Meat Science 98 (2014) 744–752

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Growth inhibition of lactic acid bacteria in ham by nisin: A model approach

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ARTICLE INFO

Article history:
Received 25 April 2014
Received in revised form 4 July 2014
Accepted 7 July 2014
Available online 13 July 2014

Chemical compounds studied in this article:
Sodium chloride (PubChem CID: 5234)
Sodium lactate (PubChem CID: 23666456)
Sodium tripolyphosphate (PubChem CID: 24455)
Sodium erythorbate (PubChem CID: 23683938)
Nisin (PubChem CID: 16219761)
Pediocin (PubChem CID: 56842033)

Keywords:
Lactobacillus sakei
Pediocin
Modified Gompertz model
Experimental design strategy

ABSTRACT

Lactic acid bacteria (LAB) have been described as spoilage organisms in vacuum-packed cooked ham. A Fractional Factorial Design was performed to investigate the relative merits of sodium chloride, sodium lactate, sodium tripolyphosphate, sodium erythorbate, nisin and pediocin, in limiting the *Lactobacillus sakei* growth in broth culture. This allowed rejection of sodium chloride, sodium lactate and sodium erythorbate (no significant effects on growth), and a Central Composite Rotatable Design broth culture study was performed comparing the effects of nisin and pediocin. From this study, nisin was identified as a more important variable for inclusion into a cooked ham model (significant effects on growth parameters: logarithmic increase in the population, exponential microbial growth rate and lag phase extension). The validation of this outcome in a model formulation of vacuum-packed sliced cooked ham (0.001%, 0.007% and 0.013% of nisin) stored for 60 days, confirmed the inhibitory effect of nisin on total LAB growth.

1. Introduction

The characteristics of microbial populations that develop in meat and meat products result from the effect of prevailing environmental conditions on the growth of the microorganisms initially present in the raw material or introduced by cross-contamination (Castellano, Belfiore, Fadda, & Vignolo, 2008). In most modified atmosphere-packaged and vacuum-packaged meat products, lactic acid bacteria (LAB) predominate (Arvanitoyannis & Stratakos, 2012). *Lactobacillus sakei* has been described by many researchers as part of the microbiota that deteriorates meat products, and especially sliced cooked ham (Han et al., 2011; Hu, Zhou, Xu, Li, & Han, 2009).

The formulations of meat products contain components that generate sensory characteristics in food, such as texture, juiciness, flavor, color and appearance. They can additionally generate positive, negative or neutral effects on microbial growth by interfering with intrinsic parameters such as antimicrobial components, water activity (Aw) and pH. Additionally, biopreservation systems, such as bacteriocinogenic LAB cultures and/or their bacteriocins, have received increasing attention, and new approaches to control pathogenic and spoilage microorganisms have been developed (Mattila, Saris, & Työppönen, 2003).

Bacteriocins can be defined as bacterially produced, small, heat-stable peptides that are active against other bacteria and to which the producer has specific immunity (Cotter, Hill, & Ross, 2005). The nisin produced by *Lactococcus lactis* subsp. *lactis* is active against Gram-positive organisms, including bacterial spores, but is not generally active against Gram-negative bacteria, yeasts and fungi (Economou, Pournis, Ntzimani, & Savvaidis, 2009). Nisin is essentially nontoxic to humans, leading to no cross-resistance with medical antibiotics, and is degraded without damage to the intestinal tract (Lindsay, 2010).

Pediocin is produced by *Pediococcus acidilactici* and is the most researched bacteriocin after nisin due to pediocin antimicrobial activity against *Listeria* strains. However, pediocin has been inadvertently or empirically used for many years as a starter culture in some food fermentations (Díez et al., 2012), which justifies further research and the use of the bacteriocin effect against different strains of Gram-positive microorganisms.

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http://dx.doi.org/10.1016/j.meatsci.2014.07.002
0309-1740/

Additionally, natural preservatives have increased the interest of consumers, who increasingly seek healthy and natural characteristics in all types of food, and especially types that already contain considerable amounts of chemical additives, such as processed meat products.

The application of statistical experimental design techniques in several process development and optimization has been extensively cited in the scientific literature (Cladera-Olivera, Caron, & Brandelli, 2004; Liu, Liu, Liao, Wen, & Chen, 2004). This methodology can be used as a tool to understand complex process and to describe the individual, cumulative and interactive effects of the test variables on the process yield and hence the process economics (Sen & Swaminathan, 2004). The Central Composite Rotatable Design (CCRD) is a statistical technique employed for multiple regression analysis by using quantitative data obtained from properly designed experiments to solve multivariate equations simultaneously (Box & Drapper, 1987; Box, Hunter, & Hunter, 1978; Box, Hunter, Hunter, & Hunter, 2005; Khuri & Cornell, 1987).

A complete application of the experimental design includes the selection of variables, by Fractional Factorial Design (FFD), followed by optimization through CCRD (Cockshott & Sullivan, 2001; Rodrigues & Iemma, 2014), as presented in this work.

Predictive microbiology is a useful tool in the study of the growth of microorganisms in food. Several researchers have used this tool to predict the logarithmic increase in the population (A), the exponential microbial growth rate (μ) and lag phase extension (λ) of microorganism growth curve. The modified Gompertz predictive model (MGM) is one of the most employed models to describe the microbial curves with good fit. Statistical indices have been applied for the evaluation of the quality of fit of the model to the experimental data, such as mean square error (MSE), bias factor, accuracy factor and determination coefficient (Chowdhury, Chakraborty, & Chaudhuri, 2007; Geitenes, Oliveira, Kalschne, & Sarmento, 2013; Sarmento, 2006; Slongo et al., 2009).

