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Research Paper

Growth Potential of *Listeria monocytogenes* in Chef-Crafted Ready-to-Eat Fresh Cheese-Filled Pasta Meal Stored in Modified Atmosphere Packaging

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ABSTRACT

This study evaluated the growth of lactic acid bacteria (LAB) in a fresh, filled-pasta meal, stored in modified atmosphere packaging and the influence of lactic acid (LA) and pH on the growth of *Listeria monocytogenes* (*Lm*). Samples were taken from three lots manufactured by a local catering company and stored at both 6 and 14°C. LAB numbers, LA concentration, pH, and the presence of *Lm* were evaluated at 1, 4, 6, 8, 10, 12, and 14 days of shelf life and the undissociated LA concentration ([LA]) was calculated. The LAB maximum cell density was greater in the products stored at 14°C than those stored at 6°C (10.1 ± 1.1 versus 5.6 ± 1.5 log CFU/g) and [LA] at 14 days was 9 to 21 ppm at 6°C and 509 to 1,887 ppm at 14°C. Challenge tests were made to evaluate the interference of LAB and [LA] on *Lm* growth. Aliquots of the samples (25 g) were inoculated at 1 to 10 days of shelf life and incubated at 9°C for 7 days, and the difference between *Lm* numbers at the end and at the beginning of the test (δ) was calculated. Logistic regression was used to model the probability of growth of *Lm* as a function of LAB and [LA]. The products inoculated at 1 day of shelf life had δ values between 4.2 and 5.6 log CFU/g, but the growth potential was progressively reduced during the shelf life. *Lm* growth was never observed in the products stored at 14°C. In those stored at 6°C, it grew only in the samples with LAB <5.7 log CFU/g. LAB interaction might thus inhibit the growth of *Lm* in temperature-abused products and limit its growth in refrigerated products. Logistic regression estimated that the probability of *Lm* growth was <10% if LAB was >6.6 log CFU/g or log[LA] was >2.2 ppm. The growth or inactivation kinetic of *Lm* was investigated with a homogenate of three samples with LAB numbers close to the maximum population density. After an initial growth, a subsequent reduction in the number of *Lm* was observed. This means that the maximum numbers of *Lm* might not be detected at the end of the product shelf life.

HIGHLIGHTS

- MAP and refrigeration were found to be a fruitful hurdle in filled-pasta meals.
- Higher growth of LAB at 14°C negatively affected the growth potential of *Listeria* sp.
- *Listeria* sp. numbers might decline after an initial growth during the shelf life.
- The maximum number of *Listeria* sp. is not always at the end of the product shelf life.

Key words: Filled pasta; Growth potential; Lactic acid bacteria; *Listeria monocytogenes*; Modified atmosphere packaging

Chef-crafted ready-to-eat (RTE) pasta meals are prepared by food industries and delivered for catering or sold in supermarkets for home consumption. They remain fresh in refrigerators for up to 3 to 4 days in air packaging. Modified atmosphere packaging (MAP) with low residual oxygen (<0.35%) can extend the shelf life of fresh, cheese-filled pasta up to five times by hindering the growth of mold and other aerobic microorganisms and reducing oxidative changes to color and flavor *(30)*. MAP generally favors the growth of psychotropic lactic acid bacteria (LAB) that are anaerobic and grow at refrigeration temperatures. Spoilage of refrigerated, fresh pasta meals under MAP usually occurs after several days, unless excessive contamination with spoilage LAB occurs before packaging or products are poorly refrigerated (>10°C) *(21, 37)*.

In RTE foods with extended shelf life, growth of psychotropic, pathogenic microorganisms, such as *Listeria monocytogenes* (*Lm*), poses a major challenge for food safety. The presence of *Lm* has occasionally been detected in soft and grated cheese used for filling pasta, but the industrially filled pasta is subjected to flash pasteurization

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($F_{70} > 40$ min) applied for starch gelatinization and to increase shelf life *(6, 30)*.

