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Shelf life and growth potential of *Listeria monocytogenes* in steak tartare

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ABSTRACT

The evaluation of the microbiological shelf-life under refrigeration conditions of steak tartare showed Total Viable Counts (excluding LAB) around 4 Log CFU/g at the beginning of the study, with a gradual increase reaching 7 Log CFU/g after 12 days. LAB represented the main microflora. A significant acidification was detected at the end of the shelf life. Total Volatile Basic Nitrogen concentration changed slightly during the shelf life. After an initial increase (until T5), all the colour indices were stable. The growth potential of *Listeria monocytogenes* was also evaluated in accordance with EURL Lm guidelines by conducting challenge tests. The growth potential calculated during the challenge test performed on three different batches indicated the absence of a significant growth ($\delta < 0.5$ Log CFU/g). When considering intermediate sampling times, a maximum increase of 0.51 Log CFU/g was detected at T5 in one batch. Finally, daily thresholds (maximum tolerable *L. monocytogenes* counts) were calculated to highlight the maximum acceptable load of remaining shelf life to avoid the overcoming of the legal limit of 100 CFU/g. Considering the worst-case scenario, a total increase of 0.89 Log CFU/g in 12 days was estimated, obtaining a "safety initial concentration" of 1.11 Log CFU/g of the pathogen.

1. Introduction

Steak tartare is a ready-to-eat (RTE) food, produced starting from raw ground meat that is usually eaten with a selection of sauces, vegetables and spices like onions, capers, pepper, Worcestershire sauce and some other seasonings. As the main ingredient of steak tartare is raw minced meat, it is clear that this product is a sensitive and very perishable food, generally characterized by a short microbiological shelf life. Indeed, it is considered to be easily susceptible to growth of spoilage organisms. It has to be considered that during the slaughtering and the subsequent production phases, the contamination of raw beef by various microorganisms is not completely avoidable.

Delhalle et al. (2016) explored the variety of general and spoilage microflora in Belgian steak tartare through metagenetic analysis collecting samples from butchers' shops, restaurants, sandwich shops and supermarkets: up to 180 bacterial species and 90 genera were identified in some samples, with seven predominant bacterial species identified as *Brochothrix thermosphacta, Lactobacillus algidus, Lactococcus piscium, Leuconostoc gelidum, Photobacterium kishitani, Pseudomonas* spp. and *Xanthomonas oryzae*.

This product was also found to be contaminated by potential pathogenic bacteria: verocytotoxigenic *Escherichia coli*, *Salmonella enterica* and *Listeria monocytogenes* may contaminate beef along the production chain with variable prevalence and concentrations (Rhoades, Duffy & Koutsoumanis, 2009). In fact, in the past, steak tartare was responsible for outbreaks: Netherlands experienced an outbreak of Shiga toxin producing *Escherichia coli* (STEC) O157 in 2008/2009 due to the consumption of contaminated steak tartare (Greenland et al., 2009), while in 1994–1995, 107 confirmed cases of *Salmonella* Typhimurium gastrointestinal illness were associated with the consumption of raw ground beef (Roels et al., 1997) in Wisconsin.

Listeria monocytogenes may also be present in raw steak tartare: in previous studies, this pathogen was found in minced beef with prevalence from 3.3% to 52% (Bohaychuk et al., 2006; Fantelli & Stephan, 2001; Scanga et al., 2000; Sheridan, Duffy, McDowell, & Blair, 1994). According to EFSA-ECDC (2017), *L. monocytogenes* was prevalent in 'RTE meat' with 2.07% of positive samples and 0.43% above 100 CFU/g. According to the European legislation (Reg. EC 2073/2005), steak tartare, that is a RTE food, must comply with the limit of 100 CFU/g throughout the whole shelf life, but this criterion can be applied if the food business operator is able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed this limit until the expiry date. Consistent with the European Union Reference Laboratory for *Listeria monocytogenes* (EURL Lm), the growth potential (δ), as the difference between the *L. monocytogenes* concentrations found at the end and at the beginning of the shelf-life in Log CFU/g, is one of the possibilities to classify the product as able or unable to support *L. monocytogenes* growth (ANSES, 2014).

