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Research article

Microbiological and physicochemical profile of Italian steak tartare and predicting growth potential of *Listeria monocytogenes*

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ABSTRACT

In the present study, growth potential of *Listeria monocytogenes* in steak tartare samples taken at retail and belonging to 13 brands marketed in Northern Italy was investigated. The samples were submitted to microbiological and chemical-physical characterization. The data obtained were used as inputs for the application of the predictive microbiology software FSSP that allows the estimation of the growth of *L. monocytogenes* during the shelf-life. Lactic acid bacteria, the main component of the microflora, gave variable counts among the brands (from 3.38 to 6.24 log CFU/g). pH and a_w values were always higher than 5.3 and 0.96, respectively, thus they could not be considered as single efficient hurdles to prevent the growth of *L. monocytogenes* according to the EC Reg. 2073/2005; the same was observed for salt content (constantly <2 %) and nitrites (not quantifiable in all the samples, even if declared in some labels). Nevertheless, the combination of all the hurdles, evaluated by predictive microbiology using critical development factors, resulted in an estimated growth <0.5 log CFU/g throughout the shelf life; this output allowed us to consider all the steak tartare analysed as unfavourable substrate for *L. monocytogenes* growth. The information obtained could be useful for tartare producers as well as for competent authority to evaluate the effective risk concerning these typology of products.

1. Introduction

Steak tartare is a very popular ready-to-eat (RTE) food, obtained from raw ground meat that is usually served with several ingredients such as raw egg yolk, vegetables, spices, sauces and other seasonings. As the main ingredient is raw minced meat, the product is extremely perishable and characterized by a short microbiological shelf life; potential meat contamination during slaughtering and the following production phases, in combination with substrate characteristics (high moisture and pH, absence of structural barriers) could theoretically lead to the presence of high counts of spoilage and pathogenic bacteria.

Delhalle et al. [1], highlighted a very complex and heterogeneous spoilage microflora in Belgian steak tartare obtained from butchers' shops, restaurants, sandwich shops, and supermarkets, recording up to 180 bacterial species and 90 genera identified with seven predominant bacterial species such as *Brochothrix thermosphacta, Lactobacillus algidus, Lactococcus piscium, Leuconostoc gelidum, Photobacterium kishitani, Pseudomonas* spp. and *Xanthomonas oryzae*. In a previous study [2], a marked increase of bacterial counts in steak tartare was evidenced during the shelf life (up to 7 log CFU/g after 12 days at 4 °C), with the predominance of lactic acid bacteria.

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This kind of meat preparation was already found to harbour pathogenic bacteria such as verocytotoxigenic *Escherichia coli*, *Salmonella enterica* and *Listeria monocytogenes*, and has been recognized as source of foodborne outbreaks caused by Shiga toxin producing *Escherichia coli* O157 and *Salmonella* Typhimurium [3–5]. *L. monocytogenes,* one of the two species considered pathogenic, has not been strictly associated with outbreaks caused by minced meat preparations, but it poses a particular concern when meat preparations are intended to be eaten raw (falling into the RTE category), as its presence has been frequently reported (from 2.07 % to 55 %) [6–11]. According to EFSA-ECDC report [7], in the EU, the overall occurrence of the pathogen in RTE beef products was 4.9 %.

According to EC Regulation N. 2073/2005, a tolerance for the presence of *L. monocytogenes* (with a threshold 100 CFU/g) could be applied to RTE foods, if the food business operator is able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed this limit throughout the whole shelf-life [12]. In this light, several predictive microbiology models have been developed in the last decades for the assessment of safe shelf-life of numerous RTE foods and are cited as useful instruments for risk assessment and management of food safety by Codex Alimentarius and EC Reg. n. 2073/2005 itself [12,13]. Predictive microbiology aims to predict and quantify the microbial growth by mathematical models, based on the physicochemical characteristics and the storage conditions of food [14]. The increasing interest in predictive microbiology studies has favoured the development of user-friendly tertiary models (software) available for free, that can be applied by food business operators in specific production fields.