Lm may present a major burden for deli dishes with prolonged storage because of its ability to grow in the presence or absence of oxygen, as well as at refrigeration temperatures *(28)*. Ripened pasta is boiled for 2 to 3 min. Nevertheless, *Lm* may contaminate precut salad and other vegetables mixed with pasta after it has boiled and cooled. Furthermore, *Lm* can be derived from the food processing environment in which the RTE pasta is made *(5, 20, 25)*. Because the preparation of RTE salads dishes involves extensive handling and the use of uncooked ingredients, they are particularly vulnerable to microbial contamination *(18)*. Summer veggie pasta salads normally contain not less than 20% fresh vegetable. The amount of *Lm* in ingredients like tomatoes (*Solanum lycopersicum*), rocket salad (*Eruca vesicaria* subsp. *sativa*), and radicchio (*Cichorium intybus* var. *foliosum*), is often very low, at or below the detection limit (presence in 25 g), in fresh products after preselection and washing *(11, 27, 38)*. However, it can grow when the final products have high water activity (a_w), mild acidic pH, and a long shelf life *(12, 31)*.

The objectives of this research, which was developed as part of a research project aimed at developing decision-support tools for the food industry *(34),* were to evaluate (i) the growth of LAB in a fresh, filled-pasta meal stored under MAP conditions; (ii) the influence of lactic acid and pH changes on the growth potential of *Lm*; and (iii) the *Lm* growth or inactivation kinetics after LAB have reached their maximum population density.

MATERIALS AND METHODS

Samples. The chef-crafted RTE filled-pasta meal used for this study was freshly prepared by a local food producer in Emilia Romagna, Italy. The selected product was prepared with ravioli filled with spinach and ricotta cheese, which was boiled 3 min, cooled, and mixed with prewashed cherry tomatoes, rocket salad (rucola), and Grana cheese and was seasoned with sunflower (*Helianthus* spp.) oil. The declared shelf life of this meal, which was packed into rigid polypropylene (PP)–ethylene vinyl–PP trays with low oxygen permeability (<100 O_2 transmission rate at 23°C, 0% relative humidity) heat-sealing film polyethylene terephthalate-ADES-PP film (Technofood Pack, Milan, Italy) with low residual oxygen (gas flushing with 50% CO_2 and 50% N_2), was 14 days. Three different lots were sampled, and 14 sample units were tested within each lot. The packaged meals were transported to the laboratory in refrigerated trucks (at 4°C) the day after production. The temperature in the boxes containing the samples was recorded using Dataloggers (model Escort iMiniPlus PDF, Cryopack EU, Maromme, France) (accuracy, ±0.3°C). At their arrival in the laboratory, two sample units were analyzed immediately (day 1 of shelf life), whereas the others were divided in two groups of six, which were stored at 6 ± 0.5 and 14 ± 0.5°C. Samples were analyzed at 4, 6, 8, 10, 12, and 14 days of shelf life (one replicate for each sampling time).

Sample preparation. Fresh, filled-pasta meal packages were opened aseptically, and products were observed to detect abnormal odors, colors, or texture defects. Part of the content was cut in small pieces with scissor and mixed before taking two 10-g subsamples for enumeration of LABs and *Lm* and one 25-g subsample for its detection. The other two portions, 25 g each, were used for the challenge tests described below. Care was taken to avoid altering the sample composition of the meal (approximately 80% ravioli, 15% cherry tomatoes, 5% rucola, Parmesan cheese, and sunflower oil). The remaining part was homogenized with a Moulinette meat mincer and tested for a_w (with a dew-point water activity meter, AquaLab Series 3, Decagon, Pullman, WA), pH (International Organization for Standardization [ISO] standard 2917 *(14)* and LA. Portions of the homogenates were also used to assess the *Lm* growth or inactivation kinetics.

Microbiological and chemical analysis. LAB were enumerated by plating spread on de Man Rogosa Sharpe agar (CM1153, Oxoid, Thermo Fisher Scientific, Basingstoke, UK), according to ISO method 15214 *(13). Lm* was enumerated on agar *Listeria* according to Ottaviani and Agosti (ALOA; Biolife, Milan, Italy) and ISO 11290-2 *(16)*. The same medium was used for the detection of *Lm* after a selective enrichment steps with half-Fraser and Fraser broth (CM0895 with supplements SR0166/SR0156, Oxoid) according to ISO method 11290-1 *(15)*. The concentration of LA was measured with the L- and D-Lactic Acid Kit (Megazyme International Ltd., Wicklow, Ireland), and the undissociated [LA] was calculated, according to pK_a and pH of the homogenates, using the Henderson-Hasselbalch equation *(39)*.

