



A simple method to evaluate the shelf life of refrigerated rabbit meat



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ABSTRACT

The shelf life of rabbit meat during refrigerated storage was investigated under industrial conditions. Rabbit carcasses were bulk packed (BP), packed under air (PUA) and under modified atmosphere (MAP) (30% O₂:40% CO₂:30% N₂). The main groups studied were mesophilic aerobes, psychrotrophic aerobes, *Pseudomonas* spp., lactic acid bacteria, yeast and moulds and *Enterobacteriaceae*. The microorganisms that showed faster growth were psychrotrophic aerobes (growth rate of $0.36 \pm 0.09 \text{ day}^{-1}$) for BP, *Pseudomonas* ($0.26 \pm 0.03 \text{ day}^{-1}$) for PUA, and lactic acid bacteria ($0.22 \pm 0.01 \text{ day}^{-1}$) for MAP, and the lag phases were 4, 4 and 8 days, respectively. The main effect of MAP was the increase in lag phase from 4 to 8 days, for lactic acid bacteria, psychrotrophic and mesophilic aerobes. The respective growth rates were similar to those observed with PUA. In addition, MAP inhibited *Pseudomonas* growth during 18 days. Considering $6 \log \text{ CFU g}^{-1}$ as maximum tolerable microbial load, the calculated values of shelf life in BP, PUA and MAP were 6, 7 and 12 days, respectively, when considering the fastest growing microorganisms.

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1. Introduction

The meat of domestic animals is consumed in large parts of the world and represents an important source of high quality protein (Simonsen, Hamm, & Rogowski, 1988). The production and consumption of rabbit meat is quite ubiquitous in some Mediterranean countries, especially there where it is associated with cultural, traditional and religious motifs (Zotte, 2002).

The nutritional and sensorial quality of meat is strongly influenced by the environment in primary production, the production method, feeding and conditions before and after slaughter, by genetic factors like weight and age (Cavani & Petracci, 2004; Miller, 2000; Ramirez et al., 2004) and by technological factors, as pre-slaughter and stunning conditions (Zotte, 2002). Compared to red meats, rabbit meat is a highly digestible, tasty, low-calorie food, and contents in fat and cholesterol are lower (Zotte, 2002). The microbiological quality is indirectly evaluated by consumers because it is based on presence or absence of off-odours and slime formation due to microbial metabolism (James, 2000).

Meat spoilage, at low temperatures in air, is mainly the result of the activity of motile and non-motile Gram negative, psychrotrophic, aerobic rods dominated by *Pseudomonas* spp. Other organisms, including *Brochothrix thermosphacta*, lactic acid bacteria

(LAB) and cold tolerant *Enterobacteriaceae* are also capable of multiplication but they usually account for a small proportion of the total flora (Rodríguez-Calleja, García-López, García-López, Santos, & Otero, 2006).

Many strategies have been tried to control microbial growth at the surface of meat and meat products. Refrigeration combined with modified atmosphere packaging (MAP) to reduce the microbial development and keep colour attractive to consumer is one of the most widespread solutions. Packaging options for raw chilled meat are air-permeable, low oxygen (O₂) vacuum, low O₂ with anoxic gases and high O₂ MAP, as recently reviewed by McMillin (2008). Phillips (1996) described an intermediate level of oxygen (e.g. 30% O₂:30% CO₂:40% N₂) to store fresh meat. The levels of O₂ are normally set as low as possible to inhibit the growth of aerobic spoilage microorganisms and to reduce the rate of oxidative deterioration of foods. However, in case of red meats, the concentration of O₂ is important to make the retention of colour (Brody, 2002; Day, 2000). The oxygen levels are much less necessary from the point of view of product appearance (level of red colour) as rabbit meat is white meat. The inhibition of aerobic bacteria and moulds is due to CO₂, used in the range of 20–40%, which increases the microbial lag phase and duplication time (Day, 2000; Farber et al., 2003). However, higher CO₂ level, or vacuum, causes a decrease in water retention capacity, leading to discolouration and toughening of the rabbit meat (Garipey, Amiot, Simard, Boudreau, & Raymond, 1986). The N₂ is an inert gas with low solubility in water and fats, without antimicrobial effect (Garipey et al., 1986). N₂ is

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used in MAP primarily to displace O₂ in order to retard aerobic spoilage and oxidative deterioration. Another role of N₂ is to act as a filler gas so as to prevent pack collapse (Day, 2000; Phillips, 1996). The efficiency of the MAP also depends on the gas permeability, mechanical properties and sealing reliability of the packaging materials (Day, 2000; Phillips, 1996).

The normal meat spoilage microflora in aerobic conditions is dominated by *Pseudomonas* spp. which is inhibited by the high CO₂ levels used in MAP or by the absence of oxygen under vacuum (Corry, 2007). The extension of shelf life by vacuum packaging or MAP is particularly effective when meat pH decreases below 6.0 as a function of glucose fermentation by lactic acid bacteria (Corry, 2007). The production of microbial metabolites during storage determines the duration of meat shelf life, usually when microbial counts reach 10⁷–10⁸ cm⁻² (Corry, 2007). Rabbit meat, although far less studied than red meat or poultry, shows the same microbial patterns, being expected to find a shelf life of 6–7 days under aerobic refrigerated storage (Badr, 2004; Berruga, Vergara, & Linares, 2005; Rodríguez-Calleja, García-López, Santos, & Otero, 2005; Rodríguez-Calleja, Santos, Otero, & García-López, 2004).

Garipey et al. (1986), Berruga et al. (2005) and Rodríguez-Calleja, Santos, Otero, García-López (2010) demonstrated that MAP is an efficient alternative to decrease microbial growth and rancidity development of fresh rabbit meat. This small number of reports justifies the evaluation of other MAP options under industrial conditions. The main objective of the present study was to evaluate the effect of different forms of packaging on the duration of shelf life of refrigerated rabbit carcasses.