The aim of the present study was the microbiological (considering the main specific spoilage population and the potential antagonistic populations that can play a role as an hurdle against the pathogenic bacteria) and chemical-physical characterization of steak tartare belonging to several different brands sold on the Italian market. Based on this characterization, the growth potential of *L. monocytogenes* was estimated by applying a predictive microbiology model included in FSSP software.

2. Materials and methods

2.1. Experimental plan

Points of sale belonging to 15 large scale retail chains were visited to identify all the brands of steak tartare present on sale and 19 different products from 10 producers were identified. The products for the analyses were selected based on the producer identification and the list of ingredients: products from different producers, and products from the same producer but with different recipes were included, whereas identical products with different market denomination were excluded. Thus, a total of 13 different products, maintained under skin packaging, were selected and named from A to M (Table 1). For each product included in the study, three batches were taken from the market in different sessions, and analyses were performed in triplicate on each batch, for a total of 117 samples analysed. All the samples were taken at the first day of sale, aiming to characterize the products in a short period after production.

Table 1
Ingredients of steak tartare brands considered in the study based on manufacturers' declaration.

Code	Beef content	Other ingredients	Antioxidants	Acidity regulators	Preservatives
A	85 %	Water, salt, dextrose, fructose, sucrose, flavorings, black pepper, garlic,	ascorbic acid, sodium ascorbate	sodium acetates	sodium nitrite
B	85 %	Water, salt, dextrose, fructose, sucrose, flavorings, white pepper	ascorbic acid, sodium ascorbate	sodium acetates	sodium nitrite
C	95 %	Extra virgin olive oil 3 %, salt, lemon juice, pepper	sodium ascorbate	–	sodium nitrite
D	91 %	Salt, pepper, dietary vegetable fiber, natural flavors, maltodextrin, sunflower oil, extra virgin olive oil,	ascorbic acid	sodium acetates, sodium citrate	sodium nitrite
E	91 %	Extra virgin olive oil, water, flavorings, salt	ascorbic acid, sodium ascorbate	–	–
F	88 %	PDO Parmesan cheese, extra virgin olive oil, salt, white pepper, dextrose,	sodium ascorbate	sodium acetates	sodium nitrite
G	92 %	Extra virgin olive oil, water, salt, dextrose, pepper	sodium ascorbate	sodium acetates	sodium nitrite
H	93 %	water, salt, natural flavors, beetroot powder, paprika extract	–	–	–
I	85 %	water, salt, dextrose, fructose, sucrose, flavorings, black pepper, garlic	ascorbic acid, sodium ascorbate	sodium acetates	sodium nitrite
J	97 %	extra virgin olive oil, salt, spices (pepper, nutmeg)	–	–	sodium nitrite
K	92 %	water, natural flavors, salt, sugars: dextrose	ascorbic acid	sodium acetates, sodium citrate	sodium nitrite
L	91 %	extra virgin olive oil, water, flavorings, salt, pepper	ascorbic acid, sodium ascorbate	–	–
M	82 %	water, cheese, salt, dextrose, natural flavors, sunflower oil, extra virgin olive oil	ascorbic acid	sodium acetates	sodium nitrite

2.2. Microbiological analyses

For microbial counts, 10 g of each sample were homogenized in 90 mL of sterile diluent solution (0.85 g/100 mL NaCl and 0.1 g/100 mL peptone), and then serial 10-fold dilutions were performed in sterile saline. We chose the following microorganisms to evaluate the microbiological status of tartare present on the market. Total mesophilic bacterial count (TVC) was determined following the ISO 4833–2:2022 method [15]. The other microbiological parameters enumerated were *Enterobacteriaceae* (ISO 21528–2:2017 method) [16], *Pseudomonas* spp. (ISO 13720:2010 method) [17], lactic acid bacteria (ISO 15214:1998 method) [18], yeasts and moulds (ISO 21527–1:2008 method) [19]. LAB counts would be also used as input data to highlight if they could exert an antagonistic activity against *L. monocytogenes*.

2.3. Chemical physical analyses

2.3.1. pH, a_w, moisture and salt content determination

pH was measured by a pHmeter (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 [20]. Moisture [21], water activity (a_w) (Rotronic Hygromer Aw-DIO, Basserdorf, CH) and salt content [22] were also determined.