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The aim of the present study was the evaluation of the microbiological and chemical-physical shelf-life of steak tartare. The growth potential of *L. monocytogenes* in accordance with EURL Lm guidelines was also evaluated to fulfil the food safety criteria for *L. monocytogenes* as reported in the EU legislation.

2. Materials and methods

2.1. Steak tartare samples

Lean beef (fat < 4%) from adult cattle (the "scottona" type and similar categories), was used by the producer, a medium scale industry in Northern Italy, to make steak tartare. Briefly, meat (93%) was minced, adding a mixture (2.5%) of salt, flavouring (plant origin extracts), beet powder and paprika extract, dissolved in tap water (7.5%); the product was mixed for 2 min in a processor and insufflated immediately with CO_2 to assure a fast temperature decrease. After production, each portion, composed of two pieces of 70g, was vacuum-skin packaged in polystyrene barrier foam trays with permeable intact films (Cryovac Sealed Air Corporation). A best-before date of 12 days at 4 °C was assigned by the producer.

2.2. Shelf life evaluation

2.2.1. Experimental design

Steak tartare samples were transported in refrigeration to the lab, stored at 4 °C and analysed at the following time intervals: 0 (day of packaging), 5, 9 and 12 days (declared expiry date). At the established dates, the microbiological and chemical-physical analyses were performed in triplicate. After the sampling for the microbiological analyses, colour parameters, pH, organic acids concentration and total volatile basic nitrogen (TVBN) were determined.

2.2.2. Microbiological analyses

For microbial counts, 10 g of each sample were homogenized in 90 mL of sterile diluent solution (0.85% NaCl and 0.1% peptone), and then serial 10-fold dilutions were performed in sterile saline. Total psychrotrophic and mesophilic bacterial counts (TVC) were determined using a spread plate technique on Plate Count Agar (Oxoid, Basingstoke, UK); plates were incubated at 10 °C for 5 days and at 30 °C for 48 h (ISO 4833–2:2013 method), respectively. The other microbiological parameters were Enterobacteriaceae (ISO 21528- 2:2017 method), *Pseudomonas* spp. (ISO 13720:2010 method), Lactic Acid Bacteria (ISO 15214:1998 method), *Brochothryx thermosphacta* (ISO 13722:2017 method), yeasts and moulds (ISO 21527–1:2008 method), and spores of sulphite-reducing Clostridia (ISO 15213:2003 method, after pasteurization of the dilutions).

2.2.3. Colour parameters

Colour parameters were determined using a Minolta Chromameter CR-400 (Minolta, Osaka, Japan) working at CIELab system. The L*, a* and b* values, which describe the intensity of whiteness/brightness, red colour and yellowness respectively, were determined at six locations on the surface of the steak tartare samples, 45 min after opening the packages. Chroma was calculated as $\sqrt{(a2^* + b2^*)}$, the hue angle (h) was calculated as h = arctan (b*/a*), where h = 0 for red hue and h = 90 for yellowish hue. Total colour differences (ΔE) between samples taken at different times were calculated as: $\sqrt{(L1^*-L2^*)^2 + (a1^*-a2^*)^2 + (b1^*-b2^*)^2}$. A ΔE value < 2 means that only experienced observers can notice the difference, 2 < ΔE < 3.5 means that unexperienced observers also notice the difference, while ΔE > 3.5 means a clear difference in colour noticed (Mokrzycki & Tatol, 2011).

2.2.4. pH value

At each sampling time, pH was measured by a pH meter (Amel Instruments, Milan, I): the sample was mixed with distilled water (max 1/2 w/w, in order to obtain a sufficiently fluid consistence), according to the MFHPB-03 method (Health Canada, 2014); three independent measurements were performed on each sample.