Challenge tests to assess *Lm* growth potential. Challenge tests were performed during the shelf life at 1, 4, 6, 8, and 10 days, with the aim of testing the growth potential of *Lm* when the numbers of LAB, pH, and [LA] were presumably different. At those times, two portions of 25 g of each sample were weighted in stomacher bio sampling blender bags (Seward, Worthing, UK) and inoculated with a mix of three *Lm* strains, characterized by different pulsed-field gel electrophoresis profiles (data not shown) previously isolated in two lots of pasta salad and one lot of cheese. The bags were then manipulated for 1 min without altering the structure of the ingredients. The challenge test was made, following the EURL *Lm* Technical Guidance Document *(3)* for conducting shelf-life studies on *Lm* in RTE foods. Each strain was subcultured twice in tryptone soya broth (CM0129, Oxoid), first at 30°C for 18 h, and then at 9°C for 3 days in order to be adapted to the product storage condition. The bacterial inoculum was prepared by diluting the mixed cultures to approximately 1×10^4 cells per mL, and 200 μL of the mixed culture were inoculated on the samples and then stored at 9 ± 1°C for 7 days. That temperature was chosen to simulate a possible worst-case scenario for storage in home refrigerators *(17, 23)*. The inoculum level and the number of *Lm* were checked after 7 days by enumeration on ALOA.

Growth and inactivation kinetics of *Lm*. In addition to the challenge tests that were aimed at assessing *Lm* growth or no-growth, additional tests were made to evaluate the growth or inactivation kinetics of *Lm* when LAB have almost reached their maximum population density. Three samples were selected: two of them were at 6 days of shelf life and had been stored at 14°C; the third was at 8 days of shelf life and had been stored at 6°C. To have a food matrix with a more-uniform distribution of [LA] and LAB numbers, those tests were made using sample homogenates. Ten aliquots of 1 g of each sample were weighted in Eppendorf tubes; seven were inoculated with 100 μL of the bacterial inoculum described above (approximately 10^3 CFU/g), and three were not inoculated. All tubes were held at 9 ± 0.1°C in a thermoblock (ThermoStat plus, Eppendorf, Hamburg, Germany). The inoculated samples were analyzed at time zero (after the

TABLE 1. *Chemical physical characteristics of pasta meals, numbers of lactic acid bacteria (LAB), and growth of* Lm[a]

