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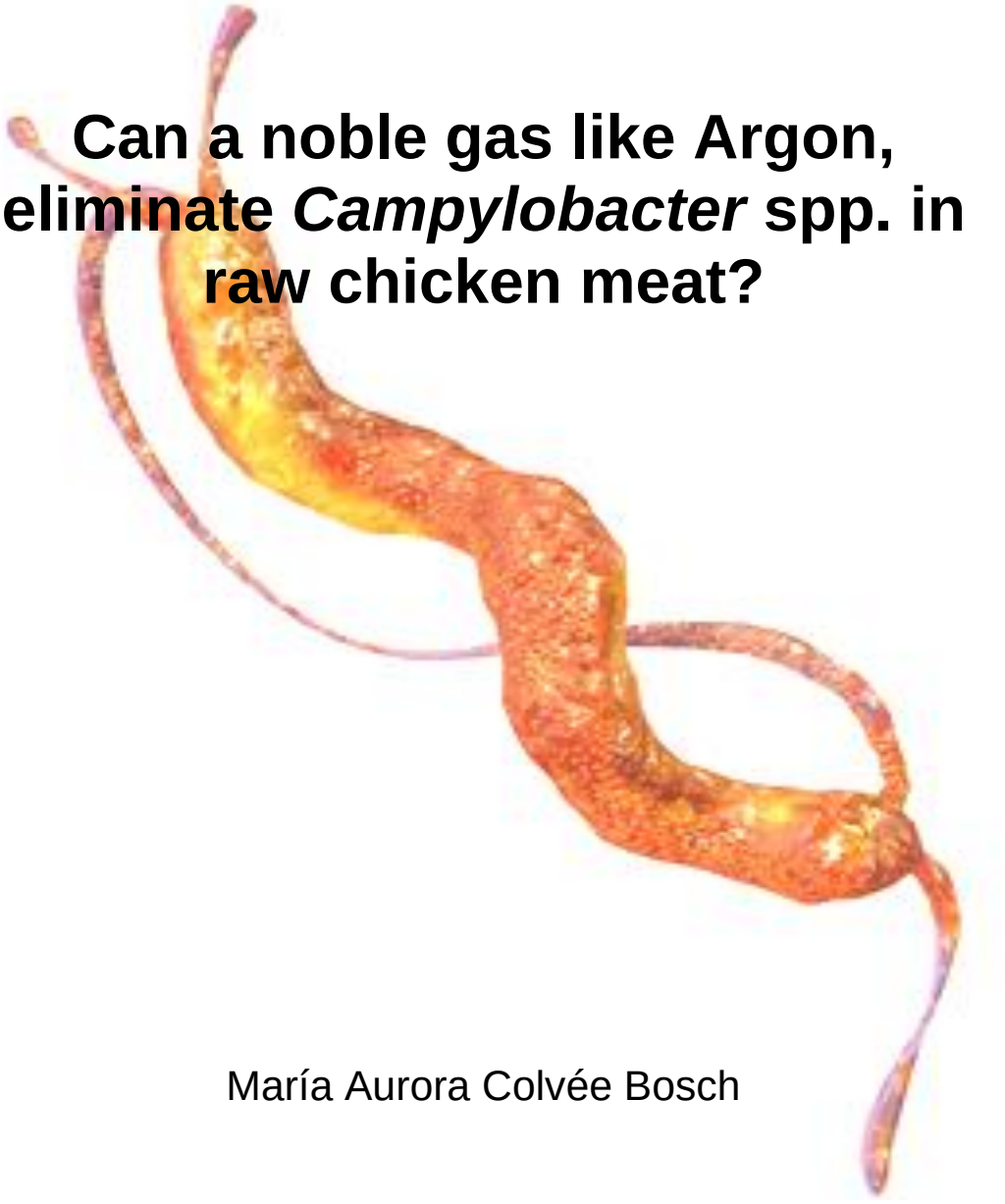

María Aurora Colvée Bosch

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GRADO EN VETERINARIA

Can a noble gas like Argon, eliminate *Campylobacter* spp. in raw chicken meat?

(B)
Trabajo original experimental

María Aurora Colvée Bosch

Clara Marín Orenga

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RESUMEN

Campylobacter es la principal causa de gastroenteritis en humanos, siendo la especie C. *jejuni* la más frecuentemente aislada en infecciones humanas (EFSA, 2015). La principal vía de infección para los humanos es el consumo de carne de pollo poco cocinada (Jacobs-Reitsma, 2000). Para poder controlar esta bacteria en el producto final, se han estudiado distintas medidas de control. Sin embargo, debido a la demanda por parte del consumidor de una carne poco procesada, la técnica más popular es el uso de atmósferas modificadas en el envasado. Por esta razón, el objetivo de este estudio es investigar y comparar el efecto de distintas atmósferas modificadas (AM): AM-A: 50%/50% N_2 / CO_2. AM-B: 50%/50% N_2 / O_2. AM-C: 30%/70% O_2 / CO_2. AM-D: 50%/50% N_2 / Ar, en la supervivencia de *C.jejuni.* Así como, determinar el efecto de estos gases en las características fisicoquímicas y sensoriales de la carne de pollo, almacenada a una temperatura de conservación de 4ºC. De acuerdo con los resultados de este estudio, el crecimiento de *Campylobacter* fue inhibido por aquellas atmósferas compuestas por un alto porcentaje de Oxígeno (>50%) y aquellas con Argón. En cuanto a los análisis fisicoquímicos, la carne de pollo envasada con la atmósfera estándar (AM-C), presentó carne de color muy pálido. Finalmente, tanto la AM-B con 50% de oxígeno como la AM-D con 50% de Argón han sido eficaces para el control de *Campylobacter*. Sin embargo, la atmósfera con Argón (AM-D) presento una mejor evaluación sensorial, por lo que resulta una alternativa idónea y novedosa para controlar esta bacteria.

Palabras clave: *Campylobacter*, atmósferas modificadas (AM), carne de pollo.

ABSTRACT

Campylobacter is the most common bacterial cause of human gastrointestinal disease, being C. *jejuni* the main species isolated in human infections (EFSA, 2015). The principal way of infecting humans is by the consumption of undercooked poultry or other food products cross-contaminated with raw poultry meat during food preparation (Jacobs-Reitsma, 2000). For the control of this bacterium in the final product different techniques have been studied. However, because of the consumer preferences for minimally processed foods, the most attractive technology is modified atmosphere packaging. For that reason the aim of this study was to investigate and compare the effect of different modified atmospheres packaging (MAP): MAP-A: 50%/50% N_2 / CO_2. MAP-B: 50%/50% N_2 / O_2. MAP-C: 30%/70% O_2 / CO_2. MAP-D: 50%/50% N_2 / Ar on the survival of *C. jejuni*. And at the same time, determine the effect of these gas mixtures on physical-chemical and sensorial qualities in raw chicken meat fillets, during storage at 4 ºC. According to the results, *Campylobacter* growth was inhibited by the application of MAPs with a high O_2 concentration (≥50%) and with Ar. In the physical-chemical analysis, the chicken meat fillets packaged in a standard poultry meat atmosphere (MAP-C) resulted in a pale chicken meat colour. In conclusion, both AM-B with 50% of oxygen and AM-D with 50% of Argon were the best treatments to control *Campylobacte*r. Nevertheless, the atmosphere composed with Argon had a better sensory evaluation; in consequence, Argon can be a good and new alternative in the control of this bacteria.

Key words: *Campylobacter*, modified atmospheres packaging (MAP), poultry meat.

