Combined application of modified atmosphere packaging and protective culture in fresh chicken legs against *Campylobacter jejuni*

Melero B.[1], Vinuesa R.[1], Osés S.M.[1], Diez A.M.[1], Gomez-Rojo E.M.[1], Wilches-Perez D.[1], Jaime I.[1] and Rovira J.[1]

[1] Burgos University, Department of Biotechnology and Food Science, Burgos, Spain

***Abstract*— In this study, a challenge test was performed to evaluate the combined effect of protective strain (*Bifidobacterium longum* PCB 133) and MAP (30% CO_2/70% N_2) on the inactivation of native microorganisms and on the *Campylobacter jejuni* inoculated strain (CECT 7572) on fresh chicken legs stored at 4ºC during 17 days. The inhibitory effect of *B. longum* PCB 133 against *C. jejuni* was not as strong in chicken legs as in vitro, however, it has been demonstrated that it has a positive effect on shelf life extension. When using MAP, chicken legs increased their shelf life in 3 days in comparison with air packed samples. When bioprotective culture is combined with MAP products expanded their shelf life 3 days more than when using MAP and 6 days more in comparison with air packaging legs.**

Keywords—**Biopreservation, *Campylobacter*, poultry.**

I. INTRODUCTION

The last report published by EFSA corresponding to the year 2009 pointed out that in this year, campylobacteriosis continued to be the most commonly reported gastrointestinal bacterial pathogen in humans in the European Union with 198,252 confirmed cases. Fresh broiler meat was once again the foodstuff with the higher proportion of Campylobacter-positive samples [1].

For this reason, several intervention strategies for reducing *Campylobacter* contamination have been applied such as carcass decontamination [2, 3]; freezing chicken carcasses [4]. Biopreservation can also be applied as a natural alternative for food preservation [5]. *Bifidobacteria* genus has the capacity of producing bacteriocins, being this one of their metabolic similarities with LAB [6, 7] mainly used in biopreservation. In 1994 a fulfilled study pointed out that *Bifidobacteria* inhibited a range of both Gram positive (*Listeria monocytogenes*, *Clostridium perfringens*) and Gram negative (*Campylobacter*, *Escherichia coli*) bacteria by the excretion of a particular metabolite because the culture media did not produce that effect. The degree of that antibacterial activity was variable, with the most potent effect generally exerted by *B. infantis* and *B. longum* [6].

The objectives of this study are: (1) to prove the capacity of MAP to extent the shelf life of poultry fresh legs. (2) to evaluate the efficacy of *Bifidobacterium longum* PCB 133 as protective culture against *Campylobacter jejuni*.

II. MATERIALS AND METHODS

A. Bacterial strains and inoculum preparation

A 100 ml inoculum of *Campylobacter jejuni* CECT 7572 (kept at -80C in 14 % of glycerol) was prepared in Ringer Solution (Oxoid, Basingstone, Hampshire, England) to raise a concentration in final product of 10^5 cfu/g.

Bifidobacterium longum PCB 133 (isolated from a new born infant) was used as a protective culture strain against *C. jejuni*. Inoculum was prepared by diluting in water a freeze-dried preparation containing 8.00 log cfu/g in order to achieve a final concentration on meat of 10^4 cfu/g.

One hundred and sixty chicken legs (right and left were selected at random in a slaughterhouse) were divided in 5 batches and submitted to the different treatments: a) control packaged in MAP (C); b) control packaged in air (A); c) chicken legs inoculated with 10^4 cfu/g of *B. longum* PCB 133 and water + MAP (B); d) chicken legs inoculated with 10^5 cfu/g of *C. jejuni* and water + MAP (J); e) chicken legs inoculated with 10^5 cfu/g of *C. jejuni* and 10^4 cfu/g of *B. longum* PCB 133 + MAP (BJ).

Two ml of *C. jejuni* inoculum were sprayed on the surface of both sides of J and BJ samples using a

hand-operated spraying. Batches B and BJ were sprayed on both sides with 2 ml of a solution of *B. longum*. With the purpose of reaching the same volume of liquid in all treated samples, batches J and B were also sprayed with 2 ml of water. Therefore, batches B, J and BJ were all sprayed with 4 ml in total. Control batches (A and C) were sprayed with 4 ml of water.

