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Anti-staphylococcal Effectiveness of Nisaplin in Refrigerated Pizza Doughs

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ABSTRACT

This study evaluated the effectiveness of nisaplin, commercial product having nisin as active component, in decreasing the staphylococcal population in refrigerated pizza doughs. The refrigerated pizza dough pieces randomly chosen were dipped in the solutions with nisaplin concentrations of 1.0 x 10^{-3} g and 1.0 x 10^{-2} g nisaplin/mL named for the treatment A and B and kept under refrigeration (7 °C ± 1 °C). On times 0, 15 and 30 days post treatment the Staphylococcus *spp. count was carried out. The results showed that both nisaplin treatments were able to reduce the* Staphylococcus *spp. count (CFU/g) in the refrigerated pizza doughs. However, only treatment B showed statistically significant reducer effect ($p < 0.05$) on the count providing a decrease of 1.0 and 0.98 log cycles, respectively, after 15 and 30 days post treatment. These data suggest that nisin could appear as promising alternative to control the survival of the pathogen microorganisms in the foods, particularly,* Staphylococcus *in the refrigerated pizza doughs.*

Key words: Nisaplin, nisin, dough, *Staphylococcus*

INTRODUCTION

There has been a trend to consume the fresh foods in respect of the frozen foods, hence the consumption of the refrigerated foods has progressively increased. The refrigerated pizza doughs are recognized as excellent substrates for the microbial growth being necessary to apply some procedures in order to control the growth and microbial survival in/on them (Cabo et al., 2001). Several authors have reported high microbial contamination in the food doughs (Stevens et al., 1992; Freitas et al., 2004).

Staphylococcus has been known as pathogen microorganisms able to survive in the refrigerated pizza doughs (Freitas et al., 2004). Capsule, peptideglycan, A protein, adhesins, outcell enzymes, outcell toxins, leukocidins and hemolysins are some virulence attributes found in *Staphylococcus* (Trabulsi et al., 2002). *S. aureus* has been most often associated to staphylococcal diseases causing gastroenteritis due its capability to synthesize a thermostable toxin (Brooks et al., 1998).

In recent years, the use of alternative compounds to be applied in food bioconservation systems has been emphasized (Brul and Coote, 1999; Devlieghere and Debevere, 2004). The biopreservation is a widely accepted food conservation system being referred as natural

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procedure able to provide the shelf-life extension and increased microbial food safety (Fiorentini et al., 2001).

Bacteriocins are proteic molecules with wide antimicrobial properties and synthesized when some microbial lineages are exposed to stressful conditions (e.g., nutrients depletion and overpopulation) (Cleveland et al., 2001). The application of nisin (a bacteriocin) in foods has been allowed in various countries to inhibit the survival of foodborne pathogens in the dairy products, canned goods, vacuum-packed meat and cold smoke salmon (Abee et al., 1994; Shefet et al., 1995; Davies, 1999; Nilsson et al., 2000). Some favorable attributes found in nisin are: i) toxicity absence; ii) naturally produced by *Lactococcus lactis*; iii) heat stability; iv) storage stability; v) degradation by digestive enzymes; vi) not proportionate undesired taste and flavor to foods; and vii) have prominent antimicrobial spectrum against Gram-positive microorganisms (Kominsky, 1999; Fiorentini et al., 2001).

This study aimed to evaluate the effectiveness of nisaplin, commercial product containing nisin as active compound, to reduce the staphylococcal population in the refrigerated pizza doughs.

MATERIAL AND METHODS

Refrigerated pizza dough samples

Twelve refrigerated pizza dough samples from two different commercial brands (PD_1 and PD_2) were randomly chosen and collected from the supermarkets of João Pessoa city, Paraíba, Brazil. These samples were cut in pieces of approximately 50 g. Two pieces of each sample were randomly chosen and kept in the plastic commercially sterile recipients under refrigeration (7 ºC ± 1 °C) until the experimental assay.