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LIST OF ABREVIATIONS

a*	±, red/green
AVEC	Association of Poultry Processors and Poultry Trade in the EU Countries
Ar	Argon
b*	±, yellow/blue
BHI	Heart Infusion Broth
BPW	Buffered Peptone Water
º C	Degrees Celsius
CFU	Colony-forming units
CO_2	Carbon doxide
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
g	Grams
h	Hours
He	Helium
ISO	International Organization for Standardization
L*	±, lightness/ darkness
MAP	Modified atmosphere packaging
mCCDA	Modified Cefoperazone Charcoal Deoxycholate agar
min	Minutes
mL	Millilitres
MRD	Maximum Recovery Diluent
n	Number of observations
N_2	Nitrogen
nm	Nanometers
N_2O	Nitrous Oxide
O_2	Oxygen
OD_{600}	Optical Density at 600 nm

P	Probability value
rpm	Revolutions per minute
s	Seconds
SD	Standard Deviation
spp	Species (plural)
UK	United Kingdom
USA	United States of America
UV	Ultraviolet
WHO	World Health Organisation
µl	Microliters

I. INTRODUCTION

Campylobacter is the most common bacterial cause of human gastrointestinal disease in developed countries. This bacterium produces 214.779 cases a year in Europe (EFSA, 2015). *Campylobacter jejuni* followed by *Campylobacter coli* are the main species responsible for the human cases (Sopwith *et al*., 2010).

In humans, the common symptoms are acute gastroenteritis, abdominal pain, fever, vomiting and headaches (WHO, 2011). Usually the infection by this bacterium is self-limiting; however several complications such as arthritis, septicemia and Guillain-Barre syndrome can occur (Nachamkin et al., 1998; Wassenaar y Blaser, 1999).

It is generally accepted that the principal way of infecting humans are poultry and poultry products (EFSA, 2015), specifically by the consumption of undercooked poultry or other food products cross-contaminated with raw poultry meat during food preparation (Jacobs-Reitsma, 2000; Corry and Atabay, 2001). In addition, it has been shown that *Campylobacter* persists on chicken skin during poultry processing because of its ability to attach to the skin and become entrapped in deeper skin layers, crevices, or feather follicles. These sites may provide a suitable microenvironment for bacteria to lodge (Gloaguen *et al*., 2011; Notermans and Kampelmacher, 1975; McMeekin *et al*., 1984; Hardy *et al*, 2013).

Considering that consumption of poultry meat has been increasing in the last two decades due to its nutritional profile, versatility and low price, the control of the microbial quality of poultry meat is a crucial concern for the food industry (Henchion *et al*., 2014; AVEC, 2013). Chicken meat is a highly perishable product even when stored in chilled conditions, and its normal shelf life is less than 5 days after slaughter (Holck *et al*., 2014). Consequently, a combination of different conservation techniques, such as chilled storage, modified atmosphere packaging (MAP), freezing and preservative methods are necessary to lower the potential of foodborne

illness and to extend the product's shelf-life (Kozačinski *et al*., 2012; Chiavaro e*t al*., 2008). However, since the consumers are demanding minimally processed foods, the most attractive technology is modified atmosphere packaging (Melero *et al*., 2012). MAP contributes to microbial and lipid oxidation stability of poultry meat and prolongs their shelf-life compared to those packaged in ambient conditions (Fraqueza and Barreto, 2009). Additionally, MAP is related to product safety leading to an effective reduction of pathogenic microorganisms like *Campylobacter* spp. (Boysen *et al*., 2007).

Several gases can be used in MAP, each one having a different role in the preservation of food products. The most common gases used in modified atmospheres are carbon dioxide (CO_2), oxygen (O_2), and nitrogen (N_2). O_2 has been widely used to maintain red fresh meat, since color is important to consumers in determining their selection of the product (Cornforth and Hunt, 2008). However, poultry meat has low concentration of myoglobin and color is not greatly influenced by the O_2 concentration (Lund et al., 2007a, b). Nevertheless, it has been reported that O_2 can enhance the growth of aerobic and also restrict the growth of strictly anaerobic microorganisms (Lund et al., 2011). Otherwise, the presence of CO_2 in the headspace of the packages inhibits microbial growth and causes a change in the microbial content to the bacteria with lower spoilage capacity (McMillin, 2008). N_2 has minimal effect on metabolic reactions due to its low solubility in water and fat, and it is used as a filler gas in meat products.

Moreover, other gases such as Argon (Ar), Helium (He), and Nitrous oxide (N_2O) are allowed in meat packaging in the European Union (EU, 1995, directive 92/02/CE). Ar is chemically inert noble gas, odourless and tasteless (Morgan 2007), and it is believed to have biochemical properties such as interference with enzymatic oxygen receptor sites and protein that cause food spoilage (Spencer et al., 2002). Regarding inhibitory activity against bacterial growth, Ar was suggested to have a better solubility in

fat, resulting in improved membrane permeability to CO_2, salts, and acids of bacterial cells (Jaime and Saltveit, 2002). These properties offer certain advantages over the N_2, O_2 or CO_2 atmospheres resulting in an increase of shelf life and freshness of packaged foods (Fachon, 2002).

II. OBJECTIVES

In this context, the aims of the present study were to investigate and compare the effect of different modified atmospheres packaging (MAP) with traditional gases (N_2, O_2 or CO_2) and innovative noble gas Ar, on survival of *C. jejuni* and determine the effect of these gas mixtures on physical-chemical and sensorial qualities in raw chicken breast fillets, during storage at 4 ºC.

III. MATERIAL AND METHODS

Bacterial strains and culture preparation

In order to assess the effect of different modified atmosphere packaging, on the survival of *Campylobacter* spp. on raw chicken meat fillets, the most frequently isolated strains *C. jejuni* from a previous study of broiler chicken carcass contamination were used. *C. jejuni* strain were plated onto Columbia Blood agar plate supplemented with 5% horse blood (Oxoid, Barcelona, Spain) and incubated at 41,5 ºC for 48 h under microaerobic atmosphere (5% O_2, 10% CO_2, 85% N_2) generated by CampyGen® (Oxoid, Barcelona, Spain). Single colonies of *Campylobacter* were transferred into brain heart infusion broth (BHI, Oxoid, Barcelona, Spain) and were incubated under microaerobic atmosphere as describe above to enrich cell numbers. Following the incubation period, the culture optical density was measured at 600 nm (OD_{600}) by spectrophotometer (UV-1, Thermo Electron Corporation, Cambridge, UK). The culture was diluted using broth (BHI), to give a final OD_{600} = 0.2 (6 log10 CFU ml^{-1})

and incubated under conditions as described above. Then, the batches of culture were serially centrifuged in 50 mL falcon tubes at 3,000 rpm for 10 min. to recover the precipitated colonies. The cell pellets were washed with maximum recovery diluent (MRD, Liofilchem ®, Barcelona, Spain) followed by centrifugation at 3,000 rpm for 10 min. The process was repeated 3 times. Finally, the cell pellets were resuspended in MRD to achieve the final concentration of 6 log10 CFU ml^{-1} (OD_{600} = 0.2) for the isolated serovar.

Preparation of samples and modified atmosphere packaging (MAP)

Chicken carcasses from the same batch were provided by a local poultry slaughterhouse (Valencia, Spain). Samples were collected in sterile plastic bags and transported to the laboratory at ambient temperature. On arriving, samples were stored at 4ºC and processed within 1 h of slaughter.