									Lm (log CFU/g) after 7 days at 9°C			G/NG
Lot	Days	°C	pH	a_w	% wps	[LA]	LAB log (CFU/g)	Inoc	R	FSSP	CB	R-F-CB
1	1	4	6.0	0.994	1.04	0.6	4.0	1.9	7.5	2.4	5.9	G-G-G
2	1	4	6.1	0.995	0.87	12.0	3.6	2.2	6.1	2.7	5.6	G-G-G
3	1	4	6.2	0.993	1.20	13.8	3.7	2.2	6.4	3.5	6.5	G-G-G
1	6	6	6.1	0.993	1.22	4.6	5.9	2.6	0.7	2.8	5.9	NG-NG-G
1	8	6	6.1	0.986	2.43	6.0	7.2	2.6	0.7	NA	5.6	NG-NG-G
1	10	6	5.9	0.986	2.37	11.6	6.8	1.9	0.7	1.9	4.9	NG-NG-G
1	4	6	6.1	0.988	2.16	14.6	5.3	2.8	6.5	3.2	5.8	G-NG-G
2	8	6	6.3	0.986	2.47	9.1	5.7	2.4	4.8	2.7	5.9	G-NG-G
2	10	6	6.3	0.986	2.39	11.9	4.7	2.8	3.2	3.4	5.8	NG-G-G
3	6	6	6.2	0.993	1.22	14.3	3.8	2.6	5.3	3.2	6.5	G-G-G
3	8	6	6.3	0.986	2.39	12.6	4.3	1.9	5.7	2.8	6.1	G-G-G
3	10	6	6.3	0.986	2.35	12.4	3.6	2.1	3.0	3.5	6.1	G-G-G
1	6	14	5.6	0.993	1.22	5.7	8.8	2.6	1.7	NA	4.2	NG-NG-G
1	8	14	5.5	0.987	2.30	10.0	8.7	2.6	0.7	NA	3.4	NG-NG-G
1	10	14	5.2	0.986	2.51	72.4	9.0	1.9	0.7	NA	2.1	NG-NG-NG
2	4	14	6.1	0.993	1.24	16.1	7.6	2.8	2.5	NA	6.2	NG-NG-G
2	8	14	5.1	0.987	2.25	542.7	10.5	2.4	2.2	NA	1.3	NG-NG-NG
2	10	14	4.7	0.986	2.43	1,676.4	10.8	2.8	1.9	NA	0.3	NG-NG-NG
3	6	14	5.4	0.993	1.22	125.6	9.5	2.6	0.7	NA	3.2	NG-NG-G
3	8	14	5.3	0.986	2.36	282.3	10.2	1.9	1.5	NA	2.0	NG-NG-NG
3	10	14	5.3	0.985	2.58	287.8	10.5	2.1	0.7	NA	1.9	NG-NG-NG

[a] wps, water phase saline; [LA], undissociated lactic acid (μg/g); Inoc, concentration at the inoculum; R, real data; FSSP and F, estimated by Food Spoilage and Safety Predictor; CB, estimated by ComBase; G, growth, ≥0.5 log CFU/g; NG, no-growth, <0.5 log CFU/g; NA, out of the range of the model (no-growth).

inoculum) and then at 2, 4, 7, 9, 11, and 14 days. The pH was measured in the noninoculated tubes at 0, 7, and 14 days.

Statistical analyses and modeling. The R statistical software (2013 version, R Foundation for Statistical Computing, Vienna, Austria) was used to analyze growth data by the logistic-regression model, implemented through the *glm* (generalized linear model) function with the "family" parameter set to "bimodal." Products were classified as able to support the growth of *Lm* when the difference between the log CFU per gram at the end of the test and the log CFU per gram at the beginning of the test (δ) was >0.5 log CFU/g. Log[LA] ppm and LAB initial concentration data were tested separately as continuous predictor variables in the logistic regression. The observed growth rates of *Lm* in the pasta meal were compared with a simulation run with the ComBase Predictor growth model for *Listeria* in presence of LA and the Food Spoilage and Safety Predictor (FSSP) for *Lm*–psychrotolerant *Lactobacillus* spp. (LAB) interaction model *(8)*.

The required input data listed in Table 1 were used. The default value for the *Listeria* physiological state ($\alpha_0 = 2.1 \times 10^{-2}$) in ComBase and the competition factor $\gamma = 1$ in FSSP were assumed. The so-called physiological state expresses that a fraction of the inoculum (α_0) that could be in the exponential phase at the observed time, without lag. The $\gamma = 1.0$ includes the assumption that LAB inhibit growth of *Lm* to the same extent that they inhibit their own growth. Log-linear and log-linear + tail were the mathematical models used to obtain the inactivation curves of *Lm (10)*. LAB growth curves were fitted with the DMFit software (ComBase 2018 version, Centre for Food Safety and Innovation, Hobart, Tasmania, Australia) to obtain growth parameters. LAB growth curves were fitted to the Baranyi model *(2)* using DMFit online version.

