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Effect of storage temperature and gas permeability of packaging film on the growth of lactic acid bacteria and *Brochothrix thermosphacta* in cooked meat emulsions

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Abstract

The effect of gas permeability of packaging film on the growth of lactic acid bacteria and *Brochothrix thermosphacta* in cooked meat emulsions stored at 0, 8 and 15 °C was investigated. The estimated parameters from Gompertz equation for the assayed temperature–oxygen permeability combinations showed LAB development to be significantly greater than those of *B. thermosphacta*. The influence of the two sources of variation (oxygen permeability of packaging film and temperature) on the growth parameters of LAB and *B. thermosphacta* was analysed showing a significant effect ($P<0.001$) of the temperature on both bacterial population while the film permeability had only a significant influence ($P<0.001$) on *B. thermosphacta* growth. Under the conditions of this study the packaging film influenced the maximum counts and growth rates of both organisms. Since the inhibition of *B. thermosphacta* occurred when the meat product was vacuum-packaged in films possessing high oxygen permeability and the effect of pH was found not to be associated with the growth inhibition, accumulation of hydrogen peroxide produced by LAB may possibly be one of the main factors responsible for *B. thermosphacta* inhibition. Shelf-life of vacuum-packaged cooked meat emulsions in high oxygen transmission rate films will be guarantied and a temperature abuse will not result in an increase of spoilage by LAB.

Keywords: Cooked sausages; Lactic acid bacteria; *B. thermosphacta*; Predictive microbiology; Temperature effect; Gas permeability effect

1. Introduction

The quality and shelf life of cooked meat foods are determined by the growth of micro-organisms. To control microbial survival and outgrowth of micro-organisms in foods, food preservation procedures are used. Vacuum-packaging has been shown to be very effective in extending the shelf-life of perishable foods such as meat products (Church and Parsons, 1995). Under these conditions the oxygen supply will be restricted, the gas phase being determined by the rate of gas permeation through the film and the rate of oxygen consumption in the package, these changes having a selective effect on the microbial population (Farber, 1991; Labadie, 1999). Storage of meat products in gas-impermeable packs restricts the growth of *Pseudomonas* so that lactic acid bacteria (LAB), *Brochothrix thermosphacta* and *Enterobacteriaceae* becomes the major component of the spoilage microflora (Taylor, 1996; Korkeala and Björkroth, 1997; Hansen and Bautista, 2000; Nychas and Drosinos, 2000).

LAB were identified as the major spoilage population of vacuum-packaged emulsion-type sausages and other processed meats stored at refrigeration temperatures (Korkeala and Björkroth, 1997; Samelis et al., 2000). *Brochothrix thermosphacta* is also found to be a numerical significant component of the microflora of

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meat and meat products stored under these conditions (Nielsen, 1983; Kotzekidou and Bloukas, 1995, Samelis et al., 2000), this bacterium changing from a major contaminant to a minor part of the final population after storage (Collins-Thompson and Rodriguez Lopez, 1980). The growth and metabolism of *B. thermosphacta* on meat and meat products depends on factors such as pH, temperature, gas environment and substrate availability (Dainty and Hibbard, 1983; Blickstad and Molin, 1983; Grau, 1980; Pin et al., 2002). LAB and *B. thermosphacta* significantly influence the quality of meat and meat products, both being associated with spoilage in this products. Under anaerobic conditions LAB may cause souring, slimy, swelling of the pack and/or greening, while *B. thermosphacta* produce mainly lactic acid, ethanol and only small amounts of short-chain fatty acids causing off-odours (Hitchener et al., 1979; Blickstad and Molin, 1983; Pin et al., 2002). Temperature is a major factor on food deteriorative reactions, especially for microbial spoilage since specific growth rate and lag phase are highly temperature dependent (Wijtzes et al., 1995; Devlieghere et al., 1998; Cayré et al., 2003). However, the bacterial development in the package is not only influenced by temperature, oxygen availability and water activity also determine the quantities and the type of micro-organisms growing on meats (Labadie, 1999).