A total of 216 pieces (For each atmosphere treatment 54 pieces, 18 inoculated with *C.jejuni* and 36 for the physicochemical and sensorial analysis that were not inoculated) of chicken breast fillets were aseptically cut to have a standardized surface area and weight (25g). Then, samples were placed into polypropylene trays (Amcor Flexibles, Barcelona, Spain) to evaluate the effect of different modified atmospheres on the control of chicken meat fillets.

Modified atmosphere conditions were obtained by flushing the trays with four gas mixtures (MAP-A: 50%/50% N_2 / CO_2. MAP-B: 50%/50% N_2/ O_2. MAP-C: 30%/70% O_2 / CO_2. MAP-D: 50%/50% N_2 / Ar.). For each MAP condition, a total of 54 chicken meat fillet samples were used (Figure 1). Thermo sealing was done in an ULMA-Smart 300 packing machine (Oñati, Spain) using a non permeable polypropylene film (Amcor Flexibles, Barcelona, Spain). The gas concentrations in all packages were measured by a gas analyser (Dansensor, Ringsted, Denmark).

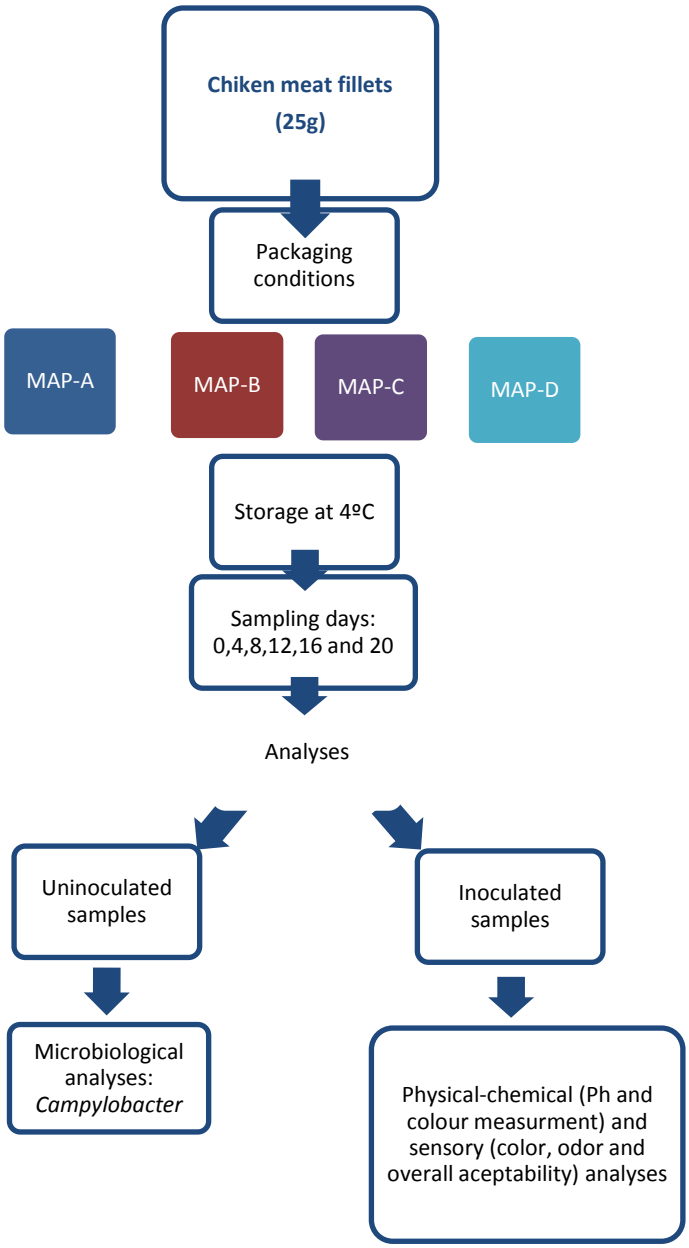

Figure 1. *Design of the experiment with modified atmospheres and chicken meat fillets. MAP conditions (MAP-A: 50%/50% N_2/CO_2. MAP-B: 50%/50% N_2/O_2. MAP-C: 30%/70% O_2/CO_2. MAP-D: 50%/50% N2/Ar).*

Inoculation of chicken meat fillets

Campylobacter culture was inoculated in 18 samples of each MAP. Previously, a septum was placed onto the film surface of each tray. Then, a syringe was introduced through the septum to spread 100 µl of inoculum onto the surface of each chicken meat fillet sample, to avoid changing inside MAP conditions. The initial concentration of bacteria on the fillets was 6.5±0.2 log10 CFU/g. Finally, samples were stored at 4 ºC without exposure to light.

Microbiological analysis

The enumeration of *C. jejuni* for the inoculated chicken meat fillets packaged was carried out at 0, 4, 8, 12, 16 and 20 days of storage (sampling days). The numbers of pathogens on three independent chicken meat fillet packages were determined by following the ISO: 10272-1 (Annex E). Starting by washing the samples in 225 mL of 0.1% buffered peptone water (BWP, AES, Valencia, Spain) and stomaching them at 230 rpm for 120 s (Stomacher®400 circulator, Seward Ltd., Worthing, UK). Then, this initial dilution was subsequently serial-fold diluted at least six times, and 100 µl of each dilution was spread onto mCCDA (AES laboratories®, Bruz Cedex, France) agar plates and incubated microaerobically at 41,5ºC for 48h. Colonies displaying *Campylobacter* characteristics in each agar were counted. Eight randomly suspect colonies (two suspect colonies per each count plate and treatment) from each sampling day of storage were analysed to confirm the validity of the counts. Cellular morphology and typical motility was evaluated for *Campylobacter* colonies confirmation using microscope view under dark ground.

Physical-Chemical Analysis

The other group of the chicken fillets meat samples packaged in each MAP (36 uninoculated samples) was use for physical-chemical and sensory analysis. After packaging, samples were stored at 4 ºC, and the

analyses were carried out at the same sampling day of storage of inoculated samples.

The pH values were determined with a portable pH meter equipped with a pH electrode (Thermo scientific). The results were expressed as the mean of three values acquired on different area of three chicken meat fillet samples (n=9) per MAP and sampling day.

Color measurements were determined with a Minolta (Model CR-300, Ramsey, N.Y., USA) on the surface of three chicken meat fillets per MAP and sampling day, approximately 30 min after opening the package. The CIEL*a*b* color space was used, in which L* (±, lightness/ darkness), a* (±, red/green), and b* (±, yellow/blue) values were determined at three different area on each chicken meat fillet. Higher L* values describe samples having lighter color, whereas lower L* values those having darker color. Positive a* values were related with samples with redder color, whereas a negative a* values describe those with greener color. A standard white calibration plate was employed to calibrate the equipment.

Sensory analysis

Acceptance test was used in the sensory evaluation. Visual quality of the chicken meat fillet samples was assessed by a total of 6 untrained judges per each MAP and sampling day. Each sample was coded, presented in random order and the attributes of appearance, odor and overall acceptability were evaluated using a five point hedonic scale as described in the *table 1*. At each sampling day, panelists were presented with freshly cut untreated chicken meat fillet as a reference.

Statistical Analysis

Statistical analysis was done using STATGRAPHICS Plus 4.1 (Manugistic Inc., Rockville, MD, USA). The differences between means were determined by least significant difference (LSD) applied after the analysis of variance (ANOVA). Significance differences were defined at $P \leq 0.05$.

