Mathematical modeling of microbial growth in refrigerated horsemeat: it's importance in the quality of meat*

Pena, I.[1]; Coll Cárdenas, F.[1, 2]; de la Sota, P.[1]; Villat, M.C.[1]; Noia, M.A.[1]; Mestorino, N.[1]

Abstract: Horsemeat is an important economic resource for our country, as Argentina is considered one of the leading world exporters of horsemeat. But there is little research about the quality of it. It was examined the effect of: (i) storage temperature (0, 4˚C) and (ii) gaseous permeability of the packaging film (polyethylene and EVA SARAN EVA for vacuum packaging) on the growth of bacteria (Total Aerobic Mesophilic Heterotrophic Bacteria, Enterobacteriaceae and *Pseudomonas sp.*) isolated from horse muscle. Microbial growth was modelled using Gompertz or the Linear Regression Models. The lowest final bacteria counts were obtained with the combination of 0˚C and vacuum packaging. At higher temperatures, 4˚C, there were no significant differences ($p<0.05$) among the final counts in relation to both films. The lowest values of μ (microbial growth rate) for the development of studied microorganisms were observed in samples stored in EVA SARAN EVA.

Keywords: *Horsemeat, Mathematical Models, Microorganisms, Refrigeration, Packaging Films.*

Modelo matemático del crecimiento microbiológico en carne equina refrigerada: su importancia en la calidad de la carne

Resumen: La carne equina es un importante recurso económico para nuestro país, ya que Argentina es considerado uno de los principales exportadores mundiales de estas carnes. Pero existen pocos datos sobre la calidad de la misma. El efecto de: (i) la temperatura de almacenamiento (0, 4ºC) y (ii) la permeabilidad gaseosa de la película de envase (polietileno y EVA EVA SARAN para envasado al vacío) sobre el desarrollo de microorganismos (Aerobios mesófilos heterótrofos totales, Enterobacteriaceae y *Pseudomonassp.)* aislados de músculo equino, fue examinado. El desarrollo microbiano se modeló usando el modelo de Gompertz o de regresión lineal. Los recuentos finales bacterianos más bajos fueron los obtenidos con la combinación de 0˚C y envasado al vacío. A temperaturas más altas, 4ºC, no se encontraron diferencias significativas ($p < 0.05$) entre los recuentos finales en función de las películas de envase. Los menores valores de μ (tasa de crecimiento microbiano) para el desarrollo de los microorganismos estudiados fueron observados en las muestras almacenadas en EVA SARAN EVA.

Palabras claves: *Carne equina, Modelos matemáticos, Microorganismos, Refrigeración, Películas de envase.*

* Recibido: 02/08/2013. Aceptado: 01/11/2013

1 Cátedra de Biofísica. Facultad de Ciencias Veterinarias, UNLP, Calle 60 y 118, La Plata 1900, Buenos Aires, Argentina.

2 Corresponding author. E-mail F. Coll Cárdenas: fcollcardenas@fcv.unlp.edu.ar.

Introducción

In prehistoric times, horsemeat was much appreciated for its typical taste and nutritional quality. Afterwards the animal was used for work and sports purposes, with few groups in the world keeping it for food. Horsemeat has a cohesive and firm structure.Muscle fibers are thin and delicate and are interspersed with fat tissue, thus providing the marble-like effect (18). The horsemeat, from young animals especially, is characterized by good tenderness due to its content of connective tissue and collagen. Besides, horsemeat is characterized by relatively good water-holding capacity.Its small content of intra-muscular fat and its low melting temperature combine to the fact that its juiciness does not differ much from other meats (14,17). The outstanding characteristic of horsemeat is it dark-red colour with slight brown tinge that results from high content of the myoglobin muscle pigment (13, 12, 16). Typical sweetish taste of horsemeat is mainly due to its high content of glycogen (11, 15) and its particular odour may be due to its volatile fatty acids content (3).

