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Research Paper

Prediction of acid lactic-bacteria growth in turkey ham processed by high hydrostatic pressure

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Abstract

High hydrostatic pressure (HHP) has been investigated and industrially applied to extend shelf life of meat-based products. Traditional ham packaged under microaerophilic conditions may sometimes present high lactic acid bacteria population during refrigerated storage, which limits shelf life due to development of unpleasant odor and greenish and sticky appearance. This study aimed at evaluating the shelf life of turkey ham pressurized at 400 MPa for 15 min and stored at 4, 8 and 12 °C, in comparison to the non pressurized product. The lactic acid bacteria population up to 10^7 CFU/g of product was set as the criteria to determine the limiting shelf life According to such parameter the pressurized sample achieved a commercial viability within 75 days when stored at 4 °C while the control lasted only 45 days. Predictive microbiology using Gompertz and Baranyi and Roberts models fitted well both for the pressurized and control samples. The results indicated that the high hydrostatic pressure treatment greatly increased the turkey ham commercial viability in comparison to the usual length, by slowing down the growth of microorganisms in the product.

Key words: high hydrostatic pressure, predictive models, lactic acid bacteria, turkey ham.

Introduction

Thermal treatment has been the basis of most processes industrially applied to ensure microbiological safety of foods. Recent consumer trends have led to technology innovation towards more healthy, nutritional and convenient foods. High hydrostatic pressure (HHP) treatment has been considered as one of the most promising nonthermal technology to preserve foods (Knorr 1993). It has been used in different food sector worldwide such as in fruit, dairy and meat products. In the meat sector, the technology offers a valuable alternative to the thermal pasteurization, especially for preserving convenience products (Rastogi *et al.*, 2007).

HHP produces morphological, biochemical and genetic changes in microorganisms, and particularly affects their membranes and cell walls (Sangronis *et al.*, 1997). It increases cell permeability and inhibits reactions and energy production by denaturing enzymes that are essential for growth and microbial reproduction (Calderón-Miranda *et al.*, 1998). The 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 and Erkmen, 2004). The capacity of microorganisms destruction or inactivation by high hydrostatic pressure varies according to the pressure 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) (Rosenthal and Silva, 1997; Calderón-Miranda *et al.*, 1998).

A cured cooked meat product is a perishable product spoiled mainly by acid lactic bacteria (LAB) which cause discoloration, slime formation, off-odors and off-flavors as the result of their metabolic activity leading to the produc-

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tion of various compounds. These microbial products in conjunction with the microbial population could be used to assess the degree of spoiled of this type of product (Mataragas *et al.*, 2007). 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 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 industrial environment is considered as the main factor which affects the shelf life of meat products, along with the storage temperature (Samelis *et al.*, 1998). Typically, the initial count of lactic acid bacteria in meat products packaged under vacuum is low, but increases during refrigerated storage and may cause evident deterioration when the count reaches 7 to 8 log 10 CFU/g (Santos *et al.*, 2005; Vermeiren *et al.*, 2005).

The prediction of shelf life allows companies to optimize their storage management and it is, due to the minimization of economic losses, one of the most important company planning issues these days (Raab *et al.*, 2008). During the last years, several models have been developed to predict the growth of the specific spoilage organism (SSO) in fresh food products such as meat and fresh meat products (Baranyi *et al.*, 1995; Mataragas *et al.*, 2006; Gospavic *et al.*, 2008). The majority of the shelf-life models for chilled products describe the growth of SSOs depending on the temperature which is generally consider the most important influencing factor of shelf life (Zwietering *et al.*, 1991; McMeekin *et al.*, 1992). Food microbiologists have sought efficient models to describe and predict microbial growth and its consequences during food storage (Baranyi and Roberts, 1994). A classic model has been used in the characterization of bacterial growth considering the three following parameters: the lag phase (λ) the maximum specific growth rate (μ) and the maximum population density within a certain growth period (A) (Baty *et al.*, 2004).

