PREDICTION OF LACTIC-BACTERIA GROWTH IN TURKEY HAM PROCESSED BY HIGH HYDROSTATIC PRESSURE

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ABSTRACT High hydrostatic pressure (HHP) is an innovative technology for food processing with lower environmental impact and product quality damage. Instead of heating, commonly used for food preservation, it uses high pressure (100 MPa to 900MPa) to ensure microbiological safety and maintain sensory and nutritional characteristics. More recently it has been investigated and industrially applied to extend shelf life of meat-based products. Traditional ham stored under refrigeration and microaerophilic conditions may sometimes present high population level of deteriorating lactic acid bacteria, which limits shelf life due to development of unpleasant odour and greenish and sticky appearance. This study aimed to evaluate the shelf life of turkey ham pressurized at 400MPa/15 minutes and stored at fridge temperatures 4, 8 and 12° C, in comparison to the non pressurized product. The population of lactic acid bacteria was considered the limiting shelf life parameter up to 107 CFU/g of product. It was found that by using the storage temperature of 4 ° C, the commercial viability of the control sample resulted in 45 days while the pressurized sample achieved 75 days, showing that the high pressure process greatly increased the product shelf life. Predictive modified using Gompertz and Baranyi & Roberts models fitted well both for the pressurized and control samples, allowing further prediction of product shelf life according to the storage conditions. These results indicated that the high hydrostatic pressure treatment may bouble the commercial viability of turkey ham, by slowing down the growth of microorganisms in the product.

Keywords: High hydrostatic pressure, predictive models, lactic acid bacteria, turkey ham.

INTRODUCTION The habits of consumers have changed over time and there is presently a higher health concern. There are nowadays greater demands for products that are easy to prepare and have greater durability, without presenting nutritional value losses in comparison to fresh food and raw materials. Therefore, it is growing the demands for

technologies that bring specific benefits, particularly in relation to increasing commercial viability without causing undesirable sensory and nutritional changes (MATHIAS, 2008).

High hydrostatic pressure (HHP) is an innovative technology and has the advantage of being a clean technology that avoids harming the environment. That is not a treatment that uses heat as the basis for preservation, but instead high pressure in the range of 100MPa to 900MPa, with optional time and temperature variation, which ensures flexibility of work in accordance with the type of food. It presents the great benefit of ensuring safe food and increasing product commercial viability, while maintaining sensory and nutritional characteristics virtually unchanged due to the processing (MATHIAS, 2008).

The HHP produces morphological, biochemical and genetic changes in the microorganisms, and particularly affects theirs membranes and cell walls (Sangronis et al., 1997). It increases the permeability of cells and inhibits reactions and energy by denaturizing enzymes that are essential for growth and microbial reproduction (Calderón-Miranda et al., 1998). The HHP treatment can ensure the destruction of up to 8 log units of certain types of bacterial cells, without altering the flavor and nutritional value of foods (Dogman & Erkmen, 2004). The capacity for destruction or inactivation of microorganisms by the high hydrostatic pressure process varies according to the level, time and temperature of pressurization, the type of microorganism and its growth stage, as well as the food composition (mainly depending on the pH and water activity) (CALDERÓN-MIRANDA et al., 1998; ROSENTHAL & SILVA, 1997).

When meat products are stored under refrigeration and microaerophilic conditions, such as vacuum or modified atmosphere packaging, lactic acid bacteria may very often predominate in the product deterioration. Since these products are commonly heated, usually within the range of 68 to 75°C, most vegetative cells are killed and recontamination of the post-heating products determines the commercial validity (Borch et al., 1996; Vermeiren et al., 2004). The recontamination after cooking, especially by the microbiota present in processing courts of industries, is considered as the main factor, along with the storage temperature, which affects the shelf life of meat products (Samelis et. al., 1998). Typically, the initial count of lactic acid bacteria in meat products packaged under vacuum is low, but increases during storage under refrigeration and can cause evident deterioration when the count reaches 7 to 8 log 10 cfu/g (Santos et. al., 2005; Vermeiren et. al., 2005). Defects caused by the deterioration include unpleasant odor, sour taste, color green and slimy appearance (CHENOLL et. al., 2007).

