GROWTH POTENTIAL OF *LISTERIA MONOCYTOGENES* IN SALTED DICED BACON

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ABSTRACT

The aim of this study was to implement challenge-tests assessing growth potential of *Listeria monocytogenes* (*L. m*) during storage of salted diced bacon, a 35 day shelf-life product. The challenge-tests were performed on 3 different batches (3 repetitions per batch) with a mixture of 3 strains of *L. m*. For the preparation of the inoculum, the first subculture was made at optimal conditions and the second one was made taking into account NaCl content and cold stresses. The initial targeted level of *L. m* was 10 cfu/g and the test units were stored at 4°C/12 days, then at 8°C/26 days.

The conclusion was that this product was unable to support the growth of *L. m*. The initial values for pH and a_w, the stresses applied to the bacteria can't be responsible for the absence of growth of *L. m*: other(s) factor(s) have to be investigated.

1. INTRODUCTION

Listeria monocytogenes (*L. m*) is a Gram-positive rod-shaped bacterium non sporeforming and motile via flagella at 30°C and below. This bacteria is present in the environment and in faeces of animals. It may be responsible for listeriosis, a serious infection caused by eating food contaminated with the bacteria. Listeriosis is a severe disease (*L. monocytogenes* may cause septicemia or meningitis) that mainly affects unborn children, the elderly and persons with compromised immune system. For pregnant women, infection can lead to miscarriage, stillbirth, premature delivery, or infection of the newborn

Concerning growth conditions in foods, *L. m* grows from the temperature -2°C to the temperature +45°C, from pH 4.2 to pH 9.6 and its growth can occur below water activity of 0.93.

It is a concern for ready-to-eat (RTE) foods because they can be contaminated by *L. m*. This bacteria can grow in many of them and RTE will not receive a heat-treatment between production and consumption.

European regulation (Regulation (EC) N°2073/2005) lays down the microbiological limit for the RTE foods; the limit is fixed at 100 cfu/g when the product is placed on the market and during the shelf-life and Annex II of this regulation specifies that food business operators shall conduct studies to evaluate the concentration of *L. m* that may be present in the product during the shelf-life under reasonably foreseeable storage conditions.

But other products contaminated by *L. m* at the end of production may support the growth of this bacteria according to their physical-chemical characteristics and may be eaten without cooking or with a cooking enabled to destroy this pathogen. For example, the consumption of diced bacon (normally used cooked) without cooking concerns 14 to 17% of the French population (Afssa, 2009).

In France, 50% of the pork breasts is transformed into diced bacon cubes (Zuliani, 2005). This production increases every year: in 2008, 16,800 tonnes were produced (FICT, 2009).

Between 1999 and 2001, 54% of the pork meat analysed was contaminated by *Listeria monocytogenes* (Giovannacci, 2002). Since, this prevalence has decreased every year and reached 25% in 2004 (ITP, personal communication) due to a better application of the good hygienic practices and HACCP principles.

In 2007, 11.22% of the pork breasts was contaminated by *Listeria monocytogenes* (IFIP, 2008, unpublished results). This level of prevalence remains however alarming in particular when the pork cuts are used for the manufacture of raw meat products such as diced bacon cubes.
During the diced bacon manufacturing there is no physical or chemical treatment sufficiently drastic to eliminate the possibly present population of *L. monocytogenes*. Consequently the prevalence of *Listeria monocytogenes* remains high for diced bacon cubes. A recent study (IFIP, 2008, unpublished results) shows a prevalence of 6.35% for these products.

The aim of this study was to assess the ability for salted diced bacon to allow the growth of *L m* throughout its shelf-life using a microbiological challenge test assessing the growth potential.

2. MICROBIOLOGICAL CHALLENGE TEST ASSESSING THE GROWTH POTENTIAL

A microbiological challenge test assessing growth potential (δ) is a laboratory test that measures the growth of a bacteria in an artificially contaminated food stored under foreseeable conditions from production to consumption.

The growth potential of *L .m* (δ) is the difference between the concentration of *L. m* expressed in log cfu/g at the end of the test and the concentration of *L .m* expressed in log cfu/g at the beginning of the test.

