INHIBITION OF LISTERIA MONOCYTOGENES IN COOKED HAM DURING STORAGE THROUGH NATURAL ANTIMICROBIALS

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I. INTRODUCTION

In the food industry, microbial contamination is a major problem, due to it is associated large economic losses and to safety of consumers. It may occur due to the appearance of bacteria, among other microorganisms, which are responsible for alterations in the quality, safety and organoleptic properties of food. *Listeria* is a standard control microorganism in the food industry since it may be pathogenic and cause a disease called listeriosis that may affect humans and animals. The food industry should to elaborate safe food at reasonable prices using techniques, treatments or ingredients which assure the innocuousness for consumers. The main objective of the present study is to verify the efficiency of different combinations of natural antimicrobial ingredients, against *Listeria monocytogenes*, to be used in ready-to-eat foods, according to the "EURL Lm Technical Guidance Document for conducting shelf-life studies on *Listeria monocytogenes* in ready-to-eat foods".

II. MATERIALS AND METHODS

A challenge Test has been elaborated, following the EURL Lm Technical Guidance Document for conducting shelf-life studies on *Listeria monocytogenes* in ready-to-eat foods (Version 3 of 6 June 2014-Amendment 1 of 21 February 2019). It has been selected the Challenge test assessing growth potential methodology, since it allows to have information on whether the studied matrix support the growth of *L. monocytogenes* under real conditions during its shelf-life time. Seven test formulations were manufactured using Good Manufacturing Practices in the Pilot Plant of PROSUR SAU, by triplicate each one. Ingredients included: pork ham meat (80 %), potato starch (15g/kg); tripolyphosphate (5 g/kg); carageenan (3 g/kg); salt (2% in the final product); and the natural ingredients composition indicated in Table 1. The cooked ham was elaborated and packed under vacuum conditions and storage at refrigerated temperature at 4ºC.

Table 1. Description of key ingredients included in the different formulations.

Identification	Ingredients
P1	Negative Control – no preservatives
P2	Cultured celery (100 ppm Nitrite); 250 ppm ascorbic acid+0,7% PRS DV-5
P3	1% NATPRE T-10 DV HS + 0.5% PRS-DV-5
P4	1% NATPRE T-10 DV LS + 0.5% PRS-DV-5 LS - 1.3% NaCl + 0.35-0.40% KCl
P5	1% NATPRE T-10 EML + 0.5% PRS-DV-5
P6	1% NATPRE T-10 EML + 0.75% PRS-DV-5

Microbiology assays: for the preparation and inoculation of the test units: cooked ham was sliced under sterile conditions. Non inoculated vacuum packages were prepared for microbiology analysis, containing three slices per package. Inoculated samples were prepared with 4 slices per packages. An initial microbial concentration of 10^2 cfu/g was inoculated per slice. Slices were surface-inoculated with the cocktail of *Listeria* and the inoculum distributed over one surface of each slice, and then stacked such that the inoculum was between the slices. Inoculated products were vacuum packaged in gas-impermeable pouches and stored at the appropriate incubation temperature (4 °C or 7 °C).

Triplicate inoculated samples were assayed for *Listeria* populations, and duplicate uninoculated samples were assayed for lactic acid bacteria populations. Microbiology analysis and storage conditions: At time 0 and at the end of study, three non-inoculated samples were analyzed PCR methodology, in order to detect a possible contamination of *Listeria* in samples. Inoculated samples were analyzed initially and after 4, 6, 8, 10, 11, 12, 13, 14, 15, 16 and 17 weeks for samples storage at 4°C. The analysis was carried out in triplicate. Data were statically analysed with the statistical package SPSS 15.0 (Statistical Package for the Social Science for Window (IBM, Armonk, New York, USA). A value of $P < 0.05$ was considered statistically significant.

