LISTERIA MONOCYTOGENES CHALLENGE TESTING IN COOKED AND COOKED-SMOKED POULTRY READY-TO-EAT PRODUCTS

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Abstract – *Listeria monocytogenes* is a foodborne pathogen and a frequent hazard in ready-to-eat (RTE) meat products. The aim of this study was to perform a microbiological challenge test in cooked and cooked-smoked poultry RTE products, in order to assess the growth potential and the maximum growth rate of *Listeria monocytogenes*. In addition, the possible inhibition effect of lactic *L. monocytogenes* was studied. The tested poultry RTE products were cooked (turkey ham, TH) and cooked-smoked (turkey sausage, TS), both sliced and packaged under modified atmosphere. Results demonstrated that *L. monocytogenes* was not able to grow on RTE cooked-smoked products, whereas in RTE cooked products it was able to grow (growth potential > 0.5 log_{10} cfu/g and maximum growth rate of 0.08 log_{10} cfu/day). The studied RTE poultry products presented inhibitory factors, such as low water activity (aw) and adequate package's CO_2 concentration that, along with good hygiene manufacture during technological process, will control this pathogen.

Key Words – safety, pathogen, foodborne, modified atmosphere, package, lactic acid bacteria, grow potential, maximum growth rate.

I. INTRODUCTION

L. monocytogenes is frequently present in soil, vegetation, feces of animals, fresh meat, raw milk and fish and it may cause, when ingested in sufficient quantity, a severe illness in humans called listeriosis, which can often be fatal. The importance of this foodborne pathogen results from its capacity to grow or survive in a chilled environment, which makes *L. monocytogenes* a significant hazard in the food chain production [1]. This is especially important for RTE products because they don't need to undergo any process that reduces or eliminates microbial contamination after listericidal treatment and before consumption. According to Hazard analysis and critical control points (HACCP) procedures, food safety should be managed by monitoring fixed critical control points (product´s extrinsic and intrinsic parameters such as temperature/time, aw and pH). Raw materials and ingredients initial contamination control, temperature control, as well as good hygiene practices implementation, will guarantee that the level of L. *monocytogenes* is below 100 cfu/g during product´s shelf-life.[1].

Challenge tests aim to provide information on the behavior of *L. monocytogenes* that has been artificially inoculated into a food or foodstuffs, under given challenging storage conditions. These tests can be performed with two different objectives: assessment of the growth potential (i.e. the ability of *L. monocytogenes* to grow in food) or estimation of the growth parameters (e.g. maximum growth rate) [2].

According to microbiological criteria [3], RTE products with pH ≤4.4 or aw ≤0.92, or with pH ≤5.0 and aw≤0.94, are automatically considered as foods unable to support *L. monocytogenes* growth. Particular microbiological characteristics are also important since lactic acid bacteria can have an important protective role for RTE products [4].

The purpose of this study was to assess how *Listeria monocytogenes* behaves in sliced RTE poultry cooked and cooked-smoked products under modified atmosphere package (MAP) during storage.

II. MATERIALS AND METHODS

Ready-to-eat poultry products

Cooked-smoked turkey sausage (TS) and turkey ham (TH) were produced taking into account a specific formulation under confidentiality agreement and hygienic manufacturing practices implemented in the factory. Products were sliced under industrial conditions and were packaged using a thermoforming ATM packaging machine with appropriate packages (upper: PET(12my)//PE/ EVOH /PE(50my); under: APET/ PE) and using modified atmosphere (20-30% CO_2 and 70-80% N_2), being kept under

refrigeration (4ºC±1ºC) and transported to the laboratory in order to be submitted to challenge tests.

Isolation of Listeria and preparation of inoculum

Listeria monocytogenes was isolated according to ISO 11290-1(1996)/ Amendment 1 (2004) [5] from raw materials and RTE products of a poultry industry, with the purpose to constitute a collection of *Listeria monocytogenes* isolates to be used as inoculum in challenge tests. The isolates of *L. monocytogenes* were identified by PCR [6].

The isolates used in challenge testing were constituted by one reference strain (*Listeria monocytogenes* ATCC U937) and two isolates from samples (one from a raw material and another from a RTE product) of the studied industry, prepared by mixing equal volumes of the three strains with an optical density (OD_{625nm}) of 0.5-0.7 in order to obtain 100-500 cfu/g in the RTE products.

Challenge tests

The protocol used was adapted from the guidelines in the technical guidance on shelf-life studies for *Listeria monocytogenes* in RTE products [2].