Respect its nutritional value horsemeat is as good as other meats and it is an excellent source of high quality protein. The high biological value of horsemeat is complemented by relatively high level of vitamins, especially B1, B2, E, PP, A, as well as mineral salts, especially phosphorus, calcium, zinc and iron (12). Besides, because of its low fat content (2.55%), its low cholesterol content, and its high percentage of oleic acid triglycerides horsemeat is easily digestible (22). It provides between 1000 and 3000 $kcal.kg^{-1}$. The composition of the equine carcass comprises 70% of muscle, 20% of bone and 10% of fat tissue. In comparison with the bovine carcass, horsemeat provides more muscle and less bone and fat tissue than cattle meat (3).

The normal bacterial microflora of fresh meat is very heterogeneous.It is mainly composed of mesophilic and psychrophilic microorganisms such as *Acinetobacter, Moraxella, Brochotrixtermosphacta, Lactobacillus, Bacillus, Pseudomonas*, and *Enterobacteriaceae*family genera such as *Escherichia coli* and *Klebsiella sp.* (10). The growth of microorganisms occurs at the expense of its soluble components, mainly carbohydrates, amino acids and lactic acid (9).

There is a little amount of studies on the storage qualities of horsemeat. However, the storage stability of this product can be deduced by consideration of the general behaviour of meat spoilage flora, the composition of the meat, and its microbiological condition at packing plants.

Vacuum packaging and refrigeration are being increasingly used as techniques to extend the useful life of perishable foods such as fresh meat cuts, using packaging films with low permeability to oxygen (7,19,4). Combining low temperature and different packages can almost completely prevent microbial growth from occurring. The application of the concept of barriers intended to prevent development of spoilage and toxin-producing microorganisms growing by mean of combined methods is gaining acceptance. These methods may not provide adequate preservation when applied individually, but when they act together they can increase significantly its effectiveness.

The need to ensure microbiological safety and quality of foods has increased the use of predictive microbiology, which is a powerful tool for predicting microorganism's growth rate under ambient conditions, and thereby determining its effective life under different conditions of time, storage temperature, pH, etc, during manufacture and distribution. One of the most frequently used mathematical models is that of Gompertz (8, 7), which describes the microorganism response under different combinations of factors (1). This model permits the estimation of parameters such as lag phase duration (LPD), specific growth rate (μ) and maximum population density (MPD) of microorganisms under such conditions (5).

The handling of meat and its associated contamination, joined to the fluctuations of both ambient and refrigerated temperature is common either in developing countries or in countries with advanced technologies.

Horsemeat is an important economic resource for our country, so Argentina is considered one of the leading world exporters of horsemeat. Among meat exports in 2006, for example, we can locate horsemeat behind beef in terms of foreign-exchange earnings, followed by other species meats as chicken, rabbit, sheep and pig.

On the course of the present year The National Service of Health and Food Quality (SENASA) from Argentina adopted Resolution 146/2010 which created the National Regulatory Framework for the Provision of horses for slaughter. According to the control of exports of fresh horse,during the first five months of 2008, 14,638 tons valued at $ 44,111,000 were exported.Those represents an increase in volume in relation to foreign exchange shipments recorded during the same period 2007 (21).

The objectives of the present work were as follows: (1) Toanalyze the effect of refrigeration temperature and gaseous permeability of the packaging film on the growth of three bacteria (Total Aerobic MesophilicHeterotrophic Bacteria, Enterobacteriaceae and *Pseudomonas sp.*) isolated from

muscle samples. (2) To mathematically model the microbial growth curves byGompertzModel.

\ Materials and Methods \

1. Samples of horsemeat

Horsemeat samples were obtained from *Longissimusdorsi* muscle of natural pH 6 with a post-mortem time of 48 h. The pH values were determined using a punctionpHmeter microcomputer pH/mv/temp Meter 6171L.

The meat samples were from meat of Freezer Indian Pampa, Export Establishment Nº351 of TrenqueLauquen, Buenos Aires province, Argentina.

Once in the laboratory, meat samples were cut aseptically into subsamples of 5 cm diameter by 1.5 cm high with a sterile scalpel.