As the high pressure technology has wide applicability in increasing the commercial viability and inactivation of microorganisms, its use in conjunction with predictive microbiology has great advantages in estimating microorganisms' growth, ensuring a much safer food. The predictive microbiology is able to provide good estimation, through the use of mathematical models and quantitative study, of microorganisms development, in food. The predictive models (mathematical modeling) can be very useful in the food industries, allowing appropriate prediction of the growth of microorganisms, and also the evaluation of the impact of each factor associated to it. It can also allow the evaluation of how an emerging technology may interfere on the validity of a certain product.

The food microbiologists have sought efficient models to describe microbial growth that may allow its prediction and consequences during food storage (Baranyi & Roberts, 1994). They have been using a classic three parameters model in the characterization of bacterial growth: the phase lag (λ) the maximum specific growth rate (μ) and maximum population density within a certain growth period (A) (Baty & Delignette-Muller, 2004).

This study aimed to model the growth of lactic acid bacteria in pressurized and unpressurized (control) turkey ham at different storage temperatures, estimating the trade product validity in each case.

MATERIAL AND METHODS

Material Turkey legs, frozen, packed in small plastic bags containing about 1.2kg per package and gathered in cardboard boxes with 15kg each for commercialization, were acquired from a company based in southern Brazil which distribution network includes Rio de Janeiro city. The company works with special cuts of frozen turkey and delivers the goods in temperature-controlled trucks, following all the basic requirements of hygiene and conservation. The goods were stored at -18°C in a refrigerator.

Methods The experimental work was carried out at Embrapa Food Technology. For the manufacture of ham, first a "toilet" was carried out on the frozen turkey thigh, using knives for removing bones, tendons, nerves, and skin, and cut the meat into smaller pieces. For the formulation additives and spices were used, purchased from the company Duas Rodas Industrial ®, which is certified ISO 9001/2000 and ISO 14001 for the manufacture of all its products. The components of the brine were weighed and diluted in cold water, by constantly stirring up to complete dissolution until it was added to the meat. The meat mixed with brine was taken to the "cutter" (*Geiger and model UM12*) in which alternating operation (2 or 3) were carried out lasting few seconds each, in order to reduce the meat into smaller pieces to obtain a more homogeneous mass. Next it was transferred to a plastic container covered with a lid and brought to the refrigerator at 5°C, where it remained for 24 hours. After this period, the mass in portion of 2,5kg in average each was placed in high temperatures resistant plastic (cook-in), enclosed in a vacuum sealer (*model 30 and Engevac gas*) and placed in stainless steel cooking forms. The cooking was carried out in an autoclave by setting and controlling the internal temperature of the product at 72°C, by using an internal temperature controller (*model ELLAB*) connected to thermocouples placed in different parts of the control. After cooking, the product was cooled in ice bath for 40 minutes and then stored in the refrigerator at 4°C for 24 hours. After that period, the turkey ham was ready to be vacuum packed and undergo high hydrostatic pressure treatment. The pieces of turkey ham were sliced (*SKYMSEN model CFI-300*) into 0,5 mm thickness slices and packaged in plastic film, being kept in cold room up to the processing time. For aseptic assurance all manipulation was carried out inside an air flow chamber with all materials previously facing the action of UV light for at least 15 minutes. Each sterile plastic bags containing sliced turkey ham (Figure 1a) and sealed under vacuum (sealing *Engevac and model 30 gas*) measured in average 10cm x 4cm.

The equipment of high hydrostatic pressure (*Stansted Fluid Power and model S-FL-850-9-W*) used was a laboratory model (Figure 1b), located in Embrapa Food Technology.

The equipment has the capacity to operate at a pressure of 100MPa to 900MPa, at temperatures between 0 to 80°C and various time intervals. The equipment was controlled through a digital panel for adjusting the pressure, time and temperature. The samples of turkey ham were placed inside the cylinder-shaped, stainless steel sample holder (Figure 1c), containing several holes through trough which circulates the pressurizing liquid, in this case 70% alcohol. At the end of the process, the chamber was opened and samples were taken from the pressurized cylinder and destined to microbiological analysis.

