Effect of the addition of starter cultures to ground meat for hamburger preparation

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Abstract

The aim of this work was to study the effect of a selected lactic acid bacteria formulation on the microbiological characteristics and colour of beef hamburgers stored at different temperatures. All hamburgers were evaluated on day-0, day-1, day-3, and day-5 for the following microbiological parameters (*Staphylococcus* spp., enterococci, *Lactococcus* spp., *Lactobacillus* spp., total mesophilic aerobes, *Pseudomonas* spp., total coliforms) according to standard methods and for colorimetric measurements performed with Colorimeter - Digital Color Picker for iOS 10, under a 6500K light, with the CIELab system. All data (geometric mean for microbiological data) were elaborated with GraphPad InStat, 3.0b and GraphPad Prism 6.0d for Mac OS X. Two-way analysis of variance (ANOVA) followed by the Tukey's multiple comparisons test was performed. The analysis of the colour proved that the addition of Lactic Acid Bacteria (LAB) does not affect the natural colour of ground meat, avoiding the risk of hiding the spoilage or fastening it. The addition of the starter has preserved the colour stability throughout the preservation period, with the same behaviour both in the hamburgers stored at 4°C and in those at 10°C after thermal abuse or not. In conclusion, the application of the proposed LAB formulation maintains hamburgers quality standards and can be a potential tool to increase their shelf-life.

Introduction

Meat products and meat preparations are very sensitive to external factors, such as temperature, and have a very sensitive substrate for the biochemical processes that occur during storage. These products are characterized by a short shelf-life and are good substrates for microbial growth.[1,2]

In food industry starter cultures are currently used in a wide variety of products in order to transform raw prime materials and to safeguard their microbial and sensory quality.[3,4] Most of the commercially available starter cultures contains different strains of the so-called Lactic Acid Bacteria (LAB). LAB can be divided in two groups: heterofermentative LAB of the *Carnobacterium*, *Leuconostoc* and *Weissella* genera which are usually more involved in meat spoilage and homofermentative LAB of the *Lactobacillus*, *Lactococcus* and *Pediococcus* genera. Therefore, commercially available meat starter cultures for meat products and preparations exclusively belong to the latter two.[5] Heterofermentative LAB species produce significant amounts of non-desirable fermentation end products, such as ropy slime, CO_2 gas, ethanol, acetic acid, butanoic acid and acetoin while homofermentative LAB produce almost exclusively lactic acid from fermentable carbohydrates present in meats, which is relatively mild and palatable.[6] However, it has been reported that *Lactobacillus* spp., under particular conditions, may also produce significant amounts of acetic acid, ropy slime and discolouration (greening) of meats.[7,8]

Lactic acid bacteria have been shown to inhibit the growth and decrease the load of foodborne pathogens such as *Escherichia coli*, *Salmonella* spp. and *Listeria monocytogenes*.[9,10] They can create a competitive environment through several different mechanisms, including the production of bacteriocins, hydrogen peroxide and weak acids such as acetic and lactic acid.[11] The antagonistic action of LAB takes place when they compete with other non-desirable bacteria for a common niche, when an extracellular agent is produced, or when the environment is modified to inhibit the growth of the other microorganism.[12]

To be acceptable for use by the meat industry, the addition of LAB cultures must not change the sensory properties of ground beef or cause *spoilage masking* within the package of the product.[13]

The aim of this study was to analyse the effect of the addition of LAB on the normal microflora present in ground beef used for the production of hamburgers and on the colour of the beef hamburgers stored at different temperatures including temperature abuse.

