Effect of Dry Buffered Vinegar on Listeria Monocytogenes Growth during Shelf Life on Ready-to-Eat Beaten Beans Dip

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Abstract

The beaten beans dip represents one of the most popular foods in Romania; it is frequently consumed, and it is on the manufacturing list of many food producers as ready to eat (RTE) meal. This category of RTE, generally, recognizes aw and pH values which permit growth of different foodborne pathogens, as *Listeria monocytogenes* (Lm). Evaluation of Lm growth potential during shelf life represents a food safety aim, according to Regulation (EC) no. 2073/2005, on microbiological criteria for foodstuffs. *Listeria monocytogenes* represents a public health threat, being the pathogen agent of human listeriosis, a severe emerging foodborne zoonosis. The purpose of the study is to assess the inhibition effect of dry buffered vinegar on Lm growth during the shelf life of beaten beans dip, in modified atmosphere packaging and refrigeration storage conditions. Based on the results, the growth of Lm is not possible, the highest value being 0.04 (less than 0.5) for the beaten beans dip with dry buffered vinegar. The use of Lm growth inhibitor represents a useful preventive measure, in the conditions of increasing the consumption of RTE type products and the potential risk of Lm multiplication.

Keywords: beaten beans dip, dry buffered vinegar, food safety, Lm, shelf life

1. Introduction

The ready to eat (RTE) foods are usually characterized by the fact that they have a long shelf life under refrigeration conditions and are intended for direct consumption, without any processing step to eliminate or reduce to an acceptable level the potential foodborne pathogens.[1] *Listeria monocytogenes (Lm)*, one of the most pathogen foodborne agents, represents a continuous challenge for RTE industry. The human listeriosis is frequently caused by RTE foods consumption which are likely to be contaminated and where Lm could survive and multiply during shelf life. The microbial shelf life of RTE food represents an important food safety issue and corresponds to the period of time during which the microbiological criteria remain within maximum limits. It has to be validated by studies, such as the challenge test, to assess whether a RTE food is able or not to support the Lm growth, according to Regulation (EC) No 2073/2005.

The ubiquitarian presence of Lm is one of the risk factors in relation with the contamination of RTE food, via raw material, processing environment, non-conform consumption and using practices of consumers. The Lm is a psychotropic bacterium that can survive at a large range of temperature as 0-45°C, and a large range of H, makes it capable of surviving in different environments and food products. There is a wide variety of RTE food associated with human listeriosis, including animal food origin and plant derived origin [2,3,4]. In the last decades, throughout the world, many recalls and outbreaks were performed in

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relation to RTE consumption and Lm contamination.
The reports of EFSA and ECDC indicate that the listeriosis affected around 2,200 people in 2015, causing 270 deaths – the highest number ever reported in the EU. Based on EFSA report on 2020, listeriosis was the fifth, most frequently reported zoonosis, evolving in humans, at the level of the European Union.[5]

2. Materials and methods

The purpose of the study is to assess the effect of dry buffered vinegar (BactoCEASE® NV DRY, Kemin,USA), as inhibitor of growth Lm. A trial was conducted to identify the presence and count Lm during the shelf life of beaten beans dip, packing in modified atmosphere and refrigeration storage conditions. For this purpose, 6 experimental batches were manufactured, based on the beaten beans dip recipe, such as: boiled white beans, cooked onion, water, sunflower oil, tomato paste, iodized salt, granulated garlic, ground black pepper. Three of them (B1, B2 and B3) were untreated with dry buffered vinegar as control batches and the other three (B4, B5 and B6) were treated with dry buffered vinegar as experimental batches. According to the technical specification of dry buffered vinegar (DBV), it was used a recommended rate of 0.75% DBV (750 g of DBV at 100 kg of beaten beans dip) for the batches B4, B5 and B6, added during the mixing stage. [6]
All batches were manufactured in the same conditions and packaging was performed in the modified atmosphere (70% nitrogen and 30% carbon dioxide). The shelf life of beaten beans dip is 15 days, at storage conditions of 2-6 °C.
The assessment of Lm behaviour during the shelf life of all 6 batches was done as challenge tests and the growth potential (Δ) was calculated in accordance with the Technical Guidance Document for performing shelf-life research on *Listeria monocytogenes* in ready-to-eat foods.[7]
Four-time intervals were carried out in relation to Lm counting, according to ISO 11290-2:2017-07 standard, such as: T1 (day 0 of shelf life), T2 (day 5 of shelf life), T3 (day 10 of shelf life) and T4 day 15 of shelf life).
For the artificial inoculation two strains of *Listeria monocytogene* were used, namely strain ATCC 13932 and strain of Listeria monocytogenes isolated from the environment of the fruit and vegetable processing plant. Twelve samples/each batch was inoculated with 1 ml inoculum, at two different locations through a special membrane. It was used as a sealing septum to protect the modified atmosphere condition. The assumed level of contamination was about 100 cfu Lm on each 1g of sample. The weight of the sample was 250 g.
The Lm counting was carried out for three inoculated samples/each batch at T1, T2, T3 and T4. The results were expressed as $\log_{10}$ cfu/g. To ensure that the inoculation of Lm was homogeneous, the standard deviation (sd) of Lm counting at the T1 for all the 3 samples/each batch was calculated. The standard deviation of Lm enumeration at T1 has to be lower than 0.3 log cfu/g.
Based on the Lm counts, the growth potential/each batch was calculated, as the difference between the highest observed Lm concentration in log10 cfu/g during the test and the initial Lm concentration in log10 cfu/g at the beginning of the test [8]. The growth potential was calculated for each batch according to the formula:

$$\Delta = log\ max - log\ i$$

log max is the highest value of the *Lm* counting obtained from these 4 sampling points.
log i is the mean value of the 3 test units analysed at day zero (T1).
The value of Lm growth potential, according to Regulation (EC) No. 2073/2005, can be use as criterion to classify the RTE food in two categories, such as:

- RTE able to support the Lm growth, other than those intended for infants and for special purpose, when Δ> 0.5 log10 cfu/g (RTE category 1.2);
- RTE unable to support the Lm growth, other than those intended for infants and for special purpose, when Δ> 0.5 log10 cfu/g (RTE category 1.3) [1].

For each batch, 3 blank samples were tested at T1 for detection of Lm, according to ISO 11290-1:2017-07 standard.
The assessment of physicochemical parameters was made at the T1 for pH value (potentiometric pH method) and water activity (aw) (according to ISO 21807:2005)/each batch.

3. Results and discussion

At T1, the results of detection analyses on blank samples have been conform, Lm being absent/25 g of product (Table 1).

Table 1. Detection of *Listeria monocytogenes* / 25g at T1

Batch no.	Sample1	Sample 2	Sample 3
B1	absent/25g	absent/25g	absent/25g
B2	absent/25g	absent/25g	absent/25g
B3	absent/25g	absent/25g	absent/25g
B4	absent/25g	absent/25g	absent/25g
B5	absent/25g	absent/25g	absent/25g
B6	absent/25g	absent/25g	absent/25g

In case of detection of Lm at the day 0 of shelf life, the assessment of Lm growth potential is possible to perform only if the level of the Lm natural contamination is lower or equal to the level of inoculum. [7]

The aw evaluation at T1 for control batches showed values between 0.9444 – 0.948 and pH values are 6.0-6.1 (Table 2).

Table 2. Determination of aw and pH values of control batches at T1

Day	Batch	aw	pH
T1	B1	0.944	6.0
	B2	0.945	6.1
	B3	0.948	6.1

For experimental batches, aw is between 0.943-0.946 and the pH values is 5.9 (Table 3). No differences between control batches and experimental batches with respect to aw and Ph value. Badvela et al. (2016) indicated in their study a similar effect, such as the dry buffered vinegar did not affect the pH values.[8] The results of Theron et al. (2007) demonstrated that buffered organic acids do not significantly change the pH of the food.[9] According to Regulation (CE) no. 2073/2005, aw and pH are intrinsic factors which can support or inhibit the Lm growth for RTE foods.

Table 3. Determination of aw and pH values of experimental batches at T1

Day	Batch	aw	pH
T1	B4	0.943	5.9
	B5	0.946	5.9
	B6	0.945	5.9

The results of Lm behaviour assessment in artificial inoculation conditions for the control batches are available in Table 4. The standard deviation of Lm enumeration at T1 for batches B1, B2 and B3 are 0.07, 0.02 and 0.02 $\log_{10}$. The inoculation step has been correctly performed, thus the results of the challenge test obtained for assessing the growth potential can be used. The Lm growth potential calculated for control batches B1, B2, B3 are 2.94,2.91 and 2.90. The highest value of growth potential Δ for control batches is 2.94. This value is higher than the criterion $0.5\log_1 a$ and based on this, the control batches were considered able to support growth of Lm during the shelf life of product. The assessment of Lm counts during the shelf life of control batches has indicated a continuous increase of Lm number starting with T2 (day 5 of shelf life).