Figure 1. (a) Vacuum packaged turkey ham; (b) High pressure equipment; and (c) sample holder containing packaged turkey ham samples.

Operating conditions of high pressure Pressure level of 400MPa for 15 minutes was used at room temperature, based on the results obtained by Slongo (2008) focusing on storage of pork ham treated by high pressure. Base on that study it was concluded that the conditions mentioned above significantly increased the commercial viability of the pork ham and preserved sensory properties, being therefore adopted as a model for the present study.

Microbiological analyses To perform the microbiological testing, samples were handled in the flow chamber cleaned, removing aseptically 5,000g and divided into sterile bags (*Nasco WHILE-PACK* ®) containing 25g each, vacuum packed and stored at 4, 8 and 12°C for 75 days. The commercial viability of turkey ham and pressurized control was determined based on the research of lactic acid bacteria (LAB) following the methodology recommended by APHA (2001). From each piece of turkey ham, either control (unpressurized) or pressurized, 25g of were aseptically sampled, placed in sterile bags and added by 225mL of peptone water (1%). Samples were homogenized for 60 seconds in *stomacher*, following by dilution and plating on agar culture of Man, Rogosa, Sharp (MRS), and incubation at 30°C for 3 to 5 days. The analyses were performed in duplicate and results were expressed in Log (N) (N: colony forming unit end [CFU/g]), until the samples reached the count of 10^7 CFU/g.

Evaluation of the predictive model The predictive models of Modified Gompertz and Baranyi were adjusted to the growth curves using software Matlab® (Math Works, Natick, MA, USA).

The Modified Gompertz Model (Gibson, Bractchell & Roberts, 1987) is defined by the equation:

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

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

The Baranyi Model (Baranyi & Roberts, 1994) is represented by the equation below, where A, B, C and D are mathematically rearranged:

$$\ln\left(\frac{x}{x_o}\right) = D + Bt + \frac{\ln\left(e^{-nBt} + e^{-C} - e^{-nBt - C}\right)}{B} - \frac{\ln\left(1 + \frac{e^{mBt + \frac{\ln\left(e^{-nBt} + e^{-C} - e^{-nBt - C}\right)}{B}} - 1}{e^{m(A-D)}}\right)}{m}$$
(2)

Constants have the following physical meaning

$$A = y_{max}, \quad B = \mu_{max}, \quad C = h_o = \lambda.\mu_{max}, \quad D = \nu \quad \text{and} \quad n = \frac{\mu_{max}}{\nu}.$$

Statistical analyses The following statistical indices were used in order to compare the performance of models: mean-squared error (MSE), regression coefficient (R^2), factor bias and accuracy factor. The lower the value of MSE is, the better is the fit of the model to experimental data (Sutherland & Bayliss, 1994). The MSE is defined in the following equation: $MSE = \Sigma (O-P)^2/n$. in which O represents the observed value, P the predictive value, n the number of degrees of freedom (number of experimental points - number of model parameters).

The factor bias is represented by the equation: BF= exp$[\Sigma((In(P/O))/n]$ and consists of an estimate for the average difference between the observed and predicted and should be close to 1. If the value is greater than 1 it indicates that the expected value is greater than that observed, but if it is less, it indicates that the predicted value is lower than that observed. The factor of accuracy is the sum of absolute differences between predictions and observations and measures the overall error of the model and is calculated by the equation: $AF = exp[(=(LNP - LNO)2/n) 0,5]$. The higher this value is, the lower is the accuracy of the estimate of the average.

RESULTS AND DISCUSSION The microbiological parameters of growth: A - logarithmic increase of the population, μ - maximum specific growth (days^{-1}), λ - duration of the lag phase (days) and time in days to reach the end of the commercial viability (CV) for the growth of lactic acid bacteria in turkey ham are presented in Table 1. These results were obtained by fitting the modified Gompertz model to the growth curves of BAL for the temperature conditions of storage, control and pressurized at 4°C,

control, and pressurized to 12°C. For the conditions of control and pressurized to 8°C, the modified Gompertz model did not provide a fit and the model of Baranyi was then used for the best fit of these curves.