RESULTS

Evolution of LAB, *Lm,* and LA. Growth of LAB was faster at 14°C than it was at 6°C, and a greater production of LA was observed, which affected the extent of the pH decline (Fig. 1 and Table 1). LAB growth reached its maximum limit at approximately 8 days, with negligible increment in lot 3 at 6°C, a maximum growth rate of approximately 0.02 log CFU/h, and final values that varied between 5.79 and 7.15 log CFU/g. At 14°C, LAB growth rate was approximately 0.05 log CFU/g/h, and the maximum cell density for LAB was significantly greater (10.11 ± 1.13 versus 5.61 ± 1.46 log CFU/g). The null hypothesis of equal cell density at 6 and 14°C was rejected (Welch *t* test with $\alpha = 0.05$, 4 df, $P = 0.0067$). Differences were observed in the extent of pH decline, which was –0.2 to –0.4 units at 6°C and as high as –1 to –1.5 units at 14°C. The differences in the final pH (6.07 ± 0.17 versus 5.05 ± 0.35) were significant (Welch *t* test with $\alpha = 0.05$, 3 df, $P = 0.0102$). The undissociated [LA] was positively correlated with LAB numbers (Kendall $\tau = 0.38$, $P = 0.016$; Spearman $\rho = 0.52$, $P = 0.018$) and negatively correlated to pH (Kendall $\tau = -0.32$, $P = 0.043$; Spearman $\rho = -0.45$, $P = 0.041$). At 14 days [LA] varied between 9 and 21 ppm at 6°C and 509 and 1,887 ppm at 14°C. [LA] variability was also detected between the lots on day 1 of shelf life. [LA] in lots 2 and 3 was 10 times higher than in the lot 1. Any off-flavor, color, or texture defects were observed during the shelf-life period of the products stored at 6°C, whereas the products that were stored at 14°C presented a slight texture

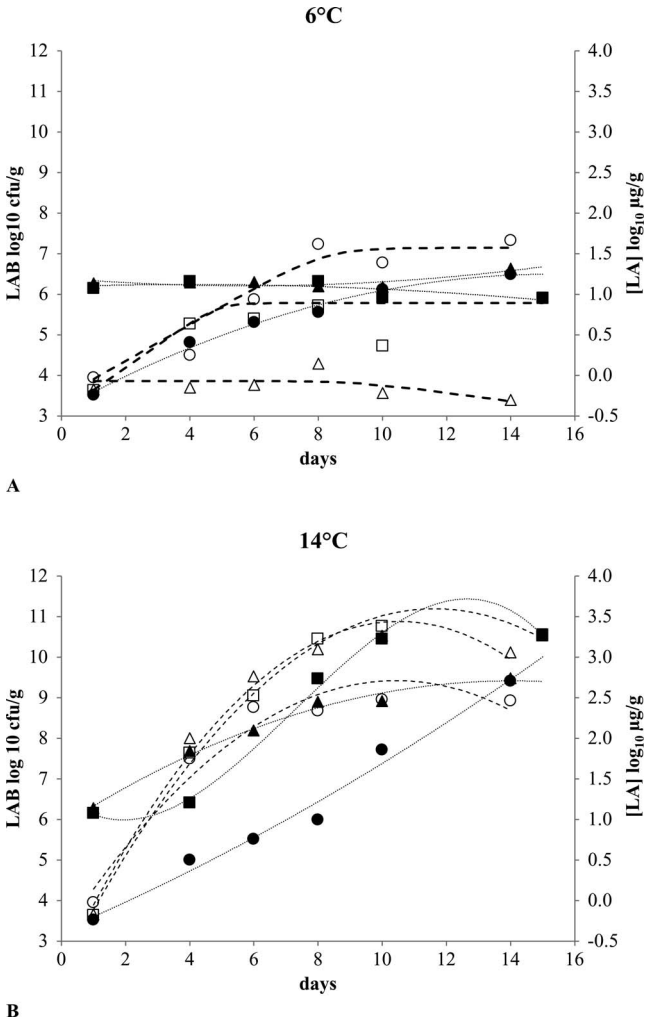

FIGURE 1. *Effect of the temperature on the growth of lactic acid bacteria (LAB) and concentration of undissociated lactic acid [LA] of filled-pasta meal stored in MAP at 6 and 14°C. LAB: ○, Lot 1; □, lot 2; Δ, lot 3. [LA]: ●, Lot 1; ▲, lot 2; ■, lot 3.*

loss of tomatoes and rocket salad at 6 days, which became evident by 10 days (spoiled products).