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

Material and Methods

Sampling

Frozen turkey legs packed in small plastic bags were purchased in a Brazilian supermarket. The packages contained about 1.2 kg each, and were grouped in cardboard boxes with 15 kg each for commercialization, the manufacturing company from South Brazil works with special cuts of frozen turkey and delivers the products in temperature-controlled trucks, following all the basic requirements of hygiene and conservation. The turkey leg packages are stored at -18 °C in freezer up to the commercialization.

Preparation of the samples

The experimental work was carried out at Embrapa Food Technology. For the manufacture of ham first a "toilet" was carried out on defrost turkey thighs using knives for removing bones, tendons, nerves, and skin, and to cut down the meat into small pieces. A commercial formulation of additives and spices purchased from the company Duas Rodas Industrial® was used in the ham preparation. Brine mixture was 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 a "cutter" (*Geiger, model UM12*) in which alternating operations (2 or 3) were carried out for few seconds each, in order to first reduce the meat into smaller pieces and then to obtain a more homogeneous mass in the end. Next it was transferred to a plastic container covered with a lid and taken to a refrigerator, where it remained for 24 h at 5 °C. After that period, the mass in portions of 2.5 kg each in average was vacuum packaged in thermal resistant plastic bag (cook-in) and placed in stainless steel cooking forms. The cooking was carried out at 72 °C in autoclave. Temperatures was monitored with a temperature indicator (*model ELLAB*) throughout thermocouples placed in different portions of the control. After cooking, the product was cooled down in ice bath for 40 min and then stored in a refrigerator at 4 °C for 24 h. After that period, the turkey ham was ready to the the high hydrostatic pressure treatment. The pieces of turkey ham were sliced (*SKYMSEN, model CFI-300*) into 0.5 mm thickness slices and vacuum packaged in plastic bags, being kept in cold room up to the processing time. For aseptic assurance all manipulation was carried out inside an air flow chamber (*Booth FLV-K, series 256-8I*) being all materials exposed to UV light for at least 15 min.

High hydrostatic pressure treatment

The high hydrostatic pressure equipment used was a laboratory model (*Stansted Fluid Power, model S-FL-850-9-W*). The equipment had the capacity to operate within a pressure range between 100 MPa to 900 MPa, and temperatures between 0 to 80 °C. The equipment was controlled through a digital panel for adjusting pressure, time and temperature. The turkey ham samples were placed inside the cylinder-shaped stainless steel sample holder, containing several holes through which circulates the pressurizing liquid, in that 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 analyses. The pressure treatment at 400 MPa for 15 min was applied at room temperature, based on the results obtained by Slongo *et al.* (2009), who investigated the pressure treatment of pork ham. According to that study the selected op-

erational conditions significantly increased the product commercial viability (cv) and preserved its sensory properties, being therefore adopted in the present study.

Microbiological analysis

To perform the microbiological testing, samples were handled inside the flow chamber being aseptically removed and divided into sterile bags (*Nasco WHILE-PACK*®) containing 25 g each. The packages were then vacuum packed and stored at 4, 8 and 12 °C for 75 days.

The commercial viability of pressurized and non treated (control) turkey ham was determined based on lactic acid bacteria growth (LAB) following the methodology described by Hall *et al.* (2001). From each piece of turkey ham 25 g of product were aseptically sampled, placed in sterile bags with the addition of 225 mL of peptone water (1%). Samples were homogenized for 60 s in *stomacher*, diluted and plated on culture agar of Man, Rogosa, Sharp (MRS) followed by incubation at 30 °C for 5 days. The analyses were performed in duplicate and results were expressed in Log (N) (N: colony forming unit per gram [CFU/g]), until microorganism growth reached the stationary phase.

Validation of the predictive modeling

The predictive models of Modified Gompertz and Baranyi were adjusted to the growth curves using the softwares Matlab® (Math Works, Natick, MA, USA) and DMFit 2.1 (Baranyi and Roberts, 1994), respectively.

The Modified Gompertz Model (Gibson *et al.*, 1987) is defined by the following equation:

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

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

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

$$\ln\left(\frac{x}{x_0}\right) = D + Bt + \frac{\ln\left(e^{-nBt} + e^{-C} - e^{-nBt-C}\right)}{B} - \frac{\ln\left(1+\dfrac{e^{mBt+\frac{\ln\left(e^{-nBt}+e^{-C}-e^{-nBt-C}\right)}{B}}-1}{e^{m(A-D)}}\right)}{m} \quad (2)$$

Constants have the following physical meaning:

$A = y_{max}$, $B = \mu_{max}$, $C = h_0 = \eta.\mu_{max}$, $D = \nu$ and $n = \frac{\mu_{max}}{\nu}$.

