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Modeling the growth of lactic acid bacteria in sliced ham processed by high hydrostatic pressure

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ABSTRACT

The main responsible for the spoilage of cooked cured meat products stored under refrigerated and anaerobic conditions are lactic acid bacteria. The application of high hydrostatic pressure (HHP) reduces the lactic acid bacterial growth extending the product shelf-life and preserving natural taste, texture, color and vitamin content. This work studied the influence of pressure level and holding time on the lactic acid bacterial growth in vacuum-packaged sliced ham. Modified Gompertz and Logistic models were used to fit experimental data obtained from post-treatment microbial counts carried out along the product storage. Samples of sliced vacuum-packaged ham treated by HHP and control samples (non-treated) were stored at 8 °C until the microorganism population reached 10^7 CFU/g. An experimental planning 2^2 with triplicate at the central point was designed to determine the influence of pressure level (200, 300, and 400 MPa) and holding time (5, 10, and 15 min) on the product shelf-life. The results have shown that the pressure intensity and the holding time significantly influenced microbial population over the product storage. Shelf-life of ham treated at 400 MPa for 15 min was extended from 19 (control samples) to 85 days.

1. Introduction

The increasing demand for processed products that preserve original sensory and nutritional characteristics has been responsible for the increasing number of researches on alternative technologies. Ohmic heating, processing by electromagnetic fields, ultrasound technology, ionizing radiation, sterilization by membrane and, especially, high hydrostatic pressure processing are among these technologies and have been highlighted in many works (Butz & Tauscher, 2002; Rosenthal & Silva, 1997; Sangronis et al., 1997).

High hydrostatic pressure (HHP) processing is a very promising preservation technology for sliced cured meat products (Hugas, 1998; Rubio, Martínez, Garcia-Cachán, Rovira, & Jaime, 2007), since it can be applied to vacuum-packaged food, avoiding product recontamination. Ham, for instance, is a high added value product that could be processed by this technology. Despite the high market value, ham shelf-life is very short and, therefore, it is of great academic and industrial interest to know how it can be extended by HHP.

Predictive microbiology has been extensively used to model bacterial growth as a function of environmental factors such as temperature, pH and a_w (Cayre, Garro, & Vignolo, 2005; McMeekin, et al., 1987) and also can be employed to predict product shelf-life (Cayre et al., 2005). Sigmoidal equations, such as Logistic and modified Gompertz models, have been widely applied to describe the growth of lactic acid bacteria (Chowdhury, Chakraborty, & Chaudhuri, 2007; Mataragas, Drosinos, Vaidanis, & Metaxopoulos, 2006).

Lactic acid bacteria (LAB) are naturally found in many vacuum-packaged meat products stored under refrigeration, e.g. ham, provoking spoilage and diminishing shelf-life. Low oxygen concentration, high water activity (normally between 0.96 and 0.98) and pH around 6.0 are some of the characteristics that favors the lactic acid bacterial growth (Cayre et al., 2005; Cayre, Vignolo, & Garro, 2003; Mataragas et al., 2006).

The use of predictive microbiology to model microbial growth along storage of ham processed by high hydrostatic pressure plays an important role to access the effects of this technology on the product shelf-life. However few studies using predictive models for

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this purpose have been carried out. Thus, the aim of this work was to investigate the influence of pressure level and holding time on the shelf-life of vacuum-packaged sliced ham treated by high hydrostatic pressure, using predictive microbiology. Modified Gompertz and Logistic models were used to fit data of lactic acid bacterial growth along obtained from counts carried out the storage of treated and non-treated (control) ham, providing the time elapsed to reach 10^7 CFU/g (shelf-life criterion), the maximum growth specific rate and the lag phase extension.

2. Material and methods

2.1. Cooked ham

Ingredients (g): pork ham 2000, water 400, salt 20, carraghenate 2.0, sodium nitrite 0.30 and spice 0.30. Meat was triturated and injected with brine containing all the ingredients. Hams were molded, pressed and cooked in steam (atmospheric pressure) until the internal temperature reached 68 °C determined with a temperature sensor inserted in the product center (CTF 9008, ELLAB A.S, Denmark). After cooking, the product was kept in an ice bath for 40 min followed by more 40 min in a freezer at −18 °C. Afterwards, the product was stored at 4 °C for 24 h in a refrigerator and, then, sliced in 0.5 mm thick slices (CFI200, Skymsen, Brazil) and vacuum-packaged in plastic pressure resistant bags containing 15 g each (about 2 slices). The plastic bags were composed by polyethylene-*co*-vinyl alcohol (EVOH) and polyethylene, and the oxygen permeability was 2 cm^3/m^2 24 h l atm and the water permeability was <7 g/24 h m^2.

