



Castro, S. M.<sup>1</sup>, Noronha, L.<sup>1</sup>, Vaz-Velho, M.<sup>2</sup>, Queirós, R.<sup>3</sup>, Silva, J.<sup>1</sup>, Saraiva, J.<sup>3</sup>, Teixeira, P.<sup>1</sup>

- <sup>1</sup> CBQF Centro de Biotecnologia e Química Fina Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto, Rua Dr. António Bernardino Almeida, 4200-072 Porto, Portugal.
- <sup>2</sup> Escola Superior de Tecnologia e Gestão Instituto Politécnico de Viana do Castelo, Avenida do Atlântico s/n, 4900-348 Viana do Castelo, Portugal.
- <sup>3</sup> Departamento de Química, Universidade de Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal.

The effect of high pressure (HP) and bacteriocionogenic lactic acid bacteria on the survival of *Listeria innocua* inoculated in *Alheira*, a naturally fermented meat sausage, typical from the North of Portugal, was evaluated in the present study. From the obtained results, HP demonstrated to be a promising technique to guarantee the safety of this product since it reduces the inoculated *L. innocua* up to admissible values. Besides, these values are maintained during refrigerated storage up to 60 days. Nevertheless, further studies are necessary to highlight the effect of both pressure and bacteriocinogenic bacteria in *L. Innocua*.





Abstract

Alheira is made of several meats, boiled in water with salt and spices, and bread, olive oil and/or fat drippings are also added to the mixture. Those are brought into a paste and stuffed into cattle intestinal casings and submitted to a dry smoke process, usually for no longer than eight days. A wide variety of microorganisms have already been isolated from Alheira, including lactic acid bacteria (LAB), and pathogens, such as *L. monocytogenes* [1]. Research on bacteriocins produced by LAB, has led to consideration of their use as natural preservatives in meat products [2]. The use of HP to reduce microbial loads has already shown great potential in several products, including meat [3].



theoria poiesi

Introducti

Experimental Procedure Alheira paste was inoculated with *L. innocua* and *Pediococcus acidilactici* with bacteriocinogenic activity against *L. Innocua* (both strains 10<sup>7</sup> CFU/g). This mixture was stuffed into artificial casings (ca. 5 g, 28 mm diameter) and further vacuum packed. Small *Alheiras* were then submitted to HP treatment (500 MPa, 5 min, 10 °C), in duplicate, followed by refrigerated storage (4 °C, the recommended storage temperature for final product). From each duplicate, three replicate samples of 1g were taken during several storage days (0, 2, 7, 14, 28, 60). Non-processed and processed small *Alheiras* were analyzed concerning the enumeration of i) *L. innocua* in ALOA medium, ii *P. acidilactici* in MRSA and iii) Total Viable Counts in TSA.





250

---- L. innocua

□ The applied HP treatments seemed to destroy *L. innocua* up to values admissible by European Regulamentation ( $10^2$  CFV / 25g of product).



Figure: 1 The effect of storage time under refrigeration in non-processed *Alheiras*. (The error bars indicate the standard deviation.)

• Concerning non-processed small *Alheiras* and with the exception of total viable counts, no differences during the storage period were observed in the enumeration of *L. innocua* and *D. acidilactici* (Figure 1)

 $\Box$  Just after HP, total viable counts had a decrease of 3.5 log CFU / g when compared to non-processed samples.

□ According to Table 1, in processed samples and up to 60 days of refrigerated storage, *L. Innocua* counts were kept at admissible values.

Table: 1. The effect of storage time under refrigeration in HP treated small Alheiras.

Storage time	Total Viable counts	P. acidilactici	L. innocuą
(days)	(Log CFV/g)	(Log CFV/g)	(Log CFV/g)
0	3.0±0.1	<2.2*	<2.2*
2	3.2±0.2	<2.2*	<2.2*
7	3.2±0.1	<2.2*	<2.2*
14	3.7±0.1	<2.2*	<2.2*
28	4.0±0.2	3.1±0.0	<2.2*

## 60 4.1±0.1 3.5±0.3

\* Values lower than the limit of the enumeration technique.

## References

[1] Ferreira, V., Barbosa, J., Vendeiro, S., Mota, A., Silva, F., Monteiro, M. J., et al. (2006). Chemical and microbiological characterization of alheira: a typical Portuguese fermented sausage with particular reference to factors relating to food safety. Meat Science, 73, 570–575.

[2] Albano, H., Pinho, C., Leite, D., Barbosa, J., Silva, J., Carneiro, L., et al. (2008). Evaluation of a bacteriocin-producing strain of *Pediococcus acidilactici* as a bio-preservative for "Alheira", a fermented meat sausage. Food Control, 20, 764-770.

[3] Campu, M. (2010). High Pressure Processing of Meat, Meat Products and Seafood. Food Engineering Reviews, 2, 256–273.

## Conclusions

 $\Box$  From the obtained results, HP seemed to be the main factor that contributes to maintain *L. Innocua* under the admissible values.

<2.2\*

■ Further studies at lower pressure values are required in order to evaluate the simultaneous effect of pressure and bacteriocinogenic LAB on the survival of *L. innocua* in *Alheiras* under refrigerated storage.

Acknowledgements Financial support for author S. M. Castro was provided by PhD fellowship, SFRH / BPD / 71723 / 2010 (FCT).