Behaviour of non-stressed and stressed *Listeria monocytogenes* and *Campylobacter jejuni* cells on fresh poultry hamburgers

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***Abstract*— The combination of modified atmosphere packaging (MAP) and protective culture to control the growth of non-stressed, and stressed *Listeria monocytogenes* and *Campylobacter jejuni* cells, was performed on fresh poultry hamburgers. Fresh poultry hamburgers were inoculated with *L. monocytogenes* (10^2 cfu/g), *Leuconostoc pseudomesenteroides* PCK 18 (10^6 cfu/g) and *C. jejuni* (10^3 cfu/g). The half of the burgers were packaged (in air and MAP) while the other half were frozen (48 h at -20 °C) to subject cells to stress. After applying stress conditions, burgers were packaged in air or in MAP (50% CO_2/50% O_2) as refrigerated ones. All samples were kept at 4 °C for 21 days. Protective culture reduced the counts of *L. monocytogenes* in all the samples, being this effect higher in those frozen samples storage in MAP. The MAP used was useful to control the growth of both *L. monocytogenes* and *C. jejuni*. The combination of a protective culture and a rich-O_2 atmosphere is a promising intervention measure to provide safe poultry hamburgers to consumers.**

Keywords—**Biopreservation, MAP, poultry.**

I. INTRODUCTION

Poultry meat products serve as reservoirs for a number of pathogens including, *Salmonella* serotypes, *Campylobacter jejuni*, *Listeria monocytogenes*, *Clostridium perfringens*, and *Staphylococcus aureus.* Poultry carcasses as well as final products can be contaminated during the slaughter and processing steps in poultry processing plants [1, 2].

Nowadays consumers are demanding safety food products, with minimal processing and the application of biopreservation as a natural alternative for food preservation is considered [3]. Biopreservation can be defined as a preservation method to improve safety and stability of food products by using determined microorganisms and/or their metabolites without changing the sensory quality [4]. A widely number of studies has confirmed that both bacteriocin and bacteriocinogenic LAB are effective against *Listeria monocytogenes* [5, 6, 7]. On the contrary, this bioprotective action is less effective in Gram negative bacteria [3]. In this sense, the application of hurdle technology, combination of different technologies to preserve the sensorial characteristics, extend the shelf life and improve the safety of the food products [3], could be an important alternative to reduce the risk of different pathogens in the same product. In this study a combination of hurdle factors like modified atmosphere packaging (MAP) and protective culture was used to evaluate the effect on stress and non-stress cells of *L. monocytogene*s and *C. jejuni* in poultry hamburgers.

II. MATERIALS AND METHODS

A. Bacterial strains and inoculum preparation

L. monocytogenes strains (CECT 5366, CECT 934, CECT 4032 and LTA0020 isolated from poultry minced meat) were grown during an overnight at 37 ºC in BHI broth (Oxoid, Basingstone, Hampshire, England) to achieve a viable cell population of 9 log cfu/ml. Then, 20 ml of cell suspension in Ringer Solution (Oxoid) were mixed with meat obtaining a final concentration of 2 log cfu/g.

Lc. pseudomesenteroides PCK 18 (isolated from Maasai milk) inoculum was prepared from a freeze-dried preparation that was diluted in water. Thus, 20 ml of cell suspension were mixed with meat, to achieve a final concentration of 6 log cfu/g.

A 20 ml inoculum of *C. jejuni* CECT 7572 (kept at -80 ºC in 14 % of glycerol) was prepared in Ringer Solution (Oxoid) to raise a concentration in final product of 3 log cfu/g.

Poultry minced meat and fat were supplied by a poultry company in Burgos (Spain). Hamburgers (80.23 % of meat, 11.46 % of fat, 5.39 % of water and 2.92 % of additives) were made in the pilot plant. Closed additive preparation (Taberner, Valencia, Spain) was diluted in water and mixed with the meat, fat and the inocula (*C. jejuni* and *L. monocytogenes*) in a mixer at medium rate for 15 minutes. Additives contained salt, vegetable fiber, antioxidants (E325, E331, E300), starch, dextrose, spices, preservatives (E221, sulphite) and colorant (E120). Half of the burger preparation was frozen (48 h at -20 h) to subject cells to stress. After 48 h *Lc. pseudomesenteroides* PCK 18 was added to the half of the meat and hamburgers were packaged in air or in MAP (50% CO_2/50% O_2). The same was done in those samples not subjected to stress.