B. Packaging and storage conditions

Two chicken legs of each batch (except batch A) 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 cm3 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 30% CO_2/70% N_2 prepared using a gas mixer WITT-Gasetechnik (WITT-Gasetechnik GmbH & Co KG, Witten, Germany). The samples were stored at 4°C to carry out microbiological, physicochemical and sensory analyses at 0, 1, 3, 6, 9, 13 and 17 days. The analysis at day 0 was prior to packaging and they are the same samples for all the batches.

C. Physicochemical analyses

pH analysis was performed after microbiological analysis. The pH was measured with a pin electrode of a pH meter (micropH2001, CRISON, Barcelona, Spain) inserted directly into the sample. The result was expressed as a mean of three separate readings.

D. Microbiological analyses

C. jejuni was detected and quantified by conventional microbiology using the International Organization for Standardization (ISO) 10272-1:2006. Typical colonies were confirmed as indicated in the European standard.

Samples homogenized in BPW (AES Laboratoire) were diluted until the require decimal dilution in culture tubes with 9 ml of BPW (AES Laboratoire) and the following analysis were carried out on agar plates: total viable count bacteria (TVC) on plate count agar (PCA, Pronadisa, Torrejón de Ardoz, Madrid, Spain) for 48 h at 30ºC; Lactic acid bacteria (LAB) on MRS agar for 48- 72 h at 37 °C, *Bifidobacterium longum* PCB113 on MRS agar supplemented with 0.5% of L- Cystein (Sigma-Aldrich, Buchs, Switzerland) (MRSC) and incubated in anaerobic conditions generated by Anaerogen™ (Oxoid, AN0025A) for 48 h at 37ºC; yeast on Sabouraud agar (SB) for 5 days at 30ºC; *Brochothrix thermosphacta* on STAA agar base (Oxoid) supplemented with STA Selective Suplement (Oxoid, SR0162) for 48 h at 25 °C.

E. Statistical analyses

Bacterial counts were converted to base-10 logarithm colony-forming units per gram of meat. Means were analyzed for differences using ANOVA procedures. Where appropriate, means were ranked using the Least Significance Difference test ($LSD_{0.05}$). Data analyses were conducted using the statistical package Statsgraphics Plus for Windows ver. For all tests, a $P \leq 0.05$ was considered significant.

III. RESULTS

The pH of all samples on day 1 after inoculation was between 6.13 - 6.36. Differences between control samples (C and A) were no significant on every day of analysis, as well as differences between inoculated samples, especially between J and BJ. However, there were significant differences between these 2 groups (inoculated and no inoculated). (Data not shown).

A significant reduction ($P < 0.05$) of *C. jejuni* is achieved by the protective culture activity in the sample where they were co-inoculated (BJ) between days 3 and 13 (Fig. 1). There were no significant differences in *C. jejuni* counts along the storage in sample B remaining more stable than in control samples (A and C).

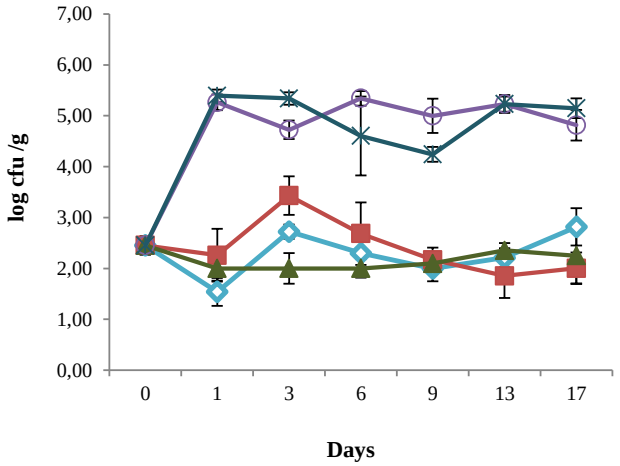

Fig. 1 Evolution of *C. jejuni* inoculated onto poultry legs subjected to different treatments. Symbols: ◊ Control C; ■ Control A; ▲Sample B; ○ Sample J; × Sample B.