IV. RESULTS

Campylobacter counts

In samples packaged under MAP-A, the results of *Campylobater* counts at inoculated chicken breast fillets were 6.73 log_{10} CFU/g, 6.17 log_{10} CFU/g, 6,39 log_{10} CFU/g, 5,91 log_{10} CFU/g, 5,86 log_{10} CFU/g and 5.911 log_{10} CFU/g for 4, 8, 12, 16 and 20 sampling day, respectively. The *Campylobacter* concentration slightly decreased in samples stored under MAP-A until 12 days of storage. After that, the *Campylobacter* counts maintained similar values, showing no significant differences at 16 and 20 days of storage at 4 ºC (P = 0.076) (Figure 2).

At the beginning of the storage, application of MAP-B (50%/50% N_2 / O_2) and standard poultry meat atmosphere (MAP-C) in chicken breast fillets helped to controlling *Campylobacter* growth. After 4 days of storage at 4 ºC, a total inhibition of *Campylobacter* was observed in samples packaged under both modified atmosphere conditions (Figure 2).

For samples packaged under MAP-D, the *Campylobacter* concentration significantly decreased, reaching values of 4.30 log_{10} CFU/g, 3.56 log_{10} CFU/g, 3.64 log_{10} CFU/g for 4, 8 and 12 sampling day, respectively *(P* = 0.00, Figure 16). After that, a total inhibition of *Campylobacter* colonies was observed till the end of the storage at 4ºC (Figure 2).

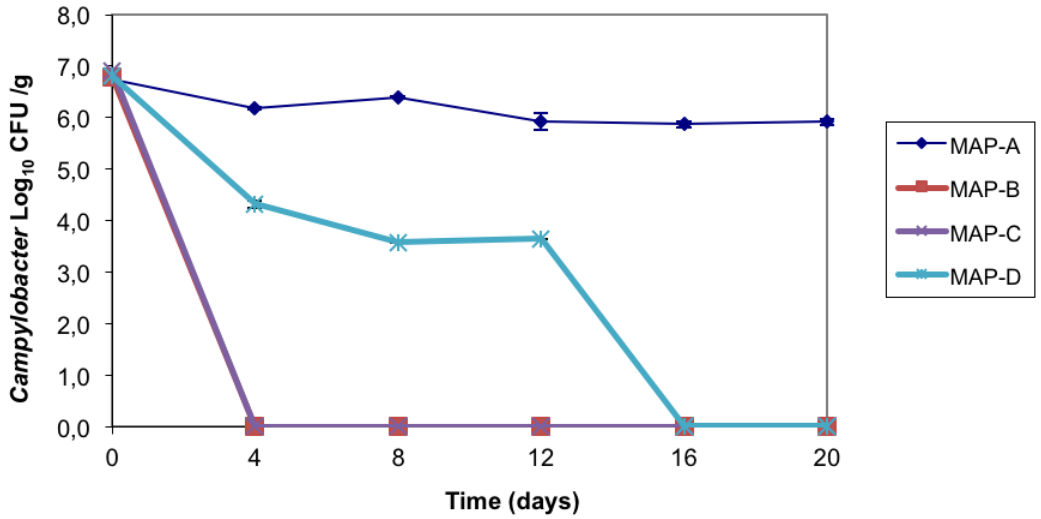

Figure 2. *Campylobacter growth (Log CFU/g ± S. D) at each sampling days, of inoculated chicken breast fillets packaged under MAP conditions (MAP-A: 50%/50% N_2 / CO_2. MAP-B: 50%/50% N_2 / O_2. MAP-C: 30%/70% O_2 / CO_2. MAP-D: 50%/50% N2 / Ar.) during 20 days of storage at 4 ºC. Vertical bars represent standard deviation.*

pH determination

The initial pH (day 0) of chicken breast fillets stored under the different MAPs studied was 5.2 ± 0.2. During the first 8 days of storage, no significantly differences on pH values were observed among the samples stored under different modified atmosphere ($P > 0.050$). After that, MAP-B showed significant difference compare to the rest of the MAPs studied ($P < 0.050$). The application of MAP-B at chicken breast fillets showed that, during the storage there was an increase of the pH value by 5.3, 5.3, 5,5, 5.6 and 5,6 for 4, 8, 12, 16 and 20 sampling day, respectively, observing significantly increase from 8 day till the end of the storage ($P = 0.000$) (Figure 3).

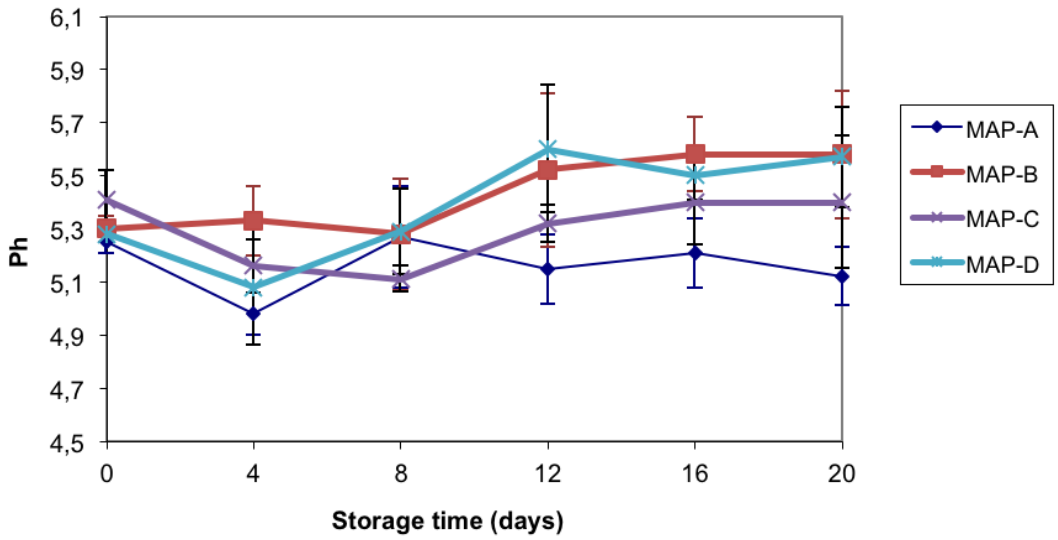

Figure 3. *Changes in pH of inoculated chicken breast fillets packaged under MAP conditions (MAP-A: 50%/50% N_2 / CO_2. MAP-B: 50%/50% N_2 / O_2. MAP-C: 30%/70% O_2 / CO_2. MAP-D: 50%/50% N_2 / Ar.) during 20 days of storage at 4 ºC. Vertical bars represent standard deviation.*

Colour measurement

The initial values of L* a* and b* parameters (day 0) of chicken breast fillets stored under MAP-A, MAP-B, MAP-C and MAP-D (standard poultry meat MAP) were 57.2±0.9, 0.6±0.2, 5.4± 0.9, respectively.

The L* value showed no significant differences during the storage for chicken breast fillets packaged under MAP-A, MAP-B and MAP-D (P > 0.050). However, the L* values of the samples packaged under standard poultry meat MAP (MAP-D) increased progressively up to day 20 of the storage (P = 0.000) (Figure 4). The a* value showed no statistical changes throughout the storage of samples packaged under MAP-A, MAP-B and MAP-D (P > 0.050). Nevertheless, chicken meat fillet samples packaged under standard poultry meat atmosphere (MAP-C) presented a significant decrease during the storage reaching lower a* values at the end of the period (P = 0.023) (Figure 5). Lastly, b* value showed no statistical changes throughout the storage of samples packaged under MAP´s studied (P > 0.050). Data not shown.