B. Packaging and storage conditions

Two hundred and fifty grams of hamburger meat were placed in white semi-rigid trays (Sanviplast, Barcelona, Spain) made of Polyethylene/Ethylene Vinyl Alcohol/Polystyrene (PE/EVOH/PS), which have oxygen and CO_2 transmission rates of 0.99 cm^3 m^{-2} day^{-1} atm^{-1} and 0.55 cm^3 m^{-2} day^{-1} atm^{-1}, respectively, at 25ºC and 75% relative humidity (RH). They were covered with a Polyethylene Tereftalato coating with Polyvinylidene chloride/Polyethylene (PETPVdC/PE) film (Amcor Flexibles, Burgos, Spain), which has oxygen and CO_2 transmission rates of 7 cm^3 m^{-2} day^{-1} atm^{-1} and 20 cm^3 m^{-2} day^{-1} atm^{-1}, respectively at 23ºC and 65% RH. The gas mixture used was 50% CO_2/50% O_2 prepared using a gas mixer WITT-Gasetechnik (WITT-Gasetechnik GmbH & Co KG, Witten, Germany). The samples were stored at 4°C for 21 days to carry out microbiological analyses. Bacterial counts were performed before and after the inoculation on day 0 and after the frozen period.

C. Microbiological analyses

Meat samples of 10 g were removed aseptically from each tray and homogenized in 90 ml of buffer peptone water (BPW, AES laboratoire, Bruz, France) for 2 min. *L. monocytogenes* was enumerated on Chromogenic Listeria Agar (Oxoid) supplemented with OCLA (ISO) Selective Supplement (SR 0226E, Oxoid) and Brillance™ Listeria Differential Supplement (SR 0228E, Oxoid), by the spread plate method. Plates were incubated at 37 ºC, for 24-48 h. *C. jejuni* was enumerated on mCCDA (Oxoid) and incubated at 41.5 ºC for 48 h in microaerobic atmosphere (5% O_2, 10% CO_2, 85% N_2) provided by CampyGen® (Oxoid). *Lc. pseudomesenteroides* PCK 18 and lactic acid bacteria (LAB) were determined on MRS agar (Oxoid). Plates were anaerobically incubated in 6 % CO_2 at 30 ºC, for 3 days.

C. jejuni detection was performed by the method described by International Organization for Standardization (ISO) 10272-1:2006.

III. RESULTS

L. monocytogenes counts remain constant in those samples packaged in air and MAP but a significant reduction was achieved when protective culture was presented both in air and MAP samples. However, in those samples subjected to stress conditions for 48 h, the reduction of *L. monocytogenes* counts was significantly higher in those samples packaged in MAP than in air (0.90 log cfu/g and 0.33 log cfu/g respectively). In this case the rich-O_2 atmosphere selected had also a bacteriostatic effect on *L. monocytogenes*.

Regarding *C. jejuni* counts the effect of the rich-O_2 atmosphere was higher than in *L. monocytogenes*. In those samples not subjected to stress conditions *C. jejuni* could not be quantified from day 2 in the sample packaged in air with protective culture (CPA) and from day 4 for all the samples. Along the storage period *C. jejuni* was only detected in those samples packaged in air. Nevertheless, in those samples submitted to cold stress *C. jejuni* was only quantified after the inoculation, but could be only detected after 48 h in frozen stressed samples. At day 1 after frozen stress, there was no detection in the sample inoculated with protective culture and packaged in MAP (CPMf). From day 2, in those samples packaged in MAP (CLMf and CPMf) *C. jejuni* was not detected.