III. RESULTS AND DISCUSSION

The results obtained during the shelf-life study are showed in the Figure 1. The P1 samples showed a progressive growth, and only after 3 or 4 weeks the counts reached between 5-6 log. The rest of samples (P2-P7) showed constant counts of *Listeria* and no growth has been observed between initial and final counts. It indicates that any of the combinations presented by PROSUR are effective to control a possible initial contamination of *Listeria* in a RTE food.

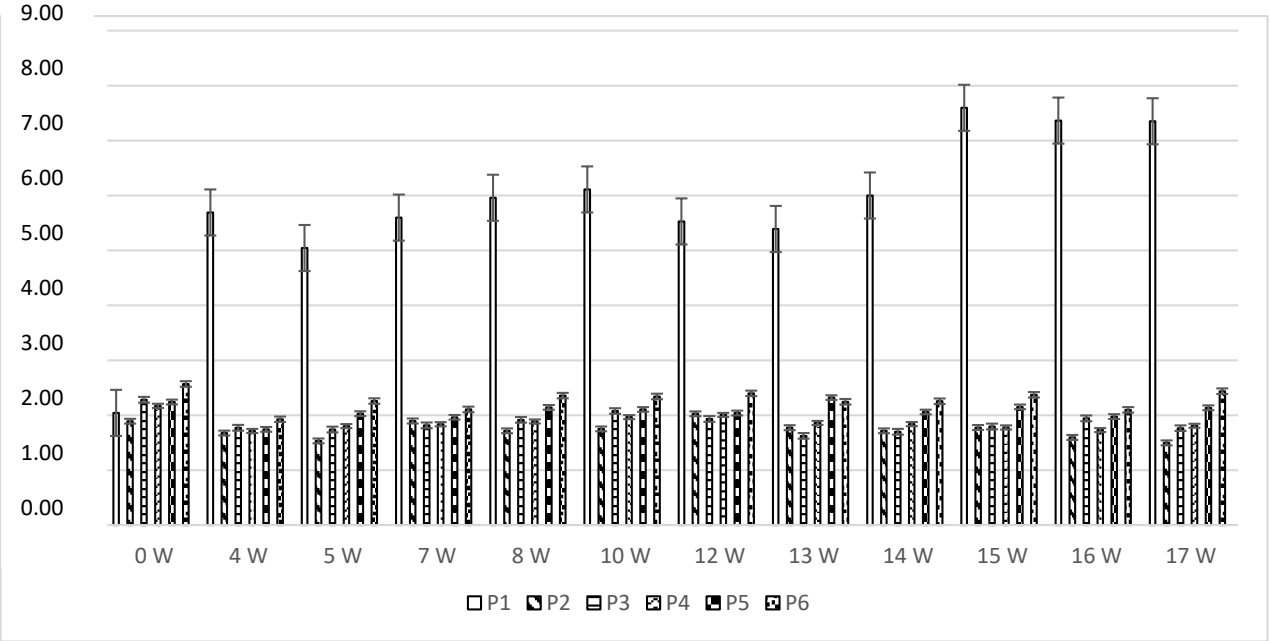

Figure 1. Count of *Listeria* cocktail (log cfu/g) in the different cooked ham elaborated and storaged at 4ºC during 17 weeks ($P<0.001$).

IV. CONCLUSION

The present Challenge test elaborated following the EURL Lm Technical Guidance Document for conducting shelf-life studies on *Listeria monocytogenes* in ready-to-eat foods, allows to conclude that the natural ingredients developed at Prosur, included in the formulation of cooked ham, helps to control, even eliminate, the initial microbial contamination of *Listeria innocua* in the raw material and inhibits its growth, for 4 ºC storage temperature, providing a Ready-to-eat food unable to support the growth of *Listeria monocytogenes*.

REFERENCES

1. Hélène Bergis, Ludivine Bonanno, Adrien Asséré, Bertrand Lombard (2021). EURL Lm TECHNICAL GUIDANCE DOCUMENT on challenge tests and durability studies for assessing shelf-life of ready-to-eat foods related to *Listeria monocytogenes*. EU Reference Laboratory for *Listeria monocytogenes* Anses -Food Safety Laboratory, Maisons-Alfort, France