2.2.5. Organic acids

Concentrations of organic acids were determined by HPLC (Tirloni, Bernardi, Rosshaug & Stella, 2019a; Tirloni, Stella, Bernardi, Dalgaard & Rosshaug, 2019b). One gram of tartare sample was homogenized with a high sear blender (Ultra Turrax, ICA T25, D) and diluted with 5 ml with water; then the samples were centrifuged at 3000 × *g* for 15 min and the supernatant was filtered through a 0.2 μm regenerated cellulose (RC) membrane (Scharlab, Barcelona, Spain).

The analysis was carried out on HPLC system consisting of a 510 HPLC pump (Waters), a 717 plus autosampler and a 480 UV detector set at 210 nm. The analyses were performed isocratically at 0.5 ml min^{-1} and 40 °C on a Rezex ROA (Phenomenex) 300 mm × 7.8 mm, 8 μm. Mobile phase was 0.005 N H_2SO_4 prepared by diluting reagent grade sulfuric acid with distilled water, filtering through a 0.45 μm RC membrane filter (Scharlab).

External commercial standards (Sigma Aldrich, St. Louis, Missouri, United States) were used for identification and quantification of acetic, citric and lactic acids. For the determination of linearity of each target organic acid, eight concentration points in triplicate were used to calculate the regression line and the coefficients of determination (R^2). Limit of detection (LOD) was calculated as ratio of 3 standard deviations of the response and the slope of calibration curve, while the limit of quantification (LOQ) was calculated as ratio of 10 standard deviations of the response and the slope of calibration curve. The percentage of recovery rate was established from the experimental response values [(blank + standard) − blank] obtained according to the calibration curves and the real concentration of the standard added.

2.2.6. Total volatile basic nitrogen

Total volatile basic nitrogen (TVBN) was determined in triplicate at each sampling time by the method reported in Reg. (EC) 2074/2005.

2.2.7. Statistical analysis

Data from microbiological (log transformed) and physical-chemical analyses performed during the different sampling times were submitted to one-way ANOVA using PRISM graph pad 6. The threshold for statistically significant differences was settled at P < 0.05.

2.3. Challenge test

2.3.1. Experimental design

Challenge tests were carried out in order to evaluate the growth potential of *L. monocytogenes* in the product. Growth of L. *monocytogenes* was evaluated by conducting independent challenge tests on three different batches in triplicate at the constant temperature of 8 °C, mimicking a likely thermal abuse (Roccato, Uyttendaele & Membré, 2017). Samples were analysed at time intervals until their expiry date: five sampling times for each challenge test were considered: days 0, 2, 5, 8 and 12 from inoculation.

2.3.2. Bacterial strains and inoculation

Samples used for challenge tests were inoculated with a mixture composed by three strains of *L. monocytogenes* (strains code 045, 085 and 112). The strains were selected according to the EURL guidelines (ANSES, 2013) from the panel supplied by the National Reference Laboratory (Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, I), based on their ability to grow in a substrate similar to steak tartare (isolated from meat and able to grow at low pH and low temperature). The strain stocks were kept frozen at −80 °C in Microbank Cryogenic vials (Pro-Lab Diagnostics U.K., Merseyside, UK). From each stock culture, a loop was transferred to Brain Heart Infusion broth (BHI) (Oxoid) incubated at 37 °C for 24h. The

