

Research Article

Thermal Diffusivities and Influence of Cooking Temperature Combined With Sodium Lactate Addition on Microbiological Characteristics of Rabbit Ham

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Abstract: In meat products as rabbit ham, is very important the addition of biopreservative agent like sodium lactate combined with cooking, represents an important role for sensorial and safety aspects. The aim of this research was determine the thermal diffusivity and impact of sodium lactate addition and cooking temperature on microbiological characteristics were determined by total count of aerobic bacteria (TAB) of ham prepared from rabbit and then stored under refrigeration. Microbiological characteristics were evaluated at two cooking temperatures (76 and 82°C) added with sodium lactate (2 and 4%) and control without sodium lactate and with 200 ppm NaNO₂. The water immersion method is used for cooking. Time-temperature profiles and thermal diffusivity values show that heat penetration in ham with 2% w/v sodium lactate is quickly than the heat penetration in 4% w/v sodium lactate and control. TAB from raw materials were 4.255 log (CFU g⁻¹) and, immediately after cooking temperatures 76 and 82°C and the addition of sodium lactate, they were 3 log (CFU g⁻¹), which had not a significant differences p<0.05 at the day of their production. Although after 7 days of storage, at 4±1°C, significant difference was not observed (p<0.05) in TAB account results.

Keywords: Cooking temperature, ham, sodium lactate, thermal diffusivity, total aerobic bacteria

INTRODUCTION

According to the technological type specifications referred to the behavior of the rabbit meat is a good raw material for ham production, because presents technological advantages in the elaboration of cooked sausage, specifically to its Water Holding Capacity (WHC) and Emulsifier Capacity (EC). Piles *et al.* (2000), established a comparison between pork, mutton, chicken and rabbit meat, where the rabbit has the highest WHC indicates that it will present the lower lost weight due to water in the final product after cooking and storage as well as, the generation of exudates. In this case WHC value most significantly on sausages with as finely ground tissue cannot control fluid protein loss, thus rabbit meat this is how rabbit meat presents a low inter filamentous space which will increase the WHC of the myofibrils what it was determined by the electrical force and the addition of substances such as the sodium chloride and, in this case also the sodium lactate will, may be increase the ionic strength, influencing at the same time the isoelectric point which

is around pH 5. Rabbit meat presented high yields determined by previous experiment (Data no show), where for total raw material for ham was obtained 64.69%, discriminated in loins (58.47%) and hindquarters (70.14%).

It is necessary to yield alternatives that allow to increase shelf life of highly perishables like meat, through the use of preservation methods that involve both, cooking process and application of biopreservative agents (Bourouni *et al.*, 2012; Samapundo *et al.*, 2010), through barrier effect that exert application of chemical preservatives agents and high temperatures, (Coppola *et al.*, 2000). This way, as part of the new biopreservation strategies Generally Recognized as Safe (GRAS), is looking for lactic acid bacteria or their metabolite to ensure food safety. Sodium lactate is used in food with no other limitation than current good manufacturing practices (Federal Register 72-7-3, Food and Drug Administration (FDA) (2012)). Kaczmarek *et al.* (2008), Casquete *et al.* (2012), Holzapfel *et al.* (1995), Bredholt *et al.* (1999, 2001), Amezquita and Brashears (2002) and Vermeiren

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et al. (2004), sodium lactate were used to evaluate their favorable effects as a control of the spoilage and pathogen bacteria. In addition the choice of sodium lactate as potential biopreservative, also was targeted a favorable effect on sensorial characteristics, because the addition of sodium lactate leads to increase the concentration of NADH having as a result the maintenance of the ferrous ions of the hem group (Kim *et al.*, 2009; Bradley *et al.*, 2010). The increase of NADH plays an important role *ante* and *post mortem* in the reduction of muscle metmyoglobin because it gets reduced subsequently to both desoximyoglobin or oxymyoglobin, thereby increasing the stability of the meat color improved with lactate; the NADH is produced by the reduction of NAD thru the dehydrogenase lactate (LDH) in the *post mortem* muscle by the conversion of the lactate to pyruvate by the increasing of the LDH activity and their regeneration to NADH (Knock *et al.*, 2006; Bradley *et al.*, 2010; Suman *et al.*, 2010).