Statistical analysis

The following statistical indices were used in order to compare the performance of models: mean-squared error (MSE), regression coefficient (R^2), bias factor and accuracy factor. The lower the value of MSE, the better is the fit of the model to experimental data (Sutherland and Bayliss, 1994). The MSE is defined according to the following equation:

$$MSE = \sum \frac{(O-P)^2}{(n-p)} \quad (3)$$

In which O represents the observed value, P is the predictive value, n is the number of experimental points and p is the number of model parameters). The *bias* factor is represented by the equation:

$$BF = \exp\left[\sum\left(\ln\frac{P}{O}\right)/n\right] \quad (4)$$

and consists of an estimate for the average difference between the observed and predicted and ideally should be close to 1. If the value is greater than 1, it indicates that the expected value is bigger than the observed one, but if it is lower, it indicates that the predicted value is smaller than the one observed. The accuracy factor is the sum of absolute differences between predictions and observations values and measures the overall error of the model, being calculated by the following equation:

$$AF = \exp\left[((LNP - LNO)2/n)0.5\right] \text{ accuracy factor} = 10^{\left(\sum\frac{\left|\log\left(\text{Value}_{predicted}/\text{Value}_{observed}\right)\right|}{n}\right)} \quad (5)$$

The higher the accuracy factor, the lower the accuracy of the average estimate.

Results and Discussion

The microbiological parameters of growth (Ross, 1996): (A, μ, λ for the lactic acid bacteria growth in turkey ham are presented in Table 1. These results were obtained by fitting the modified Gompertz model to the LAB growth curves at the storage temperature of 4 and 12 °C, both for the control and pressurized samples. For the control and pressurized samples at 8 °C, the modified Gompertz model did not provide a good fit and the Baranyi model was rather used for a better fit of the curves.

The experimental and adjusted LAB growth curves under different storage conditions, according either to the Modified Gompertz or Baranyi models, are shown in Table 2.

Table 1 - Kinetic parameters for growth of lactic acid bacteria adjusted to Modified Gompertz and Baranyi models.

	Control					Pressurized				
T (°C)	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 of lactic acid bacteria growth to modified Gompertz and Baranyi models in pressurized vacuum packed turkey ham in comparison to the control, stored at 4, 8 and 12 °C.

Predictive model	Statistical parameters			
	r^2	MSE	Bias factor	Accuracy factor
	Control stored at 4 °C			
Modified Gompertz model	0.948	0.05878	0.9989	1.0294
	Pressurized stored at 4 °C			
Modified Gompertz model	0.9586	0.11615	0.9989	1.0864
	Control stored at 8 °C			
Baranyi model	0.9768	0.09382	1	1.0396
	Pressurized stored at 8 °C			
Baranyi model	0.9595	0.1394	1.0078	1.0437
	Control stored at 12 °C			
Modified Gompertz model	0.9843	0.0254	1.00195	1.01812
	Pressurized stored at 12 °C			
Modified Gompertz model	0.9999	0.00017	0.99967	1.00203

The storage temperature proved to have a great influence on the growth of LAB. The results showed the importance of maintaining low temperatures in order to achieve greater commercial viability, and also that the use of high hydrostatic pressure highly increased commercial validity by slowing down the LAB growth. 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 achieved 45 days, while the pressurized sample lasted up to 75 days, providing 30 extra days for commercialization.

To date, most studies on the effect of high pressure treatment on the microbiota of ready-to-eat and meat products have been directed to refrigerated post processing storage at a temperature of 4 °C, instead of including temperature abuse evaluation (Kreyenschmidt *et al.*, 2009). In this study a higher temperature (12 °C) was also used aiming at reproducing possible unexpected temperature abuse that can occur in storage and to allow the prediction by the models of the product commercial validity under unfavorable conditions. It was verified that even at higher temperatures high pressure processed turkey ham showed satisfactory shelf-life, when compared with the control stored at a lower temperature, based on lactic bacteria growth.

