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ANTIMICROBIAL EFFECT OF ORGANIC ACIDS TO INHIBIT THE GROWTH OF *LISTERIA MONOCYTOGENES* INTO THE READY-TO-EAT PRODUCTS

EFECTUL ANTIMICROBIAN AL ACIZILOR ORGANICI ÎN INHIBAREA CREȘTERII *LISTERIA MONOCYTOGENES* ÎN PRODUSELE READY-TO-EAT

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ABSTRACT | REZUMAT

The food safety concern in relation with *Listeria monocytogenes* (*LM*) represents a reality and the purpose of this article is to highlight that using the organic acids represent a useful measure to inhibit the growth of LM into ready to eat products during of shelf life. *LM* infection is a foodborne zoonoses that affect humans as mild illness (the gastro-enteric form or non-invasive form) or as severe illness (the invasive form), in relation to the physiological condition of the individual. *LM* could contaminate ready-to-eat products, both of animal and non-animal origin, due to its special behaviour in relation to environmental conditions. The prevention of this disease is a continuing concern, hence the use of organic acids to preserve the RTE is a good food safety measure. In this paper, we are presenting the antimicrobial effect of organic acids to eliminate and/or inhibit the growth of *LM* in RTE products based on mayonnaise throughout the shelf life of the product. One of these is the dry buffered vinegar which contains acetic acid that has an important antimicrobial with strong antagonistic action against *LM*.

Keywords: *LM*, dry buffered vinegar, food safety, shelf life

Preocuparea privind siguranța alimentelor în relație cu *Listeria monocytogenes* (*LM*) reprezintă o realitate, iar scopul acestui articol este de a evidenția faptul că utilizarea acizilor organici reprezintă o măsură utilă pentru inhibarea creșterii *LM* în produsele ready-to-eat în perioada de valabilitate. Infecția cu *LM* este o zoonoză alimentară care, în funcție de rezistența individului afectat, induce la oameni fie boli cu evoluție blândă (forma gastro-enterică sau forma neinvazivă) fie boli cu evoluție severă (forma invazivă). Datorită comportamentului special în raport cu condițiile de mediu, *LM* poate contamina produsele ready-to-eat, atât de origine animală, cât și nonanimală. Prevenirea bolilor asociate infecției cu *LM* este o preocupare continuă a sănătății publice și, prin urmare, utilizarea acizilor organici pentru conservarea produselor ready-to-eat este o măsură bună de siguranță alimentară. În această lucrare, prezentăm efectul antimicrobian al acizilor organici pentru eliminarea și/sau inhibarea creșterii *LM* în produsele ready-to-eat pe bază de maioneză, pe toată durata de valabilitate a produsului. Unul dintre acestea este oțetul tamponat pudră care conține acid acetic și care are acțiune antimicrobiană importantă și acțiune antagonistă puternică împotriva *LM*.

Cuvinte cheie: *LM*, oțet tamponat pudră, siguranță alimentară, termen de valabilitate

Listeriosis, one of the most important foodborne zoonoses, is caused by *Listeria monocytogenes* (*LM*). The disease has a serious clinical evolution in high-risk groups such as pregnant women, elderly people (over 65 years), neonates, and immunosuppressed people, and it has caused pathologies such as meningitis, septicaemia, encephalitis, and abortions. The incidence of disease is low, with 0.1 to 10 cases per million people per year, according to the WHO, depending on the regions of the world. On the other hand, the severe clinical evolution and high rate of mortality make listeriosis a serious public health concern. In the majority of cases, the transmission mode of infection is via the consumption of contaminated foods, especially ready-to-eat (RTE) foods (3, 4, 6, 7). *LM* is a ubiquitous and relatively resistant bacteria to the action of curing agents and it could survive and multiply (8, 9, 12). However, it is widespread in the environment and represents a major hazard for a wide variety of foodstuffs, including the RTE. The development of antimicrobial substances to eliminate and/or inhibit the growth of *LM* during the extended refrigerated shelf life of RTE foods over 5 days represents a continuous challenge (11). For this category of products, as RTE products based on mayonnaise, the sources of contamination could be the production environment and/or

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raw materials. During the manufacturing flow of Boeuf salad, the *LM* recontamination after heat treatment of the ingredients represents the main issue, considering that this pathogen agent is ubiquitous, psychotropic, and relatively resistant to the action of curing agents, and this type of product is RTE. Also, the bacteria could survive and multiply during the shelf life of the product, and it could exceed the maximum level accepted of 100 ufc/g of product according to UE rules (5). The addition of organic acids, such as the dry buffered vinegar, which contains acetic acid that has an important inhibitory action against LM, is efficient in suppressing or limiting the growth of LM in RTE foods such as boeuf salad throughout the shelf life of the product (1, 13). The goal of this study is to demonstrate the antimicrobial influence of organic acids on the eradication and/or inhibition of the formation of *LM* in mayonnaise-based RTE foods during the period of the product's shelf life.