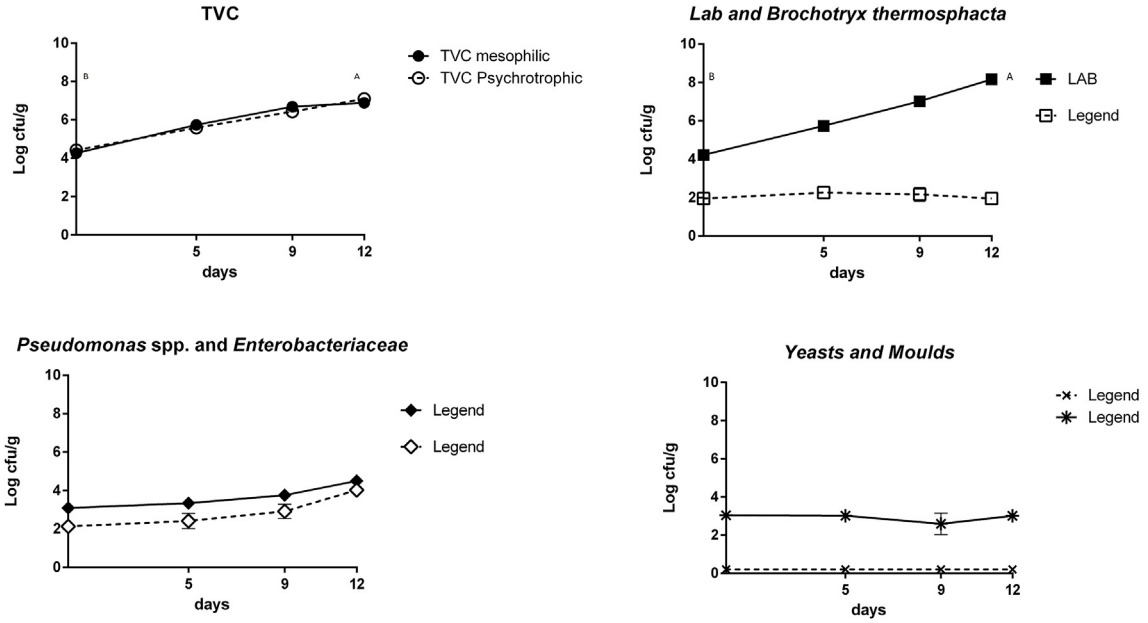

Fig. 1. Trend of total viable counts (mesophilic and psychrotrophic), Enterobacteriaceae, *Pseudomonas* spp., Lactic Acid Bacteria, *Brochothryx thermosphacta*, Yeasts and Moulds in steak tartare during the sampling period (12 days) at 4 °C. ^{A,B} Statistically significant difference among the sampling times ($P < 0.01$).

cultures were then inoculated again in BHI broth at 8 °C according to the temperature of the challenge test. The cultures were then harvested in late exponential growth phase, defined as a relative change in absorbance of 0.05–0.2 at 540 nm (Jenway 6105, Staffordshire, UK), as already described (Tirloni et al., 2019b). Afterwards, cell concentrations were determined by contrast microscopy at 1000x magnification (Motic, B310, Wetzlar, Germany), and finally, pre-cultures of individual isolates were diluted in sterile saline (0.85% NaCl) to obtain the same concentrations and mixed together in equal volume. Inoculation was performed reaching a concentration of ~2 log CFU g^{-1} of steak tartare. According to the EURL guidelines (ANSES, 2014), the inoculum volume did not exceed 1% of the product weight: the bacterial suspension was added to the steak tartare mass (~1,5 kg for each batch) and then the product was portioned (70 g) and vacuum-skin packaged according to producer practices. Samples were stored at 8 °C and analysed according to the experimental plan. Blank samples were also prepared by inoculating the same volume of sterile saline in the tartare mass; at the same sampling times reported above for inoculated units, these samples were submitted to the evaluation of natural microflora, pH and aw.

2.3.3. Microbiological analyses

Detection of *Listeria monocytogenes* was performed according to AFNOR method on tartare mass before inoculation (Association Française de Normalisation, 1998). Inoculated tartare samples were submitted to *L. monocytogenes* count in triplicate. For the analyses, the whole product share (70 g) was 5-fold diluted in pre-chilled sterile saline (0.85% NaCl and 0.1% peptone) and homogenized for 60 s in a Stomacher 400 (Seward Medical, London, UK). Appropriate 10-fold dilutions were then made with pre-chilled sterile saline and *L. monocytogenes* was enumerated by spread plating on Rapid L'mono agar (Generon, Modena, Italy) and incubated at 37 °C for 48 h (Association Française de Normalisation, 2001).

