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EVALUATION OF GROWTH POTENTIAL OF *LISTERIA MONOCYTOGENES* IN MEAT PRODUCTS READY TO EAT

EVALUAREA POTENȚIALULUI DE CREȘTERE AL *LISTERIA MONOCYTGENES* ÎN PRODUSELE DIN CARNE GATA DE CONSUM

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ABSTRACT | REZUMAT

Ready-to-eat (RTE) products containing meat, having a shelf life longer than 5 days, and under refrigeration conditions are the high-risk foods for listeriosis, especially for some risk categories that include pregnant women, neonates, immunocompromised patients, and the elderly. In this paper, the evaluation of LM growth potential in pork meatballs, and meat products is presented throughout the shelf life of the product. A RTE product is a food intended by the producer for direct human consumption without cooking or other processing effective to eliminate or reduce to an acceptable level the pathogen micro-organisms. The microbial shelf-life of a food corresponds to the period during which the food remains within predefined quantitative microbiological limits. This represents a food safety control measure that has to be validated by studies, according to the provisions of Regulation (EC) No 2073/2005, Annex II. The result of the study showed that the growth of *Listeria monocytogenes* is possible on the tested product since the highest growth potential (*Δ*) among the three batches is > 0.5. In this situation, when the limit of 100 cfu/g is likely to be exceeded at the end of the shelf-life of RTE products, the producer cannot demonstrate compliance with Regulation (EC) no. 2073/2005 and, in accordance with HACCP principles, the flow process and the defined shelf-life shall be revised and improved. This should include microbiological quality control of raw materials and other ingredients, reducing the growth potential of LMO by adding inhibitory agents.

Keywords: LM, meat products, RTE, growth potential (Δ)

Produsele Ready-to-eat care conțin carne și care au o durată de valabilitate mai mare de 5 zile, în condiții de refrigerare sunt alimentele cu risc ridicat pentru listerioză, în special pentru unele categorii de risc care includ femeile gravide, nou-născuții, pacienții imunocompromiși și persoanele în vârstă. În această lucrare este prezentată evaluarea potențialului de creștere a LM în carnea de porc, produsele din carne RTE, pe întreaga durată de valabilitate a produsului. Un produs RTE este un produs alimentar destinat de producător pentru consumul uman direct, fără gătit sau alte prelucrări eficiente pentru eliminarea sau reducerea la un nivel acceptabil a microorganismelor patogene. Perioada de valabilitate microbiană a unui produs alimentar corespunde perioadei în care produsul alimentar rămâne în limitele microbiologice cantitative predefinite. Aceasta reprezintă o măsură de control al siguranței alimentare care trebuie validată prin studii, în conformitate cu prevederile Regulamentului (CE) nr. 2073/2005, anexa II. Rezultatul studiului a arătat că este posibilă creșterea *Listeria monocytogenes* pe produsul testat, deoarece cel mai mare potențial de creștere (Δ) dintre cele trei loturi este > 0.5. În această situație, atunci când limita de 100 cfu/g este susceptibilă să fie depășită la sfârșitul duratei de valabilitate a produsului RTE, producătorul nu poate demonstra conformitatea cu Regulamentul (CE) nr. 2073/2005 și, în conformitate cu principiile HACCP, procesul de flux și durata definită a valabilității trebuie revizuite și îmbunătățite. Acest lucru ar trebui să includă controlul calității microbiologice a materiilor prime și a altor ingrediente, reducând potențialul de creștere al LMO, prin adăugarea agenți inhibitori.

Cuvinte cheie: LM, produse de carne, produse gata de consum, potențial de creștere

The infection produced by *Listeria monocytogenes* (LM) is a foodborne zoonosis that affects humans, depending on the physiological condition of the individual (2, 7). LM could contaminate ready-to-eat products (RTE) due to its special behaviour concerning environmental conditions and raw materials (8). The potential risk of LM multiplication during the shelf life of the product is a continuing concern (4, 5). Commission Regulation (EC) no. 2073/2005, on microbiological criteria for foodstuffs, sets out specific food safety criteria for LM in ready-to-eat foods, including the meat products RTE, in Annex I for categories 1.1 to 1.3 (3).

Regulation (EC) no. 2073/2005 on microbiological criteria for foodstuffs sets up in Annex I the microbio-

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logical criteria applicable for LM in RTE foods (all types of RTE foods, except those intended for infants and for special medical purposes) and a quantitative maximum limit of 100 cfu/g for criterion 1.3 (RTE foods not able to support the growth of *LM)* and for criterion 1.2 (RTE foods able to support the growth of *LM*) (3).

RTE foods which can support the growth of LM are laid down two types of criteria:

(1) the qualitative criterion – absent/25g RTE foods before the food has left the immediate control of the producer; *or*

(2) the quantitative criterion – counting method when the maximum limit is 100 cfu/g RTE placed on the market during their shelf-life. This quantitative criterion applies if the producer can demonstrate that LM will not exceed the limit of 100 cfu/g throughout the shelf-life (3).