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Material and Methods

Two batches of hamburgers (average weight of about 100g each) were prepared in the experimental processing plant of the laboratory of inspection of foods of animal origin of the department of veterinary medicine, University of Perugia. Hamburgers were prepared according to the classical method of production: minced beef was mixed with NaCl (20g/kg), prepackaged egg mixture (300ml/kg), skim milk (250ml/kg), bread crumbs and hard cheese (2g/kg), without the addition of food additives. One batch was prepared with the addition of LAB (*Lactococcus lactis* ssp. *lactis*, strain 340; *L. lactis* ssp. *lactis*, strain 16; *Lactobacillus casei* ssp. *casei*, strain 208 and *Enterococcus faecium* strain 614 in a ratio lactococci:lactobacilli:enterococci of 2:1:1; level of inoculum 10^7 cfu g^{-1}) and one without, in three replicates done in three different days. The morphological, biochemical and physiological characterisation, the growth curves at several temperatures, including refrigeration conditions, the acidifying activity of these four bacterial strains and their ability to improve palatability of dry salami along with safety considerations for the commercial probiotic *E. faecium* UBEF-41 have been reported by the authors in previous papers.[14-17] Before the inoculum, freeze-dried strains of the starter cultures were grown aerobically in Nutrient Broth (NB, Oxoid, Basingstoke, UK) at 37°C for 24 hrs. Each strain was then sub-cultured in Skim Milk (BD Difco, Franklin Lakes, NJ, USA) at 37°C for 24 hrs. The Total Viable Cell (TVC) count (on Nutrient Agar, NA, Oxoid, incubated at

37°C in air for 24 hrs) at 24 hours was approximately 1 x 10^9 cfu ml^{-1}.

For each batch, a subset of samples was stored under proper conditions (4°C) and the other at 40°C (temperature abuse conditions, to mimic inadequate storage conditions after purchase by consumer) both for 5 hours. After 5 hours, each subset was further divided into two subgroups: one maintained at 4°C and the other at 10°C, in order to simulate both a good functioning and a bad functioning refrigerator. All hamburgers were evaluated on day-0, day-1, day-3, and day-5 for the following microbiological parameters (*Staphylococcus* spp., enterococci, *Lactococcus* spp., *Lactobacillus* spp., total mesophilic aerobes, *Pseudomonas* spp., total coliforms) according to standard methods; in brief, at each analyses time, 25 g of meat were homogenised in a sterile bag with 225 ml of pepton water (PW, Oxoid, Basingstoke, Hampshire, UK) by using a stomacher. 10-fold dilutions were prepared by using sterile tubes with 9 ml of Maximum Recovery Diluent (MRD, Oxoid). Dilutions were inoculated in triplicate on different culture media. Total aerobic mesophilic microbiota was determined on Plate Count Agar (PCA, Oxoid), at 30°C for 72 h; *Lactococcus* spp. on M17 agar (Oxoid) 10% v/v lactose, at 37°C for 48 h; *Lactobacillus* spp. on Man, Rogosa and Sharpe Agar (MRS, Oxoid) pH 5.5, at 30°C for 72 h under anaerobic conditions (Gas generating kit, Oxoid); enterococci on enterococcus agar (ENT, Oxoid), at 37°C for 48 h. *Staphylococcus* spp. on Baird Parker agar (BP, Oxoid) containing Egg Yolk Tellurite (Oxoid) at 37°C for 48 h. Total coliforms on violet red bile lactose agar (VRBL, Oxoid) at 37°C for 24h. *Pseudomonas* spp. on pseudomonas agar base (PS103, Oxoid) at 37°C for 24h. The colonies were then counted on all the plates, using a colony count viewer (Petri light, PBI, Milan) and colony counter pen (Colony Count, PBI, Milan).

Moreover, colorimetric measurements were performed with Colorimeter - Digital Color Picker for iOS 10, under a 6500K light, with the CIELAB system, by taking three readings for each sample. The arithmetic means within each sampling was computed, subsequently all data (geometric mean for microbiological data) were elaborated with GraphPad InStat, 3.0b and GraphPad Prism 6.0d for Mac OS X. Two-way analysis of variance (ANOVA) followed by the Tukey's multiple comparisons test was performed.

Results

All the microbiological analysis results are reported in Table 1 (hamburgers stored at 4°C) and in Table 2 (hamburgers stored at 10°C).

The initial total aerobic mesophilic count was 6.86 cfu g^{-1} in the batch with LAB and 4.19 cfu g^{-1} in the batch without LAB. The initial enterococci count was 6.64 cfu g^{-1} and 2.10 cfu g^{-1} respectively. The initial *Lactococcus* spp. count was 6.93 cfu g^{-1} and 4.11 cfu g^{-1}. The initial *Lactobacillus* spp. count was 7.08 cfu g^{-1} and 4.15 cfu g^{-1}. The initial total coliforms count was 2.67 cfu g^{-1} and 2.76 cfu g^{-1}. The initial *Pseudomonas* spp. count was 6.24 cfu g^{-1} and 6.26 cfu g^{-1}. The initial *Staphylococcus* spp. count was 3.62 cfu g^{-1} and 3.76 cfu g^{-1}.