Total mesophilic and psychotropic viable counts were determined on blank samples (non-inoculated steak tartare); *Pseudomonas* spp., Enterobacteriaceae, Lactic Acid Bacteria, *Brochothryx thermosphacta,* yeasts and moulds were also enumerated according to the methods described in section 2.2.2.

2.3.4. Growth potential

For the calculation of growth potential (δ), according to the EURL guidelines (ANSES, 2014), the median *L. monocytogenes* counts were obtained for each batch; the following formula was applied: δ = M T12 – M T0, where M is the median value. The highest δ value among the three batches was chosen; in order to classify the product as a substrate able or unable to support *Listeria* growth, 0.5 Log CFU/g was considered as the threshold.

Taking into account the possibility of specific growth rates of *L. monocytogenes* during the different phases of the product's shelf life, daily thresholds were calculated as maximum tolerable *L. monocytogenes* counts that allowed not to overcome the 2 Log CFU/g level. Briefly, the differences between the median values for each period (T2-T0, T5-T2, T8-T5 and T12-T8) were determined for each batch. For each period, the highest difference among the three batches was chosen and used for the calculation of the specific daily increase. Finally, the values obtained were used to build a curve, determining the daily tolerable count.

2.3.5. Statistical analysis

Data from challenge tests were submitted to one-way ANOVA using PRISM graph pad 6. The threshold for statistically significant differences was settled at $P < 0.05$.

3. Results and discussion

3.1. Shelf life of steak tartare

In the present study, the shelf life of vacuum skin packaged steak tartare was determined by evaluating microbiological and chemical-physical parameters. The application of vacuum skin packaging to this product was chosen as it is known for some positive effects on raw meat shelf life, if compared to modified atmosphere packaging (MAP) and traditional vacuum packaging (Stella, Bernardi, & Tirloni, 2018). The skin packaged product is covered by a plastic film that is thermoformed simultaneously; the strict contact between the film and the meat piece reduces the formation of air pockets, thus showing advantages such as a reduction of purge production, a slower meat oxidation and an improved microbiological shelf life (Lagerstedt, Lundstrom & Lindahl, 2011; Vazquez et al., 2004).

In our study (Fig. 1), steak tartare showed total viable counts (mesophilic and psychrotrophic) around 4 Log CFU/g at the beginning of

the study (T0), with a slight increase during the shelf life, reaching values close to 7 Log CFU/g on the declared expiry date. No statistical difference was revealed in the whole period considered among mesophilic and psychrotrophic. The initial bacterial concentrations are justified by the production process of steak tartare, that is characterized by the grinding of meat and by manipulations of the operators during the deboning and sectioning phases. LAB represented the main microflora of the product, as expected for vacuum packaged meat, with an evident increase during the 12 days-storage, from ~4 Log CFU/g to values above 8 Log CFU/g ($P < 0.01$ in the whole period). As reported by Labadie (1999), the constraint of oxygen acts applying a selection on bacteria, favouring CO_2-tolerant lactic acid bacteria such as *Lactobacillus* spp., *Leuconostoc* spp., and *Carnobacterium* spp.

Considering the spoilage bacteria, a previous study by Pennacchia, Ercolini, and Villani (2011) indicated *Pseudomonas* spp., Enterobacteriaceae, *Brochothryx thermosphacta* and lactic acid bacteria were the microorganisms involved in meat spoilage, with variable contributions depending mainly on the oxygen availability. In our steak tartare samples, spoilage aerobic psychrotrophic microorganisms such as *Pseudomonas* spp. were inhibited: in fact, a slow increase was detected (from an average of 3.10–4.50 Log CFU/g in 12 days of storage at 4 °C), without reaching the values generally considered for alteration by specific spoilage organisms. The same trend was observed for Enterobacteriaceae, that showed a very limited increase reaching counts up to 4 Log CFU/g in 12 days of storage. Also *Brochothryx thermosphacta* was inhibited by the storage conditions applied, showing the absence of growth in the period considered. Yeasts were present, due to a contamination during the previous production phases, but showed to be unable to replicate in the conditions applied, while moulds and Clostridia were always below the detection limit (2 and 1 Log CFU/g, respectively). No statistical difference was revealed in the whole period considered for none of the parameters enumerated (*Pseudomonas* spp., Enterobacteriaceae, *Brochothryx thermosphacta*, Enterobacteriaceae, *Yeasts*, *moulds* and *Clostridia*).