2.3.2. Nitrites determination

Sample extraction and preparation of filtrate were conducted as follows: briefly 10 g of homogenized samples with 60 mL hot water (60 °C) were homogenized with a high sear blender (Ultra Turrax, ICA T25, D) and clarified adding in the following order 4 ml Carrez solution I and 4 ml Carrez solution II. After centrifugation for 10 min at 4000 rpm, the supernatant was filtered through filter paper (nitrate and nitrite free) and diluted to 100 ml. 20 ml of test sample was added 10 ml ammonia buffer (pH 11.0) and 2 ml sulphanilamide solution. After 5 min at room temperature, 2 ml of n-(1-napthyl)-ethyl-enediamine dihydrochloride solution was added and diluted to 100 ml. The absorbance of solution was measured at 540 nm [23].

2.3.3. Organic acids determination

Concentrations of organic acids were determined by HPLC [24]. 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 two PU-1580 HPLC pump (Jasco), a 717 plus autosampler and a 481 UV detector (Waters) 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 H2SO4 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 (R2: 0.9997, 09990 and 0.9998 for for acetic, lactic and citric acid respectively). The limit of detection (LOD: 0.21, 0.16 and 0.28 mM for acetic, citric and lactic acid respectively) and limit of quantification (LOQ: 0.39, 0.43 and 0.77 mM for acetic, lactic and citric acid respectively) were determined. LOD and LOQ were calculated by the signal-to- noise approach [25].

2.4. Application of predictive microbiology model

For the prediction of the potential growth of *L. monocytogenes*, the existing model for "*Listeria monocytogenes* and lactic acid bacteria (LAB) in lightly preserved seafood and meat products including ready-to-eat products" [26] included in the FSSP software (http://fssp.food.dtu.dk/), was applied. The primary growth model applied by the software is a Logistic model with delay and includes the interaction between *L. monocytogenes* and LAB [27], while the secondary growth model is a simplified cardinal parameter type model. The environmental parameters included in the model were temperature, atmosphere (CO_2), water phase salt/a_w, pH, smoke components/phenol, nitrite and organic acids in water phase of product (acetic acids, benzoic acid, citric acid, diacetate, lactic acid and sorbic acids) and days of assigned shelf life.

As we decided to apply the worst-case scenario, we included the simulations at 4 °C but also at 8 °C, in order to mimic a thermal abuse. The model includes also starting concentration of LAB (that information was included as mean of three replicates for each batch), *L. monocytogenes* starting concentration (it was set at 1 CFU/g equal to 0 log CFU/g) and LAG time (not included in this case).

Moreover, as the products are maintained under skin packaging, CO_2 was set at 0, applying a protective approach (CO_2 activity is only included when modified atmosphere packaging is used). Finally, when pH recorded was below the minimum validated pH value (5.6), the parameter was conservatively set at 5.6. The output obtained were detailed curves and predicted values at the storage temperature of 4 or 8 °C, with estimated μmax (maximum growth rate in the logarithmic phase), Nmax (maximum population density), time to reach a 100-fold increase in the count (for both *L. monocytogenes* and LAB) and time for LAB to reach the count of 7 log CFU/g: these data are directly extrapolated by the software. The parameter "time to reach 100 cfu/g" for *L. monocytogenes* is important according to Reg. CE 2073/2005 as the limit of the pathogen during retail in ready-to-eat foods other than those intended for infants and for special medical purposes

is 100 cfu/g: this criterion applies if the manufacturer is able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit 100 cfu/g throughout the shelf-life.

2.5. Statistical analysis

Data from microbiological (log transformed) and physical-chemical analyses were submitted to one-way ANOVA using PRISM graph pad 6, in order to reveal eventual significant differences among the brands of product analysed; Tukey's post hoc test was applied to determine differences between the mean of all brands (three. Data from yeasts and moulds counts were analysed by the exact Fisher test, considering the prevalence of samples with detectable counts (>2 log CFU/g). The threshold for statistically significant differences was settled at $P < 0.05$. Moreover, the correlation among all the parameters was calculated.