After 5 days, in the batch stored immediately at 4°C the total aerobic mesophilic count was 6.56 cfu g^{-1} in the batch with LAB and 5.88 cfu g^{-1} in the batch without LAB. The enterococci count was 6.29 cfu g^{-1} and 2.36 cfu g^{-1} respectively. The *Lactococcus* spp. count was 6.23 cfu g^{-1} and 3.80 cfu g^{-1}. The *Lactobacillus* spp. count was 6.91 cfu g^{-1} and 6.23 cfu g^{-1}. The total coliforms count was 2.00 cfu g^{-1} for both. The *Pseudomonas* spp. count was 6.11 cfu g^{-1} and 6.46 cfu g^{-1}. The *Staphylococcus* spp. count was 3.55 cfu g^{-1} and 3.51 cfu g^{-1}.

After 5 days, in the batch stored at 4°C after 5 hours of temperature abuse (40°C) the total aerobic mesophilic count was 6.50 cfu g^{-1} in the batch with LAB and 5.99 cfu g^{-1} in the batch without LAB. The enterococci count was 6.32 cfu g^{-1} and 2.70 cfu g^{-1} respectively. The *Lactococcus* spp. count was 6.37 cfu g^{-1} and 4.28 cfu g^{-1}. The *Lactobacillus* spp. count was 7.06 cfu g^{-1} and 6.24 cfu g^{-1}. The total coliforms count was 2.26 cfu g^{-1} and 1.33 cfu g^{-1}. The *Pseudomonas* spp. count was 6.39 cfu g^{-1} and 6.51 cfu g^{-1}. The *Staphylococcus* spp. count was 3.68 cfu g^{-1} and 3.80 cfu g^{-1}.

After 5 days, in the batch stored immediately at 10°C the total aerobic mesophilic count was 6.63 cfu g^{-1} in the batch with LAB and 6.01 cfu g^{-1} in the batch without LAB. The enterococci count was 6.35 cfu g^{-1} and 2.16 cfu g^{-1} respectively. The *Lactococcus* spp. count was 6.42 cfu g^{-1} and 4.04 cfu g^{-1}. The *Lactobacillus* spp. count was 7.46 cfu g^{-1} and 6.23 cfu g^{-1}. The total coliforms count was 2.30 cfu g^{-1} and 2.20 cfu g^{-1}. The *Pseudomonas* spp. count was 6.27 cfu g^{-1} and 6.37 cfu g^{-1}. The *Staphylococcus* spp. count was 3.43 cfu g^{-1} and 3.65 cfu g^{-1}.

After 5 days, in the batch stored at 10°C after 5 hours of temperature abuse (40°C) the total aerobic mesophilic count was 6.88 cfu g^{-1} in the batch with LAB and 6.01 cfu g^{-1} in the batch without LAB. The enterococci count was 6.39 cfu g^{-1} and 2.10 cfu g^{-1} respectively. The *Lactococcus* spp. count was 6.50 cfu g^{-1} and 4.31 cfu g^{-1}. The *Lactobacillus* spp. count was 7.92 cfu g^{-1} and 6.27 cfu g^{-1}. The total coliforms count was 1.33 cfu g^{-1} and 0.67 cfu g^{-1}. The *Pseudomonas* spp. count was 6.12 cfu g^{-1} and 6.43 cfu g^{-1}. The *Staphylococcus* spp. count was 3.34 cfu g^{-1} and 3.53 cfu g^{-1}.

Staphylococcus aureus was never isolated in any sample.

The results of the colorimetric analysis are shown in table 3. The initial values of the three parameters were: L=45.73±1.90, a=40.76±1.95 and b=32.42±1.44 for the hamburgers with LAB and L=38.99±1.00, a=43.53±1.64, b=31.32±0.39 for the hamburgers without LAB.

After 5 days, in the batch stored immediately at 4°C the values were L=44.33±4.04, a=8.67±2.08 and b=30.67±2.08 for the hamburgers with LAB and L=38.33±1.53, a=13.67±0.58 and b=28.67±0.58 for the hamburgers without LAB.