The key figures of growth curves under different conditions, evaluated by the modified Gompertz model and Baranyi are shown in Table 2.

The storage temperature was shown to have great influence on the growth of LAB. The parameters obtained in the temperatures studied showed the importance of maintaining low temperatures to achieve greater commercial viability, and yet it can be seen that the use of high hydrostatic pressure led to increased commercial viability and a reduction of μ. Such implications were evident from the fact that the pressurized turkey ham stored at 12°C showed greater validity when compared to the control ham stored at 8°C. It was also observed that at 4°C the commercial viability of the control sample achieved 45 days, while the pressurized sample lasted up to 75 days, assuring extra 30 days of commercial.

Table 1. Kinetic parameters for growth of Lactic Acid Bacteria adjusted to Modified Gompertz and Baranyi models.

T (°C)	Control					Pressurized				
	CV (days)	A	λ (days)	μ (day^{-1})	r^2	CV (days)	A	λ (days)	μ (day^{-1})	r^2
	Modified Gompertz Model					**Modified Gompertz Model**				
4	40	6,401	25	0,4326	0,948	75	6,767	19	0,1501	0,956
	Baranyi Model					**Baranyi Model**				
8	25	----	----	0,2387	0,976	65			0,0841	0,959
	Modified Gompertz Model					**Modified Gompertz Model**				
12	24	6,208	6	0,4521	0,984	30	7,047	5	0,317	0,999

Table 2. Statistics obtained from the fitting for modified Gompertz model and Baranyi of lactic acid bacteria population in pressurized vacuum packed turkey ham, in comparison to the control, and stored at 4, 8 and 12°C.

Predictive Model	Statistical parameters			
	r^2	*MSE*	Bias factor	Accuracy factor
	Control stored at 4ºC			
Modified Gompertz Model	0,948	0,05878	0,9989	1,0294
	Pressurized stored at 4ºC			
Modified Gompertz Model	0,9586	0,11615	0,9989	1,0864
	Controle stored at 8ºC			
Baranyi Model	0,9768	0,09382	1	1,0396
	Pressurized stored at 8ºC			
Baranyi Model	0,9595	0,1394	1,0078	1,0437
	Control stored at 12ºC			
Modified Gompertz Model	0,9843	0,0254	1,00195	1,01812
	Pressurized stored at 12ºC			
Modified Gompertz Model	0,9999	0,00017	0,99967	1,00203

To date, most studies of high pressure treatment on the microflora of ready to eat and meat products have been directed to control after processing storage temperature at 4°C, instead of including temperature abuse evaluation (Kreyenschmidt et al., 2009). In this study, we used a higher temperature (12°C), reproducing the variations that can occur in storage and by the models used to predict the validity of these unfavorable conditions. It can be concluded that, even at high temperatures, turkey ham processed by high pressure showed satisfactory validity based on lactic bacteria growth when compared with the control stored at lower temperature.