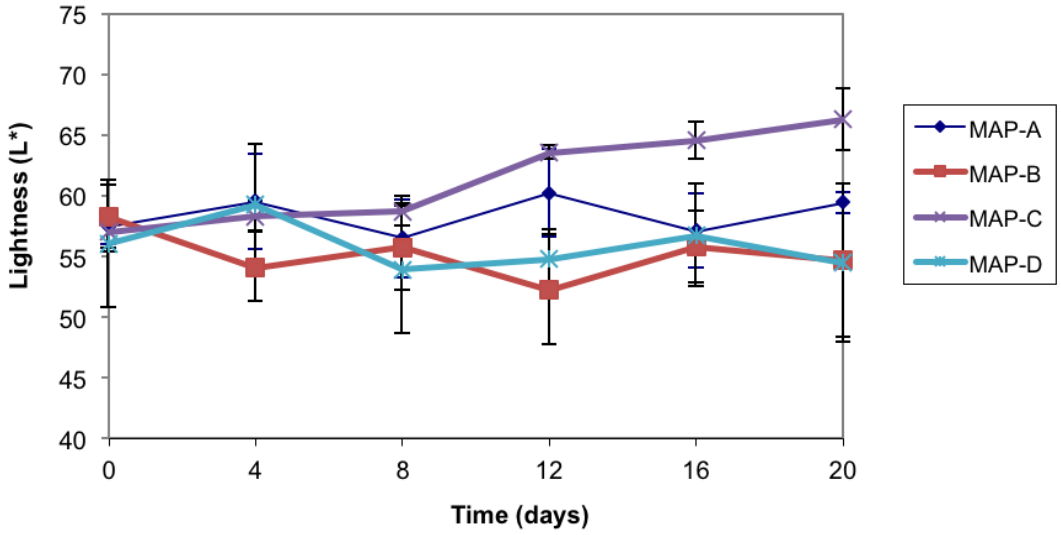

Figure 4. *Colour changes of L* values from inoculated chicken breast fillets packaged under MAP conditions (MAP-A: 50%/50% N_2 / CO_2. MAP-B: 50%/50% N_2 / O_2. MAP-C: 30%/70% O_2 / CO_2. MAP-D: 50%/50% N_2 / Ar.) during 20 days of storage at 4 ºC. Vertical bars represent standard deviation.*

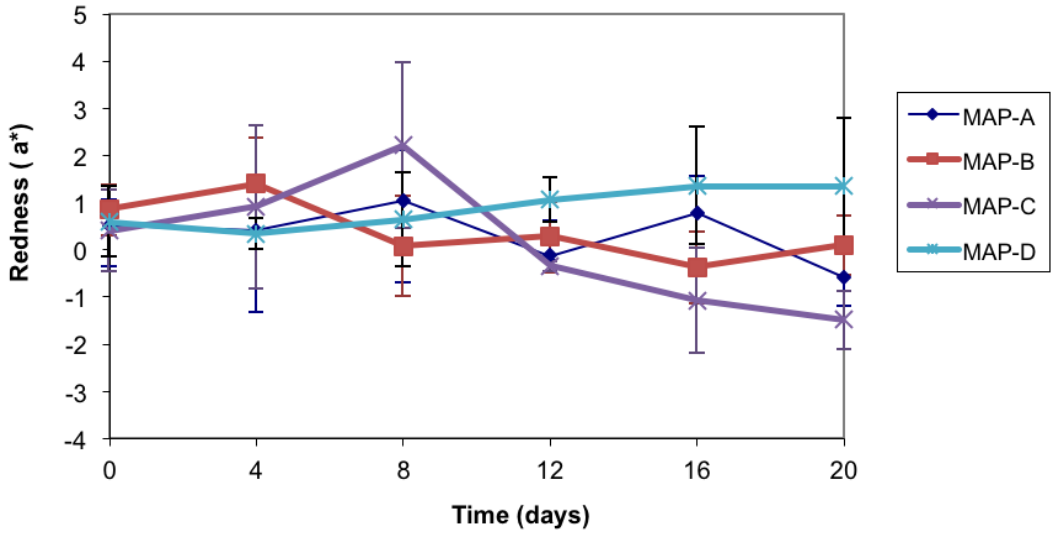

Figure 5. *Colour changes of a* values from inoculated chicken breast fillets packaged under MAP conditions (MAP-A: 50%/50% N_2 / CO_2. MAP-B: 50%/50% N_2 / O_2. MAP-C: 30%/70% O_2 / CO_2. MAP-D: 50%/50% N_2 / Ar.) during 20 days of storage at 4 ºC. Vertical bars represent standard deviation.*

Sensory analysis

Regarding the sensory evaluation, the initial scores of appearance, odor and acceptability (day 0) of chicken breast fillets stored under the different MAPs studied was 5 (the highest score) (Table 1).

Table 1, Hedonic scale for appearance, odor and overall acceptability for chicken meat fillets.

Measurement	Score	Quality description
Appearance	1	Dislike extremely; very poor, not usable.
	2	Dislike moderately; poor, excessive defects, limited marketability.
	3	Neither like nor dislike; borderline, fair, slightly to moderately objectionable defects, lower limit of appeal.
	4	Like moderately; good, minor defects, not objectionable.
	5	Like extremely; excellent, essentially free from defects, fresh-like and
Odor	1	Dislike extremely.
	2	Unacceptable; poor, stale, musty, and moldy.
	3	Fairly acceptable.
	4	Good; not objectionable, acceptable.
	5	Excellent; typical, very much acceptable.
Overall	1	Dislike extremely, very poor.
	2	Dislike moderately, poor.
	3	Neither like nor dislike; fair, limited marketability.
	4	Very good; will definitely buy.
	5	Extremely good; most definitely buy.

Appearance

Chicken breast fillets samples packaged under MAP-A, MAP-B, MAP-D were evaluated by the judges below the limit of acceptability of appearance (score as 3) (Table 1) after 12, 4 and 12 days of storage, respectively. On the other hand, samples stored under MAP-C were scored over the limit of acceptability of appearance throughout the storage period. A Significant decrease of appearance evaluation was observed among the different modified atmospheres within each sampling day ($P < 0.05$) (Figure 6).

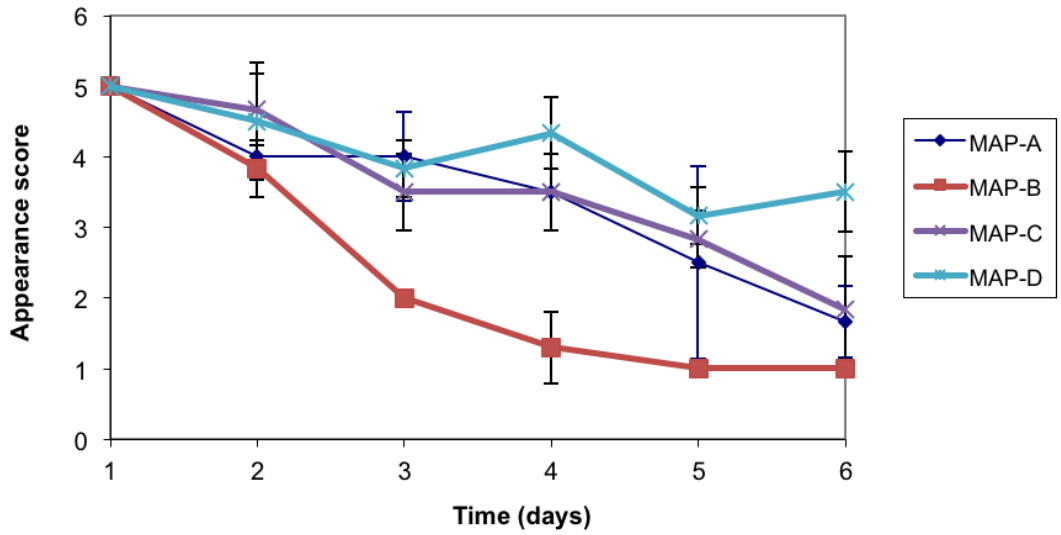

Figure 6. *Appearance score of inoculated chicken breast fillets packaged under MAP conditions (MAP-A: 50%/50% N_2 / CO_2. MAP-B: 50%/50% N_2 / O_2. MAP-C: 30%/70% O_2 / CO_2. MAP-D: 50%/50% N_2 / Ar.) during 20 days of storage at 4 ºC. The limit of acceptability of appearance evaluation (3 score) is represent by the black line. Visual appearance was based on a visual scale (5 = Like extremely; 4 = like moderately, 3 = neither like; 2 = dislike moderately; and 1 = dislike extremely). Vertical bars represent standard deviation.*