After 5 days, in the batch stored at 4°C after 5 hours of temperature abuse (40°C) the values were L=41.33±5.13, a=11.33±2.31 and b=27.67±1.15 for the hamburgers with LAB and L=39.00±7.81, a=7.67±4.16 and b=24.67±3.79 for the hamburgers without LAB.

After 5 days, in the batch stored immediately at 10°C the values were L=41.33±4.93, a=20.00±2.65 and b=25.00±2.00 for the hamburgers with LAB and L=37.00±2.00, a=22.33±0.58 and b=23.33±1.15 for the hamburgers without LAB.

After 5 days, in the batch stored at 10°C after 5 hours of temperature abuse (40°C) the values were L=37.67±4.04, a=23.00±3.61 and b=24.00±2.00 for the hamburgers with LAB and L=34.67±3.79, a=23.00±1.73 and b=20.67±2.89 for the hamburgers without LAB.

Discussion

The application of starter cultures and bio protective microbial cultures is a much discussed innovative and sustainable technology for improving the microbiological safety and overall quality of meat products, in particular pre-packaged cold cuts.[5] It could represent an alternative to chemical preservatives or to a second pasteurisation step after packaging which both have a negative sensory impact.[18]

Starter cultures allow a standardization of the product quality and considerably reduce the risk of product defects. However,

it should be kept in mind that starter cultures cannot replace good manufacturing practice which besides the selection of the appropriate raw materials with acceptable hygienic parameters also includes the implementation and control of appropriate processing conditions.[19,20]

The official authorities of food control and the big retail chains often look with suspicion at high microbial counts in meat preparations, regardless of the responsible microflora. The German Society for Hygiene and Microbiology, *e.g.*, recommends a maximum of $5x10^6$ cfu g^{-1}.[21] However, many of the pre-packaged sliced cold cuts display 10-100 times higher counts at the end of their indicated shelf lives without being recognized as spoiled by sensory panels. On the other hand, unpleasant tastes and smells (not fresh, sour) are often associated with high LAB counts.[22]

In minced meat, LAB can be added during the grinding process or during the trimming prior to grinding.[11] Our study confirms that the addition of LAB in hamburgers produced with high hygienic parameters helps to maintain the microbiology quality of the products. In addition, it is important to note that the populations of LAB in the inoculated samples did not increase over time, avoiding the risk of a bacterial over population. On the other hand, common spoilage microorganisms such as *Pseudomonas* spp. did not increase as usual in temperature abused foods.[23,24]

Table 1. Microbiota counts (log cfu g^{-1}) for hamburger stored at 4°C for 5 days.

	No abuse				Abuse			
4°C	Lab		No Lab		Lab		No Lab	
	mean	sd	mean	sd	mean	sd	mean	sd
PCA								
0	6.86[a]	0.17	4.19[b]	0.05	6.86[a]	0.17	4.19[b]	0.05
1	7.06[a]	0.01	5.21[b]	0.03	7.05[a]	0.09	5.48[b]	0.17
3	7.20[a]	0.13	5.76[b]	0.27	7.07[a]	0.08	6.02[b]	0.24
5	6.56[a]	0.03	5.88[b]	0.05	6.50[a]	0.02	5.99[b]	0.16
ENT								
0	6.64[a]	0.06	2.10[b]	0.17	6.64[a]	0.06	2.10[b]	0.17
1	6.76[a]	0.07	2.36[b]	0.10	6.71[a]	0.10	2.64[b]	0.19
3	6.48[a]	0.04	2.63	0.06	6.46[a]	0.06	1.73	1.50
5	6.29[a]	0.15	2.36[b]	0.32	6.32[a]	0.05	2.70[b]	0.17
M17								
0	6.93[a]	0.03	4.11[b]	0.08	6.93[a]	0.03	4.11[b]	0.08
1	6.97[a]	0.08	4.52[b]	0.10	6.98[a]	0.10	4.31[b]	0.22
3	6.28[a]	0.02	4.13[b]	0.31	6.25[a]	0.07	4.19[b]	0.03
5	6.23[a]	0.03	3.80	0.31	6.37[a]	0.14	4.28	0.19
MRS								
0	7.08[a]	0.14	4.15[b]	0.00	7.08[a]	0.14	4.15[b]	0.00
1	7.03[a]	0.05	4.82	0.06	6.98[a]	0.03	5.07	0.09
3	7.17[a]	0.09	5.34	0.01	7.24[a]	0.07	6.21	0.08
5	6.91[a]	0.07	6.23[b]	0.06	7.06[a]	0.04	6.24[b]	0.05
VRBL								
0	2.67[a]	0.06	2.76[a]	0.25	2.67[a]	0.06	2.76[a]	0.25
1	2.95[a]	0.17	2.91[a]	0.18	2.82[a]	0.24	2.58[a]	0.17
3	2.26[ab]	0.24	2.32[ab]	0.28	1.33[a]	1.15	2.46[b]	0.28
5	2.00[a]	0.00	2.00[a]	0.00	2.26[a]	0.24	1.33[a]	1.15
PS103								
0	6.24[a]	0.08	6.26[a]	0.05	6.24[a]	0.08	6.26[a]	0.05
1	6.15[a]	0.03	6.31[a]	0.04	6.15[a]	0.05	6.26[a]	0.05
3	6.32[a]	0.12	6.28[a]	0.06	6.39[a]	0.08	6.40[a]	0.18
5	6.11	0.13	6.46[a]	0.06	6.39[a]	0.03	6.51[a]	0.06
BP								
0	3.62[a]	0.28	3.76[a]	0.13	3.62[a]	0.28	3.76[a]	0.13
1	3.71[a]	0.20	3.87[a]	0.11	3.74[a]	0.09	3.74[a]	0.05
3	3.51[a]	0.05	3.57[a]	0.06	3.38[a]	0.11	3.65[a]	0.04
5	3.55[a]	0.10	3.51[a]	0.11	3.68[a]	0.10	3.80[a]	0.07