To guarantee safety of meat products thermal treatment evaluation is essential to allow their microbiological stability. Furthermore, it is vital to ensure the sensory quality through formulation and processing characteristics. For this reason, it is critical to establish the minimum temperature required in the food and its maintenance for 5 min in the solid thermal center which is assumed as the geometric center, mainly considering that heat is transferred by conduction and that the material has a uniform chemical composition (Akterian, 1996; Glavina *et al.*, 2006; Ayadi, *et al.*, 2009; Rinaldi *et al.*, 2012). All this to achieve a successfully stability; also the analysis of heat treatment is one of the most important physical phenomena during production and food processing. In this case for ham is “ready-to-eat” cooking is process that is used not just to modify these characteristics, also to control growth bacteria. Thereby the design thermal process through controlling time in which the cooking process of the mass of the product to be produced must be carried out.

This is to guarantee the treatment will be enough and effective to obtain a product to comply the consumer safety under legislation parameters with favorable concept on microbial characteristics. To analyze the heat treatment was determined the thermal diffusivity, this thermal property is influenced by sodium lactate addition. Thermal diffusivity, according to Singh (1992), indicates temperature rate when heat is transmitted on material. This property presents a relation between conducted and stored heat and could be determined through other thermo physical properties as, direct relation with thermal conductivity and inverse relation whit heat capacity and density.

The aim of this research was determine the thermal diffusivity and impact of cooking temperature and additional intervention of application of sodium lactate as biopreservative on microbiological characteristics were determined by total count of Aerobic Bacteria

(TAB) of ham prepared from rabbit stored under refrigeration.

MATERIALS AND METHODS

Sample preparation: The hams mixtures were prepared using 70% rabbit meat, 26.98% (samples without sodium lactate), 25.18% (samples with 2% sodium lactate) and 23.18% (samples with 4% sodium lactate) Water, 2.4% NaCl, 0.2% coloring, 0% (samples without sodium lactate), 2% (samples with 2% sodium lactate) and 4% (samples with 4% sodium lactate) sodium lactate Density (ρ) = 1.32 g. mL⁻¹, 200ppm (samples without sodium lactate) and 0% (samples with 2% and 4% sodium lactate) NaNO₂ and 0.4% Saccharose (General Codex Norm STAN 96-1981 (Rev.1 – 19910) (CODEX, 1996).

The samples cylinders were used in the experiments (height 0.06 m; diameter 0.072 m). Experimental models of meat products were prepared with rabbit pulp meat, from loins and hindquarters, free of adipose tissue, cartilage and fascia. Later, pulp meat was grinding to 5 mm particle diameter and chopping with water, sodium lactate, sodium nitrite, NaCl, coloring and Saccharose. The samples were inlayed, vacuum packaged and stored under refrigeration for 24 h and then cooked.

Determination of the thermal diffusivities: Following the Finites Differences method temperature, profiles were used to evaluate variation of concentration levels in the rabbit meat ham. T type Thermocouples were also used to measure temperature. Acquisition data system of temperatures was a Stanford Research System™ SR630 Model, Sunnyvale City, CA, USA. Geometrical centers were located in the middle from the geometric center at in the axial and radial axis, at 5 mm surface. Temperatures were measured every 30 sec until temperature reached 80°C in the geometric center. Then, it was maintained for 5 min 80±1°C.