As LAB is the main group deteriorating on vacuum-packed sliced cooked ham, due to favorable conditions (presence of substrates, low oxygen tension, pH, redox potential) the lag phase of growth curve is short, in some cases not enough to be detected, consequently the exponential phase and stationary phase are reached (Geitenes et al., 2013). When the interest is to avoid development of unwanted microorganisms the extent lag phase is the major concern, especially because LAB cause unwanted flavors, decrease in pH, milky exudates, gas production, swelling of the pack, discoloration and/or greenish color (Audenaert et al., 2010; Hu et al., 2009; Jay, 2005). For meat companies to produce a microbiologically stable product and for the consumers to purchase a product with the desired quality (firstly sensory) is of paramount importance, especially if the ingredients have a more natural appeal as bacteriocins.

This context creates a highly favorable scenario for the redesign of this category of meat products, with a focus on extended durability against growth spoilage bacteria and with a primary purpose of increasing the lag phase. It is also very important to study the possible synergistic effects among ingredients and additives in formulations of ham, with the most innovative natural preservatives being nisin and pediocin.

The aim of this study was to investigate the possibility of controlling the development of LAB on vacuum-packed sliced cooked ham by two steps. The first step employed the control of *L. sakei* growth on Man, Rogosa and Sharpe (MRS) broth by the investigation of variables sodium chloride, sodium lactate, sodium tripolyphosphate, sodium erythorbate, nisin and pediocin, using a sequential strategy of experimental design. The second step involved the validation in a food matrix (cooked ham), applying the results obtained in the broth study to shelf-life monitoring. *L. sakei* was employed in the step of the study in MRS broth since this strain was identified as one of the main spoilage LAB in vacuum-packed sliced cooked ham, in a previous work that identified LAB isolated from the product (unpublished results).

2. Materials and methods

2.1. Tests of L. sakei growth in MRS broth

2.1.1. Fractional Factorial Design (FFD)

Aiming to minimize cell growth, an FFD 2^{6-2} (five central points, total of 21 trials) was initially used (Box et al., 1978; Rodrigues & Iemma, 2014) to evaluate the effect of six variables on the parameters of growth of *L. sakei*: sodium chloride (Diana, Paraná, Brazil), sodium lactate (Purac, Gorinchem, The Netherlands), sodium tripolyphosphate (Nutrifos®, ICL, Saint Louis, USA), sodium erythorbate (Germinal, São Paulo, Brazil), nisin (Globalfood, São Paulo, Brazil) and pediocin (ALTA 2345, Kerry, Wisconsin, USA). The statistical design and the coded and real values of the variables are shown in Table 1. For comparison, along with the 21 trials of FFD, four tests called "control treatments" were performed, namely CT_1, CT_2, CT_3 and CT_4. The *L. sakei*, identified as one of the main spoilage LAB in vacuum-packed sliced cooked ham (results not published), was acquired from the collection of cultures of André Tosello Foundation (Campinas, Sao Paulo, Brazil).

2.1.2. Central Composite Rotatable Design (CCRD)

The preliminary FFD allowed the selection of statistically significant variables in relation to *L. sakei* growth, as evaluated by the parameters A, μ and λ, adjusted to the MGM. The variables without a significant effect (sodium chloride, sodium lactate and sodium erythorbate) were fixed and maintained at level −1 of the FFD, whereas the variable sodium tripolyphosphate (with a significant effect) was set and maintained at the central point (level 0) of the FFD. For the variables nisin and pediocin (both with significant effects), a CCRD with five replicates at the central point and four axial points (2^2 plus star configuration, totaling 13 trials) was performed to reduce the concentrations of nisin and pediocin applied for *L. sakei* growth inhibition (Box & Drapper, 1987; Box et al., 1978; Haaland, 1989; Khuri & Cornell, 1987). The statistical design and the coded and real values of the variables are shown in Table 2. Four control treatments were performed in parallel for comparison, namely CT_1, CT_2, CT_3, and CT_4.

2.1.3. Performance of runs of FFD and CCRD

All runs of the FFD and CCRD, described in Tables 1 and 2, respectively, were conducted in 250 mL Erlenmeyer flasks by adding 1% (2.5 mL) *L. sakei* (ATCC 15521) inoculum (10^7 CFU·g^{-1}), and filling the flasks to the total volume (250 mL) with medium (MRS broth). The pH of the runs was adjusted in the range of 6.0–6.3 by adding 0.1 mol·L^{-1} NaOH or 0.1 mol·L^{-1} HCl. The cultivation was conducted at 30 °C in an incubator without shaking (Novaética, model 403-3D, São Paulo, Brazil). Monitoring of microbial growth experiments was performed by optical density measurements (absorbance) with 2 mL aliquot in a spectrophotometer (Perkin Elmer, Lambda XLS, Beaconsfield, UK) at predetermined intervals of 1 or 2 h, with calibration for a wavelength at 600 nm. The growth time was different for each test because the cultures were followed until the stationary phase of cell growth. The tests of the FFD remained in the range of 27–49 h, and the tests of the CCRD remained in the range of 27–55 h. The responses of the FFD and CCRD were obtained using the parameters of the logarithmic increase in the population (A), the exponential microbial growth rate (μ) and lag phase extension (λ), which were obtained from the fit of the modified Gompertz predictive model (MGM).

2.2. Tests in vacuum-packed sliced cooked ham

In the second step aiming to validate the results obtained in the studies using broth, which mainly supported a reduction in the amount of nisin applied (a variable that showed significant effects in the FFD and CCRD), three test formulations of cooked ham were prepared with the addition of nisin, and a control formulation was also produced (Table 3).