Abuse: hamburger kept at 40°C for 5 hours after production to mimic inadequate storage conditions after purchase by consumer; No abuse: hamburger kept at 4°C for 5 hours after production to mimic proper storage conditions after purchase by consumer. PCA: mesophilic aerobic microbiota, ENT: *Enterococcus* spp., M17: *Lactococcus* spp., MRS: *Lactobacillus* spp., VRBL: coliform organisms, PS103: *Pseudomonas* spp., BP: *Staphylococcus* spp, sd: standard deviation. Significance is intended per row.

Data on the sensory acceptance by the consumer of non-fermented meat products with addition of LAB are scarce. It is important that proposed food additives not mask factors that consumers would typically associate with a spoiled product, so that they do not consume a product that would normally be discarded.[10] The analysis of the hamburgers' colour performed in our study confirms that the addition of LAB does not affect the natural colour of ground meat, avoiding the risk of hiding the spoilage or fastening it. The addition of the starter has preserved the colour stability throughout the preservation period, with the same behaviour both in the hamburgers stored at 4°C and in those at 10°C after thermal abuse.

Conclusions

Meat and meat products provide a concentrated source of protein of high biological value and can make a valuable contribution to human diets. However, they are also highly perishable foods which rapidly spoil and may even allow the growth of food-

Table 2. Microbiota counts (log cfu g^{-1}) for hamburger stored at 10°C for 5 days.