.*Bifidobacterium longum* PCB 133 counts are collected in Table 1. Results showed a similar behaviour between B and BJ samples until the ninth day of storage. Later on, it was observed a significant increase in BJ values, being at the end of the storage more than two logarithmic cycles higher than B value that maintained in the same line. Statistic results confirmed this parallel behaviour until day 9, establishing significant differences between both batches on day 13 and 17. Control air sample (A) was the first batch to deteriorate (Table 1).

IV. DISCUSSION

A reduction in *Campylobacter* concentration on chicken carcasses could help significantly in reducing the number of human campylobacteriosis cases. In this study the strategy tried for achieving that reduction on contamination was the use of *Bifidobacterium longum* PCB 133 as a bioprotective culture.

The bioprotective culture provided successful results since the inhibitory effect of *B. longum* PCB 133 against *C. jejuni* CECT 7572 reached a reduction of 0.5 log cfu/g at day 9, which is the limit of the product shelf life determined by sensory (Data not shown) and microbiological parameters (see below). This reduction substantiated the hypothesis of the effectiveness of *B. longum* against the pathogen and might suggest that the outer membrane of Gram negative bacteria is not as insensitive to bacteriocins as thought previously. From day 9 and onwards the counts of *C. jejuni* reached the level of the initial inoculum.

Although in this study, MAP cannot be relied on as a reduction strategy to control *Campylobacter* in chicken legs, it was demonstrated by the microbial evolution that MAP packed meat got spoiled at least 3 days later than with air packaging. Those samples inoculated with the protective culture (B and BJ) got spoiled 3 days later than the control packaged in MAP (C) and 6 days later than the control packaged in air (A). An arbitrary value for TVC of 7 log cfu/ g was taken for the upper acceptability limit of fresh chicken meat. Even though this fact helps to confirm that modified atmospheres have a major effect in prolonging the shelf life of poultry products, it should be emphasized that the injection of different gas mixtures alone will not guarantee satisfactory results. Only a combination of CO_2, low storage temperatures and satisfactory hygiene conditions can guarantee significant and reliable shelf life extension [8].

V. CONCLUSIONS

It has been proved both that *Bifidobacteriunm longum* PCB 133 has a slightly reduction effect against *Campylobacter jejuni* CECT 7572 till the end of shelf life consumption, and its positive effect on shelf life extension. This conclusion was supported by the experimental results which showed that, when using the modified atmosphere 30 CO_2 / 70 N_2 chicken legs increased their shelf life in 3 days in comparison with air packed samples. And what is more, when the treatment included the bioprotective culture it was observed that chicken legs expanded their shelf life 3 days more than when using the modified atmosphere alone, and 6 days more in comparison with the air packaging treatment.

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Table 1: Microbial evolution (log cfu/g) along the storage period

