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Influence of argon modified atmosphere packaging on the growth potential of strains of *Listeria monocytogenes* and *Escherichia coli*

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ABSTRACT

Modified atmosphere packaging (MAP) based on carbon dioxide (CO_2) – nitrogen (N_2) gas mixtures has been applied to maintain the safety and quality of ready-to-eat (RTE) meat products. The use of argon (Ar) gas as a supplement to CO_2–N_2 mixtures or as substitute for N_2 is a current approach to enhance the effectiveness of MAP. As there is limited information on the effect of Ar MAP on the growth behaviour or the survival of pathogenic bacteria in RTE foods, the aim of the present study was to assess the influence of Ar in MAP on the growth of *Listeria monocytogenes* and *Escherichia coli* strains under different conditions. For this purpose, a CO_2–N_2 (20:80) atmosphere was compared with a CO_2–N_2–Ar (30:30:40) and CO_2–Ar (30:70) atmosphere based on the assessment of bacterial growth (δ) on a gelatin-agar medium and ham. Additionally, a shelf life monitoring study was performed to evaluate the effect of these treatments on the background microflora of ham. The findings suggest that under the CO_2–N_2 MAP the product matrices supported the growth ($\delta > 0.5$ log CFU g^{-1}) of *L. monocytogenes* throughout an observation period of 21 days at 4 ± 2 °C. On the contrary, both MAP containing Ar were equally able to reduce the δ below 0.5 log CFU g^{-1}. In this regard it was irrelevant whether *L. monocytogenes* was inoculated in depth (per slice) or at the surface (top slice) of the ham. Regarding the influence of the different gas atmospheres on *E. coli* all gas mixtures applied had the capacity to reduce the δ of *E. coli* below −0.5 log CFU g^{-1}. Further, shelf-life extension could not be managed with the gas atmospheres considered.

1. Introduction

In the last decades, the maintenance of microbial safety of ready-to-eat (RTE) foods has become a central issue for food business operators (FBO) as well as for research and development divisions. Owing to the increased consumer demands, which are based on societal and demographic developments as well as the susceptibility of many RTE foods to the development of bacterial pathogens, new strategies are sought to establish microbiological safeguards in this product category (Aymerich, Picouet, & Monfort, 2008; Havelaar et al., 2010; Sofos, 2008; Weiss, Gibis, Schuh, & Salminen, 2010). In this context, zoonotic bacteria like *Listeria monocytogenes* and enteropathogens play a major role in foodborne diseases in the European Union (EU) (EFSA and ECDC, 2014; RASFF, 2014; Uyttendaele et al., 2009). *L. monocytogenes* for example is particularly challenging due to its ubiquitous occurrence and its ability to grow or survive under refrigerated conditions. Last but not least, the severances of the caused diseases are also of relevance (EFSA and ECDC, 2014; FDA/FIS, 2003).

To guarantee public health protection, the EU food legislation (regulation (EC) No 178/2002 and 852/2004) lays down general food safety requirements based on a preventive approach, including hygiene control measures and HACCP based procedures (EC, 2002, 2004). In view of the respective situation, the EU has furthermore established the regulation (EC) No 2073/2005 of 15 November 2005 (as amended) on microbial criteria for foodstuffs with specific requirements for *L. monocytogenes* in context with RTE foods that are

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able to support the growth of the pathogen. In particular, the regulation requires the FBO not only to comply with the microbial criteria and limits before the food leaves the immediate control of the producing FBO, but also to meet the criteria throughout defined shelf-life conditions. Otherwise the product has to be withdrawn from the market and corrective actions need to be taken. For setting a reasonable shelf-life and investigation of compliance, shelf-life studies - and where necessary - additional studies like predictive microbiological (mathematical) modelling, durability studies or challenge tests are suggested (EC, 2005; Everis & Betts, 2013). In the context of the regulation (EC) No 2073/2005 of 15 November 2005 (as amended), the guidance document on *L. monocytogenes* shelf-life studies for RTE foods provides FBOs with information on how to use challenge tests in order to assess the potential growth (e.g. characterized by maximum growth rate) of *L. monocytogenes* in the specific product (Anonymous, 2005; EC, 2005). Further, the technical guidance document on shelf-life studies for *L. monocytogenes* in RTE foods gives support to laboratories when conducting shelf-life studies in collaboration with FBOs (AFSSA, 2008).

Taking into account these advice, FBOs are constantly looking for new ways and methods to prevent contamination of products and to control microorganisms where contamination is likely to occur (e.g., post processing contamination during slicing and packaging) (Aymerich et al., 2008; Havelaar et al., 2010; Lin et al., 2006; Sheen, 2008; Sheen & Hwang, 2010; Sofos, 2008; Weiss et al., 2010). Since the majority of the treatments, however, do not cause complete inactivation of the microorganisms, food preservation by combined processes (hurdle technology) is widely spread (Leistner & Gorris, 1995). One of the possible and well known hurdles that microorganisms should not be able to overcome and possibility to protect the overall product quality is modified atmosphere packaging (MAP) – a two-step process in which the naturally present atmosphere in a package is removed and replaced by an artificially constituted one. Traditionally, the gases used in MAP comprise the main gases of the air, namely oxygen (O_2), carbon dioxide (CO_2) and nitrogen (N_2) (Brandenburg, 2009; Floros & Matsos, 2005). Oxygen, however, plays a minor role in the packaging of RTE foods because it promotes growth of aerobic microorganisms and may cause sensory deterioration or loss of nutritional compounds. So, for many years the MAP of choice mainly consisted of the bulk gas N_2 combined with the more or less antimicrobial gas CO_2 (Brandenburg, 2009; Farber, 1991; Floros & Matsos, 2005).