10°C	No abuse				Abuse			
	Lab		No Lab		Lab		No Lab	
	mean	sd	mean	sd	mean	sd	mean	sd
PCA								
0	6.86^{a}	0.17	4.19^{b}	0.05	6.86^{a}	0.17	4.19^{b}	0.05
1	7.09^{a}	0.06	5.65^{b}	0.05	6.96^{a}	0.05	5.69^{b}	0.04
3	6.96^{a}	0.24	6.12^{b}	0.05	7.37^{a}	0.06	6.23^{b}	0.44
5	6.63^{a}	0.10	6.01^{b}	0.10	6.88^{a}	0.25	6.01^{b}	0.14
ENT								
0	6.64^{a}	0.06	2.10^{b}	0.17	6.64^{a}	0.06	2.10^{b}	0.17
1	6.66	0.08	2.52^{a}	0.45	7.79	1.74	2.40^{a}	0.35
3	6.44^{a}	0.05	2.30^{b}	0.30	6.42^{a}	0.20	2.63^{b}	0.13
5	6.35^{a}	0.06	2.16^{b}	0.28	6.39^{a}	0.04	2.10^{b}	0.17
M17								
0	6.93^{a}	0.03	4.11^{b}	0.08	6.93^{a}	0.03	4.11^{b}	0.08
1	6.96^{a}	0.05	4.37^{b}	0.19	6.93^{a}	0.04	4.50^{b}	0.07
3	6.22^{a}	0.06	4.12^{b}	0.23	6.31^{a}	0.04	4.25^{b}	0.13
5	6.42^{a}	0.06	4.04	0.09	6.50^{a}	0.05	4.31^{b}	0.10
MRS								
0	7.08^{a}	0.14	4.15^{b}	0.00	7.08^{a}	0.14	4.15^{b}	0.00
1	7.01^{a}	0.01	4.84^{b}	0.10	6.96^{a}	0.05	4.99^{b}	0.10
3	7.33^{a}	0.07	6.20^{b}	0.04	7.45^{a}	0.08	6.36^{b}	0.09
5	7.46	0.03	6.23^{a}	0.01	7.92	0.15	6.27^{a}	0.12
VRBL								
0	2.67^{a}	0.06	2.76^{b}	0.25	2.67^{a}	0.06	2.76^{a}	0.25
1	2.96^{a}	0.16	2.91^{b}	0.19	3.00^{a}	0.07	3.04^{a}	0.17
3	2.10^{a}	0.17	2.16^{b}	0.28	1.53^{a}b	1.33	0.67^{b}	1.15
5	2.30^{a}	0.30	2.20^{b}	0.17	1.33^{a}b	1.15	0.67^{b}	1.15
PS103								
0	6.24^{a}	0.08	6.26^{b}	0.05	6.24^{a}	0.08	6.26^{a}	0.05
1	6.17^{a}	0.12	6.35^{b}	0.07	6.17^{a}	0.05	6.23^{a}	0.12
3	6.10^{a}	0.07	6.23^{b}	0.05	6.30^{ab}	0.12	6.40^{ab}	0.07
5	6.27^{a}	0.13	6.37^{b}	0.02	6.12^{a}	0.19	6.43^{b}	0.07
BP								
0	3.62^{a}	0.28	3.76^{a}	0.13	3.62^{a}	0.28	3.76^{a}	0.13
1	3.53^{a}	0.12	3.84^{ab}	0.16	3.92^{b}	0.11	3.78^{ab}	0.14
3	3.40^{ab}	0.08	3.71^{b}	0.11	3.35^{a}	0.06	3.51^{ab}	0.03
5	3.43^{a}	0.01	3.65^{a}	0.17	3.34^{a}	0.10	3.53^{a}	0.28

Abuse: hamburger kept at 40°C for 5 hours after production to mimic inadequate storage conditions after purchase by consumer; No abuse: hamburger kept at 4°C for 5 hours after production to mimic proper storage conditions after purchase by consumer. PCA: mesophilic aerobic microbiota, ENT: *Enterococcus* spp., M17: *Lactococcus* spp., MRS: *Lactobacillus* spp., VRBL: coliform organisms, PS103: *Pseudomonas* spp., BP: *Staphylococcus* spp, sd: standard deviation. Significance is intended per row.

Table 3. Mean values of CIELAB colour coordinates for hamburger stored at different temperatures. Lightness (L*), green-red coordinate (a*), and blue-yellow coordinate (b*).