For this assay, TH and TS inoculated with the mix of *L. monocytogenes* isolates were used, and repacked in a sealing machine (EVT-7-CD Tecnotrip, Barcelona) using polylaminated bags "HBX-070" (R.Bayer, Germany) under modified atmosphere (Aligal 13; Air Liquide, Production lot: 113011431575000) kept at 7ºC, during 76 days. Three different batches of the same products were used on the challenge tests.

Microbial analysis was performed at days 0, 7, 13, 27, 62 and 76. Each product had three batches (A, B and C) and from each batch, there were three inoculated and three control samples, for each analysis day.

Physicochemical characteristics and MAP conditions were also measured in one control sample of each batch at days 9, 38 and 72.

Microbiological methods: mesophilic aerobic total count (MAT, Plate count Agar, Sharlau, 48h at 30ºC), lactic acid bacteria count (LAB, Man Rogosa Sharpe pH=6.2±0.2 at 25ºC, Sharlau, 48h at 30ºC under anaerobiosis) and detection and enumeration of *L. monocytogenes* (according to ISO 11290-1 and 2:1996 [5]) were performed.

Physicochemical methods: pH was measured using a Meat pH Meter (HANNA Instruments, model HI 99163, serial number: B0064485); aw was performed using a ROTRONIC (model HygroPalm23-AW, serial number: 0061122785).

MAP CO_2 and O_2 concentration control was performed with a headspace analyzer Dansensor (model CheckPoint, serial number: 58142880)

Statistical analysis

Data were analyzed using Excel 2013 and SAS System 9.4 software.

SAS GLM procedure was used to evaluate *L. monocytogenes* behavior and physicochemical factors during the products' shelf-life, assessing polynomial regressions equations ($y=a+bx+cx^2$) and linear regressions equations ($y=a+bx$), when there was statistical significance ($p<0.05$).

To evaluate if *L. monocytogenes* and lactic acid bacteria counts had a significant correlation and also to assess the correlation coefficients, CORR Procedure of SAS was used.

The growth potential (δ) was calculated from the difference of the average concentration at "day end" and the average concentration at "day 0" ($\log_{10}$ cfu/g).

The maximum growth rate (μ_{max}) was given by $\Delta y_{a-b}/\Delta t_{a-b}$, that represents the difference of *L. monocytogenes* concentration ($\log_{10}$ cfu/g) in two days of analysis (a and b) divided by the time (days) between a and b.

III. RESULTS AND DISCUSSION

Physicochemical characteristics regarding pH and aw for both products under challenge are presented in Table 1.

TS´s pH data showed a quadratic regression with decreasing pH till day 40 and stabilization from that day forward, while TH presented a pH linear regression decreasing over time.

Regarding aw, TS showed a linear regression decreasing over time, while TH´s aw remained constant, no significant regression was found.

With such aw and pH results, these products were considered as unable to allow L. monocytogenes growth, according to microbiological criteria [3]. Aw results were below the growth limit of L. monocytogenes for both products [2]. Despite that, one of these products was able to support growth of *L. monocytogenes*.

Table 1- Physicochemical characteristics with regression equations and coefficient of determination.

Legend: Cooked-smoked turkey sausage (TS); Turkey ham (TH).

Product	Day	pH	aw
TS	9	6,27 ± 0.10	0.892 ± 0.01
	38	5.86 ± 0.12	0.838 ± 0.03
	72	5.79 ± 0.08	0.799 ± 0.00
		$y = 0.0002x^2 - 0.0233x + 6.4659$	$y = -0.0015x + 0.9012$
		$R^2 = 0.8179$	$R^2 = 0.8027$
TH	9	5.91 ± 0.29	0.826 ± 0.03
	38	5.41 ± 0.08	0.797 ± 0.00
	72	5.37 ± 0.07	0.800 ± 0.00
		$y = -0.0084x + 5.8946$	----
		$R^2 = 0.5036$	----

MAP gases percentages during products' shelf-life are presented in Table 2. In MAP TS, the O_2 concentration presented a linear regression increasing over time, while CO_2 concentration remained constant. In TH, O_2 concentration presented a linear regression increasing over time, while CO_2 concentration remained constant. The inhibitory gas CO_2 remained constant during all products' shelf-life and under previously defined concentrations (70-80% N_2 + 20-30% CO_2).

Table 2- MAP gases percentage during products shelf life with regressions equations and coefficient of determination.

Legend: Cooked-smoked turkey sausage (TS); Turkey ham (TH).