Since the last decade, potential benefits of noble gases in MAP applications are under discussion. Especially, the inert, odourless and tasteless noble gas argon (Ar) is seen as a possible alternative to N_2. Although the use of Ar (E 938) in MAP is permitted and regulated in terms of purity in the EU, its application is not wide spread yet (Day, 2007; EC, 1995, 2008; Spencer, 2005; Spencer & Humphreys, 2002). In comparison to N_2, factors like the higher density, similarity of atomic size to molecular oxygen and improved water solubility of Ar seem to allow a more sufficient removal or exclusion of O_2 from the packages and therefore may result in lower levels of residual O_2 (Spencer, 2005; Spencer & Humphreys, 2002). Furthermore, Ar is suggested to have regulating effects on enzymes (Spencer, 2005; Spencer, Schvester, & Boisrobert, 1995a, 1995b; Spencer et al., 1998). So, it is stated that less CO_2 is needed in Ar MAP than in N_2 MAP to control the microbial growth and that higher levels of antimicrobial action are achieved with a CO_2–Ar MAP than with a CO_2–N_2 MAP. In addition, MAP containing Ar should contribute to the maintenance of typical sensory attributes during shelf life of foods of plant and animal origin (Spencer, 2005). Inconsistent results, however, are found in the current body of literature regarding the growth of microorganisms on meat and meat products and the sensory improvements of the respective foods packaged under such Ar gas containing MAP.

Fraqueza and Barreto (2009) were able to show an inhibitory effect of CO_2–Ar (50:50) MAP in the range of 1 log CFU on total anaerobic and psychrotrophic counts as well as *Brochothrix thermosphacta* on turkey meat after 25 days, in comparison with CO_2–N_2 (50:50). An effect on lipid oxidation however, was not demonstrable. Tománková, Bořilová, Steinhauserová, and Gallas (2012), however, described an increase in microbial growth and olfactory impairment of poultry meat when stored under CO_2–Ar (30:70) compared to CO_2–O_2 (30:70) MAP. In addition, Herbert, Rossaint, Khanna, and Kreyenschmidt (2013) could not demonstrate a difference in microbial growth parameters, except for *B. thermosphacta*, for poultry meat stored under a CO_2–Ar (18:82) MAP in comparison with a CO_2–N_2 MAP, but a significant positive effect on sensory parameters and especially the colour of the product. Ruiz-capillas and Jiménez-Colmenero (2010) ascertained a minimal effect of CO_2–Ar (30:70) MAP on the microbiological but a positive effect on sensory parameters of pork sausages in comparison to CO_2–N_2 (20:80) MAP or vacuum packaging. Inhibition of *Carnobacterium divergens* but potential growth promotion of Enterobacteriaceae was observed in pork sausages during 28 days of storage under a CO_2–Ar (30:70) in comparison to a CO_2–N_2 (20:80) MAP (Curiel et al., 2011). Further, Parra et al. (2010) reported no significant differences between dry-cured *Iberian* ham stored under CO_2–Ar (30:70) or various CO_2–N_2 MAP ratios (40:60, 30:70 and 20:80). On the contrary, in a recent study by Pérez-Rodríguez, Zamorano, Posada-Izquierdo, and García-Gimeno (2012) focussing on post-packaging pasteurised sliced, cooked meat product it was again possible to enhance the sensory quality and also the growth inhibitory effect of a CO_2–Ar (17:83) mixture on lactic acid bacteria (LAB) in comparison to a CO_2–N_2 (22:78) MAP. However, the authors did not report any effect on the total viable count.

Since information on the effect of Ar MAP on the growth or survival of relevant pathogenic bacteria in connection with RTE foods is still limited, the purpose of the present study was to assess the influence of Ar MAP treatment on the growth of selected strains of *L. monocytogenes* and *Escherichia coli*. For this purpose, two approaches were investigated, a model test involving gelatin-agar medium as an artificial matrix, and a test on cured, pasteurised and sliced ham. Additionally, the influence of the MAP conditions on the shelf-life of the hams was evaluated.

2. Material and methods

2.1. Matrices

2.1.1. Gelatin-agar medium

For the *in-vitro* test, a sterile gelatin-agar medium consisting of 2% Gelatin Powder (Merck, DE) and 1% Agar Bacteriological (Oxoid Limited, UK) was prepared by dissolving the components in deionised water and sterilisation thereof. Subsequently, angular (120 × 120 × 17 mm) Petri dishes (Greiner Bio-One, DE) were filled with 100 g of the final medium.

2.1.2. Cured, pasteurised and sliced ham

Batches of reconstituted, cured, pasteurised (74 °C, 20 min) and sliced ham packaged in polyamide - polyethylene trays under a CO_2–N_2 (30:70) MAP with a specified total shelf-life of 22 days from the date of production on was obtained from a local FBO. Produced according to the Austrian Codex Alimentarius (Chapter B14), the product contained 19 g of protein, 0.2 g of carbohydrates and thereof 0 g of sugar, 3 g of fat and thereof 1.2 g of saturated fat, 0 g fibre and 0.8 g sodium per 100 g (Codex Alimentarius Austriacus, 2014). The specified water content of the product was 73.15% and the pH was 5.8. The 400 g packages, containing 40 slices