2. Materials and methods

2.1. Collection of carcasses

The carcasses used for this study belong to race New Zealand white rabbits at live weights between 2.25 and 2.50 kg and were slaughtered and bled in a local abattoir. The head, viscera and skin were immediately removed. The abattoir has the structure, layout, maintenance and hygiene procedures in compliance with European Union requirements. The rabbit carcasses were bulk packed (BP), packed with flow-packs but under air (PUA) and under modified atmosphere (MAP), without absorption pad and maintained under refrigeration at 4 °C ± 1 °C. In BP, polyvinyl chloride (PVC) boxes without lid were used to keep 10 carcasses/box. The rabbit were collected from three different lots, in duplicate for each day and packaging. In PUA and MAP, carcasses were individually packaged in flow-packs by means of a horizontal equipment SP100-H (Belca, Villabona, Spain). The tested flow-pack was characterised by: i) thicknesses, 45 µm; ii) transmission rate of oxygen: 3 cm³ m⁻² day⁻¹; iii) transmission rate of vapour water: 12 g m⁻² day⁻¹ (Toplex HB45, Plastopil, Israel). In PUA, no gaseous mixture was added before sealing. Under MAP, the atmosphere applied in this study is the same that was used in the company and was constituted by 30% O₂:40% CO₂:30% N₂ (Biogon CON 30:30, Linde Sogás, Lda., Alenquer, Portugal). For each kinds of packaging, three different lots of carcasses were analysed, in duplicate for each time period as described below.

2.2. Sampling scheme

The BP carcasses were examined at day 0, 3, 4, 5, 6, 7 and 8 of refrigeration. PUA samples were analysed at 0, 4, 8, 12, 14, 15 and 16 days, while MAP carcasses were analysed on days 0, 4, 8, 12, 16, 18, 19, 20 and 21. Day 0 corresponds to day of slaughter. Each sampling day, a different carcass was analysed, by aseptically removing 10 g to a sterile BagFilter (Breveté, France) containing 90 ml of tryptone

Table 1
Microbial counts in refrigerated rabbit carcasses before packaging (log CFU g⁻¹).

Group	Mean ± standard deviation (log CFU g ⁻¹)	Range (log CFU g ⁻¹)	Number of samples below detection limit/Total number of samples
Aerobic mesophilic	3.62 ± 0.59	2.87–4.87	0/12
Aerobic psychrotrophic	3.63 ± 0.86	2.46–5.25	0/12
<i>Pseudomonas</i> spp.	2.68 ± 0.85	1.00–3.99	0/12
Lactic acid bacteria	2.86 ± 0.40	2.21–3.71	0/12
Yeasts and Moulds	1.87 ± 0.97	<1.00–3.92	1/12
<i>Enterobacteriaceae</i>	1.18 ± 1.35	<1.00–3.27	6/12

salt (0.1% w/v) (Oxoid, Basingstoke, Hampshire, England) and blended in a stomacher during 30 s. The 10 g samples for microbiological analyses were composed of leg, hand, cutlet, kidney and liver, about 2 g of each part. The protocol used was adapted from Rodríguez-Calleja et al. (2005).

2.3. Microbiological analyses

Tenfold dilutions in tryptone salt were spread plated in duplicate on the following culture media. Mesophilic and psychrotrophic counts were made on plate count agar (PCA) (Biokar Diagnostics, Beauvais, France) and incubated at 30 °C or 4.5 °C for 3 and 14 days, respectively, as described by Rodríguez-Calleja et al. (2005) and Patsias, Chouliara, Badeka, Savvaiddis, and Kontominas (2006). Lactic acid bacteria were determined by sample incorporation (ISO 13408 E) on Man, Rogosa and Sharpe agar (MRS) (Biokar), incubated at 30 °C during 3 days. Catalase test was done on presumptive lactic acid bacteria. Yeasts and moulds were enumerated on Chloramphenicol Rose Bengal Agar Base (CRB) (Oxoid), supplemented with chloramphenicol (100 mg l⁻¹) and incubated during 5 days at 25 °C (Portuguese Norm 3277-1). *Enterobacteriaceae* counts were determined by sample incorporation on Violet Red Bile Glucose Agar (VRBGA) (Oxoid), incubated at 37 °C for 24 h (ISO 21528-2). From each set of countable plates, five colonies were randomly selected, isolated on plates of nutrient agar (NA) (Merck, Darmstadt, Germany) and tested for oxidase reaction and glucose fermentation in glucose agar (GA). *Pseudomonas* spp. numbers were determined after 2 days incubation at 25 °C, on *Pseudomonas* Base Agar (PBA) (Oxoid) supplemented with Ceftrimide–Fucidin–Cephalosporina (CFC) (Biokar) (ISO 13720 E). From each set of countable plates, five colonies were randomly selected, isolated on plates of NA and tested for oxidase reaction and glucose fermentation in GA.

Statistical analyses were performed using STATISTICA version 6.0 (Statsoft Inc., USA), using one-way ANOVA followed by the Fisher LSD Post Hoc test to evaluate significant differences ($p < 0.05$).

2.4. pH determination

Determination of pH was done in meat homogenates (10 g muscle per 10 ml distilled water), according to Rodríguez-Calleja et al. (2005), with a glass electrode (Schott pH) with a PHM 92 LAM meter (Radiometer, Copenhagen).

2.5. Shelf life determination

The shelf life determination was made using the counts of several microbiological indicators plotted against time, in days. Plots of log CFU g⁻¹ counts against the number of incubation days were visually examined to determine the lag phase, corresponding to the number of days of constant counts. The growth rates

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