MATERIALS AND METHODS

It was conducted through research to identify and count *LM* during the shelf life of the product, as a challenge test to evaluate the growth potential of *LM* during the shelf life of the product, and to evaluate the antimicrobial effect of dry buffered vinegar (Bacto CEASE® NV DRY, Kemin, USA) added to boeuf salad (salad with mayonnaise). The challenge test is carried out in accordance with the Technical Guidance Document for performing shelf-life research on *Listeria monocytogenes* in ready-to-eat foods (10).

According to the technical specification of Bacto CEASE® NV DRY, it contains spirit vinegar and is able to be used for RTE foods. For this purpose, we made a challenge test for three experimental batches of Boeuf salad treated with dry buffered vinegar to evaluate the inhibitory effect on the growth potential of *LM* during its shelf life in the same manufacturing conditions, the same packaging conditions, and the same storage conditions: I batch, II batch, and III batch. For these three experimental batches, 750 g of dry buffered vinegar was added to the 100 kg of boeuf salad that was added during the mixing of the ingredients. All batches were packed in a protective atmosphere (nitrogen and carbon dioxide). The weight of each sample is 250g. The storage temperature of the product is between 2°C-6°C. The product has a shelf life of 15 days.

Preparation of *Listeria monocytogenes* strains

It used two strains of *Listeria monocytogenes*: ATCC 13932 - further marked as ATCC, and *Listeria* monocytogenes isolated from the environment of processing plant (confirmed the belonging of environmental strains to serological Group II) - further marked as strain 1, strain 2. Strains ATCC and 1, 2, were inoculated on a TSYEA plate and incubated at 37 °C for 24h. After the identification, the brain–heart bullion was inoculated with each strain separately and incubated at 37 °C for 24h. Then 0.1ml of each culture was transported to another brain–heart bullion and then kept in the refrigerator at 7 °C for 7 days. On the seventh day of storing in 7 °C, the culture was tested every 2 h to determine the number of *Listeria monocytogenes* and to check if the stationary growth phase was achieved (Fig. 1). The mixed culture for the challenge test was prepared as follows:

- Strain 1. The first dilution was prepared by transferring 0.78 ml of strain suspension to 9.22 ml of diluent (saline with peptone);
- Strain 2. The first dilution was prepared by transferring 0.48 ml of strain suspension to 9.52 ml of diluent (saline with peptone);

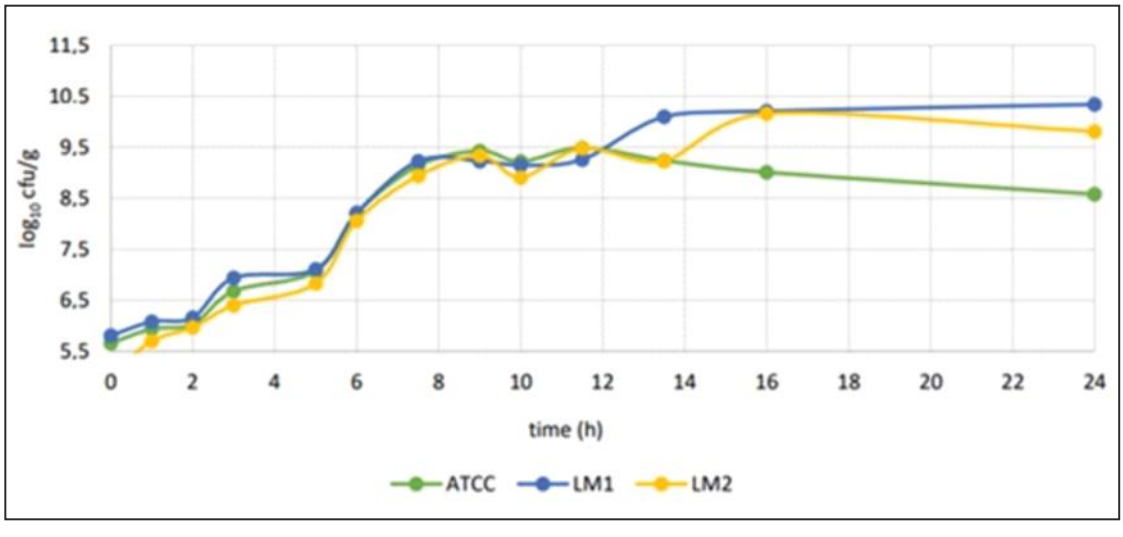

Fig. 1. The number of *Listeria monocytogenes* after incubation at 37°C for 24h

- Strain 3. The first dilution was prepared by transferring 0.85 ml of strain suspension to 9.15 ml of diluent (saline with peptone).