Cooking was analyzed as a thermal treatment that dependent of transfer phenomena. Thus, relevance is given to heat conduction within the product. This occurs in a non-stationary regime and therefore it is governed by Fourier's second law Eq. (1), which corresponds to cylindrical coordinates:

$$\frac{\partial T}{\partial t} = \alpha \frac{\partial^2 T}{\partial r^2} + \frac{1}{r} \frac{\partial T}{\partial r} \quad (1)$$

where,

α : Thermal diffusivity

T : Temperature

t : Time

r : Radius

For thermal diffusivity measurement as a thermo physical property, the variation of the solid products based upon the variation of sodium lactate concentration levels must be taken into account. The

Finite Differences method explicit forward was applied to predict the thermal diffusivity and the cooking times (Akterian, 1996; Glavina *et al.*, 2006; Ayadi *et al.*, 2009; Rinaldi *et al.*, 2012; Geankoplis, 2002). The explicit forward Finite Difference method, the thermal diffusivity of Fourier's Law is cleared beginning from the Eq. (2, 3 and 4):

$$\frac{\partial^2 T}{\partial y^2_{m,n}} \approx \frac{T_{m,n+1} + T_{m,n-1} - 2T_{m,n}}{(\Delta y)^2} \quad (2)$$

$$\frac{\partial T}{\partial t_{m,n}} \approx \frac{T_{m,n}^{p+1} + T_{m,n}^p}{\Delta t} \quad (3)$$

$$\frac{1}{\alpha} \frac{\frac{T_{m,n}^{p+1} - T_{m,n}^p}{\Delta t}}{(\Delta x)^2} = \frac{T_{m+1,n}^p + T_{m-1,n}^p - 2T_{m,n}^p}{(\Delta x)^2} + \frac{T_{m,n+1}^p + T_{m,n-1}^p - 2T_{m,n}^p}{(\Delta y)^2} \quad (4)$$

The method finite differences were used because provides temperature value in discrete points of ham, based in a several discrete instants of time. This solution presents a direct association between temperatures in a node with a near node, for this reason was evaluated temperature in any part of a sample in near points.

The determination of thermal diffusivity for each treatment, was considering on reference temperature based on time ($t+1$) (5):

$$T_r^{t+1} = T_r^t + Fo \left(T_{r+1}^t + T_{r-1}^t - 2T_r^t + \frac{2\Delta r}{r} (T_{r+1}^t - T_r^t) \right) \quad (5)$$

For determined reference temperature and time, temperature was determined by each thermal diffusivity and were evaluated which was the minor difference between experimental and determinate temperature and this respective time, this value corresponds to reference time. For this reason analytical solution was proposed on heat transfer for finite cylinder Eq. (6 and 7); assumed the superposition of infinite cylinder and infinite slab (Singh, 1992; Cengel, 2010):

$$\theta_{r,x,t} = \left(\frac{T-T_f}{T_0-T_f} \right)_{\text{finite cylinder}} = \left(\frac{T-T_f}{T_0-T_f} \right)_{\text{infinite cylinder}} \left(\frac{T-T_f}{T_0-T_f} \right)_{\text{infinite slab}} \quad (6)$$

$$\theta_{r,x,t} = \left(2 \sum_{n=0}^{\infty} \frac{e^{-\lambda_n^2 Fo}}{\lambda_n J_1(\lambda_n)} \right)_{\text{infinite cylinder}} \left(\left(\sum_{n=0}^{\infty} \frac{[2(-1)^n] e^{-\lambda_n^2 Fo}}{\lambda_n} \times \cos \left(\frac{\lambda_n r}{D} \right) \right) \right)_{\text{infinite slab}} \quad (7)$$

where, λ_n is geometry function that depends by Biot Number (Bi), r is the spatial/radial location, D is the half radius/ thickness, at/D^2 is the Fourier number (Fo), J_0 y J_1 are the Bessel functions of zero and first order, respectively. But for this case $J_0(\lambda_n)$ was assumed as 0.

Cooking procedure: A new batch of test samples were produced with the same characteristics and procedures previously standardized, it was sought to establish the effect of cooking and concentration of the sodium lactate on the total bacterial account in the rabbit meat ham evaluating the interaction of both treatments. For that the product was cooked by immersion in water at $85 \pm 0.1^\circ\text{C}$ and two final temperatures (76 and $82 \pm 0.1^\circ\text{C}$) both determined in the geometric center and maintained by 5 min. Acquisition data of temperatures was measured the same that for determination of the thermal diffusivities.

Microbiological analysis: Microbiological analysis for each treatment was for triplicate (2 variation levels of cooking temperatures and 2 variation levels of sodium lactate concentration and this control); Total Aerobic Bacteria (TAB) were quantified on raw material, immediately after cooking and after 7 days in storage refrigerated.