Figure 1 represents the growth curves of lactic acid bacteria according to storage temperatures at 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.

In pork ham treated at 400 MPa for 15 min and stored at 8 °C, Slongo *et al.* (2009) achieved a commercial viability of 85 days in comparison to the control, which lasted only 19 days. Those results are similar to the ones obtained for turkey ham in the present study using the same processing and storage conditions, in which the pressurized sample showed commercial viability of 65 days and the control sample just lasted 25 days. According to the studies by Ruiz-Capillas (2007), the high-pressure treatment at 400 MPa for 10 min applied to vacuum packaged ham pro-

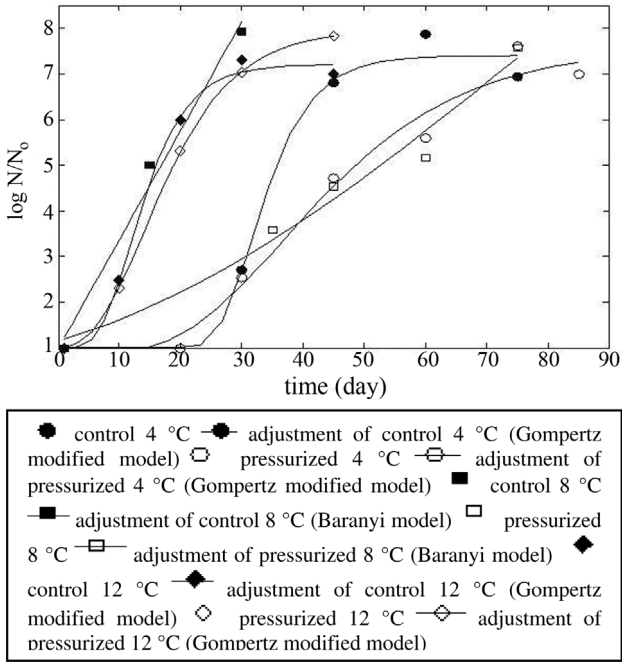

Figure 1 - Growth curves of lactic acid bacteria resulting from the fit to modified Gompertz and Baranyi models in control and pressurized turkey ham stored at different storage temperatures.

vided a commercial validity of 77 and 28 days for products stored at 2 and 12 °C, respectively. However, López-Caballero *et al.* (1999), with the same type of product but treated at 200 MPa and 400 MPa did not attain the same degree of inactivation, and the commercial viability at 3 °C resulted only in 21 days.

At higher pressures, such as those used by Slongo *et al.* (2009) with ham slices pressurized at 600 MPa for 5 min at 30 °C and stored at 5 °C for 120 days, LAB population did not increase significantly during the storage. Park *et al.* (2001) in studies with ham processed at 600 MPa for 5 min and 25 °C showed a reduction of ~ 4 log 10 CFU/g of LAB due to the processing. Garriga *et al.* (2004) reported that vacuum packaged ham treated at 600 MPa for 4 min at 16 °C showed LAB count after 30 days of 2.10 log10, and observed a significant microbial inactivation due to the pressure treatment. That also agrees with the results from Carpi *et al.* (1999), which reported 75 days of commercial viability for sliced cooked ham treated at 600 MPa for 5 min and stored at 4 °C.

Conclusion

Application of High Hydrostatic Pressure at 400 MPa and 15 min was effective to greatly delay lactic bacteria growth in turkey ham during refrigerated storage. The time required for the LAB population to achieve the limit defined for the product validity was longer for the pressurized sample stored at 12 °C even when compared to the non treated control sample stored at 8 °C. Both Modified Gompertz and Baranyi models provided good fit for the variation of lactic acid bacteria population with the storage time, showing high determinant coefficients for the regression adjustments. Modified Gompertz models presented better fit 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 proved to be a valuable tool to provide a good estimative of the product validity based on lactic bacteria growth, and high hydrostatic pressure demonstrated was very effective to delay microbial development and provide shelf life extension to the turkey ham.

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