2. Packaging and refrigerated storage of the horsemeat samples

The processed samples were packaged into two films with different values of oxygen permeability: (a) low density polyethylene (aerobic condition) of 50 µm thick, water vapour permeability WVP = 12 g m^{-2} day^{-1} atm^{-1} at 30ºC and RH = 78%, oxygen transmission rate OTR = 5000 cm^3 m^{-2} atm^{-1} day^{-1} at 23ºC, and (b) vacuum packaged EVA SARAN EVA (ESE film), being EVA ethyl vinyl acetate and SARAN a polyvinyl and polyvinylidene chloride copolymer of 50 µm thick (WVP = 7.2 g m^{-2} day^{-1} atm^{-1} at 30ºC and RH = 78%, OTR = 50 cm^3 m^{-2} atm^{-1} day^{-1}). Vacuum packaging was carried out in a Minidual equipment model MW 4980 (Schkolnik SAIC, Bs.As., Argentina). Manometric pressure in the vacuum chamber was 70 cm Hg.

Storage experiments with packaged refrigerated horsemeat were performed at 0 and 4º C ± 1. During the storage period microbial counts were determined for a maximum of 45 days.

3. Microbiological analysis

Microbiological determinations were made using sterile swabs from the surface of meat at different storage times (between 0 to 45 days). Decimal dilutions using peptone water 0.1% were then performed and 1000 µl of serial dilution were placed in specific media for each microorganism: Plate Count Agar (PCA Agar) for Total Aerobic Mesophilic Heterotrophic

Bacteria; Crystal Violet, Neutral Red bile Agar for Enterobacteriaceae and Cetrimide Agar for *Pseudomonas sp*. using the Plate Pour Procedure with incubation at 37ºC for 24-48h.

Determinations were made by duplicate. For all results, an Ionomex colony counter was used to quantify these and the counts were expressed as log N (N: Colony Forming Units. cm^{-2} ($CFUcm^{-2}$).

4. Mathematical modelling

Mathematical models allow us to describe the effects of the main factors affecting microbial growth parameters. One of the most recommended models is the Gompertz modified equation(25, 8, 1, 5). From this equation, the following derived parameters were obtained: specific growth rate $\mu = b\ c/e$ [log ($CFUcm^{-2}$) $days^{-1}$], with e = 2.7182; lag phase duration LPD = m - (1/b) [days], maximum population density MPD = a + c [log (CFU cm^{-2})].Data fits obtained from Gompertz model were analyzed by means of Systat software (24). It calculates the set of parameters with the lowest residual sum of squares (RSS) and their 95% confidence interval.

When the microbial counts in food remain constant or decrease during storage, it's possible to use the linear regression model. It was considered that microorganisms are in a lag phase when the slope gets a value lower than 0.01 (CFU $cm^{-2})^{-1}$ $days^{-1}$, or when the difference between final counts and initial ones are lower than 0.5 logarithm cycle. Lag phase was calculated as the time necessary to increase initial microbial counts in 0.5 log cycle (LPD = 0.5/μ) (5).

5. Experimental design and statistical analysis

In the experiments using horsemeat samples a full factorial analysis (2x2x3) were performed by using two storage temperatures (0 and 4°C), three counts of different microorganisms (Total Aerobic MesophilicHeterotrophic Bacteria, Enterobacteriaceae and *Pseudomonas sp*.) and two different packaging films (polyethylene and ESE). Each set of experiments was run on duplicate samples (Total number of samples= 2x2x3x2= 24).

Analysis of variance (2) and comparison tests according to the Fisher´s significant differences table (Least Significant Difference) were applied with significance levels of 0.05. It was used a Statistical computer program SYSTAT (SYSTAT Inc, version 8.0).

\ Results and Discussion \

1. Microbial growths about different conditions

Fig. 1 show the effect of temperature (0ºC) and packaging film (polyethylene or EVA SARAN EVA (ESE)) of the growth about Total Aerobic MesophilicHeterotrophic Bacteria (Fig. 1.a), Enterobacteriaceae (Fig. 1.b) and *Pseudomonas sp*(Fig. 1.c) in horsemeat surface.A higher microbial growth was observed when horsemeat was packaged in polyethylene film, because of aerobe condition of surface microflora.

In the case of Total Aerobic MesophilicHeterotrophic Bacteria (Fig. 1.a), the final counts in horsemeat samples packaged in polyethylene were lowest than 10^4 CFU cm^{-2}. On the other hand, vacuum packaged in ESE film, horsemeat samples showed lower final counts (10^3 CFU cm^{-2}).