3. Results and discussion

3.1. Chemical-physical characterization of steak tartare

In the present study, vacuum skin packaged steak tartare samples from different producers were evaluated for microbiological and chemical-physical parameters. The characterization of a high number of brands aimed to supply more complete information about the products sold by retailers. The definition of "steak tartare" includes a wide range of products, with the variable addition of different ingredients (Table 1). The meat content of the products was very variable, ranging from 82 to 95 %; water, salt and spices were added in almost all the products. In addition, oil, sugars (e.g. dextrose, sucrose) and other ingredients were included in some recipes. Additives were added in quite all the products; in particular, antioxidants (ascorbic acid/ascorbate) were added in 11 of 13 products, while acidity regulators (mainly acetates) were used in 11 brands, and preservatives (nitrites) were added to 10 products. These presence of additives could exert a variable effect on *Listeria* spp. Growth; nitrites and acetates are known to exert a preventive action towards the pathogen in different foodstuffs, whereas the effect of the addition of ascorbate could be considered negligible [28–30].

The intrinsic physicochemical characteristics of the different products considered are shown in Tables 2 and 3 pH values ranged from 5.32 to 5.76; the mean value of the different brands varied significantly ($P < 0.01$), ranging from 5.33 (brand H) to values higher than 5.65 (brand G); the variability of the values was not related to the addition of acidity regulators, as the lowest values were detected in products from brands H and J, that were not added with acetates and/or citrates, and their values were significantly lower ($P < 0.01$) than those measured in products from brands A, B, G and I that were added with acidity regulators. Considering the product as a substrate, pH alone could not be considered a single effective hurdle to define the steak tartare samples analysed as substrates that are not permissive for *L. monocytogenes* growth. Salt content ranged mainly between 1 and 2 %, but significant differences were detected among the brands ($P = 0.036$), with mean values higher than 1.7 % (brands C, D, and K) and, on the other side, five brands with mean values lower than 1 % on average (E, H, I, J and L); in any case, the values were far lower than those needed to prevent completely the growth of the pathogen [31]. A little variability in water activity value was observed (singularly from 0.967 to 0.989, mean value = 0.975 ± 0.005), without significant differences among the brands, despite a significant variability ($P < 0.01$) in moisture contents (65.37–75.18 %, on average); no evident correlation was detected between a_w and salt content values ($r = -0.14$), nor between a_w and moisture ($r = +0.20$); data are available in a supplementary file. As for pH, the a_w values observed could not be considered as single efficient hurdles for the prevention of *L. monocytogenes* growth. When considering the product composition, no evident relations were observed among the meat relative content and other parameters such as moisture or a_w values of the different products.

Lactic acid was the most abundant organic acid in the product, as expected (mean value = 9952 ± 2077 ppm, with single values ranging from 6198 to 20168 ppm in the water phase); lactic acid is recognized for its inhibitory action against pathogenic microorganisms [32]. The analyses showed a limited variability of the concentration among the brands ($P = 0.069$); this could be due to the natural presence of lactic acid in steak tartare, directly deriving from the muscle *post mortem* transformation and from the eventual growth of lactic acid-producing bacteria on meat, and not form an addition to the product. No relation between lactic acid concentration and pH was revealed ($r = -0.003$). Concentrations of acetic acid varied significantly among the brands ($P < 0.01$), ranging from values lower than the limit of quantification to a maximum value of 3898 ppm on average (mean value = 1876 ± 1356); higher concentrations were detected mainly in products that were added with acetates as acidity regulators (e.g. brands B, I and K), also if this

Table 2
Chemical physical characterization of steak tartare brands considered.

	Moisture (%)	a_w	Salt (%)	pH
A	74,43 ± 0,82	0,970 ± 0,001	1,04 ± 0,04	5,64 ± 0,20
B	74,35 ± 0,49	0,974 ± 0,003	1,32 ± 0,10	5,64 ± 0,01
C	66,68 ± 1,43	0,971 ± 0,006	1,78 ± 0,20	5,52 ± 0,12
D	68,12 ± 0,57	0,974 ± 0,002	1,89 ± 0,08	5,48 ± 0,08
E	70,07 ± 1,93	0,970 ± 0,002	0,89 ± 0,12	5,52 ± 0,10
F	71,01 ± 0,53	0,975 ± 0,007	1,34 ± 0,19	5,59 ± 0,01
G	72,30 ± 1,14	0,979 ± 0,010	1,27 ± 0,08	5,65 ± 0,01
H	72,31 ± 0,57	0,979 ± 0,003	0,86 ± 0,10	5,33 ± 0,04
I	75,18 ± 1,01	0,976 ± 0,005	0,91 ± 0,04	5,63 ± 0,18
J	72,46 ± 1,34	0,978 ± 0,005	0,81 ± 0,60	5,44 ± 0,11
K	65,37 ± 1,62	0,971 ± 0,002	1,86 ± 0,49	5,57 ± 0,04
L	70,01 ± 1,00	0,979 ± 0,008	0,92 ± 0,13	5,60 ± 0,03
M	71,20 ± 0,36	0,975 ± 0,006	1,54 ± 0,04	5,50 ± 0,14