The shelf life and the microbiological safety of a product may be influenced by different intrinsic and extrinsic factors. Intrinsic factors such as water activity (aw), pH, content of moisture, and microflora are very important in relation to the stability of a product during its shelf life. Also, extrinsic factors play an important role in food safety, such as packaging method (modified atmosphere packaging), storage condition (1, 9).

The aim of the study was to evaluate the LM growth that may be present in the product and, based on this, validate the microbiological safety of through its shelf life and food safety for human health. Also, it's assessed if a foodstuff is able or unable to support the growth of *Listeria monocytogenes* and represents a validation method for the shelf life of foodstuff based on microbiological criteria in specific storage conditions.

MATERIALS AND METHODS

The challenge test was carried out in accordance with ISO 20976-1:2019 Microbiology of the Food Chain — Requirements and Guidelines for Conducting Challenge Tests of Food and Feed Products — Part 1: Challenge tests to study growth potential, lag time, and maximum growth rate, and the provisions of the technical guide for conducting shelf-life studies on *Listeria monocytogenes* in ready-to-eat foods, version 4 of July 1, 2021 (6). For this study, we used 3 batches of meat products RTE – pork meat balls that were analysed during the shelf life regarding the growth potential of LM: batch no. 1, batch no. 2, and batch no. 3. These three batches of pork meat balls have the same recipe and low process, but they have different production dates. All three batches were made in the same production unit, using the same packaging method. The pork meat balls are thermally treated meat products RTE, obtained through frying (frying at a temperature range of 160-180°C).

The packaging of the product is done under modified atmosphere conditions (MAP- modified atmosphere packaging) with a gas mixture of 70% nitrogen and 30% carbon dioxide. The shelf life is 20 days under refrigeration conditions at 2–6 °C.

It was established five-time intervals per batch throughout the 20 days of shelf life of the product, such as T1 (first day); T2 (day 9 of shelf life); T3 (day 14 of shelf life); T4 (day 17 of shelf life); and T5 (day 20 - last day of shelf life), to count the LM on the three batches artificially inoculated.

Based on the results obtained per batch, it will calculate the growth potential (Δ). The growth potential represents the difference between the highest observed LM concentration as log10 cfu/g during the test (*log max*) and the initial LM concentration in log10 cfu/g at the beginning of the test (at T1) (*log i*).

The growth potential formula was:

Growth potential (Δ) = *log max – log i*, where:

- l*og max* represents the highest value of the *LM* (based on counting) obtained from the 4 sampling points (except the T1).
- *log I* is the mean value of the 3 test units analysed at the initial time (T1).

Interpretation:

- Δ > 0.5 log10 cfu/g, the growth of *L. monocytogenes* is possible in food throughout its shelf life.
- Δ ≤ 0.5 log10 cfu/g, the growth of *L. monocytogenes* is not possible in food throughout its shelf life.

RESULTS AND DISCUSSIONS

We carried out complementary tests per batch to evaluate the products:

- 3 blank samples / each batch for detection of *Listeria monocytogenes* /25 g (acc. ISO11290-1: 2017 – horizontal method for detection and enumeration of *Listeria monocytogenes* and *Listeria* spp. Part 1. Detection method) (Table 1).
- 3 samples / each batch for total mesophilic count at 30°C (TVC) (acc. ISO 4833-1:2014 - horizontal method of microorganism that are able to grow and form colonies in a solid medium after aerobic incubation at 30°C); the samples tested were weighted at 10g and 90 ml of dilutor was added (Table 2).
- one sample /each batch to determine the physical-chemical parameters: water activity *aw* (acc. ISO 21807:2005) and pH measurement (potentiometric method) (Table 3).

In the test units used for counting the TVC, a volume of sterile physiological water was injected, identical to the volume of the *L. monocytogenes* inoculum and the volume of the *L. monocytogenes* identical for the inoculated samples.

Table 1

Detection of *Listeria monocytogenes* /25 g on 3 black samples at T1 and T5

Batch	Day of the test	Sample 1	Sample 2	Sample 3
1	T1	absent/25g	absent/25g	absent/25g
	T5	absent/25g	absent/25g	absent/25g
2	T1	absent/25g	absent/25g	absent/25g
	T5	absent/25g	absent/25g	absent/25g
3	T1	absent/25g	absent/25g	absent/25g
	T5	absent/25g	absent/25g	absent/25g

Table 2

TVC (cfu/g) on 3 black samples at T1 and T5

Batch	Day of the test	Sample 1	Sample 2	Sample 3
1	T1	$1.6x10^4$	$1.1x10^4$	$2.2x10^4$
	T5	$4.3x10^4$	$5.3x10^4$	$1.4x10^4$
2	T1	$3.3x10^4$	$2.4x10^4$	$1.8x10^4$
	T5	$4.1x10^4$	$1.4x10^4$	$1.0x10^4$
3	T1	$6x10^3$	$4x10^3$	$7x10^3$
	T5	$1.7x10^4$	$2.4x10^4$	$6.2x10^4$

Tabel 3

Physical-chemical parameters on one sample/each batch

Day of the test	Batch	Water activity (aw)	pH
T1	1	0.934	5.9
	2	0.931	6.0
	3	0.933	6.0
T5	1	0.933	6.0
	2	0.928	6.1
	3	0.932	6.1

For the artificial inoculation, two strains of LM were used *Listeria monocytogenes* isolated from meat products and *Listeria monocytogenes ATCC 13932*.