The growth potential can be used:

- to determined if a food permits the growth of *L. m*
- to quantify the behaviour of *L. m* from production to consumption
- to set the concentration at the production according to a limit fixed for the end of the shelf-life.

The growth potential (δ) depends on many factors, the most important being:

- the inoculated strain(s),
- intrinsic properties of the food (e.g. pH, NaCl content, a_w, preservatives, associated microflora),
- extrinsic properties (e.g. temperature profile, modified atmosphere packaging),
- any injury or stress (cold stress, osmotic stress…) applied to the inoculated strain(s).

In this study, to conduct the challenge tests, the following factors were taken into consideration: product characteristics and shelf-life , number of batches, choice of the strain(s), preparation of the inoculum, number of test units to be prepared per batch, inoculation of the test units, storage conditions, physical-chemical characteristics and microbiological analyses.

3. MATERIALS AND METHODS

3.1 Product characteristics and shelf life

Salted diced bacon was manufactured with pork breasts in a French plant. The process from the raw material to diced bacon included the following steps: tumbling and brining, steaming, dicing and packaging. Thus the breasts were tumbled during 10h with brine. Final NaCl concentration in meat was about 3%. Breasts were then steamed at 48°C for 2h30. Finally the breasts were diced (8x8x30 mm).

The food was packaged under modified atmosphere: CO_2: 50%, N_2: 50% and had a shelf-life of 35 days.

According to another study (unpublished results), at the end of the production the physical-chemical of salted diced bacon was 5.71 - 5.84 for pH, around 0.957 for a_w and lactic acid bacteria concentration ranged from around 10^2 cfu/g.

So, according to these characteristics, salted diced bacon was able to support *L. m* growth during the shelf-life determined by the manufacturer for reasonable conditions (4°C/12 days plus 8°C /26days). As this food

is susceptible to be consumed after a cooking unable to ensure a complete destruction of *L. m* or even without cooking according to a French survey (Afssa, 2009), it seemed relevant to calculate the growth potential of this pathogen from the production to the consumption.

3.2 Number of batches

Three different batches of the same product were tested to take into account the variability of the production. The batches 1, 2 and 3 were produced respectively in May, August and September 2008.

3.2 Choice of the strains

The tests were performed with a mixture of 3 strains to account for the variation in growth among the strains. One was a reference strain (strain ATCC 19116) isolated from chicken brain and the 2 others were isolated from smoked diced bacon.

3.3 Preparation of the inoculum

Two subcultures were made. The first one was made at a temperature favourable to optimal growth of *L m.* Each strain was subcultured in TSB (Tryptone Soja Broth) and at a temperature (37°C) for a sufficient time for the organism to reach the beginning of the stationary phase. This first subculture was mainly aimed at getting the cells in the same physiological state.

The second one was made under conditions reproducing the stresses encountered by the bacteria in naturally conditions: this second subculture was made in TSB with 2.5% of NaCl and at pH = 6 until the stationary phase. Then it was placed at –10°C for 48 h to reproduce freezing before slicing.

Each of the 3 subcultures was adjusted to the same concentration and then, the subcultures were combined in equal quantity.

Successive dilutions of the mixed culture are made in physiological water to obtain a concentration in the foodstuff of, approximately, 10 cfu/g.

3.4 Number of test units to be prepared per batch

The number of test units per batch is shown in table 1. Each test unit weighted 100 g.

(Note: the whole experiment required destructive sampling for microbiological procedures).

Table 1: Number of test units per batch

	D0	**D38**
Concentration of *L. m*	3	3
Check of the absence of *L. m* in blank samples	3	3
Concentration of the Total Count Flora (TCF)	3	3
Physical-chemical characteristics	3	3

D0 is the day of inoculation and D38 is the end of the shelf-life.

Six tests units were prepared to calculate the growth potential of *L. m* from D0 to D38.
Six test units were prepared to check for the absence of *L. m* in the foodstuff, the so called “blank samples”, were not inoculated.
Six test units were prepared to determine the TCF concentration.
Six test units were prepared to determine the physical-chemical characteristics.