	Days						
	0	1	3	6	9	13	17
Total Viable Counts							
C	$^{a}4{,}45_{A}$	$^{b}4{,}87_{A}$	$^{c}5{,}35_{A}$	$^{d}7{,}01_{C}$	$^{e}8{,}03_{B}$	$^{f}8{,}63_{B}$	$^{g}8{,}96_{B}$
A	$^{a}4{,}45_{A}$	$^{b}5{,}01_{AB}$	$^{c}6{,}72_{C}$	$^{d}8{,}17_{D}$	$^{e}9{,}00_{C}$	$^{f}9{,}60_{C}$	$^{g}9{,}94_{C}$
B	$^{a}4{,}45_{A}$	$^{b}4{,}88_{A}$	$^{c}5{,}36_{A}$	$^{d}6{,}30_{A}$	$^{e}7{,}11_{A}$	$^{f}8{,}15_{A}$	$^{g}8{,}96_{B}$
J	$^{a}4{,}45_{A}$	$^{b}5{,}32_{B}$	$^{c}6{,}02_{BC}$	$^{d}7{,}00_{C}$	$^{e}7{,}88_{B}$	$^{f}8{,}81_{B}$	$^{g}9{,}12_{B}$
BJ	$^{a}4{,}45_{A}$	$^{b}4{,}87_{A}$	$^{c}5{,}47_{AB}$	$^{d}6{,}54_{B}$	$^{e}7{,}33_{A}$	$^{f}8{,}13_{A}$	$^{g}8{,}35_{A}$
Lactic Acid Bacteria							
C	$^{a}4{,}4_{A}$	$^{b}3{,}80_{A}$	$^{a}4{,}82_{A}$	$^{c}6{,}09_{AB}$	$^{d}7{,}45_{B}$	$^{e}8{,}50_{B}$	$^{e}8{,}36_{A}$
A	$^{a}4{,}4_{A}$	$^{b}3{,}95_{AB}$	$^{c}5{,}37_{AB}$	$^{d}7{,}00_{C}$	$^{e}7{,}50_{B}$	$^{f}8{,}47_{B}$	$^{f}8{,}32_{A}$
B	$^{a}4{,}4_{A}$	$^{a}4{,}25_{ABC}$	$^{b}5{,}27_{AB}$	$^{c}5{,}89_{A}$	$^{d}5{,}57_{A}$	$^{e}7{,}87_{A}$	$^{e}8{,}12_{A}$
J	$^{a}4{,}4_{A}$	$^{a}4{,}59_{C}$	$^{b}5{,}51_{B}$	$^{c}6{,}33_{B}$	$^{d}7{,}61_{B}$	$^{e}8{,}32_{B}$	$^{e}8{,}19_{A}$
BJ	$^{a}4{,}4_{A}$	$^{a}4{,}49_{BC}$	$^{b}5{,}45_{AB}$	$^{c}6{,}06_{A}$	$^{d}6{,}82_{A}$	$^{e}7{,}85_{A}$	$^{e}7{,}89_{A}$
Bifidobacterium longum PCB 133							
B	$^{ab}3{,}87_{A}$	$^{abc}4{,}08_{A}$	$^{c}4{,}26_{AB}$	$^{abc}4{,}00_{A}$	$^{bc}4{,}15_{AB}$	$^{abc}4{,}08_{A}$	$^{a}3{,}74_{A}$
BJ	$^{a}3{,}87_{A}$	$^{b}4{,}30_{A}$	$^{c}4{,}55_{B}$	$^{a}3{,}96_{A}$	$^{a}4{,}06_{A}$	$^{d}5{,}02_{B}$	$^{e}6{,}19_{B}$
Brochrothrix thermosphacta							
C	$^{a}2{,}65_{A}$	$^{b}3{,}84_{BC}$	$^{c}4{,}2_{A}$	$^{d}6{,}7_{C}$	$^{e}7{,}43_{B}$	$^{f}8{,}4_{B}$	$^{f}8{,}13_{BC}$
A	$^{a}2{,}65_{A}$	$^{b}3{,}45_{A}$	$^{c}5{,}92_{C}$	$^{d}7{,}34_{D}$	$^{e}7{,}78_{B}$	$^{f}8{,}34_{B}$	$^{f}8{,}11_{BC}$
B	$^{a}2{,}65_{A}$	$^{b}3{,}55_{AB}$	$^{c}4{,}28_{A}$	$^{d}6{,}31_{B}$	$^{e}7{,}09_{AB}$	$^{f}7{,}74_{A}$	$^{g}8{,}34_{C}$
J	$^{a}2{,}65_{A}$	$^{b}3{,}88_{C}$	$^{c}5{,}22_{B}$	$^{c}5{,}75_{A}$	$^{d}6{,}57_{A}$	$^{e}8{,}54_{B}$	$^{f}7{,}74_{AB}$
BJ	$^{a}2{,}65_{A}$	$^{b}3{,}42_{A}$	$^{c}4{,}45_{A}$	$^{d}5{,}96_{A}$	$^{e}7{,}39_{AB}$	$^{e}7{,}44_{A}$	$^{e}7{,}54_{A}$

Means in the same line with different lower case letter are significantly different (P<0.05)
Means in the same column with different capital letter are significantly different (P<0.05)

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