The assumed level of each strain was 8.3 x 10^3 cfu/ml. The targeted concentration in the whole matrix is 100 cfu/g (50–200 cfu/g). A mixture of 0.3 ml of each strain was prepared in Eppendorf tubes. Actual value obtained for contamination: 2.23x10^4 cfu/g. The samples were inoculated at two different locations through a special membrane. The volume of the inoculum did not exceed 1% of the mass of the whole matrix; the maximum volume of the inoculum was 1 ml.

Inoculation of samples

Three samples were inoculated out of each batch, and each sample was inoculated with 0.33 ml of ATCC strain suspension, and the same for strains 1 and 2. The assumed level of contamination was about 100 cfu per 1g of sample. The samples were protected against a change in the composition of the atmosphere by using a sealing septum. The samples were tested at determined time intervals (T0, T5, T10, and T15) to count *LM*, according to the ISO 11290-2:2017-07 standard. For each time, there were tested 3 samples inoculated with *LM*. Three blank samples were tested for counting and detecting *LM* at T0 and T15. For each batch, 3 out of 12 inoculated samples were tested for counting *LM*, according to the ISO 11290-2:2017-07 standard,right after the inoculation.The other samples were stored at 7 °C, 10 days and then at 10 °C, 5 days.

RESULTS AND DISCUSSIONS

According to the Technical Guidance Document for performing shelf-life research on *LM* in ready-to-eat foods, a challenge test for determining maximum growth rate is a microbiological laboratory-based study that measures the growth rate of a single *LM* strain in artificially contaminated food stored at a constant temperature. By charting the natural logarithm of the bacterial population vs. time, the maximum growth rate is determined from the exponential phase of a growth curve of *LM* produced at a constant temperature. The greatest growth rate (max) is the slope of the linear phase (2). The results of tests on contaminated samples are available below (Fig. 2).

To calculate the growth potential (δ) of *LM* for each batch, the concentration of LM (in $\log_{10}$ cfu/g) for each sample was determined over time (3 samples at T0, T5, T10, and T15) during the shelf life of the product. It is essential to check for each experimental batch at T0 if the standard deviation of the three *LM* enumerations is ≤ 0.3 $\log_{10}$ cfu/g. The calculation formula of the growth potential is provided by the standard EN ISO 20976-1 and the EURL Lm Technical Guidance Document: *"the difference between the decimal logarithm of the highest concentration of the target microbial population ($\log_{max}$) and the decimal logarithm of the initial concentration of this microbial population ($\log_i$)"* (10). The maximum difference between these values is the growth potential of RTE food and based on this it is considered able to support the growth of *LM* if the *δ* is higher than 0.5 $\log_{10}$cfu/g, or it isn't able to support the growth if the *δ* is lower than 0.5 $\log_{10}$ cfu/g.

The results of determination of the growth potential for each batch are available in Tables 1–3.

For each series tested on the "last day", the standard deviation between $\log_{10}$was calculated and the results meet the assumed criterion <0.3$\log_{10}$. Batch 1 - standard deviation SD=0.05. Batch 2 - standard deviation SD=0.04. Batch 3 - standard deviation SD=0.05

The criteria of evaluation:

- δ > 0.5 $\log_{10}$ cfu/g, the growth of *L. monocytogenes* is possible in food
- δ ≤ 0.5 $\log_{10}$ cfu/g, the growth of *L. monocytogenes* is not possible in food.