The presence of *Lm* was sporadically detected in enriched samples (i.e., below the limit of quantification) in two packs of pasta meal that were stored at either 6°C (i.e., lot 2 at 4 days) and 14°C (i.e., lot 1 at 10 days). Other samples of the same lots examined at the end of the shelf life were negative for the presence of the bacterium.

***Lm* growth in products experimentally contaminated during postprocessing storage.** Table 1 reports the characteristics of filled-pasta meals, the number of LAB, and *Lm* at the time of inoculum (1, 4, 6, 8, and 10 days of shelf life) and its number after 7 days of storage at 9°C. Increments in the number of *Lm* were in a range between 3.9 and 5.5 log CFU/g with the samples inoculated on day 1 of the shelf life. Conversely, the samples that were stored at 14°C did not support growth of *Lm*. When the inoculum was made in the samples stored at 6°C at 4 to 10 days of shelf life, *Lm* grew only in the samples that presented low LAB numbers (<5.7 log CFU/g) at the time of the inoculum. The estimates produced by the ComBase growth model for *Listeria* in presence of LA and by the FSSP *Lm*–psychrotolerant *Lactobacillus* spp. (LAB) interaction model are also reported in Table 1.

The effects of LAB number and [LA] on the growth potential for *Lm* are shown in Figure 2. The logistic regression curves fitted the data well (i.e., *P* value (>|*z*|) for [LA] = 0.0980; *P* value (>|*z*|) for LAB = 0.0488). Logistic regression estimated that the probability of *Lm* growth was <10% if LAB was >6.6 log CFU/g or log[LA] was >2.2 (approximately 160 ppm).

Kinetics of *Lm* inactivation for pasta homogenates contaminated when LAB numbers were close to their maximum density. Figure 3 shows the growth and inactivation kinetics observed in the challenge tests made with the pasta homogenates. When the inoculum was made in the samples stored at 14°C for 6 days, an immediate decline was observed in the number of *Lm* (–2 or –2.5 log CFU/g in 4 to 8 days), whereas a biphasic kinetic was observed in the homogenate of the sample that had been stored at 6°C for 8 days before the inoculum. The final pH values after 7 days of incubation at 9°C were 4.47, 4.50, and 4.83.

DISCUSSION

Effects of temperature abuse on the growth of LAB and *Lm*. The results of this study highlight that changes associated with the growth of LAB in filled-pasta meals that

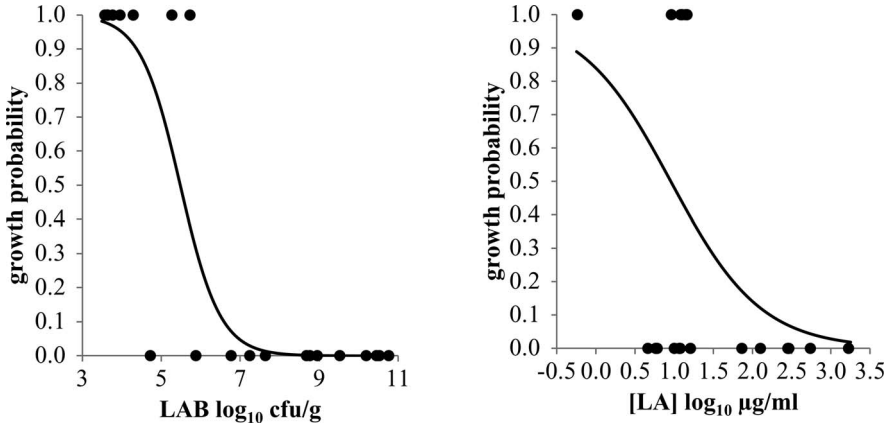

FIGURE 2. *Data and predicted values from the logistic regression model. When the growth potential (δ) is >0.5 log CFU/g, the products were classified as "able to support the growth of* Lm.*"*