Experimental assay: nisaplin treatment

Independent variable of this study was the nisaplin content (10^6 IU g^{-1} of nisin, Aplin and Barret Ltd. Dorset, UK). The effect of nisaplin at two different concentrations was analyzed: 1 x 10^{-3} g nisaplin/mL (10^3 IU nisin/mL) and 1 x 10^{-2} g nisaplin/mL (10^4 IU nisin/mL) named treatment A and treatment B, respectively. Nisaplin solutions were prepared in the sterile 0.02 N HCL prior the experimental assay (Shefet et al., 1995). Nisaplin treatment was carried out by dipping the refrigerated pizza dough pieces for one minute in 100 mL of different nisaplin solutions. After draining, each piece was put into a plastic commercially sterile recipient and kept under refrigeration (7 ºC ± 1 °C). Control assay was carried out by dipping the pizza dough pieces in 100 mL of sterile 0.02 N HCl and the next steps were the same described for nisaplin treatment.

Microbiological analyses

On 0, 15 and 30 days post nisaplin treatment the *Staphylococcus* spp. count was carried out (Vanderzant and Splittstoesser, 1992). Initially, each refrigerated pizza dough piece was weighed and prepared serial dilutions (10^{-1} – 10^{-4}) using 0.01 % (w/v) sterile peptone water. Afterwards, 0.1 mL of each dilution was inoculated by spread plate procedure into sterile Baird-Parker Petri dishes (added of egg yolk emulsion and potassium telurite to 1 % v/v). After incubation at 37 °C for 48 h the colonies of *Staphylococcus* ssp. were counted. The colonies which were circular, black, small, even, convex and with perfect borders surrounded by opaque halo were considered *Staphylococcus* spp. typical colonies. All the assays were performed twice and the results regarding the *Staphylococcus* spp. count and reduction were expressed in log CFU/g and log cycles, respectively.

Statistical analysis

An experimental delineation 2 x 3 x 3 (two pizza dough kinds, two different nisaplin concentrations and control assay, three times post nisaplin treatment) was used. Student t-test was used to verify statistically significant differences ($p < 0.05$). For this, the Statistical Analysis System (SAS) Institute was used.

RESULTS AND DISCUSSION

Nisaplin effect on *Staphylococcus* spp. count in the refrigerated pizza doughs is shown in Fig. 01. The treatment B (1.0 x 10^{-2} g nisaplin/mL) presented statistically significant inhibitory effect ($p < 0.05$) on the staphylococcal count. It was able to cause a reduction of 1.1 and 0.98 log cycles on the count after times 15 and 30 days post-treatment (PT), respectively, when compared to the control assay. The treatment B showed *Staphylococcus* spp. count on 0 day PT of 4.91 log CFU/g, followed for counts of 4.09 and 3.90 log CFU/g on 15 and 30 days PT, respectively.

The treatment B provided a highest reduction in the number of *Staphylococcus* until the time 15 days PT suggesting the occurrence of a selection of more resistant *Staphylococcus* strains to the applied treatment. Possibly, from 15 days PT on, the ones could have taken larger relative proportion in the total number of reminiscent *Staphylococcus* cells and, possibly, provided a smaller reduction in the staphylococcal population as found on 30 day PT. Some food-borne pathogens when exposed to inimical procedures could exhibit an initial exponential kill proceeded by a "tail section" that indicate an apparently increased resistance in the later stages of the exposure. These survivors were likely to be physiologically protected front the stress and were not mutants (Rowan, 1999).

The treatment A (1.0 x 10^{-3} g nisaplin/mL) presented decreasing effect on the staphylococcal population in the refrigerated pizza doughs on 0 and 15 days PT; however it was not statistically significant ($p < 0.05$). The refrigerated pizza doughs exposed to the treatment A showed a *Staphylococcus* spp. growth curve similar to that found for the control assay along the evaluated times.

Other authors have noted the role of bacteriocins from *Lactobacillus plantarum* BN in improving the shelf-life of the refrigerated bovine meat (Fiorentini et al., 2001) and effectiveness of the concomitant application nisin/EDTA in inhibiting the growth of Gram-negative bacteria (*Salmonella tiphymurium. S. infantis, S. heidelberg, S. choleraesuis*, *E. coli* O157:H7) (Stevens et al., 1992; Cutter and Siragusa, 1995). Shefet et al. (1995) found inhibitory effect of nisin when applied in combination of ETDA and citric acid on *S. typhimurium* in chicken meat.

The *Staphylococcus* spp. average count found for each brand of refrigerated pizza dough when exposed to treatment B on 0, 15 and 30 days PT are shown in Table 1. It was found that the anti-staphylococcal activity of nisaplin was depending on the pizza dough brand and time PT. Anti-*Staphylococcus* spp. activity was noted only after 30 days PT and it was more intense in the assay with PD_2 (difference of 1.28 log cycles between the counts in PD_1 and PD_2). On 30 days PT, a statistically significant difference ($p < 0.05$) between the *Staphylococcus* spp. counts found in PD_1 and PD_2 was noted. Larger or smaller nisin antimicrobial effectiveness depends on the bacterial species, bacterial growth phase, substrate composition and environment conditions as pH and storage temperature (Moreno et al., 2000).