Odor

Chicken breast fillets samples packaged under MAP-A, MAP-B, MAP-C and MAP-D were evaluated by the judges below the limit of acceptability of odor (score as 3) (Table 1) after 12, 4 and 8 days of storage, respectively. The odor evaluation for all the samples packaged under MAPs studied decrease significantly during the storage period ($P < 0.05$) (Figure 7).

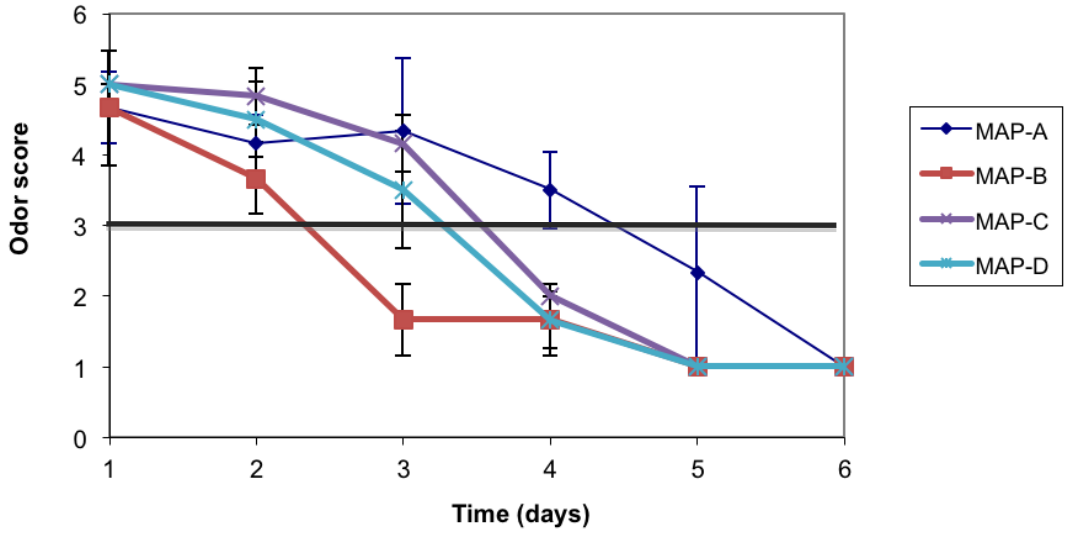

Figure 7. *Odor score of inoculated chicken breast fillets packaged under MAP conditions (MAP-A: 50%/50% N_2 / CO_2. MAP-B: 50%/50% N_2 / O_2. MAP-C: 30%/70% O_2 / CO_2. MAP-D: 50%/50% N_2 / Ar.) during 20 days of storage at 4 ºC. The limit of acceptability of odor evaluation (3 score) is represent by the black line. Odor evaluation was based on a visual scale (5 = Excellent; 4 = good, 3 = fairly acceptable; 2 = unacceptable; and 1 = dislike). Vertical bars represent standard deviation.*

Overall Acceptability

Chicken breast fillets samples packaged under MAP-A, MAP-B and MAP-D were evaluated by the judges below the limit of overall acceptability (score as 3) (Table 1) after 12, 4 and 8 days of storage, respectively. Whereas, samples stored under MAP-C were scored over the limit of acceptability of appearance throughout the storage period. A Significant decrease of overall acceptability evaluation was observed among the different modified atmospheres within each sampling day ($P < 0.05$) (Figure 8).

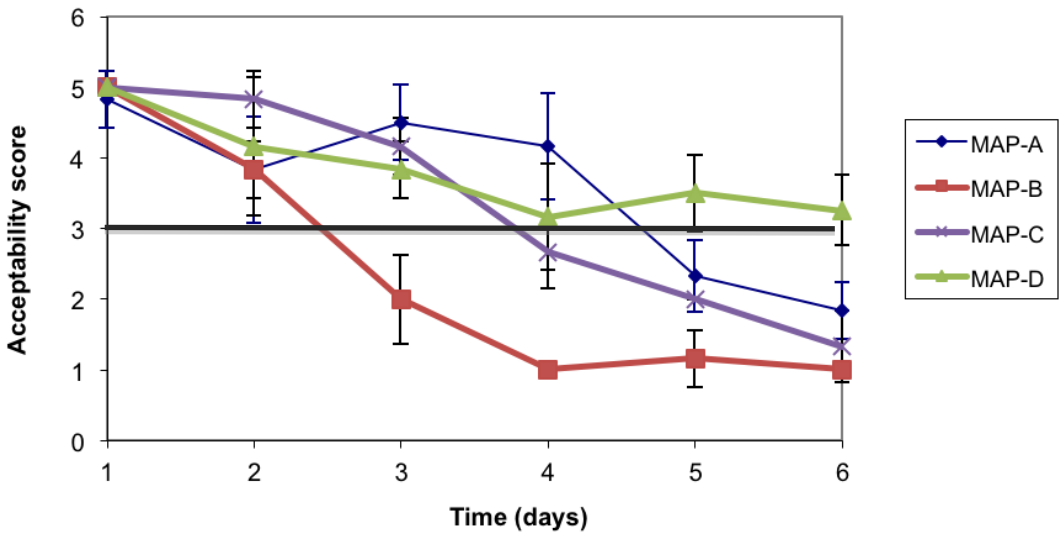

Figure 8. *Overall acceptability score of inoculated chicken breast fillets packaged under MAP conditions (MAP-A: 50%/50% N_2 / CO_2. MAP-B: 50%/50% N_2 / O_2. MAP-C: 30%/70% O_2 / CO_2. MAP-D: 50%/50% N_2 / Ar.) during 20 days of storage at 4 ºC. The limit of acceptability (3 score) is represent by the black line. Overall acceptability evaluation was based on a visual scale (5 = Extremely good; 4 = very good, 3 = neither like; 2 = dislike moderately; and 1 = dislike extremely). Vertical bars represent standard deviation.*

V. DISCUSION

As previously described, a chicken carries several bacteria internally and externally, and is an excellent vehicle for food-borne pathogens, especially for *Campylobacter* .This pathogen has been known to persist on chicken skin during poultry processing (Gloaguen *et al.,* 2011) and for that reason the control of microbial poultry meat quality is a major concern for the food industry (Henchion *et al.,* 2014). In addition, as consumers demand minimally processed foods, the most attractive technology to control this bacterium is MAP (Melero *et al.,* 2012). MAP contributes to the microbial and lipid oxidation stability of poultry meat and prolongs its shelf life compared to those packaged in ambient conditions (Fraqueza and Barreto, 2009). MAP is also related to a safe product, and effectively reduces pathogenic microorganisms like *Campylobacter* (Boysen *et al.,* 2007).

