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Effect of different modified atmospheric packaging (MAP) gaseous combinations on *Campylobacter* and the shelf-life of chilled poultry fillets

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ABSTRACT

Studies were undertaken to investigate the effect of different modified atmospheric packaging (MAP) gaseous combinations on *Campylobacter* and the natural microflora on poultry fillets. Skinless chicken fillets were stored in gaseous mixtures of 10%, 30%, 50%, 70% and 90% CO_2 balanced with N_2, 80:20% O_2:N_2 and 40:30:30% CO_2:O_2:N_2 and control conditions (air) at 2 °C. Samples were analysed periodically for (previously inoculated) *Campylobacter*, total viable counts (TVC) (mesophiles), TVC (psychrophiles), *Enterobacteriaceae*, *Pseudomonas* and lactic acid bacteria (LAB) over 17 days of storage. The carbon dioxide solubility was determined by monitoring the changes in the headspace volume over time using a buoyancy technique and performing calculations based on volumetric measurements and the Henry's constant. Henry's constant was also used to estimate the oxygen solubility in the chicken fillets. The presence of O_2 in the MAP gaseous mixtures increased the rate of *Campylobacter* decline on poultry fillets but in general the counts obtained in aerobic versus anaerobic packs were not significantly ($P > 0.05$) different. CO_2 inhibited the growth of TVC, TEC, LAB and *Pseudomonas* but only at MAP gaseous combinations containing 50–90% CO_2 where concentrations of up to 2000 ppm CO_2 were recorded in the fillets after 5 days. Under these conditions a shelf-life in excess of 17 days at 2 °C was obtained. Although, dissolved O_2, at levels of 33 ppm in 80:20% O_2:N_2 packs after 3 days, reduced *Campylobacter*, it also favoured the growth of the other microbes on the chicken. The optimum gaseous mixture for achieving the combined objectives of reducing *Campylobacter* and extending shelf was therefore 40:30:30 CO_2:O_2:N_2, which achieved a shelf-life in excess of 14 days.

1. Introduction

Poultry is an important source of *Campylobacter*, the primary cause of bacterial gastroenteritis in the developed world. The most recent European Food Safety Authority (EFSA) baseline survey reported a 98.3% *Campylobacter* prevalence on raw poultry carcasses in Ireland (EFSA, 2010). Although, biosecurity measures on broiler farms and interventions throughout the slaughter plant are continually being addressed, levels of *Campylobacter* contamination remain high (Haughton et al., 2010). To date, much research has focused on pre-harvest and processing interventions with few investigations on the use of modified atmospheric packaging (MAP) to control *Campylobacter* and extend shelf-life (Byrd et al., 2011). Furthermore, in the few studies that have been published, no data is provided on the amount of oxygen (O_2) and carbon dioxide (CO_2) dissolved in the product.

Poultry is a highly perishable food which deteriorates after 4–10 days post slaughter even under chilled conditions (Jimenez et al., 1997; Patsias et al, 2006a,b). MAP is a well established method to extend the shelf-life of fresh and processed chilled foods (Charles et al., 2006; Devlieghere et al., 1998; Devlieghere and Debevere, 2000; Rotabakk et al., 2010) and is used to prolong the shelf-life of poultry fillets by suppressing aerobic spoilage bacteria such as *Pseudomonas* (Sade et al., 2013). However, psychrotrophic

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facultative anaerobic bacteria such as LAB, that are less sensitive to CO_2, will grow under MAP conditions. *Enterobacetriaceae* will also grow in the presence of CO_2 but to a lesser extent than LAB (Sade et al., 2013). Data on the effect of different MAP gaseous combinations on the survival of *Campylobacter* on poultry fillets is limited (Byrd et al., 2011) and the optimum gaseous combination in terms of the elimination of *Campylobacter* while maximising shelf-life is unknown (Patsias et al., 2006b). In theory high O_2 concentrations should inhibit *Campylobacter* spp. which are microaerobic and grow optimally at about 5% O_2 and this has been demonstrated by Byrd et al. (2011). However, O_2 supports the growth of other bacteria including spoilage organisms like *Pseudomonas* and lactic acid bacteria (LAB) which produce slime, souring and/or off-odours when counts reach 10^{7-8} CFU/g (Nychas et al., 2008; Charles et al., 2006).

MAP is a non-thermal method of food preservation that uses 3 gases; nitrogen (N_2), Oxygen (O_2) and carbon dioxide (CO_2). N_2 is an inert gas with no antimicrobial activity but the anoxic atmospheres created when using this gas will select for anaerobic, aerotolerant *Lactobacilli* (Thippareddi and Phebus, 2007). Its primary function is as a filler and to prevent pack collapse. O_2 inhibits the growth of anaerobic bacteria but the principle, antimicrobial effect is due to the presence of CO_2. Although the use of CO_2 enriched modified atmospheres to extend shelf-life has been well documented (Gill et al., 1990), data for use in food safety risk assessments and shelf-life modelling studies are scarce. CO_2 readily passes through the bacterial cell membranes and four possible bacteria related mechanisms for inhibition have been suggested including: [1] the formation of carbonic acid within the bacterial cell resulting in decreased intracellular pH and reduced enzyme activity (Wolfe, 1980); [2] specific inhibition of decarboxylating enzymes (King and Nagel, 1975); [3] non-specific inhibition of susceptible non-decarboxylating enzymes (Ranson et al., 1960) and [4] alteration of membrane properties that inhibits membrane functions (Sears and Eisenberg, 1961).

