7].

Based on the above, the aim of this study was to select the most effective method of applying Nisaplin as an antimicrobial agent to preserve pork meat samples after artificial contamination by *Lactobacillus sakei* ATCC 15521.

L. sakei was chosen as the contaminating strain because lactobacilli, especially *L. sakei*, are considered spoilage bacteria of vacuum-packed fresh meat products [8, 9]. It was cultivated overnight at 37 °C in De Man, Rogosa and Sharpe (MRS) medium (Roth®, Karlsruhe, Germany).

Pork meat used in the experiments was purchased in a local market in Vienna, Austria, and transported on ice to the laboratory. Samples were aseptically cut into 25-g pieces and submitted to 30-min exposure to UV radiation on both surfaces to eliminate any possible microbial contaminants. After this treatment, each sample surface was artificially contaminated by spraying 500 μL of a suspension of *L. sakei* with 0.3-optical density (OD) at 600 nm (Hitachi U-5100, Tokyo, Japan), corresponding to 8×10^6 CFU/mL.

To test which of the two selected methods of antimicrobial application (dipping or spraying) was the most efficient, 500 μL of 1.0% (*w*/*v*) Nisaplin solution was directly applied on both sample surfaces after artificial contamination with *L. sakei*. Nisaplin solution was prepared in sterile distilled water and filtered through membranes with 0.22-μm pore diameter (Millipore, Billerica, MA, USA) before use. The dipping method consisted in submerging the meat sample into a vat containing the Nisaplin solution [10], while in the spraying method, it was applied by means of a spray gun. After Nisaplin application with either method, samples were

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allowed to stand for 30 min at room temperature to drain away the excess coating and dry them. Then the samples were immediately vacuum-packaged (Vacuboy, Komet Plochingen, Germany) in suitable plastic sealed bags (180 × 225 mm) and stored at 4 °C for a shelf life of 0 (d0), 2 (d2), 5 (d5), and 7 (d7) days. Sterile distilled water was used instead of any antimicrobial substance as a control.

Since antimicrobial substances may display a synergistic effect when used in combination [11] and to select the best application method, Nisaplin and bacteriocin-like inhibitory substance (BLIS) produced by *Pediococcus pentosaceus* ATCC 43200 were also tested together, at the same concentration (50%, *w*/*v*), according to both methods. To obtain BLIS, *P. pentosaceus* was cultivated in 500-mL Erlenmeyer flasks containing 300 mL of MRS medium at 30 °C for 10 h without agitation. The fermented broth was then centrifuged at 4,470×*g* at 4 °C for 15 min, had its pH adjusted to 6.0–6.5 by addition of 1.0 N NaOH, and filtered through membranes with 0.22-μm pore diameter (Millipore) to remove residual cells of *Pediococcus* and heated to 70 °C for 25 min to inactivate proteases. The supernatant was used in its crude form, and, since the antimicrobial was neither purified nor characterized, it was named BLIS.

L. sakei growth was monitored during cold storage at 4 °C. For this purpose, after addition of 225 mL of 0.3% sterile saline to the meat samples within the bags, homogenization for 2 min in a Lab-Blender 400 (Seward Stomacher, Worthing, UK) and serial tenfold dilution with sterile saline, 10 μL of each dilution were placed onto the surface of plates containing 10 mL of solidified MRS-agar (1%, *w*/*v*) medium and incubated at 37 °C for 24 h. The counts of *L. sakei* colonies, made in triplicate and expressed in CFU/g, were converted to logarithms after calculating mean values. All the spraying and dipping tests were also performed in triplicate.

Mean values were submitted to analysis of variance (ANOVA) by the Statistica Software 10.0 (TIBCO Software Inc., Palo Alto, CA, USA) using the Tukey's post-hoc test, and differences were considered significant at $p < 0.05$.

Nisaplin was effective as preservative of pork meat during 7-day shelf life at 4 °C under vacuum-packing, being able to inhibit *L. sakei* growth, regardless of the method applied. Using the dipping method, Nisaplin was in fact responsible for a 1.70-log reduction of *L. sakei* count at the end of the storage period compared with the control (5.78 logCFU/g; Table 1), but it completely suppressed *L. sakei* growth when administered through the spraying one (Table 2). In other words, the inhibitory effect of Nisaplin on *L. sakei* growth was prolonged when applied by spraying, because this method allows for a better antimicrobial diffusion on the meat and, consequently, a more extensive coating of sample surface. The absence of *L. sakei* growth even at the start of shelf life was because cells were plated as quickly as possible after nisin addition. Accordingly, the spraying method showed improved

Table 1 Growth of *Lactobacillus sakei* ATCC 15521, expressed in logarithm with base 10 of colony forming units per gram (logCFU/g) on artificially contaminated pork meat after application of Nisaplin according to the dipping method. Samples were vacuum-packaged and stored at 4 °C for a shelf life of up to 7 days

Shelf life (days)	Control	Nisaplin
0	5.56 ± 0.09^{a}	4.08 ± 0.07^{b}
2	$5.70 \pm 0{,}07^{a}$	4.08 ± 0.08^{b}
5	5.72 ± 0.07^{a}	4.26 ± 0.11^{b}
7	5.78 ± 0.14^{a}	4.08 ± 0.08^{b}