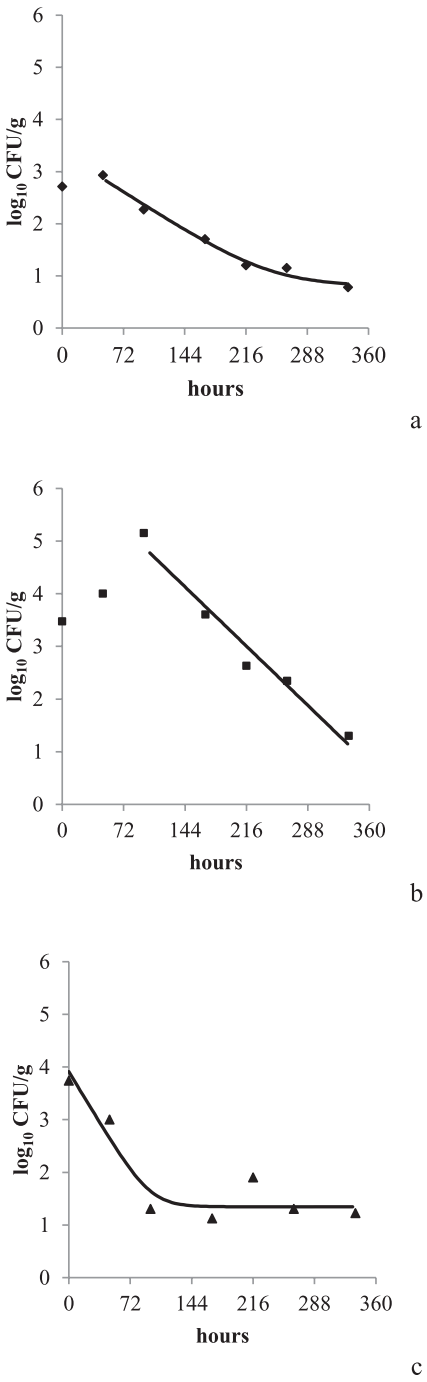

FIGURE 3. *Growth and inactivation kinetics of* Lm *at 9°C in pasta homogenates. (a) ◆, Lot 1, inoculated at 6 days of shelf life (14°C); LAB = 8.8 log CFU/g; pH 5.6; [LA] = 6 μg/g; inactivation model, log-linear + tail: log(N) = log[($10^{3.36}$ − $10^{0.80}$) × exp(−0.06 × t) + $10^{0.80}$]; R^2_{adj} = 0.98. (b) ■, Lot 2, inoculated at 8 days of storage at 6°C; LAB = 5.7 log CFU/g, pH 5.7; [LA] = 13 μg/g; inactivation model, log-linear: log(N) = (6.38 − 0.04 × t)/ln(10); R^2_{adj} = 0.96. (c) ▲, Lot 3, inoculated at 6 days of shelf life at 14°C; LAB = 9.5 log CFU/g; pH 5.4; [LA] = 125 μg/g; inactivation model, log-linear + tail: log(N) = log [($10^{3.91}$ − $10^{1.35}$) × exp(−0.06 × t) + $10^{1.35}$]; R^2_{adj} = 0.85.*

affect the growth potential of *Lm*. Storage above refrigeration temperatures (i.e., 14°C) in MAP resulted in greater growth of LAB and was associated with LA production and a pH drop that gave rise to high undissociated [LA]. LAB grew faster and reached greater cell densities in products stored at the higher temperature. The samples stored at 6°C remained unspoiled up to the end of the shelf life, whereas the samples stored at 14°C showed softening of tomatoes and rocket after 6 to 8 days. Slower growth of LAB and considerable lot-to-lot variability were observed at 6°C. LAB constitute only a minor portion of the initial population of fresh vegetables, but different stages of ripening have a significant effect on the growth kinetics of LAB *(26),* whereas they are in numbers between 1.7 and 6.8 log CFU/g (mean, 3.6 to 3.7) in the Ricotta cheese that is normally compartmentalized in the ravioli *(32)*. These values are very close to those detected in this study in the products at 1 day of shelf life. The variability in the growth of LAB at 6°C could not be attributed to the differences in the formulation of the products. The differences in the LAB numbers at the time of the inoculum affected the growth potential of *Lm*. Differences between actual data and *Lm* numbers estimated by the predictive models FSSP and ComBase were observed. The differences might be related to the presence of organic acids other than LA (FSSP) or to the need to adapt the values of the factors α_0 (ComBase). In addition, the ComBase *Listeria* growth model assumes a constant [LA]. The FSSP interaction model was developed for chilled seafoods and meat products. Nevertheless, it failed to predict the growth of *Listeria* in only 2 of 21 cases.