The pH value of the steak tartare was quite stable during the first part of the shelf life, whereas a significant acidification ($P < 0.01$) was detected at T8 and T12, presumably due to the growth of LAB (Table 1). Anyway, all the values were in the normal range for raw beef; thus, the acidification of the product didn't represent a critical factor in determining its shelf life. Confirming this trend, the quantification of organic acids showed the main presence of lactic acid from T0, presumably due to the starting loads of LAB (~4 Log CFU/g) with an evident and significant increase from T9 for lactic and acetic acids, coupled with a significant growth of these bacteria (7–8 Log CFU/g) ($P < 0.05$).

Considering TVBN determinations, initial values were in the normal range for minced beef (just above 20 mg N/100 g). A slight increase from T0 till the end of the storage period was observed but always with moderate values, and no evident deterioration due to excessive proteolysis was observed during the trial. It is possible that the development of the LAB population, favoured by the skin packaging conditions, exerted a bio-protective action that inhibited an efficient growth of spoilage bacteria, thus limiting the formation of TVBN.

According to Lorenzo and Gomez (2012), when considering the shelf life of a meat product, also case life that is related to colour shelf life or display life should be taken in consideration. In our case, it has to be noted that the colour of steak tartare was influenced by the recipe of the product: indeed, beet powder was added among the ingredients to enhance the red colour, in order to contrast the reversible browning of meat pigment (deoxymyoglobin) due to the anaerobic environment. The colour parameters showed a significant modification during the first part of the shelf life (Table 1), as a significant increase of all the parameters (lightness, red and yellow index) was observed from T0 to T5, followed by a stable trend until the end of the shelf life; the same trend was observed for Chroma and Hue Angle. The potential perception of a general colour difference was evaluated as ΔE (difference from samples taken at different sampling times): the values calculated were all > 3.5 (that is the difference from the original colour) indicated an initial evident modification, much higher than the threshold of consumers' perception, due to the effect of packaging, followed by a colour stabilization.

In the light of the information supplied by the various analytical parameters (microbiological, chemical and colorimetric ones), the shelf life duration proposed by the producer seemed to be justified, considering the usual storage conditions for steak tartare.

3.2. Growth potential of L. monocytogenes

Assessing the ability of foods to support/not support the growth of *L. monocytogenes* in RTE foods is a real challenge as the products are produced starting from many different formulations that determine specific chemical-physical characteristics having a repercussion on bacterial growth.

L. monocytogenes may be a natural contaminant of raw minced meat; during our study, the non-inoculated samples didn't show the presence of the pathogen: this result is a mandatory condition for performing a valid growth potential determination, according to the EURL guidelines (ANSES, 2014). Steak tartare, as already shown by the shelf life study conducted, was characterized by TVC at T0 around 3–4 Log CFU/g, showing an increase up to 7 Log CFU/g after 5–8 days of storage at 8 °C ($P < 0.01$). This high microflora concentration was mainly composed by LAB, that may act as potential competitors. As reported firstly by

Table 1
Chemical-physical parameters of steak tartare during the shelf life at 4 °C.