The determination of the physical-chemical characteristics and associated microflora were necessary in order to compare the products submitted to challenge testing to the products routinely produced by the factory. And, moreover, the determination of the concentration of the associated microflora can bring some information, about possible interactions between *L. m.* and associated microflora. Such interactions may influence the growth of *L. m.*

3.5 Inoculation of the test units

The inoculation was made on the whole commercial unit of the foodstuff through 2 septums (airtight adhesive foam rubber PBI-Dansensor, Denmark).in order not to break packaging atmosphere. The septum sticked on the packaging, permitted to introduce the inoculum with a syringe through the packaging without modifying the gas condition. Each sample was inoculated with 1 ml of the inoculum, not more in order to affect as less as possible the properties of the product.

The contamination of the salt diced bacon was supposed to be uniform; so, a strong shaking of the units was made after distribution of the inoculum with a syringe.

The test units addressed to the determination of TCF and physical-chemical characteristics were not inoculated with *L. m*, but sterile physiological solution is injected instead.

3.6 Storage conditions

The main stages of the cold chain were: transportation from the plant to the arrival to the display cabinet, retail (display cabinet) and consumer storage. Thanks to a previous study (Afchain et *al.*, 2005), we had enough information available on the cold chain to select the following storage conditions.

For the 1st stage (from the manufacture until the arrival to the display cabinet), storage temperature was fixed at 4°C and storage duration was equal to 12 days. For the 2nd stage (at retail) and the 3rd stage (consumer storage), storage temperature was fixed at 8°C and storage duration was 26 days.

3.7 Physical-chemical characteristics and microbiological analyses

The physical-chemical characteristics (pH and a_w) were measured according to the standard methods.

pH was performed with a pH meter (Orion 210A CG 818) according to the NF V 04-108 method and a_w was performed with a water activity meters (AquaLab) according to the International Standard Method NF EN ISO 21807.

Detection of *L. m* was performed by the "ALOA One Day" method (validated method AFNOR n° 10/3-09/00 and International Standard Method NF EN ISO 11290-1). Twenty-five grams of the food were diluted at 1:10 in Fraser 1:2 broth. After 24 h of incubation at 30°C, 0.1 ml of homogenate was spread over ALOA agar which was then incubated for 24 h at 37 °C.

Enumeration of *L. m* was performed with the "ALOA Count method" (validated method AFNOR n°. 10/3-09/06 and International Standard Method NF EN ISO 11290-2). Ten grams were homogenized with 90 ml of tryptone salt solution using a Stomacher blender. Ten-fold serial dilutions were made in tryptone salt diluent. One ml of each decimal dilution was pour plated into one plate of agar designed for *Listeria* growth according to Ottavioni and Agostini (ALOA) (AES Laboratories). Plates were then incubated at 37 °C for 24 h and 48 h. The presumptive isolates were not confirmed.

Total Count Flora was enumerated in pour plate count agar after incubation at 30°C for 72 h according to NF EN ISO 4833 (Anonymous, 2003).

RESULTS AND DISCUSSION

The results of physical-chemical and microbiological analyses are shown in Tables 2 to Table 4.

Table 2: Physical-chemical analyses and enumeration of *L. m* and TCF for batch 1

Physical-chemical characteristics				**Enumeration of the bacteria**			
D0		**D38**		**D0**	**D38**	**D0**	**D38**
pH	**a_w**	**pH**	**a_w**	***L. m* (log cfu/g)**		**TCF (log cfu/g)**	
5.89	0.946	5.62	0.945	<1	<1	3.32	7.23
5.81	0.949	5.62	0.936	1	<1	3.28	7.20

5.81	0.949	5.73	0.950	1.3	1	3.63	7.23

TCF: Total Count Flora

Tables 3: Physical-chemical analyses and enumeration of *L. m* and TCF for batch 2

Physical-chemical characteristics				**Enumeration of the bacteria**			
D0		**D38**		**D0**	**D38**	**D0**	**D38**
pH	**a_w**	**pH**	**a_w**	***L. m* (log cfu/g)**		**TCF (log cfu/g)**	
5.85	0.953	5.5	0.952	1.78	0.96	3.48	7.89
5.84	0.951	5.5	0.950	1.3	1.26	3.53	7.93
5.87	0.948	5.51	0.954	1.48	1.56	3.42	8.12