Biphasic kinetics (growth and inactivation) of *Lm* were observed in the pasta homogenates, especially in lot 2. A similar behavior in the products on the market would mean that higher numbers of *Lm* could be detected at an intermediate shelf life, before LAB have reached their maximum density, and that can influence the probability of detection of *Lm* that is present at low levels. The sporadic detection of *Lm* in the two lots of pasta meals, where its presence was detectable at only 4 and 10 days, but not later, was always below the limit of quantification (i.e., 10 CFU/25 g), which could be related to nonhomogeneous contamination in the lots, but also to this biphasic kinetics.

This study showed that interaction with LAB can inhibit the growth potential of *Listeria* after temperature abuse of refrigerated meals. Other studies with cheese have reported that the *Listeria* growth can be suppressed by interaction with LAB, and that inhibition was greater in the samples purchased closer to the expiration date compared with those close to the production date *(19)*. In a recent article, De Cesare et al. *(9)* observed that *Lm* was able to grow in different formulations of pasta salads stored at 4, 6, and 12°C under air or MAP. *Listeria* growth was observed even when LAB reached their stationary phase, but an inhibitory effect was observed when the LAB stationary phase was reached early (i.e., at 3 to 6 days of shelf life). In the present study, the formulation of the pasta meal was probably the key factor that affected the rapid growth of LAB, especially at 14°C in MAP. Competitive inhibition of *Lm* by LAB has been related to the production of organic acids and bacteriocins *(1, 7, 24, 35)*. Undissociated LA was evaluated to be the most important growth inhibitor of *Lm* in nature-ripened, Dutch-type Gouda cheese *(35),* and minimal inhibitory [LA] (and other organic acids) for *Lm*

under conditions relevant to cheese were assessed *(36)*. The minimal inhibitory, undissociated [LA] was estimated to be 5.0 mM in the pH range of 5.2 to 5.6 at 14°C, which is commonly used during cheese ripening. In the present study, the growth potential of *Lm* was affected at much lower concentration of undissociated LA (Fig. 2). Indeed, with [LA] = 158 ppm (≈1.8 mM), the growth probability was estimated to be 10%.

Influence of ingredients on the organic acid profile. A potential weakness of this study is that [LA] was measured in whole-sample extracts; therefore, it could have been derived by the LAB metabolic activity and by the cherry tomatoes that were used in the recipe. A study has analyzed the organic acid profile of tomato juice *(22)* and reported that the average [LA] was in the range 121 to 219 ppm, and other organic acids, such as malic and acetic, were also present in relevant concentration. At lower pH values, the presence of undissociated lactate and other organic acids exerts inhibitory effect on the growth of *Lm (33),* but it cannot be easily demonstrated that the organic acid concentration and *Lm* are distributed uniformly, although a uniform distribution was certainly obtained in the homogenized samples. The different amounts of cherry tomatoes that were possibly used in the recipe might have influenced the LA concentration detected on day 1 of this study in the different lots. Composition and differences in the number of LAB on day 1 of shelf life might also have influenced the LAB maximum population density, with differences up to 3 log CFU/g at 6°C and 1.6 log CFU/g at 14°C. Furthermore, the moisture content of the fresh MAP, pasteurized pasta is different for the dough and for the filling *(30)* approximately 30 and 47%, respectively. Finally, because LAB constitute the predominant element of the microbial flora in tomatoes *(29),* their acidity and sugar content (i.e., glucose and fructose) *(4)* may have positively affected the concentration of undissociated LA.

Thus, product formulation, other than MAP and refrigeration temperature influences the number of LAB and their ability to grow.

In conclusion, refrigerated fresh cheese-filled pasta meal in MAP was able to support the growth of *Lm,* but the interaction with LAB progressively reduced its growth potential. Importantly, LAB maximum density changes with temperature, and variability between the lots was observed.

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