Table 3
organic acids determination in steak tartare brands considered. Organic acids: LOD acetic acid 4.56 ppm, citric acid 44.19 ppm, lactic acid 21.62 ppm; LOQ: acetic acid 23.42 ppm, citric acid 82.61 ppm, lactic acid 69.36 ppm.

	Acetic acid (ppm)	Citric acid (ppm)	Lactic acid (ppm)
A	2222 ± 1016	1199 ± 1039	14675 ± 4788
B	3898 ± 275	2329 ± 63	12630 ± 475
C	805 ± 140	1738 ± 180	14035 ± 1465
D	2124 ± 1186	2031 ± 2553	15933 ± 877
E	2900 ± 2576	1995 ± 1936	16125 ± 3443
F	944 ± 857	<LOQ	14539 ± 1003
G	696 ± 603	<LOQ	14694 ± 251
H	796 ± 366	<LOQ	13122 ± 476
I	3135 ± 496	1534 ± 1360	12182 ± 1626
J	1091 ± 531	<LOQ	12460 ± 3358
K	3062 ± 1632	3379 ± 1932	13022 ± 624
L	1046 ± 36	359 ± 621	13529 ± 350
M	1675 ± 100	1050 ± 85	12929 ± 1112

was not a constant finding (products form brand G, with the lowest acetic acid concentration, were added with acetates). As stated before, the different concentration of acetic acid didn't reflect in a difference in pH values (r = +0.32). Acetic acid is known for its efficient antimicrobial action, that is related to its ability to reduce the pH and consequently to damage the bacterial cell walls [33]. Citric acid was also detected in all the brands with a significant variability ($P < 0.01$), with values from the limit of quantification to a maximum value of 3379 ppm on average (mean value = 1523 ± 1188). Higher concentrations were detected in products where citrates were added (brands D and K, with mean values higher than 2000 and 3000 ppm, respectively), also if this result was not constantly found. Previous studies showed a weak antimicrobial effect of citric acid [34]; thus it could not be regarded as an efficient hurdle in this substrate. The concentrations of organic acids detected in the products were far lower than the MIC values calculated in similar pH conditions by previous studies [35,36]. Thus, these compounds did not seem to represent single efficient hurdles to prevent the growth of *L. monocytogenes* in steak tartare, once a contamination eventually occurs.

Finally, although reported in the ingredients list of many of the brands considered, nitrites were never detected or quantified in all the samples, also if their addition was declared in 10 out of 13 brands. Thus, the activity of these compounds as hurdles in the products could be considered as negligible.

3.2. Microbiological characterization of steak tartare

The data obtained from the microbiological evaluation of steak tartare samples are shown in Fig. 1. TVC showed very heterogeneous results among producers, with mean counts ranging from 4.16 (brand C) to 6.17 log CFU/g (Brand M); the comparison between producers showed a significant variation ($P = 0.002$); in particular, brand C showed significantly lower count if compared to other brands (L, M: $P < 0.05$; F, H: $P < 0.01$). Lactic acid bacteria represented almost all the bacterial population of the product, with values that in some cases exceeded TVC counts (as typical of substrates with abundant LAB population, that is favoured by specific incubation conditions); mean LAB counts obtained from the different brands showed a significant variability ($P < 0.01$), with values ranging from