25 ± 0.1 g for each sample mixed for 30 s with 250 mL peptone water 0.1% (BD Bioxon, Mexico), diluted with peptone water 0.1% and plating on PCA (BD Bioxon, Mexico) for triplicate. Later plates were incubated $37 \pm 1^\circ\text{C}$ to 48 h and counted.

Statistical analysis: The experiment was designed with six treatments (concentration of sodium lactate and cooking temperatures) in a complete randomized experiment design with factorial arrangement that would allow evaluating interactions between effects of the addition of sodium lactate and applied temperatures. The results were subjected to ANOVA to screen for interaction among two concentration of sodium lactate (2 and 4%) and control with 200 ppm sodium nitrite and two cooking temperatures maintained for 5 min (76 and 82°C) in the geometric center.

The values for evaluated thermal diffusivities were determined for each treatment four times and total bacteria count were determined for each treatment three times and entered into the experimental design as the response. ANOVA by the JMP program (SAS Institute) was used, to evaluate the probability that sodium lactate and cooking temperature significantly affected the bacteria total count and F test also apply DMSH test (Tukey) ($p < 0.05$) by contrast there were significant differences in the variation levels in the sodium lactate concentration and cooking temperatures. Before apply F test was used statistical handling employee for the

anomalous data analyzed, refers to the Grubbs test with $p<0.05$ contrasted against test t-student. A confidence level of $p<0.05$ as the highest level (i.e., test concentration) and as zero the lowest resulting in a highly significant effect upon the response.

RESULTS AND DISCUSSION

Time-temperature profiles and thermal diffusivity: The main objective here was to determine thermal diffusivity thought of time-temperature profiles for each sodium lactate concentration. About the time-temperature profiles, was observed two linear regions for all treatments. This behavior could be cooking promotes changes on ham structure associated to meat proteins denaturation (Carciofi *et al.*, 2002); the coagulation of meat proteins begin at about 40°C and is complete at 55°C. At 60°C the major parts of the sarcoplasmic and myofibrillar proteins are denatured; and this phenomenon is accompanied by phase change of fat between 30 to 40°C from solid to liquid state and a severe decrease in water holding capacity (Mittal *et al.*, 1989).

Thermal diffusivities for rabbit meat ham for time references (Table 1) based in time-temperature profiles, the treatment with 2% w/v sodium lactate, was presented the mayor thermal diffusivity (Table 1), proportional to faster heat transfer penetration; this phenomenon which is consistent with the differences with the differences in composition could be associated to increasing of heat transfer rate respect decreasing of solids and also, because the most important factor on thermophysical properties according to Perussello *et al.* (2013) was moisture content.

On the other hand, sodium lactate addition causes decrease water activity (a_w) and WHC, it is possible that this effect cause increment on thermal diffusivity. The sodium lactate addition has effect in ham system on pH and ionic strength that influence change interactions between proteins and water; because water binding have been based on electrostatic repulsions and uneven distribution of ions within myofibrillar proteins (Puolanne and Peltonen, 2013).

Table 1: Thermal diffusivities by finites differences method for each treatment obtained

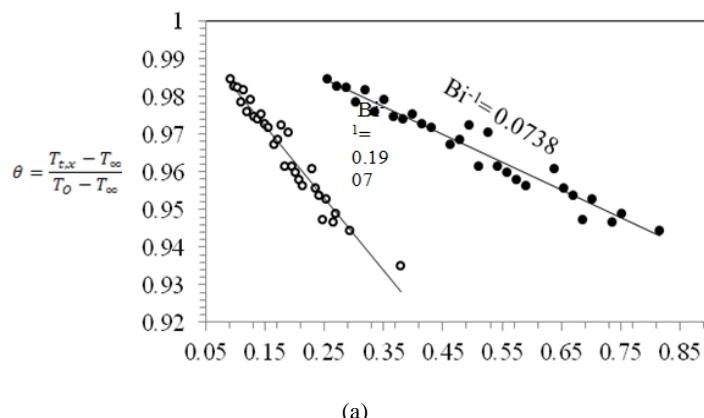
Treatments	Time (s)	Thermal diffusivity (m^2/s)
Control	t_{ref} 660	6.9069×10^{-7}
A	t_{ref} 2130	1.0537×10^{-6}
B	t_{ref} 2340	3.8376×10^{-7}