Table 4. The growth potential of control batches

Batches	T1		T2		T3		T4		Growth potential (Δ)/batch (*log10 cfu/g)*	Growth potential (Δ)/control batches
	Log_{10} cfu/g	Average ±s	Log_{10} cfu/g	Average	Log_{10} cfu/g	Average	Log_{10} cfu/g	Average		
B1	2.79 2.84 2.70	2.78±0.07	3.77 3.85 3.78	3.8	4.69 4.96 4.72	4.79	5.77 5.68 5.70	5.72	5.72 -2.78=2.94	2.94
B2	2.83 2.87 2.86	2.85±0.02	3.83 3.78 3.85	3.82	4.85 4.92 4.78	4.85	5.40 5.70 6.10	5.76	5.76-2.85=2.91	
B3	2.88 2.93 2.92	2.91±0.02	3.81 3.78 3.80	3.79	4.78 4.86 4.88	4.84	5.00 5.80 5.83	5.71	5.81-2.91=2.90	

* Average of Lm $\log_{10}$ cfu/g; **standard deviation

The results of Lm behaviour assessment in artificial inoculation conditions for the experimental batches B4. B5 and B6 are available in Table 5. The standard deviation of Lm enumeration at T1 for batches B4,

B5 and B6 are 0.04, 0.04 and 0.03 log_{10}. Based on this evaluation, it is considered that the inoculation stage has been correctly performed, thus the results of the challenge test obtained for assessing the growth potential can be used. The Lm growth potential calculated for experimental batches are 0.2, 0.15 and 0.22. The highest value of growth potential Δ for the experimental batches is 0.22. This value is lower than the criterion $0.5log_{10}$ and based on this, the experimental batches are considered able to inhibit growth of Lm during the shelf life of the product.

Table 6. The growth potential of experimental batches

Batches	T1		T2		T3		T4		Growth potential (Δ)/batch (*log10 cfu/g)*	Growth potential (Δ)/control batches
	Log_{10} cfu/g	Average ±sd	Log_{10} cfu/g	Average	Log_{10} cfu/g	Average	Log_{10} cfu/g	Average		
B4	2.72 2.78 2.7	2.73±0.04	2.40 2.32 2.25	2.32	3.0 2.85 2.95	2.93	2.75 2.59 2.6	2.64	2.93 –2.73 = 0.2	0.22
B5	2.76 2.75 2.68	2.75±0.04	2.34 2.28 2.32	2.31	2.82 2.9 3.0	2.9	2.46 2.6 2.72	2.59	2.9 - 2.75 = 0.15	
B6	2.8 2.73 2.75	2.76±0.03	2.46 2.38 2.5	2.44	3.1 2.87 2.98	2.98	2.68 2.64 2.71	2.67	2.98-2.76 = 0.22	

* Average of Lm log_{10} cfu/g; **standard deviation

The assessment of Lm counts during the shelf life of experimental batches has indicated a decreasing of Lm number at T2 (day 5 of shelf life). During shelf life of all the three experimental batches (treated with 0.75% DBV) a bacteriostatic effect induced by the organic acid added was observed. The study of Lavieri et al. (2014) showed that DBV has bacteriostatic effect, not a bactericidal one, for contaminated food with Lm.[10] The research of Badvela et al. (2016) indicated a similar effect, such as the bacteriostatic effect of DBV at different concentrations of 0.5-0.9% of DBV in food. [8]

The study of Butler et al. (2018) on chicken breast demonstrated the inhibitory effect of Lm growth during the shelf life of DBV treated products (various concentration between 0.4%-1.5%) [11].

4. Conclusions

The results of assessment of the growth potential and survival of *Listeria monocytogenes* during a 15-day shelf-life with initial inoculum of 100 cfu /g in Beaten beans dip confirmed that the treatment with dry buffered vinegar inhibited the growth of L. monocytogenes, compared with the control batches (untreated with dry buffered vinegar). The growth of Listeria monocytogenes was inhibited on the experimental batches where the Δ = 0.22 log10 cfu/g. Based on these results, the beaten beans dip treated with 0.75% dry buffered vinegar is classified as RTE unable to support the growth of *Listeria monocytogenes,* during the 15 days of shelf life.

The use of dry buffered vinegar represents a practical measure in ready-meal industry, considering the ubiquitous character of Lm and the various sources of contamination. Additionally, the dry buffered vinegar as inhibitor represents a practical measure, in compliance with EU regulations on *Listeria monocytogenes* in RTE foods.

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