TCF: Total Count Flora

Table 4: Physical-chemical analyses and enumeration of *L. m* and TCF for batch 3

Physical-chemical characteristics				**Enumeration of the bacteria**			
D0		**D38**		**D0**	**D38**	**D0**	**D38**
pH	**a_w**	**pH**	**a_w**	***L. m* (log cfu/g)**		**TCF (log cfu/g)**	
5.79	0.961	5.4	0.959	1	<1	4.51	8.09
5.80	0.962	5.43	0.963	1.3	1.26	5.28	8.61
5.77	0.955	5.4	0.959	1	<1	5.04	8.48

TCF: Total Count Flora

According to the results presented in the tables 2, 3 and 4, at D0, mean values and standard deviations (SD) obtained for pH were: 5.84 (SD = 0.04) for batch 1, 5.85 (SD = 0.01) for batch 2 and 5.79 (SD = 0.01) for batch 3. Mean values obtained for a_w were: 0.948 (SD = 0.001) for batch 1, 0.951 (SD = 0.002) for batch 2 and 0.959 (SD = 0.003) for batch 3. Then, the results showed a good intra-batch homogeneity.
At D0, the mean values obtained for the 3 batches were 5.83 for pH (SD = 0.026) and 0.950 for a_w (SD = 0.005). The results showed a good inter-batches homogeneity, too.

The initial values of the Total Count Flora (tables 2, 3 and 4) were rather high (3.28 to 5.28 log cfu/g) at D0 and this flora had a growth rate high enough to reach 7.20 to 8.61 log cfu/g at the end of the test.

The *L. m* growth potential (δ) was calculated for batch 2 from the results in table 3 as the difference between the median of the log cfu/g at D38 (in grey) and the median of the log cfu/g at D0 (in grey) as prescribed in the guidance document written by EU CRL *L. m* (Beaufort *et al*., 2008). According to this document, if δ equals or is lower than 0.5 log cfu/g, then it is assumed that the food is incapable of supporting the growth of *L. m*. If δ is higher than 0.5 log cfu/g, then it is assumed that the food is able to support the growth of *L. m*.
For batch 1:
- mean value for *L. m* was 1.15 cfu/g (SD = 0.21) at D0 and the only result at D38 was 1 cfu/g.
- mean values for TCF were 3.41 cfu/g (SD = 0.16) at D0 and 7.22 (SD = 0.01) at D38.

For batch 2:
- mean values for *L. m* were 1.52 cfu/g (SD = 0.24) at D0 and 1.26 cfu/g (SD = 0.30) at D38
- mean values for TCF were 3.48 cfu/g (SD = 0.04) at D0 and 7.98 cfu/g (SD = 0.10) at D38.

For batch 3:

- mean values for *L. m* were 1.10 cfu/g (SD = 0.17) at D0 and 1.09 cfu/g (SD = 0.15) at D38
- mean values for TCF were 4.94 cfu/g (SD = 0.32) at D0 and 8.39 cfu/g (SD = 0.22) at D38.

For batch 1 and 3, the growth potential for *L .m* couldn't be calculated but concentrations at D38 were lower or equal to those at D0.

For batch 2, growth potential for *L. m* was equal to - 0.22 log cfu/g.

Then, the conclusion from the challenge tests suggested that this product was unable to support the growth of *L. m*. The initial physical-chemical values for pH (5.77 to 5.89) and aw (0.946 to 0.955), the stresses applied to the bacteria during the process couldn't be responsible for the absence of growth of *L. m.* The level of TCF as a cause of non growth could be investigated.

On the contrary, the probability module of the predictive software Sym'Previus (French Software) we used to predict the behaviour of *L. m* according to pH, water activity and temperature profiles values generated high finals concentrations. These values were: 3.09 log cfu/g for batch 1, and 5.52 log cfu/g for batch 2 and 5.70 log cfu/g for batch 3.

Further studies should be implemented to help understanding which additional factors need to be considered to explain the absence of growth of *L. m* in this product and to investigate the possibility of growth for *L. m* during a longer storage time.

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