2.2. Experimental design

An experimental design was planned using the treatment pressure and holding time as variables. The pressure range varied from 200 to 400 MPa and the holding time from 5 to 15 min.

The design consisted of seven trials 200 MPa/05 min; 200 MPa/15 min; 400 MPa/05 min; 400 MPa/15 min and three replications at the central point (300 MPa/10 min). Ham shelf-life, lag phase extension and maximum specific growth rate were the searched responses. For all the experiments, the pressurization temperature was 27 ± 5 °C.

2.3. High hydrostatic pressure (HHP) treatment

The samples of vacuum-packaged sliced ham were treated by high hydrostatic pressure (HHP) in the 500 ml and 7 mm diameter chamber of the equipment Stanted Fluid Power (S-FL-850-9-W, England). The device was able to operate under pressures ranging from 100 to 450 MPa and temperatures ranging from 0 to 80 °C. The pressurization fluid used in this work was ethanol aqueous solution (0.70 L/L). Pressure was increased at 7 MPa s^{-1} and the decompression time was 30 s. The temperature of the pressurization medium increased by 5 °C while pressurizing, returning to the initial temperature after 3.5 min. The treated samples were stored together with the non-treated samples (control) at 8 °C, until the end-point defined as product shelf-life (10^7 CFU/g). Lactic acid bacteria (LAB) counts were carried out in duplicate at given times during storage.

2.4. Microbiological analyses of ham

Ten grams of each sample were taken and placed in sterile plastic bags with 90 ml of NaCl solution (0.85 g/100 ml). After 2 min in a stomacher blender (Nova Ética, 130/1, São Paulo, Brazil), appropriate decimal dilutions were pour-plated (1 ml) on De Man, Rogosa, Sharp (MRS) Agar medium (Biolife, Milano, Italy) for lactic acid bacteria and incubated at 30 °C for 3–5 days. The results were expressed as log (N/N_0), where N is the LAB concentration at time t (CFU/g) and N_0 is the LAB concentration at time zero (CFU/g).

2.5. Predictive model evaluation

The growth curve of LAB, log $(N/N_0) \times$ time, for each HHP treatment, was fitted by the modified Gompertz and Logistic models, by nonlinear regression, using Matlab® software (Math Works, Natick, MA, USA). The modified Gompertz model (Gibson, Bratchell, & Roberts, 1987) is given by Eq. (1),

$$\log\left(\frac{N}{N_0}\right) = A \times \exp\left\{ -\exp\left[\frac{\mu \times e}{A}(\lambda - t) + 1\right]\right\} \quad (1)$$

where λ is the lag phase extension (days); μ is the exponential microbial growth rate ($days^{-1}$); A the logarithmic increase of population and t is the storage time.

The Logistic Model is presented by Eq. (2),

$$\log\left(\frac{N}{N_0}\right) = \frac{A}{(1 + \exp(D - B \times t))} \quad (2)$$

where D is a dimensionless parameter; and B is the specific growth rate at the half-time value of the exponential phase (day^{-1}) and A is the logarithmic increase of microbial population (Gallo, Pilosof, & Jagus, 2007; Giannuzzi, Pinotti, & Zaritzky, 1998). From these parameters, μ and λ were derived as given by Eqs. 3 and 4.

$$\mu = \frac{A \times B}{4} \quad (3)$$

$$\lambda = \frac{(D - 2)}{B} \quad (4)$$

The predictive models used in this work are based on the post-treatment growth of LAB over storage at 8 °C.

2.6. Statistical analyses

The following statistical indicators were used to compare the performance of the models: mean square error (MSE), correlation coefficients (r^2), bias factor (BF) and accuracy factor (AF). The lower the MSE value (Eq. (5)) the better is the goodness-of-fit of each model.

$$\mathrm{MSE} = \frac{\sum(O - P)^2}{n} \quad (5)$$

O represents the observed value, P the predicted value and n is the degree of freedom (number of experimental points − number of model parameters).