Regarding the effect of the MAPs applied against *Campylobacter* in this study, the application of gas mixtures with a high oxygen concentration and with Ar showed significant control of bacterium growth throughout storage (MAP-B and MAP-D). *Campylobacter* growth was not affected by the application of an anaerobic atmosphere (MAP-A), which brought about high microbiological counts throughout storage. These findings agree with other studies, which reported the effect of anaerobic atmospheres on *Campylobacter* survival on chicken meat. Boysen *et al.* (2007) observed that *C. jejuni,* inoculated onto chicken fillets, survived significantly longer under anaerobic MAPs (100% N_2 and 70%/30% N_2/CO_2) than in an aerobic poultry atmosphere (70/30% O_2/CO_2). This confirms that CO_2 afforded a protective effect on *Campylobacter* survival. A similar effect was also described by Wesley and Stadelman (1985) as a result of reducing the oxygen concentration in an ambient atmosphere. Rajkovic *et al.* (2010) also showed that the survival of *C. jejuni* strains inoculated onto chicken legs was scarcely affected by 80% CO_2 when compared to 80% O_2. Other authors observed that after exposure to O_2 (an adverse environmental

condition), *C. jejuni* cells became slightly elongated and less coiled, and lost their spiral morphology to result in coccoid formation (Non-Culturable form). This therefore affected their growth on selective media given the absence of culturable cell numbers.

The effectiveness of an atmosphere with Ar (MAP-D) was worse compared to high O_2 concentration atmospheres (MAP-B and MAP-C). However at the end of storage, no significant differences were observed among those atmospheres that completely inhibited *Campylobacter* growth. To our knowledge, it has not been evaluated the effect a MAP with Argon has against *Campylobacter* strains. Other studies that have been conducted with fresh cut-products have reported that the beneficial effect of Ar is due to the formation of an inert gas hydrate called clatharate, which reduces intracellular water activity by diminishing organic matter leaching and microorganism movements into deeper tissues (Wu, Zhang and Adhikari, 2012; Wu, Zhang and Wang, 2012). Nevertheless, further studies are required to obtain more results about the effect of Ar gas against *Campylobacter* survival on chicken meat.

In the physical-chemical analysis, the chicken meat fillets packaged in a standard poultry meat atmosphere showed that the pH values of the studied MAPs were generally similar during storage. Several authors (Vongsawasdi *et al.,* 2008; Melero *et al.,* 2012; Herbert *et al.,* 2013) have also reported no significant variations in the pH values of meat chicken samples under different gas mixture conditions during storage.

According to the colour analysis, the samples packaged in a standard poultry meat atmosphere showed significantly higher L* and lower a* values during storage, which resulted in a pale hue of chicken meat (MAP-C). Several authors have indicated that pale hue of chicken meat is related to greater lightness (L*), lesser redness (a*) and no variations in yellowness (b*) (Petracci *et al.,* 2004; van Laack *et al.,* 2000; Qiao *et al.,* 2001). Therefore, meat paleness (L*) does not correlate highly with the b*values as observed in previous studies carried out on pale broiler breast

meat (Fletcher, 1999; van Laack *et al.,* 2000; Qiao *et al.,* 2001). Despite our results, Boysen *et al.* (2007) observed that filleted chicken packaged in an aerobic atmosphere maintained a red hue throughout shelf life (7 days) compared with those packaged under anaerobic conditions (70%/30% N_2/CO_2 and 100% N_2).

The other studied MAPs showed no significant differences in colour (L*, a* and b*) during storage, and similar values were maintained throughout storage. Similarly, Petracci *et al.* (2004) reported that the typical range of L* values for broiler meat lies between 50 and 56. Higher variability was also observed for a* (range: 0 to 13) and b* (range: −3 to 12) values on chicken meat samples.

During the sensorial evaluation, the MAP-D packaged samples were scored as the best treatment for appearance and overall acceptability among the studied MAPs. For the odour evaluation, judges preferred MAP-A, which was evaluated as the best treatment. Ruiz-Capillas and Jimenez-Colmenero (2010) observed a positive sensory acceptability made by judges for the fresh pork sausages packaged in 30%/70% CO_2/Ar. Herbert *et al.* (2013) also described a sensory benefit for chicken breast fillets stored in 15%/60%/25% Ar/O_2/CO_2, which resulted in the better retention (preservation) of the natural pink colour of the studied samples. However, Tomankova *et al.* (2012) showed that the poultry meat samples packaged in MAP 70%/30% Ar and O_2 gave an unpleasant aroma and lower acceptability compared to those packaged as 70%/30% O_2/CO_2 MAP.

In contrast of what was observed in MAP-D, MAP-B was scored as the worst treatment for appearance, odour and overall acceptability. Storage of poultry meat in high oxygen atmospheres has been found to cause loss of quality (Vukasovic, 2014). An increased oxygen concentration can induce lipid oxidation and cause rancid off-flavours. Rancid aromas often present very low threshold values and can be easily detected by consumers (Campo *et al.,* 2003). High oxygen levels can also cause

intermolecular cross-linking, and provoke tenderness, juiciness and lower nutritional values of meat due to loss of essential amino acids and reduced digestibility (Vukasovic, 2014).

VI. CONCLUSION

According to the results, *Campylobacter* growth was inhibited by the application of MAPs with a high O_2 concentration (≥50%) and with Ar. Nevertheless, CO_2 packaging did not show any effect on controlling *Campylobacter* growth. In the physical-chemical analysis, the chicken meat fillets packaged in a standard poultry meat atmosphere (MAP-C) obtained significantly higher L* and lower a* values at the end of storage and resulted in a pale chicken meat colour. The other studied MAPs gave no significant differences among their colour values. In the sensorial evaluation, MAP-D was evaluated as the best for appearance and overall acceptability among the studied MAPs. In odour evaluation terms, judges preferred the chicken meat fillet samples packaged with a high CO_2 concentration (MAP-A). The samples packaged with a high oxygen concentration (MAP-B) obtained the lowest scores for appearance, odour and overall acceptability. In this context, both MAP-B with 50% of oxygen and MAP-D with 50% Argon, were able to eliminate *Campylobacter* in raw chicken meat fillets. However, the atmosphere with Argon had a better sensory evaluation. These results highlight the noble gas Argon as a new and attractive alternative in the control of this bacterium, and also the necessity of more studies related with this innovative gas.

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VIII. REFERENCES

AVEC (Association of Poultry Processors and Poultry Trade in the EU Countries). Annual Report 2013.

Boysen L, Knøchel S, Rosenquist H. Survival of *Campylobacter jejuni* in different gas mixtures. FEMS Microbiol. Lett. 2007; 266 (2):152-157.

Campo MM, Nute GR, Wood JD, Elmore SJ, Mottram DS, Enser M. Modelling the effect of fatty acids in odour development of cooked meat in vitro: part I – sensory perception. Meat Sci 2003; 3:367-375.

Chiavaro E, Zanardi E, Bottari B, Lanieri A. Efficacy of different storage practices in maintaining the physicochemical and microbiological properties of fresh pork sausage. Journal of Muscle Foods 2008; 19(2): 157-174.

Cornforth D, Hunt M. Low oxigen packaging of fresh meat with carbin monoxide: meat quality, microbiology and safety. American Meat Science Association. White Paper Series. No 2. 2008.

Corry JE, Atabay HI. Poultry as a source of *Campylobacter* and related organisms. J. Appl. Microbiol 2001; 90:96–114.

