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## Joint application of antimicrobial agents on microbial flora chilled meat cattle. Use of mathematical models.

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### Abstract

Objectives of this work were: i) to study the effect joint application of antimicrobial agents on microbial flora chilled meat cattle; ii) to model mathematically this microbial growth and iii) validate the models with own experimental results. Samples were irradiated with UVC light for 5 minutes and then were added a solution of oregano oil and lactic acid (1:1). Untreated samples were considered as control. Gompertz model was appropriate to quantify microbial growth in all conditions studied. Meanwhile, *Pseudomonas sp* in treated samples showed no development, having to apply the linear regression model.

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### 1. Introduction

Application of mathematical models to quantify and predict microbial growth in meat is useful tool because the control thereof is critical. One of the more frequently used models is that of Gompertz which describes the microorganism response under different factor combinations and that allows to consider parameters such as LPD,  $\mu$  and MPD of the micro-organisms.

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As the industry develops new technologies to produce higher hygienic quality for increasingly competitive markets, systems must be designed to allow safeguards to be implemented in processing procedures (Coll Cárdenas, Giannuzzi & Zaritzky, 2008). In order to extend the useful life, various technologies have been implemented that acting jointly have a greater action, than if used separately, as is the case of UVC light, organic acids such as lactic acid and essential oils.

UVC light is a potent bactericidal agent, is not ionizable and when it is absorbed by proteins and nucleic acids, affects genetic material of microorganisms inducing changes in cell proliferation. Presently, UVC radiation technology is used as an alternative to chemical sterilization in food products (Chun et al., 2010; Pena et al., 2013).

Lactic acid is an acceptable decontaminant because it is a natural, non-toxic, produced naturally in meat products, and it offers the possibility of reducing the contamination of carcasses, cuts and beef products.

EOs and their components, commonly used as flavouring in the food industry, also present interesting antibacterial, antifungal and antioxidant properties (Sánchez-González et al., 2011). Plant-derived EOs have shown remarkable antimicrobial potency against spoilage and pathogenic microorganisms in meat (Jayasena & Jo, 2013). *Origanum vulgare* L. has been known as having many therapeutic properties and its antimicrobial activity has currently received a renewed interest (Souza et al., 2007).

The objectives of this work were: i) to study the effect of the combined application of antimicrobial agents on samples of beef with polyethylene low density films and stored at refrigeration temperatures, on development of spoilage microorganisms; ii) to model mathematically this microbial growth and iii) validate the models with own experimental results.

## 2. Materials and Methods

### Meat samples and storage conditions:

Beef samples (n=28) were obtained from *Longissimus dorsi* muscle of natural pH 5.8, from steers, carcass weighing up to 240 kg, with post-mortem time of 48 h at 4°C. The pH values were determined using a peachimeter of puncture Meter 6171L. The samples were cut aseptically into subsamples of 5.0 cm diameter by 1.0 cm high with a sterile scalpel (approximately 10g of meat sample). Then were placed in Petri dishes, splitting into two groups, one to be treated (irradiated with UVC light for 5 minutes ( $D=0.5567 \text{ J cm}^{-2}$ ) and were added of 1ml a solution of oregano oil (*Origanum vulgare* L) and lactic acid (1:1) and other non-irradiated considered as Control. The dose was determined by a digital radiometer. Samples were packaged with polyethylene low density films of 50  $\mu\text{m}$  thick and stored in controlled refrigeration cameras to 4°C, for 24 days.

### Microbiological analysis

Microbiological determinations were made using sterile swabs from the surface of meat at different storage times (0 to 24 days). Decimal dilutions using peptone water 0.1% were then performed and 1000  $\mu\text{l}$  of serial dilution were placed in specific media for each microorganism: Plate Count Agar (PCA Agar) for Total Aerobic Mesophilic Bacteria; Crystal Violet, Neutral Red Bile Agar for Enterobacteriaceae and Cetrinide Agar for *Pseudomonas sp.* using the Plate Pour Procedure. Spread plates were incubated aerobically at 37°C for 24h. Determinations were made in 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  $\text{cm}^{-2}$  (CFU  $\text{cm}^{-2}$ )).