	No abuse				Abuse			
4°C	Lab		No Lab		Lab		No Lab	
	mean	sd	mean	sd	mean	sd	mean	sd
L								
0	45.73^{a}	1.90	38.99^{a}	1.00	45.73^{a}	1.90	38.99^{a}	1.00
1	43.26^{a}	0.62	37.49^{a}	1.33	42.30^{a}	1.30	37.26^{a}	2.37
3	42.67^{a}	6.03	34.00^{a}	2.65	42.00^{a}	2.00	36.00^{a}	3.61
5	44.33^{a}	4.04	38.33^{a}	1.53	41.33^{a}	5.13	39.00^{a}	7.81
a								
0	40.76^{a}	1.95	43.53^{a}	1.64	40.76^{a}	1.95	43.53^{a}	1.64
1	34.68a	1.24	36.41^{a}	3.76	28.29	0.88	23.04	2.89
3	10.00^{ab}	2.65	13.67^{b}	0.58	8.33^{a}	2.08	11.00^{ab}	4.58
5	8.67^{ab}	2.08	13.67	0.58	11.33^{ab}	2.31	7.67^{b}	4.16
b								
0	32.42^{a}	1.44	31.32^{a}	0.39	32.42^{a}	1.44	31.32^{a}	0.39
1	30.35^{a}	2.08	27.28ab	1.20	25.45^{bc}	1.05	22.25^{d}	1.38
3	31.00^{ab}	4.36	26.00^{b}	4.00	30.00^{a}	1.00	27.33^{ab}	3.06
5	30.67^{a}	2.08	28.67^{b}	0.58	27.67^{ab}	1.15	24.67^{ab}	3.79
	No abuse				Abuse			
10°C	Lab		No Lab		Lab		No Lab	
	mean	sd	mean	sd	mean	sd	mean	sd
L	mean	sd	mean	sd	mean	sd	mean	sd
0	45.73^{a}	1.90	38.99^{a}	1.00	45.73^{a}	1.90	38.99^{a}	1.00
1	38.57^{a}	3.00	37.98^{a}	0.37	41.98^{a}	3.51	36.80^{a}	1.97
3	39.00^{a}	3.46	33.67^{a}	3.06	40.67^{a}	0.58	32.33^{a}	3.51
5	41.33^{a}	4.93	37.00^{a}	2.00	37.67^{a}	4.04	34.67^{a}	3.79
a								
0	40.76^{a}	1.95	43.53^{a}	1.64	40.76^{a}	1.95	43.53^{a}	1.64
1	28.22a	3.36	30.07a	2.49	21.92b	3.16	21.63b	1.99
3	12.67^{a}	4.04	15.33^{a}	0.58	11.33^{a}	3.79	14.67^{a}	2.31
5	20.00^{a}	2.65	22.33^{a}	0.58	23.00^{a}	3.61	23.00^{a}	1.73
b								
0	32.42^{a}	1.44	31.32^{a}	0.39	32.42^{a}	1.44	31.32^{a}	0.39
1	27.36^{a}	4.41	24.65^{a}	0.50	25.97^{a}	2.38	25.21^{a}	3.47
3	26.00^{ab}	3.46	27.33ab	1.53	29.33^{a}	2.08	23.67^{b}	4.04
5	25.00^{a}	2.00	23.33^{a}	1.15	24.00^{a}	2.00	20.67^{a}	2.89

Abuse: hamburger kept at 40°C for 5 hours after production to mimic inadequate storage conditions after purchase by consumer; No abuse: hamburger kept at 4°C for 5 hours after production to mimic proper storage conditions after purchase by consumer. Significance is intended per row.

borne pathogenic microorganisms if no suitable preservative actions are taken. The addition of LAB beside good manufacturing practices can help to maintain the safety of these products without altering their sensory quality and the consumer perception.