	T0	T5	T9	T12
pH	$5.50^{A} \pm 0.03$	$5.57^{A} \pm 0.01$	$5.45^{B,a} \pm 0.01$	$5.39^{B,b} \pm 0.03$
L	$27.41^{B,b} \pm 0.98$	$30.49^{a} \pm 0.61$	$30.22^{A} \pm 0.13$	$31.03^{A} \pm 1.16$
A	$17.03^{B} \pm 1.66$	$23.90^{A} \pm 1.11$	$23.37^{A} \pm 0.69$	$24.13^{A} \pm 0.69$
B	$6.47^{B} \pm 0.67$	$9.73^{A} \pm 0.47$	$9.07^{A} \pm 0.26$	$9.88^{A} \pm 0.30$
hue-angle	20.79	22.17	21.21	22.28
Chroma	18.22	25.80	25.07	26.08
ΔE	–	7.87	7.30	8.58
TVBN	21.93 ± 1.12	26.11 ± 2.94	27.41 ± 7.38	24.49 ± 0.52
Organic acids (ppm)				
Citric	< LOD	< LOD	< LOD	< LOD
Acetic	$< LOD^{B,b}$	$< LOD^{B,b}$	1.266 ± 1790^{a}	5.097 ± 2579^{A}
Lactic	20.891 ± 849^{B}	21.647 ± 647^{B}	25.500 ± 118^{A}	24.747 ± 170^{A}

^{A,B} Statistically significant difference among the sampling times ($P < 0.01$).
^{a,b} Statistically significant difference among the sampling times ($P < 0.05$).
LOD = Limit of detection.
TVBN: Total volatile nitrogen.

Jameson (1962), the main microflora should be present in high amount to exert its mechanism of action through different ways (competition for nutrients, production of bacteriocins and organic acids, production of hydrogen peroxide …). In steak tartare, LAB rapidly increased in load reaching values above 7 Log CFU/g after 5–8 days and values around 8 Log CFU/g at the end of the experimental time considered (LAB T0: ~3–4 Log CFU/g; LAB T12: 7,48-8,56 Log CFU/g). *Pseudomonas* spp., Enterobacteriaceae, *Brochothryx thermosphacta* showed very limited loads at T0 and a gradual growth just at the end of the storage period, without statistical differences in the period considered. Yeasts and moulds did not show any significant growth in the samples.

The challenge test considered also the main chemical-physical parameters. Water activity showed, as expected, values constantly permissive for *Listeria* growth (0.97–0.99), while pH showed initial values around 5.5. According to Regulation (EC) 2073/2005 on microbiological criteria, this condition cannot be automatically considered as unable to support the growth of *L. monocytogenes* in ready-to-eat products (the concomitant presence of pH below 5.0 and aw below 0.94 should be achieved to give a sure growth prevention). Thus, the combination of microbiological and chemical-physical characteristics did not allow predicting how *L. monocytogenes* would grow in this substrate. A marked acidification of the samples was observed during the trial, reaching values between 5,03 and 5,12 at the end of the trial: this result was justified by the fast growth of LAB, thanks to the high storage temperature.

Table 2 shows the results of *L. monocytogenes* counts performed in the three different batches, with the respective Median values. Considering the worst batches (batches 1 and 2), a growth potential (δ = T12 –T0) of 0.38 Log CFU/g was obtained: according to the EURL guidelines, this value can be used to indicate the absence of significant growth of the pathogen (δ < 0.5 Log CFU/g).

When considering intermediate sampling times (as suggested by the same guidelines), the results indicated the absence of a significant growth in batches 2 and 3, whereas in batch 1 a maximum increase of 0.51 Log CFU/g was detected at T5 (Fig. 2). Thus, to keep safe, we cannot consider the product as unable to support the growth of *L. monocytogenes* unless it is supposable that many hurdles act to contrast the replication of the pathogen.