Vacuum package was a determinant factor on counts of Enterobacteriaceaethat was inhibited in the growths during the 45 days ofthe experience. Similar results were reported by Coll Cárdenas *et al.* (2008) to work with the same conditions of temperature and packaging, but in beef.

Fig. 2 shows the effect of a higher temperature, 4ºC, and packaging film (polyethylene or ESE) on the growth of the same bacteria on horsemeat surface. In all these cases microbial growth was observed.The meaning of this is that neither the temperature nor the packaging films were determinant factors as to inhibit bacterial growth. In Figure 2a the development of Total Aerobic MesophilicHeterotrophic Bacteria with counts greater than 10^5CFU cm^{-2} in the surface of the meat samples packaged in polyethylene is represented.

Similar results were reported by Pérez Chabela *et al.*,1998 (20), they found Total Aerobic MesophilicHeterotrophic Bacteriacounts of 10^4 CFU g^{-1} after 5 days of storage at 4°C.In ESE,counts were below10^5 CFUcm^{-2}.

In samples packaged under aerobic condition both Enterobacteriaceae and *Pseudomonas sp* showed counts of 10^4 CFU cm^{-2}, while in vacuum-packaged meats, the results were slightly lower (3.90 and 3.86 log CFU cm^{-2}).

In Figure 3 the combined effect of the treatment conditions (temperature, packaging film) on final counts of Total Aerobic MesophilicHeterotrophic Bacteria, Enterobacteriaceae and *Pseudomonas sp* from the surface of the meat samples is presented.

If we compare these values, it can be observed that the combination of 0°C and vacuum packaging permit the obtention of the lowest final counts

for the bacteria tested. At higher temperatures (4°C) there are no significant differences ($p<0.05$) between the final counts in relation to both films.

Vacuum-packaging and refrigeration are increasingly being used as two techniques for enhancing shelf-life of perishable foods such as cuts of fresh meat, using low-oxygen permeable packing materials (19, 5, 6). Here we demonstrate its usefulness in processing horsemeat.

2. Mathematical modelling

Table 1 shows the Gompertz´s parameters and its derivatives for the microbial growth of analyzed bacteria from the surfaces of the meat samples packaged in both films and stored at 0°C.

A good fit of the experimental results with respect to the modelwas observed.

For Total Aerobic MesophilicHeterotrophic Bacteria counts, vacuum packaged in ESE films samples could only be modeled by the Gompertz equation, which reached LPD of 7.85 days. While, Enterobacteriaceae and *Pseudomonas sp* were modeled by the linear regression model, showing good determination coefficients (r^2: 0.91 and 0.81, respectively).

For samples packaged in polyethylene, the highest values of μ were observed in the case of *Pseudomonas sp* (0.37 log (CFU cm^{-2}) days $^{-1}$).

Table 2 shows the application of Gompertz Model to microbial development on meat surfaces of packaged samples in both films and stored at 4°C. It was not necessary to use the linear regression model in any case.

The lowest values of μ (0.15, 0.19 and 0.09 log(CFU cm^{-2}) day^{-1}) for the development of Total Aerobic MesophilicHeterotrophic Bacteria, Enterobacteriaceae and *Pseudomonas sp.,* respectively were observed in samples stored in ESE. These parameters were higher for packaged in polyethylene filmssamples, showing differences between 0.06 and 0.90 units for those in the other film.

Respect the values of MPD, it was observed that Total Aerobic MesophilicHeterotrophic Bacteria counts were the highest ones (5.45 log CFU cm^{-2}) in samples packaged with polyethylene.

\ Conclusion \

Although our country is one of the main exporters of horsemeat, due to cultural reasons the human consumption of such meat is very low, as well as there are few experimental studies performed for this purpose.

However the high demand for horsemeat with high quality standards by the international market, justifies the investment in the production of horses for meat, the development of related industries and their by products and its promotion. And justifies also, increment the basic and applied research about aptitudes of horsemeat for human consumption, health, quality and useful life of this product. All this will result in a high impact in the maximum consolidate the existent market and will allow grasp and capitalize new markets.