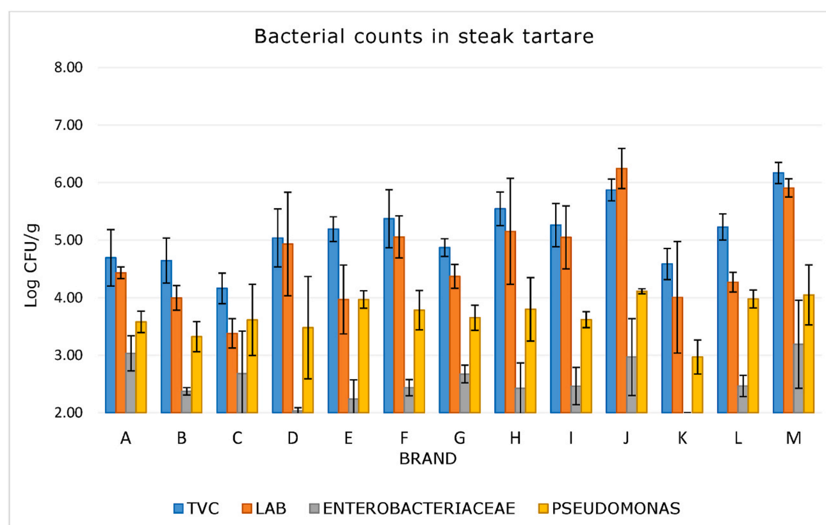

Fig. 1. Bacterial counts in steak tartare (mean of three batches in triplicate).

3.38 (brand C) to 6.25 log CFU/g (brand J). In agreement with these data, also in our previous study [2], steak tartare showed total viable counts around 4 log CFU/g with LAB that represented the main microflora of the product. A moderate contamination by *Pseudomonas* spp. was observed, with mean counts ranging from 2.97 (brand K) to 4.11 log CFU/g (brand J); a significant difference ($P < 0.05$) was only observed between brand K samples and the others. *Enterobacteriaceae* were enumerable in most of the samples analysed; mean values obtained from the different brands varied significantly ($P < 0.01$), from values near 2 log CFU/g (brands D and K) to values close to 3 log CFU/g (brands J and M). Yeasts counts were detectable in most samples, with mean values ranging from 2.07 (brands F and G) to 3.38 log CFU/g (brand L); finally, moulds were sporadically evidenced (only products from six brands), with the highest mean value detected in brand C (3.03 log CFU/g). A slight correlation among the microbiological parameters was observed; the r values observed for TVC towards the specific microbial populations were 0.42 (LAB), 0.45, (*Pseudomonas* spp.), 0.36 (*Enterobacteriaceae*) and 0.33 (yeasts). Summarily, the brands with higher TVC counts tended to show high counts for all bacteriological parameters (e.g. brands F, H, L, M), and on the opposite, low counts were obtained for all the parameters in some brands (A, B, K). The variability observed among the different products could be due to the contamination level of the meat used; this is influenced by the hygiene of slaughtering/deboning procedures and by the time period between slaughtering and production. Also if this information was not available, the use of vacuum packed beef stored for some days, as allowed by the EC Reg. 853/2004 [37], could be hypothesized in some brands (e.g. J and M), characterized by high LAB counts (EC, 2004). In steak tartare, an efficient competition of LAB towards *Listeria monocytogenes* could presumably occur, as these microorganisms could find favourable conditions during the storage of the product, as already shown in previous studies [2,38].

3.3. Application of predictive microbiology model

The evaluation of the ability of foods to support the growth of *L. monocytogenes* in RTE foods is a challenge as the products are

Table 4
Kinetics parameter predicted for LAB by FSSP software.