The bias factor (BF) is an index of the model performance calculated by $\mathrm{BF} = \exp[\sum((\ln(P/O))/n)]$. A bias factor greater than 1.0 indicates that the predicted value is greater than the observed value. Conversely, a bias factor less than 1.0 indicates that the predicted value is lower than the observed value. Perfect agreement between predictions and observations leads to a bias factor of 1.0.

The accuracy factor is the sum of the absolute differences between predictions and observations, it measures the overall model error and is calculated by $\mathrm{AF} = \exp[\sum((\ln P - \ln O)^2/n)^{0.5}]$. The higher this value, the lower the accuracy of the average estimation.

Table 1
Statistical indexes obtained from fit Modified Gompertz (MG) and Logistic (LM) models to growth curves of lactic bacteria in pressurized and non-pressurized (control) sliced vacuum-packaged ham stored at 8 °C

Trails	Modified Gompertz model				Logistic model			
	r^2	MSE	Bias factor	Accuracy factor	r^2	MSE	Bias factor	Accuracy factor
1	0.999	0.004	0.939	1.200	0.998	0.017	1.055	1.130
2	0.987	0.159	1.039	1.282	0.986	0.170	1.025	1.189
3	0.998	0.057	0.965	1.081	0.997	0.107	0.974	1.079
4	0.997	0.043	1.080	1.205	0.982	0.012	1.085	1.160
5	0.991	0.114	0.985	1.123	0.994	0.079	1.017	1.155
6	0.992	0.126	0.969	1.124	0.994	0.093	1.007	1.105
7	0.996	0.062	0.969	1.129	0.992	0.121	0.969	1.159
Control	1.000	0.001	1,118	1.254	0.999	0.009	1.191	1.373

3. Results and discussion

Statistical indexes obtained for each trial are presented in Table 1. Both models were able to describe microbial growth in ham, but Gompertz model presented a slightly superior performance. Therefore, microbial growth parameters (Table 2) and product shelf-life were calculated using MG model. Levels of 10^7 CFU/g have been pointed by some authors as the safety limit for cooked meat products (Ruiz-Capillas, Carballo, & Colmenero, 2007).

Fig. 1 shows the curves of lactic acid bacterial growth in treated and non-treated (control) samples. The lag phase extension is very important to the product commercialization. As can be seen, it varied from 11 (control samples) to 55 days (samples treated at 400 MPa for 15 min). Fig. 1 and Table 2 show that the shelf-life of the control sample was limited to 19 days, at 8 °C, due to microbial growth. The shelf-life of samples treated by the most severe conditions (400 MPa/15 min) increased up to 85 days. Even the less severe conditions (200 MPa for 5 min), was responsible for the extended lag phase and product shelf-life up to 13 and about 47 days, respectively. Thus, the application of HHP to the vacuum-packaged sliced ham significantly increased shelf-life. Although it is well known that some strains of lactic acid bacteria are responsible for slime in vacuum-packaged ham (Cayre et al., 2003, 2005; Kotzekidou & Bloukas, 1996), in this work, this kind of alteration was observed only in control samples.

Although the increased lethality of the treatments carried out at higher pressures may also have resulted from higher temperatures this study did not aim to access the role of each variable of the HHP processing but to demonstrate that the product shelf-life can be extended.

Ruiz-Capillas et al. (2007) treated the HHP processing of 400 MPa for 10 min to a vacuum-packaged sliced ham. They obtained shelf-lives of 77 and 28 days, for products stored at 2 and 12 °C, respectively. As expected, the product storage temperature was a very important parameter to determine the product shelf-life. Statistical analysis were performed on data presented in Table 2 using the software Statistical 6.0. The statistical parameter p value ($p < 0.05$) was used to determine the significance of pressure and holding time on the product shelf-life and on the growth parameters (μ and λ). The results are reported in Table 3.

Even the less severe conditions revealed to diminish microbial growth be able to products treated under 200 MPa for 5 min had then lag phase and shelf-life extended to 13 and about 47 days, respectively. Slongo et al. (2007a) noticed that the sensory characteristics of pressurized sliced ham were similar to the non-pressurized.

The effects of pressure, holding time and the interaction between them were significant for the lag phase extension (λ). The more influent factor was the pressure intensity: by increasing pressure from 200 to 400 MPa, the lag phase length increased almost 30%.