The assumed level of contamination was about 100 cfu per 1g of sample. T1 was the day of artificial inoculation, carried out two days after the production date. The samples were inoculated at two different points through a special membrane. After, it carried out the protection of samples against the change in the composition of the atmosphere by using a sealing septum.

The samples were tested at established time intervals (T1, T2, T3, T4, and T15) for enumeration of *L. monocytogenes*, according to ISO 11290-2:2017-07. At T1 and T5, three inoculated samples were tested per batch. At T2, T3, and T4, one inoculated sample/batch was tested.

Table 4

Level of contamination (Cfu/g), *Listeria monocytogenes* concentration (Log_{10}), and growth potential (Δ) of Batch No. 1

Day of the test	cfu/g	Log_{10}	Δ of batch
T1	$6.1x10^2$	2.79	5.72-2.78 = 2.94
	$6.9x10^2$	2.84	
	$5.0x10^2$	2.70	
		average log_{10}=2.78	
T2	$5.9x10^3$	3.77	
T3	$4.9x10^4$	4.69	
T4	$9.2x10^4$	4.96	
T5	$5,9x10^5$	5.77	
	$4.8x10^5$	5.68	
	$5.0x10^5$	5.70	
		average log_{10}=5.72	

Table 5

Level of contamination (Cfu/g), *Listeria monocytogenes* concentration (Log_{10}), and growth potential (Δ) of Batch No. 2

Day of the test	cfu/g	Log_{10}	Δ of batch
T1	$6.7x10^2$	2.83	5.76-2.85 = 2.91
	$7.4x10^2$	2.87	
	$7.2x10^2$	2.86	
		average log_{10}=2.85	
T2	$6.7x10^3$	3.83	
T3	$7.0x10^4$	4.85	
T4	$9.4x10^4$	4.97	
T5	$5.4x10^5$	5.73	
	$5.7x10^5$	5.76	
	$6.1x10^5$	5,70	
		average log_{10}=5,76	

Table 6

Level of contamination (Cfu/g), *Listeria monocytogenes* concentration (Log_{10}), and growth potential (Δ) of Batch No. 3

Day of the test	cfu/g	Log_{10}	Δ of batch
T1	$7.5x10^2$	2.88	5.81-2.91 = 2.90
	$8.5x10^2$	2.93	
	$8.3x10^2$	2.92	
		average log_{10}=2.91	
T2	$6.4x10^3$	3.81	
T3	$7.6x10^4$	4.88	
T4	$1.0x10^5$	5.00	
T5	$6.3x10^5$	5.80	
	$6,1x10^5$	5.79	
	$6.8x10^5$	5.83	
		average log_{10}=5,81	

Considering the obtained results of the challenge test carried out on the three batches of pork meat balls, the LM growth potential of the tested product can be assessed. The growth potential for each batch is more than 0.5 log10 cfu/g, and the highest Δ value among the 3 batches is 2.94 log10. The growth potential is > 0.5 log10 cfu/g. Also, these results can be used to classify the foodstuff according to the provisions of Regulation (EC) No. 2073/2005, as follows:

- RTE is able to support the LM growth (other than those intended for infants and for special medical purposes) during the shelf life – when Δ> 0.5 log10 cfu/g; or
- RTE is unable to support the LM growth (other than those intended for infants and for special medical purposes) during the shelf life – when Δ0.5 log10 cfu/g (3).

The organoleptic parameters of the product for all tree batches were adequate throughout the shelf life, under conditions of storage in the thermal regime of refrigeration. The product tested (pork meat ball) is able to support the growth of *Listeria monocytogenes* throughout the shelf life (20 days), in refrigeration conditions (2-6°C), based on the results obtained on the three batches analysed. According to Reg. (EC) No. 2073/2005, this RTE food supports LM growth and is classified into category 1.2 of Regulation (3).

CONCLUSIONS

The growth of *Listeria monocytogenes* was possible on tested product since the highest growth potential (*Δ*) among the three batches was > 0.5 log10 cfu/g. When the limit of 100 cfu/g is likely to be exceeded at the end of the shelf-life of RTE products, the producer cannot demonstrate compliance with Regulation (EC) No. 2073/2005 and, in accordance with HACCP principles, the flow process and the defined shelf-life shall be revised and improved. This should include microbiological quality control of raw materials and other ingredients of pork meat balls, reducing the growth potential of LMO by adding inhibitory agents of LM growth.

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