Use of bio-protective cultures to improve the shelf-life and the sensorial characteristics of commercial hamburgers

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Abstract

The use of mixtures of bio-protective cultures, like *Lactobacillus sakei* subsp. *carnosus/Lactobacillus sakei* + *Staphylococcus xylosus* (1/1 ratio), *Lactococcus lactis* spp. *lactis/Lactobacillus sakei* + *Staphylococcus xylosus* (1/1 ratio), and *Lactobacillus sakei* subsp. *carnosus/Lactobacillus sakei* + *Staphylococcus xylosus* (1/2 ratio), inoculated in beef hamburger packaged in modified atmosphere and stored at 4 ± 2 °C, determined a better microbiological and chemical-physical quality of the products. In particular, they inhibited the growth of *B. thermosphacta* resulting in no white slime on the products as well as they determined a low concentration of total volatile basic nitrogen (TVB-N). Moreover, the bio-protective cultures influenced the flavour and the odour of the hamburgers. For this reason, the shelf life of the products added with starter cultures could be extended up to 12 days.

Keywords: Hamburger, bio-protective cultures, shelf life.

1. Introduction

Muscle meat from healthy animals (Nychas et al., 2008) is usually free of microorganisms but is susceptible to microbial contamination by both pathogenic and spoilage bacteria, even up to the moment of cooking and consumption (Andritsos et al., 2012; Papadopoulouet al., 2011; Papadopoulou et al., 2012). The potential source of contamination depends on the condition of the animals before, during and after slaughter and the transportation, by marketing and consumer handling of the meat. Microorganisms such as *Pseudomonas* spp., *Brochothrix thermosphacta*, *Shewanella putrefaciens*, coagulase-negative cocci and *Enterobacteriaceae* can cause spoilage (Papadopoulou et al., 2012; Nychas et al., 2008; Xu et al., 2010; Russo et al., 2006). Contamination can also be caused by psychrotrophic and pathogenic species such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium perfringens*, *Campylobacter jejuni* and *Yersinia enterocolitica* and by enteropathogenic bacteria such as *Escherichia coli* and *Salmonella* spp. (Nastasijevic et al., 2009; Cloak et al., 2001; Stock & Stolle, 2001). Minced meat used for hamburger production, in particular, is a potentially hazardous substrates for bacterial growth and has a very short shelf life (Andritsos et al., 2012). The storage temperature and the packaging may influence the microbial quality of minced meat (von Holy & Holzapfel, 1988), as well as the effect of the type of retail outlet sampled and the season of analysis (Andritsos et al., 2012). It is well documented that *Pseudomonas* spp. dominates the microbial population of meat stored under aerobic conditions, while *B. thermosphacta* becomes the main spoilage microorganism for meat packaged in modified atmosphere (MAP) (Russo et al., 2006). *Enterobacteriaceae* and lactic acid bacteria (LAB) also contribute with *B. thermosphacta* to the spoilage of minced meat packaged in air, under vacuum or in MAP (Papadopoulou et al., 2012; Xu et al., 2010). The use of MAP, which contains oxygen and carbon dioxide, is intended to preserve and increase the shelf life of hamburgers. During refrigerated storage, the presence of high concentrations of oxygen (40-80%) causes the transformation of myoglobin into oxymyoglobin, a process that results in the bright red colour of meat (Lambert et al., 1991). The

carbon dioxide (20-30%) in MAP inhibits the growth of aerobic spoilage and pathogenic bacteria (Zakrys et al., 2009). The presence of oxygen maintains the attractive appearance of the burgers for a few days (Paleari et al., 2004; Scanga et al., 2000), but the burger colour eventually darkens due to the growth of aerobic bacteria (Zhao & Wells, 1994). Generally, high concentrations of CO_2, used in MAP, inhibits the growth of microorganisms, but higher concentrations are necessary to prevent the growth of aerobic spoilage bacteria (Paleari et al., 2004). This however results in a corresponding reduction in O_2 concentrations (< 60%) that further may lead to a loss of the bright red colour of meat (Paleari et al., 2004). For these reasons a proper balance of the two gasses is needed. Commercial hamburgers packaged in MAP and stored at refrigeration temperature have a shelf life of 7 days, as based on the expiry date assigned by the producers. Recently, combinations of hygienic quality control and protective technology have been used to improve and extend the shelf life of meat and meat products by limiting the growth of spoilage and pathogenic bacteria. Among the main protective technologies, bio-protective cultures are of particular concern (Comi et al., 2011; Vasilopoulos et al., 2010). Aim of this study was the evaluation of different mixtures of bio-protective cultures to improve the microbiological quality, the physical-chemical parameters and sensory attributes of beef hamburgers in order to extend their shelf life in MAP.