Values are the means of triplicates. Different letters in the same line mean statistically significant difference by the Tukey's test ($p < 0.05$)

effectiveness after simultaneous application of Nisaplin and BLIS produced by *P. pentosaceus* (NB) compared with the dipping one (Table 3). Although the NB combination did not display any synergistic effect, the spraying method led to a reduction of *L. sakei* counts over the shelf life of up to 1 week (1.62–2.19 logCFU/g), compared with the control, significantly larger than that obtained by dipping (1.00–1.42 logCFU/g). These results confirm that the spraying method may be successful in applying antimicrobials in pork meat and that NB is not a promising combination, its inhibitory effect (Table 3) being weaker than that obtained with Nisaplin alone (Table 2).

The lantibiotic nisin has been the focus of several studies in combination with other antimicrobials [12]. It has been proposed that the stronger bactericidal effect of a bacteriocin when used in mixture with other antimicrobials may be due to the ability of these to kill cells resistant to one bacteriocin [13]. Nisin proved to exert potentiated action in combination with other antimicrobials such as chemical preservatives, phenolic compounds, other natural antimicrobial proteins, antibiotics, and organic acids [11, 14]. However, Gram-positive bacterial species differ considerably in their sensitivity to bacteriocins, and the degree of inhibition appears to depend on the genus, species, and strain [13]. The effectiveness of

Table 2 Growth of *Lactobacillus sakei* ATCC 15521, expressed in logarithm with base 10 of colony forming units per gram (logCFU/g), on artificially contaminated pork meat after application of Nisaplin according to the spraying method. Samples were vacuum-packaged and stored at 4 °C for a shelf life of up to 7 days

Shelf life (days)	Control	Nisaplin
0	4.72 ± 0.08^{a}	AG^{b}
2	4.82 ± 0.09^{a}	AG^{b}
5	4.67 ± 0.07^{a}	AG^{b}
7	4.72 ± 0.05^{a}	AG^{b}

AG, absence of *Lactobacillus sakei* ATCC 15521 growth. Values are the means of triplicates. Different letters in the same line mean statistically significant difference by the Tukey's test ($p < 0.05$)

Table 3 Growth of *Lactobacillus sakei* ATCC 15521, expressed in logarithm with base 10 of colony forming units per gram (logCFU/g), on artificially contaminated pork meat after application of Nisaplin combined with BLIS produced by *Pediococcus pentosaceus* ATCC 43200 (NB) by the dipping and spraying methods. Samples were vacuum-packaged and stored at 4 °C for a shelf life of up to 7 days

Shelf life (days)	Control	Dipping NB	Spraying NB
0	5.93 ± 0.13^{a}	4.84 ± 0.11^{b}	4.31 ± 0.14^{c}
2	6.19 ± 0.13^{a}	4.78 ± 0.08^{b}	4.06 ± 0.11^{c}
5	6.22 ± 0.16^{a}	4.80 ± 0.09^{b}	4.03 ± 0.15^{c}
7	5.93 ± 0.14^{a}	4.70 ± 0.07^{b}	4.08 ± 0.10^{c}

Values are the means of triplicates. Different letters in the same line mean statistically significant difference by the Tukey's test ($p < 0.05$)

bacteriocins also depends upon environmental factors such as pH and temperature, interactions with food components, preparation, inactivation, or uneven distribution of bacteriocin in the matrix (i.e., agar medium, liquid medium, food) [14]. Therefore, possible reasons of the less efficiency of NB treatment compared to the use of Nisaplin alone could be that nisin and BLIS may belong to different classes of bacteriocins, with considerable differences in their amino acid sequences [13, 15], and that some negative environmentally induced interaction occurred between them.

In general, the dipping method offers advantages when products require a total coating for it provides good uniformity around a complex and rough surface. However, it poses several problems including coating dilution, build-up of trash or dirt, and microbial growth in the dipping tank [10]. Coating applications by this method are usually thick, which may affect product respiration and storage features [10, 16], and dilute the outer layer of food surface impairing its functionality [17]. In contrast, the spraying method provides a more uniform coating layer over the food surface and makes multiple successive applications possible [10, 17–19], using aqueous solutions or suspensions [19].

Spray applications have been used in many food processes [20] and edible coatings [10]. For instance, bovine gelatin was applied successfully to coat beef tenderloins, pork loins, salmon fillets, and chicken breasts. Indeed, spray coating increased the shelf life, and the color of products was preserved [19].

In the absence of studies comparing methods for direct application of antimicrobials on meat products to inhibit or even suppress the growth of spoilage bacteria, this study highlights a significant improvement of inhibition of *L. sakei* growth on pork meat when Nisaplin, either alone or in combination with BLIS, was applied according to the spraying method compared with the dipping one. As a concluding remark, coating application by spraying was shown to be the best choice for direct application of Nisaplin (nisin) in vacuum-packed pork meat. The next effort will focus on the shelf life of antimicrobials alone or in combination to preserve foods artificially contaminated by spoilage bacteria and fungi.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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