Nisin inhibitory action on *Staphylococcus* species occurs in two stages. The first stage involves unspecific interaction between the nisin molecule and target bacterial cytoplasmic membrane characterized as reversible and pH-dependent phenomenon. At the second stage, the nisin molecule and target bacterial cytoplasmic membrane present a strong attraction leading to the formation of the channels with 0.1-0.2 µm diameter in the bacterial cytoplasmic membrane. The Simultaneous cytoplasmic membrane depolarization provides fast flux of essential molecules (K^+ ions, aminoacids, ATP) resulting in cell lyses (Moreno et al., 2000).

The results obtained in this study indicated potentiality of nisin as alternative antimicrobial compound to use in the food conservation. Nisaplin, commercial product having nisin as active component, was able to provide statistically significant reduction of *Staphylococcus* spp. in the refrigerated pizza doughs.

Table 01 - *Staphylococcus* spp. count average (log CFU/g) in refrigerated pizza doughs submitted to nisaplin treatment B (1.0 x 10^{-2} g nisaplin/mL) and stored at 7 °C.

Pizza dough	Times post nisaplin treatments (days)		
	0	15	30
PD_1[a]	4.85	5.04	4.98
PD_2[b]	4.97	5.08	3.70

[a] pizza dough brand 1; [b] pizza dough brand 2

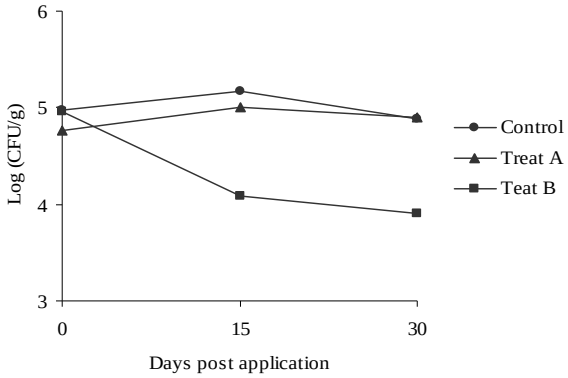

Figure 01 – *Staphylococcus* spp. count average (log CFU/g) in refrigerated pizza dough submitted to two different treatments using nisaplin as interfering variable and stored at 7 °C

Nisaplin, commercial product having nisin as active component, was able to provide statistically significant reduction of *Staphylococcus* spp. in the refrigerated pizza doughs. Nisin, and possibly other bacteriocins, could act on/in the microbial cell through the unconventional mechanisms providing inhospitable conditions to the microbial survival. Moreover, some studies could be carried out to evaluate the antimicrobial effectiveness of nisin in pizza doughs when applied jointly with other antimicrobial procedures used in the food conservation.

RESUMO

Este estudo avaliou a efetividade de nisaplin, produto comercial tendo nisina como componente ativo, em diminuir a população estafilocócica em massas de pizza refrigeradas. Pedaços de massa de pizza refrigerada foram randomicamente escolhidos e mergulhados em soluções de nisaplin com concentração de 1.0 10^{-3} g e 1.0 10^{-2} g nisaplin/mL, nomeados, respectivamente, tratamento A e B, e mantidos sob refrigeração (7 °C ± 1°C). Nos tempos 0, 15 e 30 dias pós-tratamento foram feitas as contagens de *Staphylococcus* spp. Os resultados mostraram que ambos os tratamentos diminuíram a contagem (UFC/g) de *Staphylococcus* spp. em massas de pizza refrigeradas. Entretanto, somente o tratamento B mostrou um efeito redutor estatisticamente significante ($p < 0.05$) sobre a contagem de *Staphylococcus* spp. causando uma diminuição de 1.0 e 0.98 ciclos logarítmicos, respectivamente, nos tempos 15 e 30 dias pós tratamento. Estes dados sugerem que nisina poderia apresentar-se como uma promissora alternativa para controlar o a sobrevivência de microrganismos patógenos em alimentos, particularmente, espécies de *Staphylococcus* em massas de pizza refrigerada.

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