### Mathematical modeling

One of the most recommended models is the Gompertz modified equation. From this equation, the following derived parameters were obtained:  $\mu = b.c/e [\log (\text{CFU cm}^{-2}) \text{ days}^{-1}]$ , with  $e = 2.7182$ ;  $\text{LPD} = m - (1/b) [\text{days}]$ ;  $\text{MPD} = a + c [\log (\text{CFU cm}^{-2})]$ . Data fits obtained from Gompertz model were analyzed by means of Systat software (Systat Inc.12.0). It calculates the set of parameters with the lowest residual sum of squares 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  $0.01 (\text{CFU cm}^{-2})^{-1} \text{ days}^{-1}$ , or when the difference between final counts and initial ones are lower than 0.50 log cycle. Lag phase was calculated as the time necessary to increase initial microbial counts in 0.50 log cycle ( $\text{LPD} = 0.50 / \mu$ ) (Coll Cárdenas et al., 2008).

Statistical analysis of experimental measurements (analysis of variance and pair wise comparisons) was computed using Systat software. Differences in means and F-tests were considered only when  $P < 0.05$ .

**3. Results and discussions**

Fig. 1 show the effect combined of UVC light, oregano oil and lactic acid solution and storage temperature (4°C) of the growth about Total Aerobic Mesophilic Bacteria (Fig. 1.a), *Pseudomonas sp* (Fig. 1.b) and Enterobacteriaceae (Fig. 1.c) in meat surface. Microbial growth in treated and non-treated samples showed significant differences ( $P < 0.05$ ).

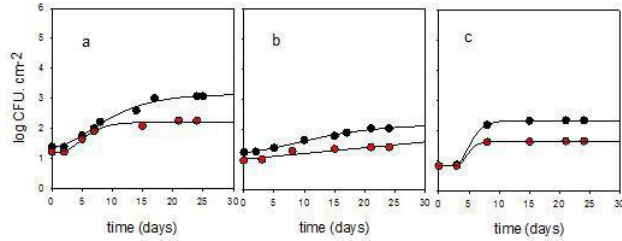


Fig. 1: Effect of UVC light, solution of oregano oil and lactic acid and storage temperature (4°C) on the growth of Total Aerobic Mesophilic Bacteria (a); *Pseudomonas sp* (b) and Enterobacteriaceae (c) in Treated (●) and Non-treated (●) samples. Full lines correspond to Gompertz or linear models (●,●).

Final counts of Total Aerobic Mesophilic Bacteria (Fig. 1.a) showed a good growth reaching final results of 3.08 log CFU cm<sup>-2</sup> in the Control samples. Meanwhile in the Treated samples, counts were 2.27 log CFU cm<sup>-2</sup>. In terms of the development of *Pseudomonas sp* (Fig. 1.b), microbial growth in Non-treated samples was 2.04 log CFU cm<sup>-2</sup> while in the others were not observed development during the day experience. In the case of Enterobacteriaceae (Fig. 1.c) microbial growth in both samples was also observed presenting a 0.69 log CFU cm<sup>-2</sup> difference between final counts at the end of 24 days.

The values of Gompertz parameters as well as the Derived parameters from the application of Mathematical models for Total Aerobic Mesophilic Bacteria, *Pseudomonas sp.* and Enterobacteriaceae respectively are shown in Table 1. In general terms, the model represents accurately microbial growth for all conditions studied in this work. To corroborate this observation, relative error, (%) with respect to experimental data (Fig. 1, symbols) was calculated. For all conditions tested, predicted microbial growth has an error less than 3 %.

Table 1. Application of Mathematical models for Total Aerobic Mesophilic Bacteria, *Pseudomonas sp.* and Enterobacteriaceae in Treated and Non-Treated Samples.

	Gompertz Parameters			Derived Parameters			
	a	c	b	m	μ	LPD	MPD
Total Aerobic Mesophilic Bacteria							
Treated Samples	1.21±0.07	1.00±0.09	0.43±0.15	4.63±0.65	0.14	2.34	2.21
Non-Treated Samples	1.30±0.15	1.84±0.22	0.18±0.04	6.62±0.91	0.14	1.30	3.14
<i>Pseudomonas sp</i>							
Treated Samples	-----	-----	-----	-----	0.01	26.3	r <sup>2</sup> 0.86
Non-Treated Samples	1.18±0.08	1.00±0.16	0.12±0.03	8.40±0.99	0.04	0.53	2.18
Enterobacteriaceae							
Treated Samples	0.82±0.01	0.82±0.01	1.00±0.13	4.47±0.31	0.30	3.47	1.64
Non-Treated Samples	0.81±0.01	1.51±0.01	0.70±0.02	4.73±0.09	0.39	3.30	2.33

