EFFECTIVENESS OF HIGH HYDROSTATIC PRESSURE ON REDUCES *Listeria monocytogenes* POPULATION IN INOCULATED DRY-CURED HAM

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Introduction

Listeria monocytogenes, a psychotropic pathogenic micro-organism, can cause a severe human infection called listeriosis. The disease is a significant health treat primarily to the YOPI (Young, Old, Pregnant and Immuno-compromised patients) group (Batt, 1999). Although the prevalence of listeriosis is low (2-15 cases per 10^6 inhabitants) (Lou and Yousef, 2000) the high resistance of the pathogen makes it therefore dangerous.
L. monocytogenes is of particular concern because of its ability to grow at refrigeration temperatures and at reduced water activity (a_w) values that are characteristic of dry-cured ham (Morales et al., 2006).
Nowadays, hams are sliced and vacuum-packaged at the processing plants. This operation may result in contamination by *L. monocytogenes* (Morales et al., 2006). Thus, alternatives to post-packaging treatments such as high hydrostatic pressure (HHP) are being considered. HPP is of special interest in high quality meat products, as dry-cured ham, which can not be heat-treated to reduce microbial load (Andrés et al., 2006).
On the other hand, Andrés et al. (2004) found that HHP treatment had a slight effect on the colour of this product.
The aim of the present work was to examine the effect of two HHP treatments on the survival of *L. monocytogenes* inoculated onto slices of dry-cured hams prior to vacuum-packaged and the effect of HHP treatment on the colour of this product along the storage time.

Materials and Methods

20 packages of 100 g of slice dry-cured ham were divided into four treatment groups (Non-inoculated, inoculated no-HPP, inoculated-500MPaHPP and inoculated-600MPaHPP). The samples were inoculated with a 20h culture of four strains of *L. monocytogenes* (CECT 932, CECT 934, CECT 4032, CECT 5366) at 10^3-10^4 cfu/g, spread onto the surface with a sterile swap, packaged under-vacuum and kept at 4ºC for 5h before HPP treatment with Wave 6000/300 equipment (NC Hyperbaric, Burgos, Spain). The inoculated samples were processed at two pressures (500 and 600 MPa) during 3 min at 20ºC and kept at 4ºC for 75 days. At 0, 10, 50 and 75 days samples were collected and analysed to detect the survival of *L. monocytogenes*. The analysis were done in quadruplicate and performed according to the European norm. For counting, 25g of product were incubated in 225 ml of Half Fraser (CM0895, OXOID, Basingstoke, Hampshire, England) supplemented with Half Fraser selective (SR0166E, OXOID) for 1 hour at room temperature and two plates of ALOAcount (AEB620088, AES, CHEMUNEX, Bruz cedex, France) were inoculated with 1 ml in depth of 1/10 dilutions using Ringer (BR0052G, OXOID) and then incubated at 37ºC from 24 to 48h (UNE-EN-ISO 11290-1:1998). For detection, the 25g of sample with Half Fraser was incubated during 24h at 37ºC and after incubation, 1 ml of this suspension was incubated for 24-48h at 37ºC in 10 ml of Fraser and another 1 ml was inoculated on ALOA plates (AEB520080, AES), and incubated at 37ºC for 24-48h (UNE-EN-ISO 11290-2:1996) in order to count and detect *L. monocytogenes*.
Colour was measured with a Konika Minolta spectrophotometer (Sakai-ku, Sakai, Osaka, Japan) in two lots of vacuum-packaged non-inoculated hams. One lot was not HPP treated (control) and the other one was treated at 600MPa. Both lots were kept at 4ºC, and samples were taken on days 0, 10, 50 and 75 by triplicate. The illuminant used was D65 and the standard observer position was 10º. The colour measurements carried were lightness (L*), rednss (a*) and yellowness (b*).