Product	Day	O_2 (%)	CO_2 (%)
TS	9	0.20 ± 0.08	25.40 ± 1.55
	38	0.30 ± 0.24	24.07 ± 1.77
	72	0.73 ± 0.29	23.40 ± 1.65
		$y = 0.0086x + 0.0705$	----
		$R^2 = 0.4737$	----
TH	9	0.20 ± 0.08	26.23 ± 1.59
	38	0.17 ± 0.17	26.03 ± 0.90
	72	0.60 ± 0.29	24.47 ± 0.63
		$y = 0.0065x + 0.0631$	----
		$R^2 = 0.3557$	----

MAP could have an inhibitory effect on *L. monocytogenes*, however it is always important to remember that this pathogen is a facultative anaerobe, so it can be a potential hazard for MAP RTE products.

Microbiological characterization results for the studied RTE products are presented in Figure 1 and 2.

L. monocytogenes counts in TS remained constant, while in TH the growth was demonstrated on a quadratic regression, increasing till day 30 and stabilizing from that day forward.

Figure 1. Microbiological characteristics of cooked-smoked turkey sausage (TS).

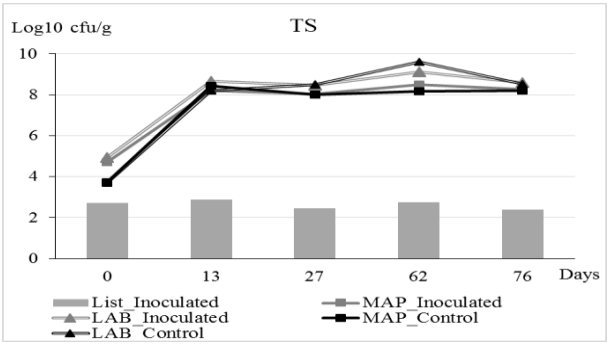
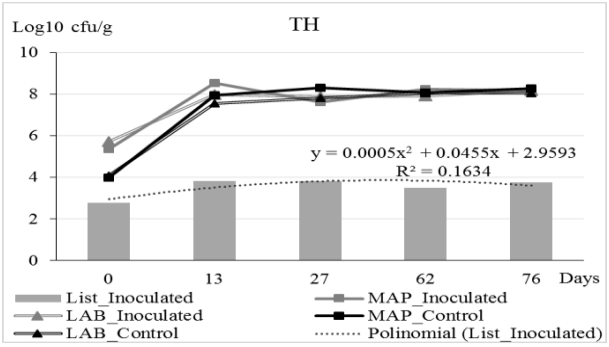


Figure 2. Microbiological characteristics of Turkey ham (TH).



L. monocytogenes growth potential (δ) in the studied products was: -0.33 $\log_{10}$ cfu/g for TS and 0.99 $\log_{10}$ cfu/g for TH. This means that cooked-smoked products (TS) were not able to support *L. monocytogenes* growth, while in turn cooked product (TH) was [2]. In food products able to support *L. monocytogenes* growth (δ higher than 0.5 $\log_{10}$), the δ could be used to set up the pathogen's concentration at the shelf-life's beginning in order to respect the limit of 100

cfu/g (2 $\log_{10}$) at shelf-life's end (Initial concentration = 2 log – δ) [2], then the maximum initial concentration for TH is 1.01 $\log_{10}$ cfu/g (=10 cfu/g), in order to respect the microbiological criteria limit. No 2-$\log_{10}$ increase (from 1 to 100 bacteria) was detected during TH shelf-life.

L. monocytogenes maximum growth rate results are presented in Table 3.

Table 3- Maximum growth rate ($\log_{10}$ cfu/day).

Legend: Cooked-smoked turkey sausage (TS); Turkey ham(TH).

Product	*Listeria monocytogenes* ($\log_{10}$ cfu/day)			
	Δy_{0-13} /Δt_{0-13}	Δy_{13-27} /Δt_{13-27}	Δy_{27-62} /Δt_{27-62}	Δy_{62-76} /Δt_{62-76}
TS	0.01	-0.03	0.01	-0.03
TH	0.08	0.00	-0.01	0.02

The maximum growth rate for TS was 0.01 $\log_{10}$ cfu/day and occurred between day 0-13 and day 27-62. The maximum growth rate for TH was 0.08 $\log_{10}$ cfu/day and occurred between days 0-13.

Mesophilic aerobic total and lactic acid bacteria counts presented by both products under study were high. It seems that lactic acid bacteria were dominant and this could be one of the reasons why *L. monocytogenes* growth was under control in this challenge test [4], however it was not possible to demonstrate any correlation between *L. monocytogenes* and lactic acid bacteria count.

IV. CONCLUSION

While *L. monocytogenes* was able to grow in RTE poultry cooked products, it was not in cooked-smoked poultry products. It was not detected any 2-$\log_{10}$ increase during shelf-life of cooked poultry products. The studied products revealed *L. monocytogenes* inhibitory and limiting factors such as aw, pH and microbial competition due to lactic acid bacteria that could explain the control of the pathogen growth in these challenge tests.

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