As can be seen from these values, microbial growth in both samples showed significant differences ( $P < 0.05$ ). *Pseudomonas sp* in Treated Samples did not show development and the Gompertz Parameters could not be calculated, since a linear regression model was applied. This bacterium presented a good correlation between this dates ( $R^2 > 0.8$ ). In the case of Total Aerobic Mesophilic Bacteria, while  $\mu$  values between both samples were similes ( $0.14 \log(\text{CFU cm}^{-2}) \text{ days}^{-1}$ ), LPD was 1.42 times higher in Treated samples (2.34 days). This derived parameter in *Pseudomonas sp* was very longer reaching a difference of 25.78 between both samples. The highest values of  $\mu$  occurred in Enterobacteriaceae reaching a value of  $0.39 \log(\text{CFU cm}^{-2}) \text{ days}^{-1}$ .

Isohanni & Lyhs (2009) showed that UVC light used alone or combined with others techniques produced reduction of microbial counts in broiler meat using higher doses about us, but the use of these doses in meat may be limited because it can produce oxidation of fatty acids. In the case of EOs, Apostolidis, Kwon & Shetty (2008) also observed as in our case, that using EOs combined with sodium lactate produced reduction of microbial counts in ground beef.

#### 4. Conclusions

The results in this work indicate that the use of techniques combined as UVC light with solution of oregano oil and lactic acid in chilled meat are effectives for produce a reduction of microbial counts. In all cases studied (Total Aerobic Mesophilic Bacteria; *Pseudomonas sp* and Enterobacteriaceae) of Treated samples were observed a good reduction of final counts about the untreated samples. Gompertz model and linear regression model were appropriate to quantify microbial growth in all conditions studied. From the results, it can infer that the combined application of these obstacles is efficient to extend the useful life of this food.

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#### References

1. Apostolidis, E.; Kwon, Y. & Shetty, K. Inhibition of *Listeria monocytogenes* by oregano, cranberry and sodium lactate combination in broth and cooked ground beef systems and likely mode of action through proline metabolism. *International Journal of Food Microbiology*. 2008, 128, 317–324.
2. Coll Cárdenas, F.; Giannuzzi, L. & Zaritzky, N. Mathematical modelling of microbial growth in ground beef from Argentina. Effect of lactic acid addition, temperature and packaging film. *Meat Science*, July 2008; 79, 3; 509-520.
3. Chun, H.; Kim, J.; Lee, B.; Yu, D.; Song, K. Effect of UVC irradiation on the inactivation of inoculated pathogens and quality of chicken breasts during storage. *Food Control*, 2010, 21, 276–280.
4. Isohanni, P. & Lyhs, U. Use of ultraviolet irradiation to reduce *Campylobacter jejuni* on broiler meat. *Poultry Science*, 2009, 88, 661–668.
5. Jayasena, D. & Jo, Ch. Essential oils as potential antimicrobial agents in meat and meat products: A review. *Trends in Food Science & Technology*, December 2013; 34, 2; 96–108.
6. Pena, I.; Coll Cárdenas, F.; de la Sota, P.; Villat, M.; Noia, M.; Mestorino, N. Mathematical modeling of microbial growth in refrigerated horsemeat: It's importance in the quality of meat. *Revista Ciencia Veterinaria*, Fac Cs Veterinarias, UNLPAM, Noviembre 2013;15(1); 65-76.
7. Sánchez-González, L.; Vargas, M.; González-Martínez, Ch.; Chiralt, A.; Cháfer, M. Use of Essential Oils in Bioactive Edible Coatings. *Food Eng. Rev.* 2011; 3:1–16
8. Souza, E.; Stamford, T.; Lima, E.; Trajano, V. Effectiveness of *Origanum vulgare* L. essential oil to inhibit the growth of food spoiling yeasts. *Food Control*, May 2007; 18, 5; 409–413.

#### Nomenclature

$\mu$ : specific growth rate  
 LPD: lag phase duration  
 MPD: maximum population density  
 EOs: Essential oils  
 CFU: Colony Forming Units