Different holding times did not cause significant differences on the μ value. The pressure intensity and the interaction between pressure and holding time were significant. The increase of pressure and holding time was responsible for the reduced μ values.

The results showed that the pressure intensity was the most significant factor affecting the shelf-life extension and the growth parameters of LAB in vacuum-packaged sliced ham processed by HHP technology. Garriga, Grebol, Aymerich, Monfort, and Hugas (2004) also observed an important microbial inactivation after treating vacuum-packaged sliced ham by HHP technology. They concluded that this process could also contribute for preserving the product sensory characteristics. Besides, the results of preference tests revealed that the pressurized sliced hams performed very well among consumers (Slongo et al., 2007b).

Table 2
Microbial growth parameters (μ and λ) and product shelf-life (t_{sl}) obtained by fitting Modified Gompertz model to LAB growth curves of sliced vacuum-packaged ham treated by HHP and non-treated samples (control), stored at 8 °C

Trails	Factors		Responses		
	Pressure	time	t_{sl}[a] [day]	μ [Day^{-1}]	λ [Day]
1	200	05	47	0.24	13
2	200	15	45	0.21	14
3	400	05	80	0.13	30
4	400	15	85	0.21	55
5	300	10	61	0.21	30
6	300	10	55	0.19	22
7	300	10	58	0.21	26
Control	–	–	19	1.00	11

[a] t_{sl} –time to reach 10^7 CFU/g – end of product shelf-life.

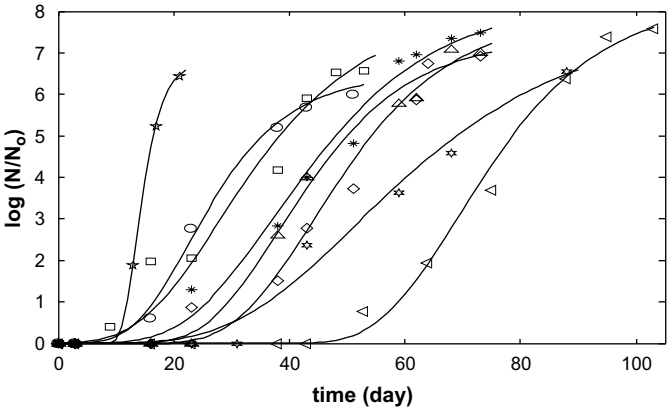

Fig. 1. LAB growth curves in sliced vacuum-packaged ham submitted to different HPP treatments (*– control; ■ – 200 MPa/05 min; □ – 200 MPa/15 min; ○ – 300 MPa/10 min; ● – 300 MPa/10 min; - 300 MPa/10 min; ◆ – 400 MPa/05 min; ▲ – 400 MPa/15 min) and stored at 8 °C. Data were fitted by Modified Gompertz model (MG).

Table 3
Influence of pressure (P) and holding time (PT) on product shelf-life and on the growth parameters (μ and λ) of LAB growth in vacuum-packaged sliced ham, stored at 8 °C

Factors	Responses					
	λ (day)		t_{sl}[a](day)		$\mu = (\text{dias}^{-1})$	
	Effect	p Value	Effect	p Value	Effect	p Value
P	29.5	0.0047	36.6	0.0067	−0.055	0.013
PT	13.2	0.0426	1.35	0.697	0.025	0.096
$P \times$ PT	12.1	0.0530	3.05	0.613	0.055	0.013

[a] time to LAB count reach 10^7 CFU/g in vacuum-sliced packaged ham.

4. Conclusions

The application of HHP technology to vacuum-packaged sliced ham increased the product shelf-life based on LAB population.

Both Gompertz and Logistic models were able to describe microbial growth in ham, but Gompertz model revealed a slightly superior performance. Through this model, shelf-lives of products treated by different pressures and holding times could be accessed.

The pressure intensity was the most significant factor influencing ham shelf-life and the growth parameters of LAB in vacuum-packaged sliced ham treated by HHP technology. The most severe treatment used in this study (400 MPa for 15 min) increased the product shelf-life from 19 days (non-pressurized sample) to almost 85 days. This result clearly showed that HHP is a very useful technology to extend the shelf-life of vacuum-packaged sliced ham.

Further studies are necessary to access the influence of high pressure on physical (texture, color) and chemical (moisture, pH, lipid oxidation, a_w) properties of pressurized ham.

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