In the present work it has been shown that by using mathematical models predicted good prediction of the different microorganism's behaviour could be accomplished. Microorganisms growing can be prevented with various combined technologies by way of obstacles, reducing bacterial growth on food surfaces, which could contribute to ensure food quality and enlarge useful life of this product.

From the behavior observed in microbial horsemeat can conclude that this food turns out to be a product suitable for human consumption.

Acknowledgments

The authors gratefully acknowledge to Freezer Indian Pampa, Export Establishment Nº351 of TrenqueLauquen, Buenos Aires province and the financial supportgiven by the National University of La Plata.

\ References \

1 Andrés, S.; Giannuzzi, L.; Zaritzky, N.E. 2001. Mathematical modelling of microbial growth in packaged refrigerated orange juice treated with chemical preservatives. *J. Food Sci.* 66, 724-728.

2 ANOVA. 1989. *Super ANOVA: Assessable General Linear Modelling*. Berkley, California. AbacusConcepts Inc.

3 Coll Cárdenas, F.; Noia, M.; Ostrosky, R.; Ferster, A.; García, M.; Fernández, J.; Prieto, L.; Tejerina, H. 2006. Variación del pH en diferentes cortes de carne equina. *La Industria Cárnica Latinoamericana*. 143, 64-66.

4 Coll Cárdenas, F.; Giannuzzi, L.; Zaritzky, N.E. 2007. Modelling microbial growth in meat broth with added lactic acid under refrigerated storage. *Int. J.Food Sci. Technol.* 42, 175-184.

5 Coll Cárdenas, F.; Giannuzzi, L.; Zaritzky, N.E. 2008. Predictive Equations to Assess the Lactic Acid and Temperature on Bacterial Growth in a Model Meat System. *In:* Gutiérrez Lopez, G.; Barbosa-Cánovas, G.; Welti-Chanes, J. y Parada Arias, E. (eds). *Food Engineering: Integrated Approaches*, Ch 24, p. 345-358.

6 D'Agata, M.; Nuvoloni, R.; Pedonese, F.; Russo, C.; D'Ascenzi, C.; Preziuso, G. 2010. Effect of Packaging and Storage Time on Beef Qualitative and Microbial Traits. *J. Food Quality*.33, 352-366.

7 Giannuzzi, L.; Pinotti, A.; Zaritzky, N. 1997. Mathematical modelling of microbial growth in packaged refrigerated beef stored at deferent temperatures. *Int. J. Food Microbiol.* 39 (1–2),101-110.

8 Gibson, A.M.; Bratchell, N.; Roberts, T.A. 1988. The efect of sodium chloride and temperature on rate and extent of growth of Clostridium botulinum type A in pasteurized pork slurry. *J. Appl. Bacteriol.* 62, 479-490.

9 Ingram, M.; Simonsen, B. 1980. Meat and meat products. *In:* International Commission on Microbial Specifications for Foods (eds).*Microbial Ecology of Foods.* Vol. 2. Academic Press Inc., New York, U.S.A., 333-409.

10 International Commission on Microbiological Specifications for Foods. 1983. *In: Microbial Ecology of Foods.* Ch.15, Meat and meat products. Blackie Academic and Professional, London, United Kingdom.

11 Kondratowicz, J.; Bak, T. 1998. Change in the weight and taste quality of horsemeat frozen by means of liquid carbon dioxide and the ventilation method during 3-month cold storage. *Nat. Sci.* 1, 229-239.

12 Kondratowicz, J.; Sobina, I.; Domanska, P. 2000. Analysis of the changes in the quality of horsemeat frozen by means of liquid carbon dioxide and the ventilation method during 3-month cold storage. *Nat. Sci.* 5, 177-186.

13 Kondratowicz, J.; Podlejska, Z. 2000. Wplywdodatkutluszczunaturalnegonaskladchemicznyiwlasciwoscifizykochemicznemiesakonskiegozamrozonegoroznymimetodami. *Biul.Nauk.* 8, 187-195.

14 Kondratowicz, J.; Kowalko, P. 2001. Zmianymasy I jakosesensorycznamiesakonskiegomrozonegoprzyuzyciuskroplonegodwutlenkuweglaimetodaowiewowa w czasie 6-miesiecznego przechowywaniachlodniczego. *Chlodnictwo.* 6, 43-46.