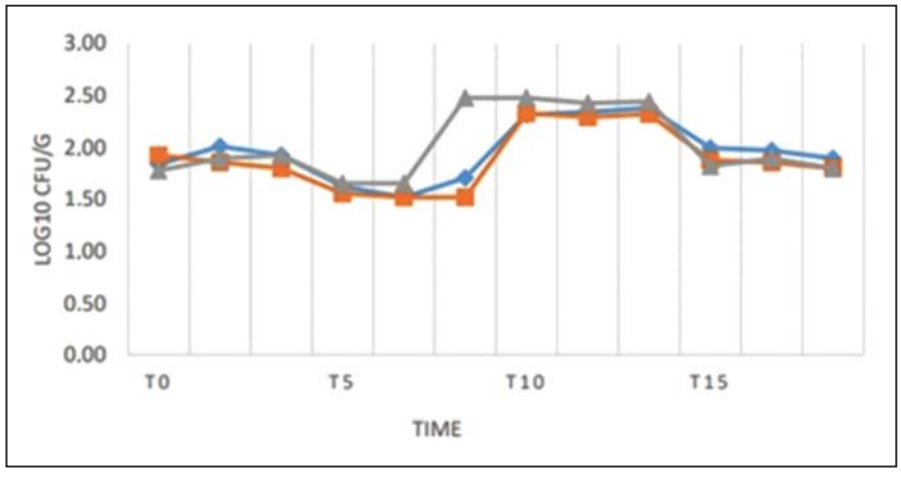

Fig. 2. Graphic of $\log_{10}$ cfu/g for contaminated samples

Table 1

Determination of the growth potential for batch I. The growth potential of batch I is 0.04

Batch	sample	day	Number of colonies (cfu/g)	log_{10}	Growth potential (δ)
I	1	T0	6.90E+01	1.84	0.04
	2		1.02E+02	2.01	
	3		8.40E+01	1.92	
	1	T5	4.20E+01	1.62	
	2		3.30E+01	1.52	
	3		5.10E+01	1.71	
	1	T10	2.07E+02	2.32	
	2		2.19E+02	2.34	
	3		2.40E+02	2.38	
	1	T15	9.90E+01	2.00	
	2		9.30E+01	1.97	
	3		7.80E+01	1.89	

Table 2

Determination of the growth potential for batch II. The growth potential of batch II is 0.00

Batch	sample	day	Number of colonies (cfu/g)	log_{10}	Growth potential (δ)
II	1	T0	8.40E+01	1.92	0.00
	2		7.20E+01	1.86	
	3		6.30E+01	1.80	
	1	T5	3.60E+01	1.56	
	2		3.30E+01	1.52	
	3		3.30E+01	1.52	
	1	T10	2.13E+02	2.33	
	2		1.95E+02	2.29	
	3		2.10E+02	2,32	
	1	T15	7.50E+01	1,88	
	2		7.20E+01	1.86	
	3		6.30E+01	1.80	

Table 3

Determination of the growth potential for batch III. The growth potential of batch III is -0.02

Batch	sample	day	Number of colonies (cfu/g)	log_{10}	Growth potential (δ)
III	1	T0	6.00E+01	1.78	-0.02
	2		7.80E+01	1.89	
	3		8.40E+01	1.92	
	1	T5	4.50E+01	1.65	
	2		4.50E+01	1.65	
	3		3.00E+02	2.48	
	1	T10	3.00E+02	2.48	
	2		2.67E+02	2.43	
	3		2.76E+02	2.44	
	1	T15	6.60E+01	1.82	
	2		7.80E+01	1.89	
	3		6.30E+01	1.80	

Table 4

The growth potential of *LM* δ_{max}

The final number*		Growth potential δ_{max}	The maximum value in the first day*	
[cfu/g]	[log_{10} cfu/g]	[log_{10} cfu/g]	[log_{10} cfu/g]	[cfu/g]
100	2.00	0.04	1.96	9.10E+01

* The maximum value on the initial day is the amount of *Listeria monocytogenes* against the criterion of 100 cfu/g at the end of the shelf-life

The highest growth potential (δ) among the three batches is 0.04. Values for *Listeria monocytogenes* to be achieved by the product on the following days after production so as not to exceed 100 cfu/g on the last day of storage. The growth of *Listeria monocytogenes* is not possible in the tested samples. The efficiency of the highest value of the obtained growth potential is less than 0.5 and it is 0.04 (Table 4)

CONCLUSIONS

The aim of this study was to determine the growth potential and survival of *LM* during a 15-day shelf-life study at 7°C and 10°C with an initial inoculum of 100 cfu/g for Boeuf salad treated with dry buffered vinegar. The growth of *Listeria monocytogenes* is not possible in the Boeuf salad treated with dry buffered vinegar since the maximum growth potential is δ = 0.04 $\log_{10}$ cfu/g. In accordance with Commission Regulation (We) No. 2073/2005, category 1.3, the calculated growth potential indicates that the tested product is classified as "ready-to-eat foods unable to support the growth of *LM*, other than those intended for infants and for special medical purposes". If the storage and transportation conditions are maintained, the product tested, with the initial concentration of *LM* at the level of 9.10E+01 cfu/g or lower, should maintain the concentration conforming to the criterion of 100 cfu/g at the end of the shelf-life. The challenge study is a useful method to validate the product formulation set up to control the survival or growth of *LM* during the shelf life of the product.

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