EFSA (European Food Safety Authority and European Centre for Disease Prevention and Control). The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2013. EFSA J 2015; 13(1): 3991.

EU. Directive No. 95/2/CE. Commission Decision 20/02/1995, on food additives other than colours and sweeteners. Off. J. L 1195; 61:0001–0040.

Fachon N. Modification de l'atmosphére de conservation (sous vide, gaz). Cours International de Microbiologie et Maitrise de la Sécurité des Aliments. Institut Pasteur de Lille, Lille, France, 2002.

Fletcher DL. Broilers breast meat color variation, pH, and texture. Poult Sci. 1999; 8:1323-1327.

Fraqueza MJ, Barreto AS. The effect on turkey meat shelf life of modified-atmosphere packaging with an argon mixture. Poult. Sci 2009; 88(9):1991-1998.

Gloaguen M, Picherot J, Santolini G, Salvat S, Bougeard, Chemaly M. Prevalence of and risk factors for *Campylobacter* spp. contamination of broiler chicken carcasses at the slaughterhouse. Food Microbiol 2011; 27(8):992-999.

Hardy B, Crilly N, Pendleton S, Andino A, Wallis A, Zhang A, Hanning I. Impact of rearing conditions on the microbiological quality of raw retail poultry meat. J. Food Sci 2013; 78(8):1232-1235.

Henchion M, McCarthy M, Resconi VC, Troy D. Meat consumption: Trends and quality matters. Meat Sci 2014; 98(3):561-568.

Herbert U, Rossaint S, Khanna M, Kreyenschmidt J. Comparison of argon-based and nitrogen-based modified atmosphere packaging on bacterial growth and product quality of chicken breast fillets. Poult. Sci 2013; 92(5):1348-1356.

Holck AL, Pettersen M K, Moen MH, Sørheim O. Prolonged shelf life and reduced drip loss of chicken filets by the use of carbon dioxide emitters and modified atmosphere packaging. J Food Prot 2014; 77(7):1133-41.

ISO 10272-1 (Annex E). 2006. Microbiology of food and animal feeding stuffs–Horizontal method for detection and enumeration of *Campylobacter* spp. Part 1: Detection Method. International Organization for Standardization, Geneve, Switzerland.

Jacobs-Reitsma W. *Campylobacter* in the food supply. 2nd edition. ASM Press, Washington, 2000.

Jaime P, Saltveit ME. Postharvest changes in broccoli and lettuce during storage in argon, helium, and nitrogen atmospheres containing 2% oxygen. Postharvest Biol. Technol 2002; 26:113–116.

Kozačinski L, Cvrtila Fleck Z, Kozačinski Z, Filipović I, Mitak M, Bratulić M, Mikuš T.. Evaluation of shelf life of pre-packed cut poultry meat. Vet. Arhiv 2012; 82:47-58.

Lund M, Hviid M, Skibsted L. The combined effect of antioxidants and modified atmosphere packaging on protein and lipid oxidation in beef patties during chill storage. Meat Sci 2007a; 76: 226-233.

Lund M, Heinonen M, Baron C, Estevez M. Protein oxidation in muscle food: a review. Mo Nutr Food Res 2011; 55(1): 83-95.

Lund M, Lamentsch R, Hviid M, Jensen O, Skibsted L. High.oxygen packaging atmosphere influences protein oxidation and tenderness of porcine longissimus dorsi during chill storage. Meat Sci 2007b; 77: 295-303.

McMeekin TA, Thomas CJ, Pennington PI. Contamination and decontamination of poultry carcass neck tissue. J. Food Safety 1984; 6: 79-88.

McMillin K W. Where is MAP Going? A review and future potential of modified atmosphere packaging for meat. Meat Sci 2008; 80(1):43-65.

Melero B, Diez AM, Rajkovic A, Jaime I, Rovira J. Behaviour of non-stressed and stressed *Listeria monocytogenes* and *Campylobacter jejuni* cells on fresh chicken burger meat packaged under modified atmosphere and inoculated with protective culture. Int. J. Food Microbiol 2012; 158(2):107-112.

Morgan N. (2007). Argon—the noble protector. Gasworld. Available in: http://www.gasworld.com/argon-the-noble-protector/1706.article.
Accessed January 2015.

Nachamkin I, Allos BN, Patton CM. *Campylobacter* species and Guillian-Barré syndrome. Clin. Microbiol 1998; 11: 555-567.

Notermans S, Kampelmacher EH, Van Schothorst M. Studies of different sampling methods for the determination of bacterial counts from frozen broilers. Appl Bacteriol 1975; 39(2):125-31.

Petracci M, Betti M, Bianchi M, Cavani C. Color variation and characterization of broilers breast meat during processing in Italy. Poult. Sci 2004; 83:2086-2092.

Qiao M, Fletcher DL, Smith DP, Northcutt JK. The effect of broilers breast meat color on pH, moisture, water-holding capacity, and emulsification capacity. Poult. Sci 2001; 80: 676-680.

Rajkovic A, Tomic N, Smigic N, Uyttendaele M, Ragaert P, Devlieghere F. Survival of *Campylobacter jejuni* on raw chicken legs packed in high-oxygen or high-carbon dioxide atmosphere after the decontamination with lactic acid/sodium lactate buffer. Int. J. Food Microbiol 2010; 140(2-3):201-206.

Ruiz-Capillas C, Jiménez-Colmenero F. Effect of an argon-containing packaging atmosphere on the quality of fresh pork sausages during refrigerated storage. Food Contr 2010; 21:1331-1337.

Sopwith W, Birtles A, Matthews M, Fox A, Gee S, James S *et al.* Investigation of food and environmental exposures relating to the epidemiology of *Campylobacter* coli in humans in Northwest England. Appl. Environ. Microbiol 2010; 76, 129–135.

Spencer KC. Method of preservation foods using noble gases. US patent number 6,342,261. 2012. American Air Liquide, assignee.

Tomankova J, Bonilova G, Steinhauserova I, Gall L. Volatile organic compounds as biomarkers of the freshness of poultry meat packaged in modified atmosphere. Czech J. Food Sci 2012; 30(5):395-403.

Van Laack RLJM, Liu CH, Smith MO, Loveday HD. Characteristics of pale, soft, exudative broilers breast meat. Poult. Sci 2000; 79: 1057-1061.

Vongsawasdi P, Wongwicharn A, Khunajakr A, Dejsuk N. Shelf-life Extension of Precooked Chicken Fillets by Modified Atmosphere Packaging. Kasetsart. J. Nat. Sci 2008; 42:127-135.

Vukasovic T. European meat market trends and consumer preference for poultry meat in buying decision making process. World's Poultry Science Journal 2014; 70(02):289-302.

Wassenaar TM, Blaser MJ. Pathophysiology of *Campylobacter jejuni* infections of humans. Microbes Infect 1999; 1:1023–1033.

Wesley RD, Stadelman WJ. The effect of carbon dioxide packaging on detection of *Campylobacter jejuni* from chicken carcasses. Poult. Sci 1985; 64(4):763-4.

WHO (World Health Organization). (2011). Available in: http://www.who.int/mediacentre/factsheets/fs255/en/. Accessed January 2015.

Wu ZS, Zhang M, Adhikari B. Application of high pressure argon treatment to maintain quality of fresh-cut pineapples during cold storage.J. of Food Eng 2012; 110:395-404.

Wu ZS, Zhang M, Wang S. Effects of high pressure argon treatments on the quality of fresh-cut apples at cold storage", Food Control 2012; 23:120-127.