Effect of nisin-loaded pectin nanoparticles on the survival of *Listeria innocua* in a meat model

Borges, A.[1]; Krivorotova, T.[2,3], Sereikaite, J.[2], Barreto A.S. [1] & Fraqueza, M.J.[1]

[1]Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Av. da Universidade Técnica, Polo Universitário, Alto da Ajuda, 1300-477 Lisbon, Portugal. ana.borges@gmail.com; mjoaofraqueza@fmv.utl.pt

[2]Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Vilnius, Lithuania.

[3]Department of Polymer Chemistry, Vilnius University, Vilnius, Lithuania

Abstract – The objective of this study was to evaluate the impact of free nisin and nisin-loaded pectin nanoparticles on the growth rate of *Listeria innocua* in a pork meat model subjected at different temperatures mimicking different fermentative conditions (1st step at 7ºC and 2nd step at 20ºC) during 96 hours in order to study further application on fermented meat sausage processing. A significant inhibitory effect on *Listeria innocua* was observed using both nisin and nisin-loaded pectin nanoparticles with a reduction of 1.5 log cfu/g when compared with the meat model only inoculated with *Listeria innocua*. Nisin-loaded nanoparticles showed a good antilisterial capacity similar to free nisin when applied in meat.

Key Words – Free Nisin, *Listeria innocua*, Nisin-loaded pectin nanoparticles, safety, meat model.

I. INTRODUCTION

Listeria monocytogenes human infection has resulted in numerous major foodborne outbreaks worldwide [1]. This pathogen has the capacity to grow at temperatures ranging from 0 to 45°C, tolerate salt and grow at a relatively low pH so it is difficult to control their grow in food (Ruiz *et al*., 2010). In addition to that, it is difficult to prevent its contamination in meat products only by applying good hygienic and sanitation practices in processing facilities due to its ubiquity nature and persistence linked to biofilm forming ability [2]. *L. monocytogen*es is easily killed by standard cooking techniques, however there is a link of this pathogen with ready-to-eat meat products being recognized as potential vehicles to cause human listeriosis. Therefore, controlling the growth of these bacteria in this kind of foodstuff is of utmost importance for food industry [3]. An emergent approach of controlling it is the use of antimicrobial peptides known as bacteriocins [4, 5]. Nisin, a bacteriocin produced by *Lactococcus lactis*, exhibits antibacterial activity against a wide range of food-borne Gram-positive pathogens, including *Listeria* [4, 6]. Nisin has a long record of safe use and has been approved as a natural biopreservative for use in a wide variety of foods including processed cheese, dairy products and canned foods [6, 7]. Despite of that, and aside from interacting with food components namely its sensitivity to a series of proteases, nisin may be adversely affected by processing and storage conditions such as pH and temperature of the product [7, 8]. Considering all this questions, nowadays, the major issue with the use of nisin in meat products is determining the proper usage level and appropriate application method. To enhance the stability and prolong the efficacy of nisin in food systems, different strategies were developed, namely systems that protect nisin from the interaction with food components to ensure the stability of this antimicrobial peptide during food processing and storage period [6].

The aim of this study was to determine anti-*Listeria* and general antimicrobial properties of nisin-loaded pectin nanoparticles compared with free nisin on a pork meat model inoculated with *L. innocua*, in order to use these compounds as a potential biopreservative in dry fermented meat products with different temperature steps of fermentation. It was used nisin-loaded pectin nanoparticles prepared in a simple and low cost way based on the complexation method [6].

II. MATERIALS AND METHODS

Preparation of the *Listeria innocua* CECT 910T inocula and growth conditions

Listeria innocua CECT 910T used in this study were maintained as stock culture at -80ºC in Brain Heart Infusion broth (Scharlau, Spain) containing 15% (v/v) glycerol.

Culture was prepared by growing the isolate in TSA for 24 hours at 37ºC and a culture suspension was performed in NaCl 0.9% with a optical density adjusted to an OD_{625} of 0.5, which matches approximately to 10 log CFU/ml.

Meat samples preparation and inoculation procedure

The meat was minced (1x1cm) being twenty-five grams aseptically weighted in sterilized bags. Each one of the bags corresponds to an analysis time of the test (0, 24, 48, 72 and 96 hours). The meat model was stored at 7°C for 48h days and then the temperature was changed to 20°C and stored till 96 hours.

The study was conducted with a meat model under different conditions: 1- control raw meat; 2- raw meat inoculated with *Listeria innocua* 910 CECT; 3- raw meat inoculated with *Listeria innocua* CECT 910 and free nisin; 4- raw meat inoculated with nisin loaded particles; 5- raw meat inoculated with *Listeria innocua* CECT 910 and nisin loaded particles.

In condition 2, 3 and 5, the bag with raw meat was inoculated with 1 ml of a suspension of *Listeria innocua* CECT 910 in order to obtain approximately 7 log CFU/g, considering a high level of contamination.