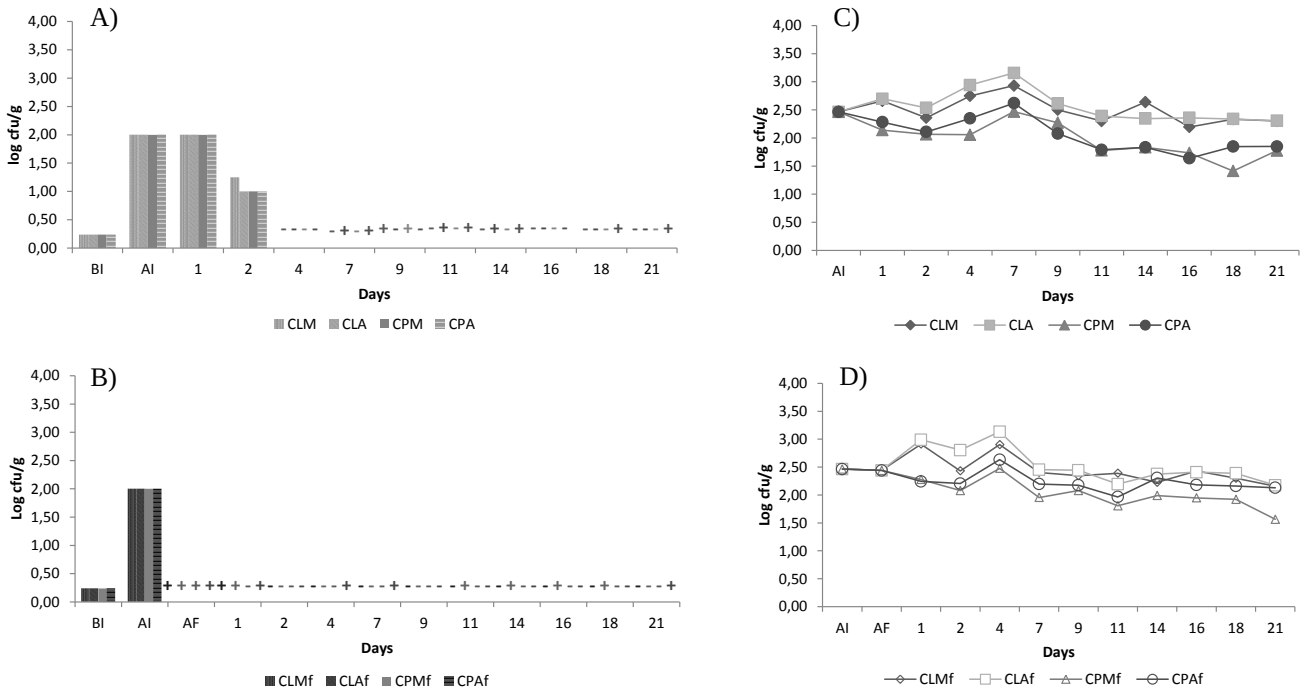

Fig 1. Evolution of non-stressed (A) and stress (B) cells of *Campylobacter jejuni* cells on fresh poultry hamburgers packaged in air or MAP. Enumeration of non-stress (C) and stress (D) cells of *Listeria monocytogenes* on fresh poultry hamburgers packaged in air or MAP. CLM: co-inoculation of *C. jejuni* and *L. monocytogenes* non-stress cells in MAP; CLA: co-inoculation of *C. jejuni* and *L. monocytogenes* non-stress cells in air; CPM: co-inoculation of *C. jejuni*, *L. monocytogenes* and *Lc. pseudomesenteroides* PCK 18 non-stress cells in MAP; CPA: co-inoculation of *C. jejuni*, *L. monocytogenes* and *Lc. pseudomesenteroides* PCK 18 non-stress cells in air; CLMf: co-inoculation of *C. jejuni* and *L. monocytogenes* stress cells in MAP; CLAf: co-inoculation of *C. jejuni* and *L. monocytogenes* stress cells in air; CPMf: co-inoculation of *C. jejuni*, *L. monocytogenes* and *Lc. pseudomesenteroides* PCK 18 stress cells in MAP; CPAf: co-inoculation of *C. jejuni*, *L. monocytogenes* and *Lc. pseudomesenteroides* PCK 18 stress cells in air.

The selected atmosphere kept the level of *Lc. pseudomesenteroides* PCK 18 inoculum constant along the storage, and extended the lag phase of LAB in those samples no inoculated with protective culture (Data not shown).

IV. DISCUSSION

Nowadays, food technologists are more interested in the "Hurdle Technology" that combine different technologies to preserve the sensorial characteristics, extend the shelf life and improve the safety of the food products [3]. In this study a combination of hurdle factors like MAP and protective culture was used to evaluate the effect on stress and non-stress cells of *L. monocytogene*s and *C. jejuni*.

The effect of freezing stress on *L. monocytogenes* was not as significant as on *C. jejuni* as it was previously reported [8]. *C. jejuni* was reduced in 2 log cfu/g after 48 h of freezing stress but was detectable during all the storage showing the capacity of survival both on refrigerate and freeze temperatures as other authors have described [9, 10]. In this study, the reduction of *L. monocytogenes* counts was achieved by the protective culture activity being higher in those stress cells (0.68 log cfu/g non-stress and 0.90 log cfu/g stress cells) at the end of the study. A widely number of studies have reported a positive effect on *L. monocytogenes* reduction by the application of protective culture [6, 7] *Lactobacillus sakei* CTC494 inoculated on raw chicken breast obtained a further

1.5-3 log reduction of *Listeria* spp. [5]. However, there was not effect of *Lc. pseudomesenteroides* PCK 18 activity on *C. jejuni*.

The use of MAP to extend food products shelf live can also contribute to reduce the counts of *C. jejuni* as it was observed in this study. Along the storage period *C. jejuni* was detected only in those samples packaged in air atmosphere. It has been reported that only rich-O_2 atmosphere has a positive effect on the reduction of *C. jejuni* [11, 12].

V. CONCLUSIONS

In this study it has been proved the positive effect of the application of *Leuconostoc pseudomesenteroides* PCK 18 and a rich-O_2 atmosphere to control and reduce the counts of *Listeria monocytogenes* and *Campylobacter jejuni* in fresh poultry hamburgers. The effect of this combination was higher in those cells subjected to freezing stress, thus this effect is more effective in those samples that simulate the real situation of pathogen cells in a poultry processing plant.

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