Control: 200 ppm NaNO₂ without sodium lactate; A: 2% sodium lactate without NaNO₂; and B: 4% sodium lactate without NaNO₂ (% w/w on formulation of rabbit meat ham)

Experimental values obtained of the thermal diffusivity were confirmed by Ochoa *et al.* (2006), who found a value of $4.0499 \times 10^{-7} m^2/s$ based on the chemical composition, primarily in the amount of water, fat and freezing point. It is noted that the higher value of thermal diffusivity is presented in the concentration of 2% sodium lactate which as it was clarified presented the higher amount of water compared against the two concentrations given that appearing the sodium lactate solution in 60% indicates a higher water amount in the sample than the contained in the NaNO₂ in its solid state.

Knowledge about thermal diffusivities is employed for cooking time predictions. As a result, heat transfer for multidimensional stationary conduction occurs in radial and axial dimensions and analyzed samples were considered finite cylinder both limits were combining infinite cylinder for radial dimension and flat plate for axial dimension. For this reason, each concentration of sodium lactate presented lineal regression between temperatures relation (geometric center temperature, heat transfer fluid and initial temperature) and Fourier number (relation between time, thermal diffusivity and characteristic length), curve slope corresponds to inverse from Biot number that is the relation between conduction and convection (Fig. 1) (Cengel, 2010; Geankoplis, 2002; Bird *et al.*, 2002).

Influence of cooking temperatures and sodium lactate on microbiological characteristics: The main objective here was to measure the influence of cooking temperature by water immersion and sodium lactate concentration on microbiological quality, rabbit meat was evaluated for aerobic mesophilic bacteria incubated at $35 \pm 0.1^\circ C$ during 48 ± 4 h, samples from it were obtained $4.255 \log CFU.g^{-1}$, while for anaerobic



(a)