Results and Discussion

Table 1 shows the results of *L. monocytogenes* survival in dry-cured hams. In control samples no *L. monocytogenes* appeared at any sampling time. In inoculated samples counts of *L. monocytogenes* decreased along the time. In non-HPP samples counts decreased 1.1 log cfu/g in 75 days, and in all cases *L. monocytogenes* was detected in all samples analysed. HPP treatments determined a reduction of *L. monocytogenes* counts just after pressure application. This reduction was around 1.5 log cfu/g at 500 MPa and around 3 log cfu/g at 600 MPa. Although initially, treatment at 500 MPa was less effective on *L. monocytogenes*, after 10 days of storage both pressure levels guaranteed the safety of product with respect to *L. monocytogenes*. Some conditions of the

ham slices, such as the low a_w and pH values, seem unfavourable for the recovering of pressure-injured cells, which would gradually die (Morales et al, 2006).

Table 1: Results of counts (log cfu/g) and detection of *L. monocytogenes* along storage in HPP treated and non treated samples

Time(days)	*0*		*10*		*50*		*75*	
Sample	C	D	C	D	C	D	C	D
Control	<1	-	<1	-	<1	-	<1	-
Non-HPP	3.70	+ (4)	3.06	+ (4)	3.00	+ (4)	2.60	+(4)
HPP (500MPa)	2.44	+ (4)	<1	+ (3)	<1	-	<1	-
HPP (600MPa)	<1	+ (1)	<1	+ (1)	<1	-	<1	-

C: count; D: detection
-: negative; +: positive (number of positive samples)

High pressure treatment did not determine differences in L*, a* and b* values (data not shown) respect to control samples at any storage time. The resistance of the nitrosylmyoglobin pigment might be probably the explanation to the colour stability of cured meat products treated at HPP (Rubio et al., 2007). However, b* value decreased significantly along the time in treated and not treated samples. While in control samples this change occurred in the first 10 days, in HPP treated samples did not occur till 75 day. This decrease of yellowness did not agree with data reported by other authors. Andrés et al. (2004) found in vacuum-packaged hams that HPP treatment (600MPa) decreased a* and L* values, while b* value were not significantly affected by pressure. In another work Andrés et al. (2006) observed an increase of L* value after HPP treatment (400MPa), that they explained by structural changes on proteins on the surface of the dry-cured ham. Due to the contradictory results found, further studies have to be done to know how HPP treatment affects dry-cured ham colour.

Conclusions

HPP treatment at 600 MPa for 3 min was able to reduce by more than 2.70 log cfu/g a pool of four *L. monocytogenes* strains inoculated in dry-cured ham. Besides, in dry-cured hams, refrigeration temperatures seem to produce along storage a slight reduction in *L. monocytogenes* survival. Furthermore, colour was not affected by HPP, only b* values changed. Taking into account, that commercial dry-cured hams present initial contamination lower than the inoculation level used in this study, and the European Union regulations have established a limit of 100 cfu/g for ready-to-eat foods, it can be concluded that 600 MPa HPP treatment is enough to obtain safe commercial products.

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References

Andrés, A.I., Cava, R., Ventanas, J., Thovar, V., and Ruiz, J. (2004). Sensory characteristics of Iberian ham: influence of salt content and processing conditions. Meat Science, Vol. 68, pp. 45-51.

Andrés A.I., Adamsen C.E., Moller J.K.S., Ruiz J. and Skibsted L. (2006). High-pressure treatment of dry-cured Iberian ham. Effect on colour and oxidative stability during chill storage packed in modified atmosphere. European Food Research Technology, Vol. 222, pp. 486-491.

Batt C.A. (1999). In introduction. Encyclopaedia of Food Microbiology. Edited by Robinson R.K., Batt C.A. and Patel P.D. Academic press, 2000.

Lou Y. and Yousef A.E. (1999). In characteristics of *Listeria monocytogenes* important to food processors. Edited by Ryser E.T. and Marth E.H. (1999). *Listeria*, listeriosis and food safety (second edition). Marcel Dekker.

Morales P., Calzada J. and Nuñez M. (2006). Effect of high-pressure treatment on the survival of *Listeria monocytogenes* Scoot A in sliced vacuum-packaged Iberian and Serrano cured hams. Journal of Food Protection, Vol. 69 (10), pp. 2539-2543.

Rubio B., Martínez B., García-Cachán M.D., Rovira J. and Jaime I. (2007). Effect of high pressure preservation on the quality of dry-cured beef "cecina de León". Innovative Food Science and Emerging Technologies, Vol. 8, pp. 102-110.