References

1. Grispoldi L, Popescu PA, Karama M, Gullo V, Poerio G, Borgogni E, et al. Study on the growth and enterotoxin production by Staphylococcus aureus in canned meat before retorting. Toxins 2019;11:291.
2. Cenci-Goga BT, Karama M, Hadjichralambous C, Sechi P, Grispoldi L. Is EU regulation on the use of antioxidants in meat preparation and in meat products still cutting edge? Eur Food Res Technol 2020;246:661-8.
3. Caplice E, Fitzgerald GF. Food Fermentations: Role of Microorganisms in Food Production and Preservation. Int J Food Microbiol 1999;50:131-49.
4. Lücke FK. Utilization of Microbes to Process and Preserve Meat. Meat Sci 2000;56:105-15.
5. Krockel L. The Role of Lactic Acid Bacteria in Safety and Flavour Development of Meat and Meat Products. Intech Open 2013. doi: 10.5772/51117
6. Cenci-Goga BT, Sechi P, Iulietto MF, Amirjalali S, Barbera S, Karama M, et al. Characterization and growth under different storage temperatures of ropy slime-producing Leuconostoc mesenteroides isolated from cooked meat products. J Food Prot 2020;83:1043-9. doi: 10.4315/JFP-19-521.
7. Björkroth J, Ridell J, Korkeala H. Characterization of Lactobacillus sake Strains Associated with Production of Ropy Slime by Randomly Amplified Polymorphic DNA (RAPD) and Pulsed-Field Gel Electrophoresis (PFGE) patterns. Int J Food Microbiol 1996;31:59-68.
8. Egan AF. Lactic Acid Bacteria of Meat and Meat Products. Antonie van Leeuwenhoek 1983;49:327-36.
9. Cenci-Goga BT, Karama M, Sechi P, Iulietto MF, Grispoldi L, Selvaggini R, et al. Fate of selected pathogens in spiked "salame nostrano" produced without added nitrates following the application of NONITTM technology. Meat Sci 2018;139:247-54.
10. Smith LJ, Mann E, Harris K, Miller MF, Brashears MM. Reduction of E. coli O157:H7 and Salmonella spp. in ground beef using lactic acid bacteria and the impact on sensory properties. J Food Prot 2005;68:1587-92.
11. Hoyle AR, Brooks JC, Thompson LD, Palmore W, Stephens TP, Brashears MM. Spoilage and Safety Characteristics of Ground Beef Treated with Lactic Acid Bacteria. J Food Prot 2009;72:2278-83.
12. Aguirre M, Collins MD. A review: lactic acid bacteria and human clinical infection. J Appl Bacteriol 1993;75:95-107.
13. U.S. Department of Agriculture FSaIS. Guidance on the Procedures for Joint FSIS and Food and Drug Administration (FDA) Approval of Ingredients and Sources of Radiation Used in the Production of Meat and Poultry Products. Document ID: FSIS-GD-2013-0016. July 5, 2013. Available from: https://www.fsis.usda.gov/wps/portal/fsis/topics/regulatory-compliance/guidelines/2013-0016 Accessed: May 2020. 2003.
14. Cenci-Goga BT, Clementi F, Di Antonio E. Behaviour of lactic and non

lactic microflora during production and ripening of farm manufactured Pecorino cheese. Ann Microbiol 1995;45:219-36.
15. Cenci-Goga BT, Karama M, Sechi P, Iulietto MF, Novelli S, Selvaggini R, et al. Effect of a novel starter culture and specific ripening conditions on microbiological characteristics of nitrate-free dry-cured pork sausages. It J Animal Sci 2016;15:358-74.
16. Cenci-Goga BT, Karama M, Sechi P, Iulietto MF, Novelli S, Selvaggini R, et al. Growth inhibition of selected microorganisms by an association of dairy starter cultures and probiotics. It J Animal Sci 2015;14:246-50.
17. Clementi F, Cenci-Goga BT, Trabalza-Marinucci M, Di Antonio E. Use of selected starter cultures in the production of farm manufactured goat cheese from thermized milk. It J Food Sci 1998;10:41-56.
18. Krockel L, Dederer I, Troeger K. Starter and Protective Cultures for Meat Products. Fleischwirtschaft 2011;91:93-8.
19. Hui YH. Hazard Analysis and Critical Control Point System. In: Hui YH (ed). Handbook of Meat and Meat Processing, 2nd Ed. CRC Press, 2012. pp. 741-767.
20. Leistner L, Gorris LG. Food Preservation by Hurdle Technology. Trends Food Sci Technol 1995;6:41-6.
21. [Verffentlichte Mikrobiologische Richt und Warnwerte zur Beurteilung von Lebensmitteln (Stand: November 2011) Eine Empfehlung der Fachgruppe Lebensmittelmikrobiologie und -hygiene der Deutschen Gesellschaft fr Hygiene und Mikrobiologie (DGHM).] Available from: http://www.dghm.org/m_275. [Website in German].
22. Kröckel L. [Mikrobiologisch-genetische Ressourcen bei Fleisch. Biodiversität und Nachhaltige Nutzung bei der Herstellung von Fleisch-Erzeugnissen.] Biologische Vielfalt und Ernährungsqualität 2010;1:24-6. [Article in German].
23. Cenci-Goga BT, Karama M, Sechi P, Iulietto MF, Novelli S, Mattei S. Evolution under different storage conditions of anomalous blue coloration of Mozzarella cheese intentionally contaminated with a pigment-producing strain of Pseudomonas fluorescens. J Dairy Sci 2014;97:6708-18.
24. Rossi C, Serio A, Chaves-López C, Anniballi F, Auricchio B, Goffredo E, et al. Biofilm formation, pigment production and motility in Pseudomonas spp. isolated from the dairy industry. Food Control 2018;86:241-8.