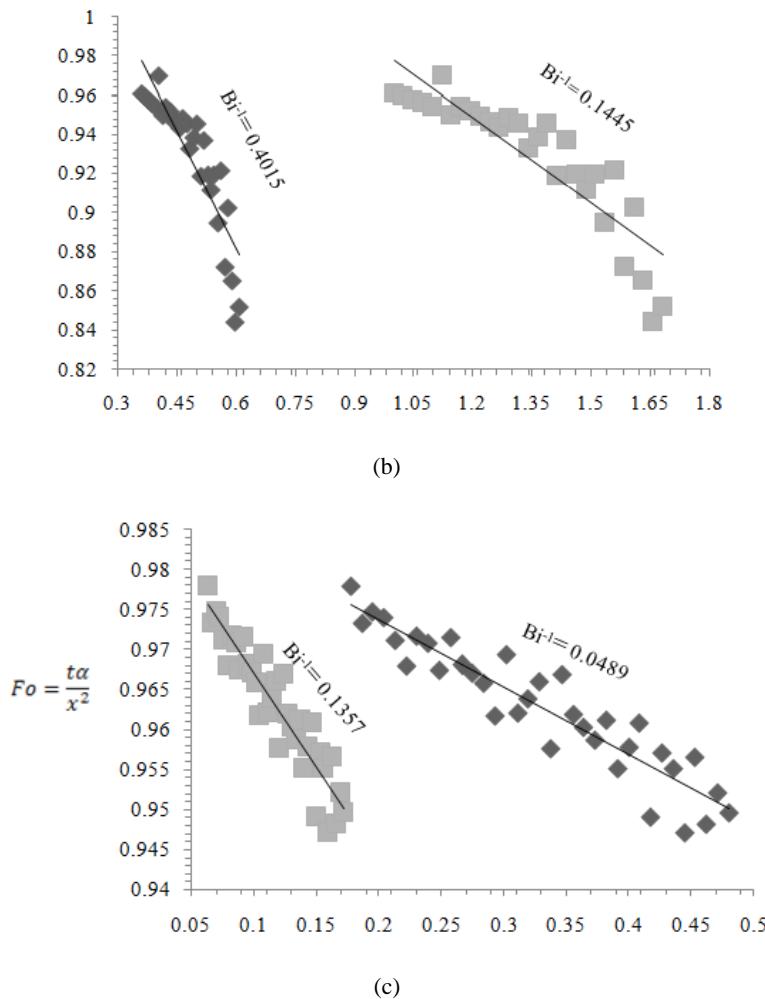


Fig. 1: Curves of heat transfer for multidimensional stationary conduction by samples 200ppm NaNO₂ without sodium lactate; (a): 2% sodium lactate without NaNO₂; (b): 4% sodium lactate, without NaNO₂; (c): Axial dimension (○), radial dimension (●)

Table 2: Mesophilic bacteria plate count ($\log \text{CFU.g}^{-1}$) ($35\pm 1^\circ\text{C}$) from rabbit meat ham

Time sampling	Cooking temperature* ($^\circ\text{C}$)	Incubation condition	Treatments		
			Control	A	B
Immediately after cooking	76	AE	3.622 \pm 0.487 ^a	3.752 \pm 0.046 ^a	3.595 \pm 0.483 ^a
		ANA	3.160 \pm 0.039 ^c	3.350 \pm 0.302 ^c	3.123 \pm 0.098 ^c
	82	AE	3.473 \pm 0.073 ^a	3.699 \pm 0.001 ^a	3.532 \pm 0.128 ^a
		ANA	2.997 \pm 0.066 ^c	3.292 \pm 0.364 ^c	3.469 \pm 0.085 ^c
7 days after refrigeration	76	AE	4.701 \pm 0.027 ^b	4.636 \pm 0.025 ^b	4.636 \pm 0.041 ^b
	82		4.675 \pm 0.011 ^b	4.625 \pm 0.053 ^b	4.574 \pm 0.053 ^b

*: Cooking temperature maintained for 5 min at thermal center; Incubation condition: aerobiosis (AE) and for anaerobic condition (ANA); Control: 200 ppm NaNO₂ without sodium lactate; A: 2% sodium lactate without NaNO₂; and B: 4% sodium lactate without NaNO₂ (%m/m on formulation of rabbit meat ham); ^{a,b}: Means, \pm standard error, within a column and rows, no common superscript are significantly different ($p<0.05$) determined by Tukey test

mesophilic bacteria incubated at $35\pm 0.1^\circ\text{C}$ per 48 ± 4 h, the count was $4.146 \log \text{CFU/g}$, where initial microbial total count is critical as the sodium lactate works as a bacteriostatic and therefore it will not hide a defective initial quality reason why the respective protocols of uniform disinfection under the good manufacture practices.

Microbiological quality was evaluated in the finished product for aerobic and anaerobic mesophilic

bacteria (Table 2), immediately after cooking with the two corresponding temperature levels at 76 and $82\pm 0.1^\circ\text{C}$. Then, the hams were inlay, 48 hours from the addition of NaNO₂ or sodium lactate, they were massaged during which was kept care to avoid altering the bacterial flora of the samples.

According to the analysis of variance ($p<0.05$), there were no significant differences between thermal treatment temperatures and sodium lactate

concentrations for neither aerobic mesophilic bacteria measured immediately after cooking. However, it had an impact on microbiological characteristic on meat rabbit ham since total viable count presented an interaction between variables of the sodium lactate concentrations and the reached cooking temperature maintained in the thermal center.

There were no significant differences ($p<0.05$) in regard to anaerobic mesophilic bacteria count, evaluated immediately after cooking, concerning the thermal treatment temperatures and the sodium lactate concentration, the interaction between variables is not significant. However, total microbial load decreased as temperature at the thermal center of 76°C to $82^{\circ}\text{C} \pm 1^{\circ}\text{C}$ decreased during the cooking, as expected, but this did not show a significant difference between them on bacteria aerobic and anaerobic count.