Predictive modeling has been extensively used mainly to predict bacterial growth as a function of environmental factors such as temperature, pH and a_w (McMeekin et al., 1987; Zwietering et al., 1994; Rosso et al., 1995) and more recently to predict fungus growth (Panagou et al., 2003). Most of the research in this area has focused on modeling the effect of intrinsic and extrinsic parameters on the growth/inactivation of food pathogens (Pond et al., 2001; Castillejo-Rodríguez et al., 2002). Predictive modeling can also be applied to predict the shelf-life of foods, this being based on a prediction of the growth of the responsible spoilage bacteria in specified product. Sigmoidal curve such as Gompertz function has often been used to model microbial numbers on foods as a function of time. Combined with different statistical approaches, the Gompertz equation can be used to describe single and multiple effects of different growth conditions (Skinner and Larkin, 1994; Linton et al., 1996; Cayré et al., 2003).

In this study, the growth of LAB and *B. thermosphacta* on cooked meat emulsion vacuum-packaged under different oxygen permeability films and stored at different temperatures were monitored. Four growth parameters were estimated by fit the experimental data to primary growth model and the effect of oxygen permeability of packaging film and temperature on each were analysed.

2. Materials and methods

2.1. Sausage samples and storage

Cooked sausages were prepared in a local meat processing plant by traditional techniques. The composition of the food product as supplied by the manufacturer was 46% beef; 35% pork; 15% pork fat; 2% NaCl, powder milk 0.8%; nitrite and nitrate 0.02%; binding and flavoring additives 1%. Two separate batches were prepared by thoroughly mixing the ingredients. The mix was then emulsified, filled into natural casings and cooked to a core temperature of 75 °C this procedure resulting in sausages that were 13 cm long and 2 cm in diameter with an average weight of 35 g. Immediately after cooking samples were taken and transported to the laboratory under refrigeration conditions and vacuum-packaged using three packaging films: (a) Maraflex, oxygen transmission rate of $19\,cm^3\,m^{-2}\,24\,h^{-1}\,atm^{-1}$ at 25 °C and 75% Relative Humidity (RH) and (b) Triplon, oxygen transmission rate of 70 and $150\,cm^3\,m^{-2}\,24\,h^{-1}\,atm^{-1}$ at 25 °C and 75% RH. The films were supplied by ENVARIL S.A.I.C.I.F., Argentine. The packages were sealed to a final vacuum of 0.95 mm of Hg using a RAPI-VAC S-750 vacuum machine (SERVIVAC S.R.L Argentine). Same samples were immediately analysed (day 0) while the remaining were stored at 0 °C for 75 days, and 8 and 15 °C for 52 days. A new package was opened at each sampling time. The data presented are the result of two experiments.

2.2. Microbiological analysis and pH determination

Microbial evaluation of cooked sausages was performed by aseptically transferred 10 g of sample to a Stomacher bag containing 90 ml of sterile 0.1% (w/v) peptone-water and homogenized in a Stomacher (Lab-Blender 400, Seward, London, UK) at room temperature for two minutes. Serial decimal dilutions of the samples were prepared and duplicate 0.1 ml aliquots of appropriate dilutions were spread on agar plates. Total LAB were determined on MRS agar (Merck, Darmstadt, Germany) after incubated at 30 °C for 72 h while *B. thermosphacta* was enumerated on selective Streptomycin Thallous Acetate Agar base (STAA) whit full selective supplement (Oxoid, Basingstoke, England) and incubated at 25 °C for 48 h. Two separate determinations were performed and results were expressed as log colony forming units per gram ($cfu\,g^{-1}$). For pH determination 10 g of sample were blended with 90 ml distilled water for 30 s. Readings of pH values were taken using a digital pH meter (Orion Model 525A, Boston, Massachussets, USA).