	Batch	μmax (h^{-1})		Time to 100 fold (days)		Time to reach 7 log/CFU/g (days)		Nmax (log ufc/g)	
		4 °C	8 °C	4 °C	8 °C	4 °C	8 °C	4 °C	8 °C
A	**1**	0.0245	0.0648	7.8	2.9	10.4	3.9	7.39	8.50
	2	0.0192	0.0601	10.0	3.2	12.4	4.0	6.97	8.50
	3	0.0324	0.0698	5.9	2.8	7.7	3.6	8.20	8.50
B	**1**	0.0200	0.0594	9.6	3.2	14.9	5.0	6.42	8.50
	2	0.0159	0.0553	12.1	3.5	16.7	4.8	6.23	8.50
	3	0.0194	0.0585	9.9	3.3	15.7	5.2	6.26	8.50
C	**1**	0.0251	0.0627	7.6	3.1	13.1	5.3	6.44	8.50
	2	0.0215	0.0583	8.9	3.3	15.8	5.8	5.94	8.49
	3	0.0182	0.0561	10.5	3.4	20.7	6.7	5.20	8.46
D	**1**	0.0150	0.0538	12.8	3.6	16.2	4.5	6.05	8.49
	2	0.0118	0.0466	17.5	4.4	8.4	2.1	7.19	8.50
	3	0.0110	0.0449	17.1	4.3	23.1	5.7	5.95	8.46
E	**1**	0.0174	0.0585	11.0	3.3	20.6	6.1	4.99	8.34
	2	0.0216	0.0662	8.9	2.9	12.5	4.1	6.51	8.50
	3	0.0078	0.0407	24.7	4.7	31.9	6.1	5.15	8.05
F	**1**	0.0194	0.0605	9.9	3.2	11.4	3.7	7.51	8.50
	2	0.0267	0.0668	7.3	2.9	5.7	2.3	8.44	8.50
	3	0.0210	0.0603	9.2	3.2	9.0	3.2	7.96	8.50
G	**1**	0.0251	0.0647	7.7	3.0	9.9	3.8	7.96	8.50
	2	0.0324	0.0715	5.9	2.7	7.3	3.3	7.23	8.50
	3	0.0241	0.0645	8.0	3.0	11.5	4.3	6.16	8.46
H	**1**	0.0239	0.0654	8.1	2.9	10.6	3.9	7.33	8.50
	2	0.0262	0.0679	8.3	3.2	3.1	1.2	8.45	8.50
	3	0.0296	0.0703	6.5	2.7	6.8	2.9	8.26	8.50
I	**1**	0.0302	0.0685	6.4	2.8	5.7	2.5	8.38	8.50
	2	0.0209	0.0621	9.4	3.2	6.9	2.3	7.99	8.50
	3	0.0265	0.0663	7.3	2.9	9.3	3.7	7.68	8.50
J	**1**	0.0341	0.0707	6.1	3.0	2.8	1.3	8.50	8.50
	2	0.0243	0.0656	Nr	Nr	1.4	0.5	8.50	8.50
	3	0.0186	0.0596	11.3	3.5	4.9	1.5	8.43	8.50
K	**1**	0.0157	0.0539	12.3	3.6	17.5	5.1	6.33	8.50
	2	0.0132	0.0488	14.6	3.9	29.5	8.0	4.75	8.46
	3	0.0162	0.0514	11.9	3.8	12.6	4.0	4.75	8.46
L	**1**	0.0252	0.0657	7.7	2.9	6.4	3.7	6.84	8.50
	2	0.0235	0.0648	8.2	3.0	11.8	4.3	6.34	8.49
	3	0.0252	0.0670	7.6	2.9	12.2	4.6	6.61	8.50
M	**1**	0.0247	0.0620	8.2	3.3	4.4	1.8	8.48	8.50
	2	0.0201	0.0592	10.0	3.4	5.9	2.0	8.37	8.50
	3	0.0189	0.0582	11.2	3.6	4.7	1.6	8.39	8.50

LAB: lactic acid bacteria; μmax (maximum growth rate in the logarithmic phase), Nmax (maximum population density).