Total microbial count decreased as sodium lactate concentration increased in the samples; however, it presented lower total microbial load with the application of nitrites not representing a significant difference $p<0.05$. Additionally, product vacuum packaging was not carried out favoring the growth of BAL by the high conditions of CO_2 ; which making an association of the significant presence of BAL is manifested by the remarkable visualization of unpleasant colors, bouquet and in general sensory characteristics, as well as the increase of viscosity and excessive generation of exudates which was not present in accordance to the qualitative observations made.

In turn, it was observed that sodium lactate had a minor effect on the total viable mesophilic count in anaerobic conditions that can be considered mainly lactic acid bacteria (Table 2); this is possibly due to the fact that sodium lactate is salt from the lactic acid which is a metabolite explaining less reduction of $\text{CFU} \cdot \text{g}^{-1}$. However, there were no statically significant differences ($p<0.05$) implying that its effect is not considered as different from the nitrates to prevent bacteria presence and its spoilage effect in the cooked meat product.

Total bacterial increase count can be seen after 7 days in refrigerated storage ham, of about a logarithm. The analysis of variance ($p<0.05$) showed no statistically significant differences in the plate count results of aerobic mesophilic bacteria, regarding to the temperature achieved and maintained for 5 min in the thermal center. By contrast there weren't significant differences in the variation levels in the sodium lactate concentration, according to the DMSH test (Tukey) ($p<0.05$). In the analysis of the total bacterial count it was visualized that given this difference, the sample with 4% of sodium lactate showed a better performance, in this case decreased a logarithm the aerobic mesophilic exponential growth after 7 days of refrigerated storage. Likewise also showed an interaction between the concentration of sodium lactate and the cooking temperature ($p<0.05$), which

corresponds to the total bacterial count from the cooking day of the samples.

The main reason why lots added with sodium lactate presented a better behavior than the one with nitrate, which formulation was controlled for other components to do not intervene on this variable, upon microbial control, may be because sodium lactate maintains pH values which impacts in a significantly way on the microbial growth and in the same way decrease generation of exudates which may facilitate the development of microorganisms (Knock *et al.*, 2006; Bradley *et al.*, 2010).

Following this line of thought, the addition of sodium lactate (4%) in meat products, reduces the aqueous activity of 0.97 initial theoretical value to 0.96 (Wit and Rombouts, 1999), indicating this is a highly perishable product, which action could be primarily based on the effect on microbial metabolism and enabling to extend its usage life according to other products of the same type. In fact, it is observed that sodium lactate compared with sodium nitrite increases the latency phase of microorganism. This is observed in the significant difference $p<0.05$ between the pattern concentration and the 4% of sodium lactate, as its inhibitory activity is in function of reducing the logarithmic phase working as a bacteriostatic and not as a bactericidal, in the sanitary quality of the sample in the refrigerated one, which could be because the sodium lactate enables a higher permeability in the membrane given the conditions of pH and therefore, within the bacterial cells are protons accumulation and lactic acid that favor the control in the growth of bacterial cells.

CONCLUSION

In conclusion cooking time varied significantly ($\alpha = 0.05$) in terms of thermal diffusivity as shown to be directly related to the solutes presence. For those without sodium lactate and 200 ppm of NaNO_2 (healing salts), were of 20.38 min; for the 2% of lactate and without healing salts of 15.09 min and for the 4% of sodium lactate without sodium nitrite of 34.5 min. Thus it was determined through mathematical handling the influence of the present solutes in the cooking time, likewise employing sodium lactate to 2% reduces the cooking time. The use of sodium lactate in these formulations of hams did not show a significant difference in the microbiological results, as there were no significant differences between the used levels on immediately after cooking samples and after 7 days of refrigerated storage at $4 \pm 0.1^{\circ}\text{C}$. The temperature of 76°C reached in the thermal center and maintained for 5 minutes, did not have a significant difference ($p<0.05$) respect of 82°C , even that in both cases were obtained microbiological results from samples on refrigerated storage during 7 days at 4°C allowing them to comply with actual legislation.

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