In condition 3, the bag with 25g of raw meat was inoculated with a 1 ml of 0,4mg/ml free nisin suspension to obtain a final concentration of 0,016mg/g of meat. The same procedure was done with 0,4mg/ml nisin-loaded nanoparticles suspension (prepared at pH 6.0 using low methoxyl pectin and according to Krivorotova *et al.* [7]) in conditions 4 and 5 to obtain a final concentration of final concentration of 0,016mg/g of meat.

Assays were done in triplicate for each condition in study contemplating the same procedures.

Microbiological analysis

The samples were analyzed to monitor the dynamic changes in the main microbial groups responsible for ripening of fermented sausages and their hygienic quality. Microbiological analysis was performed 1 hour after inoculation (time 0), 24h, 48h, 72h and 96h, for total aerobic microorganisms at 30ºC, *Listeria* spp., *Pseudomonas* spp. and lactic acid bacteria (LAB) counts according with the methods proposed by ISO (International Organization of Standardization).

Statistical Analysis

For data analysis was used the Microsoft Excel 2011 program and Statistical Package for Social Sciences (SPSS) software, version 21. Free nisin and nisin-loaded nanoparticles inoculation and time and temperature storage effects were evaluated using one-way analysis of variance (ANOVA) and Tukey test. The results were considered significantly different with $P < 0.05$.

III. RESULTS AND DISCUSSION

The figure 1 presents the evolution over the time (0h, 24h, 48h, 72h and 96h) of *Listeria innocua* growth inoculated in a meat model with free nisin and nisin-loaded nanoparticles. The initial counts of *Listeria innocua* on meat at time 0 were reduced in 1.39 log $cfu.g^{-1}$ under the effect of free nisin and in 1.26 log $cfu.g^{-1}$ under the effect of nisin-loaded nanoparticles. The growth of *Listeria innocua* at 7ºC after 48h of storage was significantly ($p<0.05$) inhibited when free nisin or nisin-loaded nanoparticle were added to meat. This listerial inhibitory effect was maintained in both study conditions after 48h at 7ºC. When the temperature was changed from 7ºC to 20ºC it was noticed an increase of *Listeria innocua* counts for all the conditions in study and his growth rate under the effect of free nisin and nisin-loaded nanoparticles were the same. After 7ºC and 20ºC cycles of temperature the *Listeria* final counts obtained on meat with free nisin and nisin-loaded pectin nanoparticles

where approximately the same, both presenting 1.49 log cfu.g^{-1} reduction when compared with the meat model inoculated only with *Listeria*. The antilisterial activity obtained in this study was similar in meat samples with free-nisin when compared to the nisin-load pectin nanoparticles.

Total microbial counts were not affected by the presence of nisin-loaded pectin nanoparticles in meat. Also, under the study conditions was not observed any significant reduction of LAB counts in meat with nisin loaded pectin nanoparticles which could be suitable for use in fermented meat products.

The *Listeria* inhibitory effect of nisin-loaded carbohydrate nanoparticles were also studied by Bi *et al.* [12] who reported a higher affectivity for solutions of free nisin. In our study were observed effects for both nisin-loaded pectin nanoparticles and free nisin witch could be profitable for future application of this systems since nisin will be protected without loss of its effectiveness. Further studies should be addressed in other to clarify the length of effectiveness during time of meat storage.

IV. CONCLUSION

When applied in a meat model, nisin-loaded pectin nanoparticles showed a good antilisterial capacity which was similar to the effect obtained with free nisin. After 5 days of study both have the same capacity of inhibiting *Listeria innocua* growing. In this context, in future studies we are interested in characterize the action of nisin-loaded pectin nanoparticles in meat model beyond 96 hours and see if its performance is superior to the demonstrated for free nisin, taking into account that nanoparticles are supposed to be protected from enzyme's substrate and slowly release nisin at a constant rate.

The data obtained in this study provides useful insights on the influence of the free nisin and nisin-loaded pectin nanoparticles on the survival and⁄or growth of *L. innocua* demonstrating that this peptides could be used as a natural preservative to reduce and control *Listeria spp.*, particularly *L. monocytogenes*, on fermented meat sausage processing.

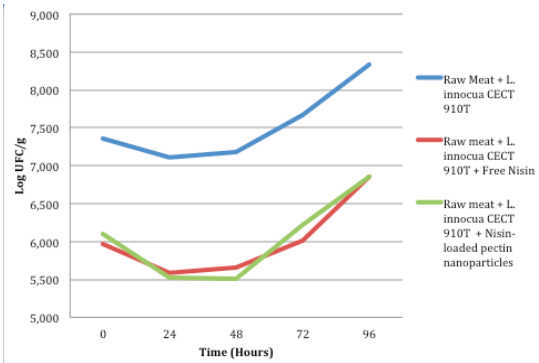

Figure 1 – Influence of free nisin and nisin-loaded nanoparticles on *Listeria innocua* CECT 910Tgrowth inoculated in a meat model.

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