produced starting from different ingredients that are strictly related to specific chemical-physical characteristics having a repercussion on *Listeria* growth. In this study, different products labelled as "steak tartare" were analysed, showing a certain variability in composition and chemical-physical parameters. As reported in section 3.1, pH and a_w values are not sufficiently low to make the steak tartare samples unable to support the growth of *L. monocytogenes* according to Regulation (EC) 2073/2005 [12], considering both the single parameters (pH below 4.4 or a_w below 0.92) or their combination (pH below 5.0 and a_w below 0.94). Nonetheless, the combination of multiple hurdles could be hypothesized in most brands (e.g. addition of acetic acid, high LAB counts): in such condition, the application of a predictive microbiology software including a high number of parameters represents a useful option, that is also mentioned by Regulation (EC) 2073/2005 [12]. This opportunity could be used by producers as well as by competent authority to obtain a rapid information on the ability of a product to support *Listeria* growth. In this light, the application of "*Listeria monocytogenes* and lactic acid bacteria (LAB) in lightly preserved seafood and meat products including ready-to-eat products" included in the FSSP software gave estimated μmax, maximum population density at a storage temperature of 4 and 8 °C for both *L. monocytogenes* and LAB. Fortunately, *L. monocytogenes* didn't find favourable conditions for growth in steak tartare samples, whichever brand was considered: indeed μmax value and Nmax were always equal to 0, indicating an infinite lag time, and the estimated time for 100-fold increase was not reached during the shelf life of the products. The same prediction was obtained at both temperatures (4 or 8 °C). This result highlighted the synergic role of the hurdles; as shown in section 3.1, the chemical-physical parameters, such as pH, a_w, and organic acids, could not be considered as single effective hurdles against *L. monocytogenes* growth, whereas their combination exerted an evident inhibition; it has also to be considered that in some cases, the real action of pH as hurdle should be stronger than that estimated, as only values equal or higher than 5.6 can be used as inputs for the software. The role of chemical-physical parameters should be considered in combination with the natural presence of lactic acid bacteria, whose antagonistic activity is well documented and is called "Jameson effect"; Jameson [39], observed *Escherichia coli* inhibition by *Salmonella* when the maximum population density of *Salmonella* spp., present at a higher concentration in the substrate, was reached.

Table 4 shows the software outputs obtained for LAB population: the growth of these microorganisms during the shelf life of steak tartare was predicted in all the products; an evident difference was observed among the brands, in particular considering the maximum population density at 4 °C, with values ranging from 4.75 log CFU/g (brand K) to values near 8.50 log CFU/g (stationary phase of bacterial growth, brands J and M. As expected, Nmax at 4 °C showed a correlation with the count of LAB obtained from the analyses (r = 0.78), while a lower correlation was obtained at 8 °C (r = 0.19). The time needed to reach a count of 7 log CFU/g, that can be considered an effective hurdle for the pathogen growth, ranged from 1.4 to 31.9 days at 4 °C and from 0.5 to 8.0 days at 8 °C; also this estimated parameter was correlated to the LAB count, both at 4 °C (r = 0.74) and 8 °C (r = 0.94). The estimated growth rate (μmax) ranged from 0.013 to 0.027 h^{-1} at 4 °C and from 0.048 to 0.068 h^{-1} at 8 °C: some differences were evidenced among the product brands, with significantly lower rates in brands D and K towards the others. The differences observed were caused by a combination of factors; considering the influence of each single potential hurdle, the highest correlations with μmax were observed for moisture (r = 0.48 and 0.56 at 4 and 8 °C, respectively) and salt concentration (r = −0.46 and −0.60). A lower but evident correlation was observed also for citric (r = −0.40 and −0.42) and acetic acid (r = −0.53 and −0.56); indeed, brands D and K showed high salt concentration, low moisture and high citric acid concentration (see Table 2). A low correlation was observed for lactic acid concentration and pH, showing the scarce impact of the general acidification of the substrate on LAB growth.

4. Conclusion

The present study allowed characterizing several brands of steak tartare from a physicochemical and microbiological point of view, showing the variability of the product sold on the Italian market and allowing to understand if the substrates considered were able or not to support bacterial growth. This product, that can be easily contaminated by *L. monocytogenes*, was characterized by the simultaneous presence of several hurdles, whose synergic activity gave an estimated complete inhibition of the pathogen growth, both in optimal refrigeration and in abuse conditions. The data obtained highlighted the role of product characterization and of the software applied of predictive microbiology in determining the correct classification and management of this popular Ready To Eat foodstuff. The use of this software for the assurance of tartare safety would be extremely advantageous especially in the reformulation by the manufacturers, with important social and economic profits.

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CRediT authorship contribution statement

Erica Tirloni: Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Cristian Bernardi:** Methodology, Investigation, Formal analysis. **Viviana Fusi:** Formal analysis. **Carlo Angelo Sgoifo Rossi:** Writing – review & editing, Formal analysis, Data curation. **Simone Stella:** Writing – original draft, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

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