According to the Reg. (EC) 2073/2005, the application of the threshold limit of 100 CFU/g is related to the demonstration, with satisfaction of the competent authority, that the product will not exceed this limit throughout its shelf life. Moreover, "the operator may fix intermediate limits during the process that should be low enough to guarantee that the limit of 100 CFU/g is not exceeded at the end of the shelf life". In this situation, it is clear that it would be convenient for the producer to know the highest tolerable daily load allowing to stay below this threshold along the 12 days of assigned shelf life. Data obtained from the challenge tests, considering the worst-case scenario (highest Median increase among the batches for each inter-sampling period), allowed to estimate a total increase of 0.89 Log CFU/g (0.42 + 0.13 + 0.17 + 0.17 Log CFU/g for the four-time intervals). In this light, a starting concentration of at least 1.11 Log CFU/g (13 CFU/g) would be needed to overcome the final load of 2 Log CFU/g (Table 3). It has to be stated that if proper hygiene is maintained during the production, contamination *L. monocytogenes* levels lower than 1 Log CFU/g could be easily assured by the producer. Moreover, the intermediate thresholds calculated confirmed that even if a presence of *L. monocytogenes* was found during the shelf-life, that would not mean a subsequent overcome of the threshold limit within the 12 days of shelf-life; thus, an enumeration of alive *L. monocytogenes* cells would be preferable to give a correct information to the Food Business Operator and to the Competent Authority.

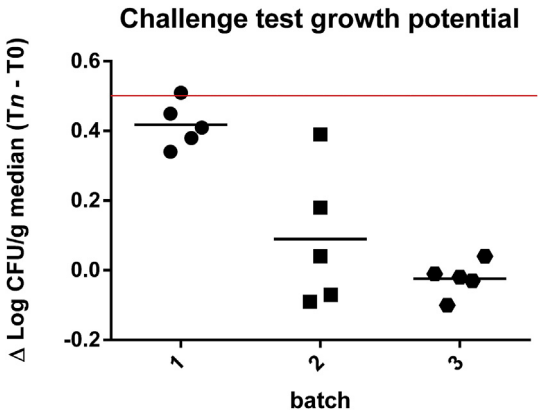

Fig. 2. Growth potential of inoculated *Listeria monocytogenes* in three batches of steak tartare maintained in thermal abuse (8 °C).

Table 2
L. monocytogenes counts (Log CFU/g) and growth potential in steak tartare.

Batch 1	T0	T2	T5	T8	T12
A	**1.60**	2.20	1.48	1.48	**1.98**
B	1.54	**2.02**	2.46	**1.88**	1.30
C	1.95	2.00	**2.11**	2.02	2.08
δ	-	*+0.42*	*+0.51*	*+0.28*	*+0.38*
Batch 2	T0	T2	T5	T8	T12
A	**2.15**	2.24	2.20	**2.36**	2.15
B	2.16	1.60	2.04	2.56	**2.53**
C	1.98	**2.06**	**2.19**	1.85	2.98
δ	-	*−0.09*	*+0.04*	*+0.21*	*+0.38*
Batch 3	T0	T2	T5	T8	T12
A	**2.51**	2.53	2.61	**2.50**	2.45
B	2.41	**2.55**	**2.41**	2.56	**2.50**
C	2.54	2.61	2.41	2.49	2.64
δ	-	*+0.04*	*−0.10*	*−0.01*	*−0.01*
Growth potential					+0.38

The values in bold are referred to the median value among the three replicates.

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Table 3
Daily load of *Listeria monocytogenes* necessary to overcome the threshold of 2 Log CFU/g in 12 days of storage at 8 °C.

	Log CFU/g	CFU/g	Daily increase (Δ Log CFU/g/day)
12th day	2.00	100	*0.043*
11th day	1.96	91	*0.043*
10th day	1.92	82	*0.043*
9th day	1.87	75	*0.043*
8th day	1.83	68	*0.057*
7th day	1.77	59	*0.057*
6th day	1.72	52	*0.057*
5th day	1.66	46	*0.043*
4th day	1.62	41	*0.043*
3rd day	1.57	37	*0.043*
2nd day	1.53	34	*0.210*
1st day	1.32	21	*0.210*
T0	**1.11**	**13**	-

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2019.108807.

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