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Modelling the fate of *Listeria monocytogenes* in beef meat stored at refrigeration temperatures under different packaging conditions

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Abstract

The objective of this study was to model the fate of *L. monocytogenes* inoculated in beef at two concentrations (2.5 and 4.0 log CFU/g), packaged under aerobic, vacuum and three modified atmosphere combinations – 70%O_2/20%CO_2/10%N_2, 50%O_2/40%CO_2/10%N_2 and 30%O_2/60%CO_2/10%N_2, and refrigerated at a normal temperature (4ºC) and at a mild abuse temperature (9ºC). An omnibus model based on the three-parameter Weibull equation proved statistically that *L. monocytogenes* survives better in vacuum (VP) than in aerobic conditions, although without significant difference in its ability to survive in the temperature range between 4ºC and 9ºC. Furthermore, regardless of the refrigeration temperature, the presence of CO_2 in package atmosphere exerted a bactericidal effect on *L. monocytogenes* cells, being approximately 1.5 log of reduction when storage time reached 10 days. Since the pathogen can survive in VP/MAP beef at refrigerated storage, there is a need of maintaining its numbers below 100 CFU/g before packaging by placing efforts on the implementation of control measures during processing.

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Keywords: Listeria monocytogenes; Packaging; Weibull; survival; predictive microbiology

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1. Introduction

Listeria monocytogenes is a Gram-positive anaerobic facultative food-borne pathogen which is widely distributed in nature [1]. This bacteria is able to survive in environments with pH between 4.0 and 9.6 [2] and at temperatures below freezing, growing from 1ºC to 45ºC [3]. Refrigerated meats, ready-to-eat meat (RTE) foods, milk and cheeses, cold-cut meats, smoked fish and seafood, have been implicated in isolated cases of listeriosis [4,5]. A recent systematic review and meta-analysis on the incidence of pathogens in Portuguese meats revealed that incidence average of *L. monocytogenes* in beef meat is 17.6%, and in meat products is 8.8% [6]. Although the concentrations are unknown, and may as well be low, these high prevalence estimates may represent a considerable risk due to the common practice among consumers of eating rare meat (i.e., undercooked) which could not destroy *L. monocytogenes*, if present. According to European Communities Commission [7], *L. monocytogenes* must not be exceeded the limit of 2 log CFU/g at the end of the shelf-life in RTE. It is assumed that the temperature, the ultimate pH, the film permeability and gaseous composition of packaging are important parameters that affect the growth of microorganisms in packaged raw meat [8]. It is well known that the composition of MAP systems can be an effective way to restrict the growth of spoilage aerobic organisms [8,9]. But, the extended shelf-life of refrigerated meats under VP and MAP conditions has raised concerns about the survival dynamics of *L. monocytogenes*. The aim of this work was to assess, by means of predictive microbiology modelling, the effect of packaging on the dynamics of *L. monocytogenes* inoculated at low and high level in beef meat stored at a normal refrigeration temperature (4ºC) and a mild abusive temperature (9ºC).

2. Material and methods

2.1. Preparation of meat samples

Longissimus dorsi (LD) muscles were obtained from carcasses of eight Portuguese bulls (9 to 11 months) at 24 h *post mortem*. Muscles were cut into pieces of approximately 200 g and two samples of each piece were immediately (24h *post mortem*) investigated for the presence of *Listeria monocytogenes* according to ISO 11290-1 [10]. If at least one positive-sample were detected, all piece cuts were totally excluded from the inoculation experiments. Meat samples were prepared by removing a layer of ~1cm from the meat surface, and aseptically cut in small meat pieces.

2.2. Preparation of inoculum and inoculation procedure

L. monocytogenes (ATCC 7973) stock culture was cultured on tryptone soy agar (TSA, England) slants at 4°C, which were replaced every 30 days. A single colony was transferred from the slants to 10 ml Brain Heart Infusion (BHI) broth (Oxoid CM225, England) (37°C, 24 h), followed by a second activation step in BHI (37°C, 18 h) to achieve a viable cell population of 9 log CFU/ml. The culture was centrifuged at 10,000× g for 10 min at 4°C. The supernatant was decanted and the sediment suspended in 0.1% peptone water (Merck, Germany). The washing step was repeated twice. The suspension of washed cells was diluted in a sterile 0.1% peptone solution to obtain an optical density of 0.5 (600 nm, 10 mm). Serial dilutions were performed to yield approximately 2.5 or 4 log CFU/g.

Each piece of beef was placed into individually package and dropped in the center with 20 µl bacterial suspension of *L. monocytogenes*. The entire procedure was repeated to obtain a low inoculation level (2.5 log CFU/g) and a high inoculation level (4.0 log CFU/g). Each bag of beef was massaged manually and then bags were heat-sealed.

2.3. Packaging

Inoculated samples were packed in air (A), vacuum (VP) and modified atmosphere packaging $70\%O_2/20\%CO_2/10\%N_2$ ($MAP_{70/20}$), $50\%O_2/40\%CO_2/10\%N_2$ ($MAP_{50/40}$) and $30\%O_2/60\%CO_2/10\%N_2$ ($MAP_{30/60}$). For air-packaged samples, meat cuts were accommodated in a tray overwrapped with polyethylene film, while for VP and MAP samples, meat cuts were individually packaged in COMBITHERM bags (WIPAK Walsrode, HAFRI). Meat samples were packaged using a SAMMIC V-420 SGA machine, with a gas-to-meat ratio of ~3:1. Samples were stored at 4±0.5ºC and 9±0.5ºC, and examined for *Listeria monocytogenes* counts at days 1, 3, 7, 10, 14, 21 and 28, depending on the packaging system.