15 Kondratowicz, J. 2001a. Effect of natural fat addition on changes in the weight and sensory quality of horsemeat frozen according to different methods. *Nat. Sci.*8, 183-192.

16 Kondratowicz, J. 2001b. Wplywroznichmetodmrozenianazmianyskladupodstawowego I wybranichwlasciwoscifizykochemicznichmiesakonskiegowczasie 6-miesiecznegoprzechowywaniachlodniczego. *Rocz. Inst. Przem. Miesn. Tluszcz.* 38, 61-69.

17 Kondratowicz, J. 2002b. Change in the weight and taste quality of horsemeat frozen by means of liquid carbon dioxide and the ventilation method during 6-month cold storage. *Pol. J. Nat. Sci.* 10 (1), 187-195.

18 Kortz, J.; Gardzielewska, J. 1998. Miesneuzytkowaniekoni. *Kon. Pol.* 2, 10-11.

19 Osmanagaoglu, O. 2002. Behaviour and biological control of bacteriocin producing Leuconostocs associated with spoilage of vacuum-packaged sucuk. *Turk. J.Vet.Anim. Sci.* 27, 471-480.

20 Pérez Chabela, M.L.; Rodríguez Serrano, G.M.; Lara Calderón, P.; Guerrero, I. 1998. Microbial spoilage of meats offered for retail sale in Mexico City. *Meat Sci.* 51, 279-282.

21 SENASA. 2010. Available at: http://www.senasa.gov.ar/index html.php. Accessed December 5, 2010.

22 Stanislawczyk, R.; Znamirowska, A. 2005. Changes in physico-chemical properties of horsemeat during frozen storage. Acta Sci. Pol.*Technol. Aliment.* 4 (2), 89-96.

23 Whiting, R.C. 1995. Microbial modelling in foods. *Crit. Rev. Food Sci.Nutr.*35, 467-494.

24 Wilkinson, L. 1990. *SYSTAT, The System for Statistics.*Evanston, I.L.

25 Zwietering, M.H.; Jongenburger, I.; Rombouts, F.M.; Van'tRiet, K. 1990. Modelling of the bacterial growth curve. *Appl. Environ.Microbiol.* 56, 1875-1881.

Table N° 1: Mathematical modelling of growth of *Total Aerobic Mesophilic Heterotrophic Bacteria* (Total Aerob. Mesoph. Het. Bact.), Enterobacteriaceae and *Pseudomonas sp* in horsemeat packaged in polyethylene and ESE films and storage at 0ºC.

Film	*Microorganisms*	*Gompertz Parameters*				*Derivated Parameters*		
		a	*c*	*b*	*m*	*μ*	*LPD*	*MPD*
ESE film	Total Aerob. Mesoph. Het. Bact.	2.02± 0.01	0.98± 0.01	0.47± 0.02	6.32± 0.05	0.11	7.85	3.00
	Enterobacteriaceae r^2 0.91	-------	-------	-------	------	0.01	45	------
	Pseudomonas sp r^2 0.81	-------	-------	-------	------	0.01	45	------
Polyethylene	Total Aerob. Mesoph. Het. Bact.	1.01± 0.13	3.12± 0.19	0.14± 0.02	14.70± 1.38	0.16	4.19	4.13
	Enterobacteriaceae	0.98± 0.02	1.83± 0.03	0.39± 0.03	5.72± 0.17	0.26	3.16	2.81
	Pseudomonas sp	2.00± 0.01	1.42± 0.01	1.90± 0.01	9.68± 0.01	0.37	9.16	3.42

a: log (CFU cm^{-2}), c: log (CFU cm^{-2}), b: $days^{-1}$, m: days, μ: log (CFU cm^{-2})$days^{-1}$, MPD: (log (CFU cm^{-2}), LPD: (days).

Table N° 2: Mathematical modelling of growth of *Total Aerobic Mesophilic Heterotrophic Bacteria* (Total Aerob. Mesoph. Het. Bact.), Enterobacteriaceae and *Pseudomonas sp* in horsemeat packaged in polyethylene and ESE films and storage at 4ºC.