Figure 2 represents the growth curves of the lactic acid bacteria according to storage temperatures of 4, 8 and 12°C, applying predictive models of modified Gompertz and Baranyi. The curves of microbial growth presented in overall good fit, giving important information about the potential growth of lactic bacteria and commercial viability of turkey ham for each storage temperature.
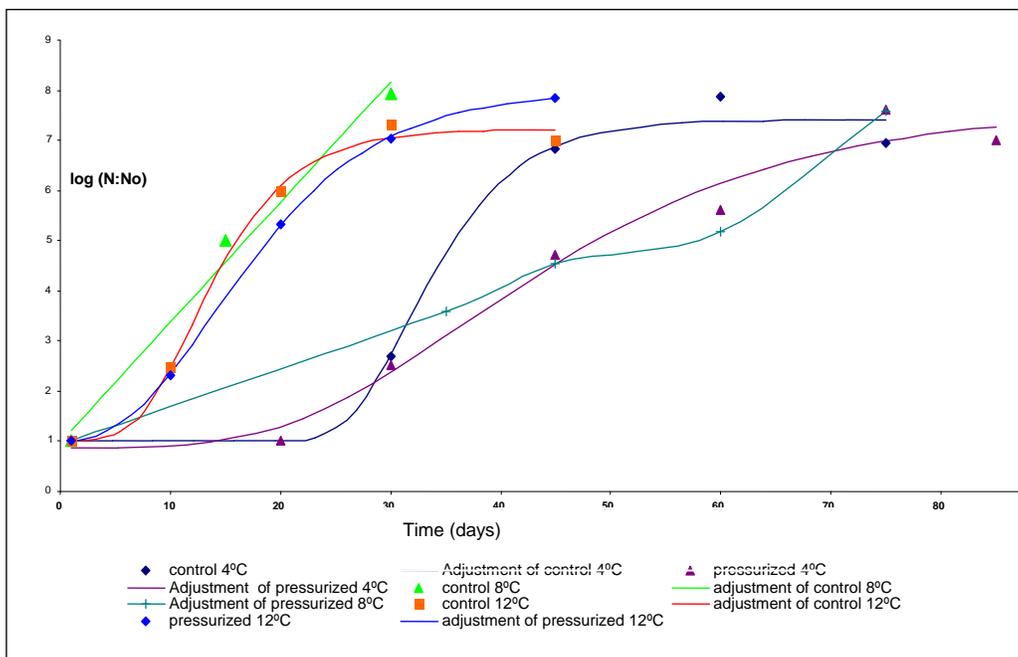

Figure 2. Growth curves of lactic acid bacteria in control and pressurized turkey ham at different storage temperatures.

In ham treated at 400MPa for 15 minutes at a temperature of 8°C, Slongo et. al. (2008) obtained commercial viability of 85 days compared with control, which lasted only 19 days. Those results are similar to the presently obtained with turkey ham using the same conditions, in which the pressurized sample showed commercial viability of 65 days and a control sample reached commercial viability in 25 days. According to the studies by Ruiz-Capellas (2007), the high-pressure treatment of 400MPa for 10 minutes to vacuum packaged ham provided a commercial validity of 77 and 28 days for products stored between 2 and 12°C, respectively. However, López-Caballero et al. (1999), with the same type of product but treated at pressures between 200MPa and 400MPa, did not attain the same degree of inactivation and commercial viability achieved at 3°C was only 21 days.

At higher pressures, such as those used by Slongo (2008) with ham slices pressurized at 600MPa for 5 minutes at 30°C and stored at 5°C for 120 days, lactic acid bacteria population growth had not increase significantly during storage period. Park et. al. (2001) in studies with ham processed at 600MPa for 5 minutes and 25°C showed a reduction of ~ 4 log 10 cfu/g of lactic bacteria due to the processing. Garriga et al. (2004) reported that vacuum packaged ham treated at a pressure of 600MPa for 4 minutes at 16°C showed lactic bacteria count after 30 days of 2.10 log10, and observed a significant microbial inactivation after pressure treatment. That also agrees with the results from by Carpi et al. (1999), which reported an increase up to 75 days in the commercial viability of sliced cooked ham treated at 600MPa for 5 minutes, when stored at 4°C.

CONCLUSION Application of High Hydrostatic Pressure at 400MPa and 15 minutes was effective to greatly delay lactic bacteria growth in turkey ham. The time required for the microbial population to achieve the limit presently defined for the product validity was lower for the stored pressurized sample at 12°C even when compared to the control stored at 8°C. Both Modified Gompertz and Baranyi models provided good fitness for the microbial population variation with the storage time, showing high determinant coefficients for the regression adjustments. Modified Gompertz models presented better fitting for the lactic bacteria growth for both pressurized and control sample, either stored at 4°C or 12°C, while Baranyi model presented a better fit for samples stored at 8°C. Predictive microbiology showed to be a valuable tool to provide a good estimative of the validity of the product based on lactic bacteria growth, and high hydrostatic pressure proved to be very effective to delay microbial development and allow an extended product shelf life.

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