Regardless, the bacteriostatic effect of CO_2 within MAP is primarily influenced by CO_2 absorption into the food and several studies have measured the solubility of CO_2 in perishable foods (Jakobsen and Bertelsen, 2004, 2006; Jakobsen and Risbo, 2009; Rotabakk et al., 2010), although poultry data is lacking. Different methods have been used in these studies including a comparison of initial and final pressures (Devlieghere and Debevere, 2000) and modified titration (Gill, 1988) but continuous monitoring requires a non-destructive approach such as the buoyancy force based method (Rotabakk et al., 2007). A minimum head space concentration of 20–30% is required to achieve bacterial inhibition (Stiles, 1991a,b) and the poultry industry therefore typically uses 40–100% CO_2 balanced with N_2.

The objectives of this study were to investigate the effect of different gaseous combinations on inoculated *Campylobacter* and the natural microflora on chilled poultry fillets and to characterise the different MAP treatments in terms of dissolved carbon dioxide and oxygen in chicken fillets throughout a given storage period.

2. Materials and methods

2.1. Culture preparation

Five *Campylobacter* strains, two strains of *Campylobacter jejuni* (1136DF, 11168 NCTC) and three strains of *Campylobacter coli* (2124GF, 323BC, 1354 DF) were used in this study. Strains were stored at −80 °C on ceramic beads (TSC, Heywood, UK). Inocula were prepared separately by aseptically transferring a bead from the stock cultures to 30 ml Hunts broth (Nutrient broth (Oxoid, Basingstoke, UK) and Yeast Extract (Oxoid, Basingstoke, UK), 5% lysed horse blood and 0.4% *Campylobacter* growth supplement FBP) and incubating at 42 °C for 48 h under microaerobic conditions (Biomerieux, Marcy l'Etoile, France). From the resultant suspension, 1 ml of each was used to inoculate 5 × 100 ml Hunts broth and these were incubated under microaerobic conditions at 42 °C for a further 24 h. Cells were recovered by centrifugation (10 min at 2655*g*), washed 3 times in maximum recovery diluent (MRD; Oxoid Basingstoke, UK), resuspended in 10 ml MRD, mixed and the volume of MRD increased to 500 ml, which gave a cell suspension containing approximately 8 $\log_{10}$ CFU/ml. Cell suspension concentrations were assessed by preparing 10 fold dilution series and plating 0.1 ml dilutions onto modified charcoal cefoperazone deoxycholate agar medium (mCCDA, Oxoid, Basingstoke, UK) plates in duplicate.

2.2. Sample preparation

Three hundred and forty chicken breast fillets were collected from the poultry processing plant immediately after chilling. The samples were transported to the laboratory at 4 ± 1 °C. They were then divided into 3 groups, group 1 and 2 each containing 160 fillets and group 3 containing 20. Group 1 and 3 fillets remained uninoculated while the 160 fillets in group 2 were inoculated using the *Campylobacter* cocktail prepared above. Each fillet was independently immersed in the freshly prepared *Campylobacter* suspension for 15 s and left at room temperature for 15 min to allow for bacteria adhesion. Using this method each fillet was inoculated with approximately 4.5 $\log_{10}$ *Campylobacter* per g. The weights of all samples were taken prior to packaging.

Groups 1 and group 2 fillets were each divided into 8 groups (labelled A to H) of 20 samples each. Group 1A and 2A samples were used as the control and packaged in air. Groups B to H had the following gaseous combinations; (B) 90/10% (N_2:CO_2), (C) 70:30% (N_2:CO_2) (D) 50:50% (N_2:CO_2) (E) 30:70% (N_2:CO_2) (F) 10:90% (N_2:CO_2) (G) 80:20% (O_2/N_2) or (H) 40:30:30% (CO_2/O_2/N_2). At times 1, 2, 3, 4, 5, 7, 9, 11, 14 and 17 days 2 samples were removed from groups 1 and 2 (eg. 1A 2A, 2B, etc.), the gaseous composition was analysed (see Section 2.6) prior to microbiological analysis (see Section 2.4). Group 3, two packs were removed from each group immediately after packaging and used repeatedly for volumetric analysis over the 17 days (see Section 2.5).

2.3. Packaging and storage

All samples were packed into EVOH semi-rigid trays 110 × 150 × 46 mm (Versatile Packaging, Ireland), gases were pumped in and heat sealed using a MECAPAC 500 MAP machine (Mecaplastic Bagnolet, France). The trays (had an oxygen transmission rate of 0.15 cm^2/Pck d bar) were covered with a 76 mm antifog high barrier film with an oxygen transmission rate of 0.8 ml/m^2/24 h (at 23 °C, 0% RH) and packed in a refrigerated room environment (<4 °C). During packaging the air was removed and flushed with food grade gas mixtures of CO_2 and N_2 (10%, 30%, 50%, 70%, 90% CO_2, balanced with N_2), 80:20% O_2:N_2 and also 40:30:30% CO_2:O_2:N_2 (BOC, Ireland). The gas/product ratio was 5:1. All samples were stored at 2 °C without light exposure for up to 17 days.

2.4. Microbiological analysis

All chicken samples in group 1, i.e., uninoculated control group, were analysed immediately for *Campylobacter* spp., total viable counts (TVC (mesophiles, 30 °C)), TVC (psychrophiles, 6.5 °C), total *Enterobacteriaceae* counts (TEC), lactic acid bacteria (LAB) and *Pseudomonas* spp. and on days 1, 2, 3, 4, 5, 7, 9, 11, 14 and 17. Group 2 samples were only analysed for *Campylobacter* spp. For microbial