Film	*Microorganisms*	*Gompertz Parameters*				*Derivated Parameters*		
		a	*c*	*b*	*m*	*μ*	*LPD*	*MPD*
ESE film	Total Aerob. Mesoph. Het. Bact	2.99± 0.04	1.93± 0.06	0.21± 0.02	11.54± 0.55	0.15	6.54	4.92
	Enterobacteriaceae	2.00± 0.04	1.74± 0.06	0.29± 0.04	15.87± 0.29	0.19	11.6	3.74
	Pseudomonas sp	2.01± 0.09	1.56± 0.16	0.16± 0.04	14.07± 1.30	0.09	7.82	3.57
Polyethylene	Total Aerob. Mesoph. Het. Bact	2.47± 0.11	2.98± 0.19	0.28± 0.04	7.20± 0.51	0.21	3.63	5.45
	Enterobacteriaceae	0.95± 0.37	3.27± 0.56	0.21± 0.07	5.46± 1.20	0.25	0.91	4.22
	Pseudomonas sp	1.50± 0.01	2.84± 0.01	0.36± 0.01	9.39± 0.02	0.99	6.66	4.34

LPD: (days).
a: log (CFU cm-2), c: log (CFU cm-2), b: days-1, m: days, m: log (CFU cm-2)days-1, MPD: (log (CFU cm-2),

Fig. N° 1: Effect of temperature (0ºC) and gaseous permeability of the packaging film on the microbial growth of TotalAerob. Mesoph. Het. Bact. (a), Enterobacteriaceae (b) and *Pseudomonas sp* (c) in horsemeat. Full lines correspond to Gompertz or linear models. ●: horsemeat with polyethylene film at 0ºC; ○: horsemeat with ESE (vacuum packaging) at 0ºC.

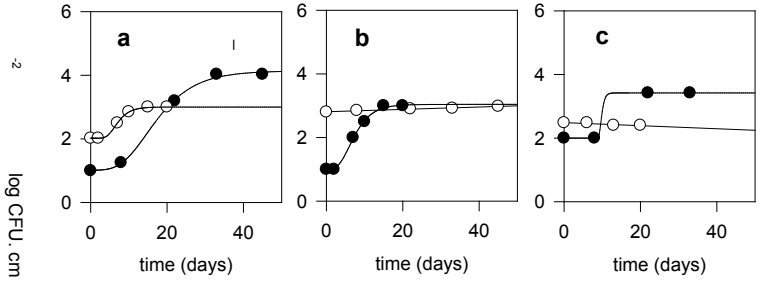

Fig. N° 2: Effect of temperature (4ºC) and gaseous permeability of the packaging film on the microbial growth of Total Aerob. Mesoph. Het. Bact. (a), Enterobacteriaceae (b) and *Pseudomonas sp* (c) in horsemeat. Full lines correspond to Gompertz or linear models. ■: horsemeat with polyethylene film at 4ºC; □: horsemeat with ESE (vacuum packaging) at 4ºC.

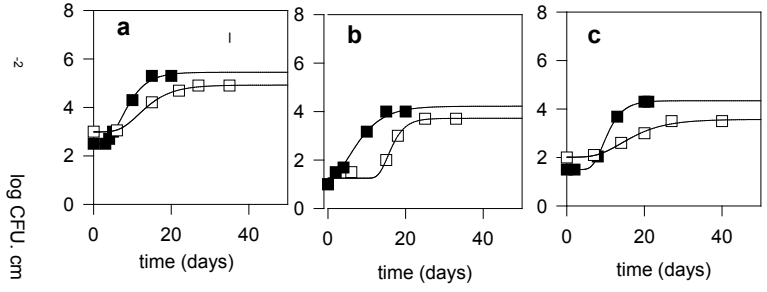

Fig. N° 3: Finals Counts of Total Aerob. Mesoph. Het. Bact. (a), Enterobacteriaceae (b) and *Pseudomonas sp* (c)in horsemeat at: (1) 0ºC in ESE film; (2) 0ºC in polyethylene film; (3) 4ºC in ESE film and (4) 4ºC in polyethylene film.