2. Materials and Methods

2.1. Sample preparation, storage condition and sampling methods

Meat cut from different anatomical parts of adult cattle were ground, mixed and divided into 4 batches of 50 kg each. The first batch, representing lot 1, was formed into patties, directly packaged and used as control. The other batches were inoculated with a mixture of LAB and coagulase-negative, catalase-positive cocci (CNCPC) at a final concentration of 10^5 CFU/g of product before

104 being formed into patties. Lot 2 was inoculated with a mix of *Lactobacillus sakei* subsp.
105 *carnosus*/*Lactobacillus sakei* + *Staphylococcus xylosus* at a ratio of 1/1. Lot 3 was inoculated with
106 *Lactococcus lactis* ssp. *lactis*/*Lactobacillus sakei* + *Staphylococcus xylosus* at a ratio of 1/1. Lot 4
107 was inoculated with *Lactobacillus sakei* subsp. *carnosus*/*Lactobacillus sakei* + *Staphylococcus*
108 *xylosus* at a ratio of 1/2. The burgers were packed in MAP, consisting of 70% O_2 and 30% CO_2, and
109 placed inside 15 x 10 x 3 cm rectangular trays of 200 μm in thickness made of PET/PE/EVOH/PE
110 ANTIFOG - EVOH. The trays were laminated with a top film consisting of APET/PE/EVOH/PE.
111 The packaged burgers were stored at 4 ± 2 ºC for 12 days in artificial light. At 0, 6, 9 and 12 days,
112 10 boxes were collected for microbiological, and physical-chemical analyses. All analyses were
113 conducted in duplicate on three replicates at each sampling point.
114
115 *2.2. Bacterial strains, preparation and inoculation of hamburgers*
116
117 *Lactobacillus sakei*, *Lactobacillus sakei* subsp. *carnosus*, *Lactococcus lactis* ssp. *lactis* and
118 *Staphylococcus xylosus* used in this experiment were obtained from the Italy branch supplier of Chr.
119 Hansen, Denmark. The lyophilised cultures were resuspended in peptone water [0.1% sodium
120 chloride and 0.7% peptone (Oxoid, Italy)] and left for 1 h at room temperature to rehydrate.
121 Subsequently, appropriate dilutions were made, and 1 ml of each dilution was placed in MRS agar
122 (de Man-Rogosa-Sharpe agar, pH 6.2, Oxoid, Italy) and incubated at 30°C for 48-72 h in a
123 microaerophilic conditions (gas pack anaerobic system, BBL, Becton Dickinson, USA). A
124 suspension of 10^7 CFU/ml was used to directly inoculate the ground meat (hamburgers), and the
125 final bacterial cell concentration was approximately 10^5 CFU/g hamburger.
126
127 *2.3. Microbiological analysis*
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The Total Viable Count (TVC) was enumerated onto Plate Count Agar (Oxoid, Italy) that was incubated at 30ºC for 48-72 h; LABs were grown in De Man Rogosa Sharpe (MRS) agar (Oxoid, Italy),incubated in microaerophilic conditions at 42ºC for 48 h; yeasts and moulds were grown on Malt Agar (MA) (Oxoid, Italy),incubated at 25ºC for 72-96 h; *Escherichia coli* was grown in Violet Red Bile Agar (VRBGA) (Oxoid, Italy), incubated at 44ºC for 24 h; Coagulase positive staphylococci were grown on Baird-Parker agar medium (BP) (Oxoid, Italy), supplemented with egg yolk tellurite emulsion (Oxoid, Italy) and incubated at 35ºC for 24-48 h after confirmation with a coagulase test. *Brochothrix thermosphacta* was enumerated in streptomycin-sulfate-thallous acetate-cycloheximide agar (SSTAA, Oxoid, Italy) with selective supplement SR 151 (Oxoid, Italy), following incubation at 22ºC for 48-96 h. Sulphite-reducing clostridia were quantified in Differential Reinforced Clostridia Medium (DRCM) (VWR, USA), incubated at 37ºC for 24-48 h in an anaerobic jar with an anaerobic kit (gas pack anaerobic system, BBL, Becton Dickinson, USA). *Campylobacter jejuni*, *Campylobacter coli* (ISO 10272), *Salmonella* spp. (ISO 6579), *Listeria monocytogenes* (ISO 11290-1), and *Yersinia enterocolitica* (ISO 10273) were detected according to the recommended methods for the microbiological analysis of foods (Lombardy Region – Official Bulletin of the Lombardy Region, 4th Suppl. Extraordinary No. 24, June 17th 1995 and methods OM 7/12/93).

2.4. pH measurements

The pH value was measured in 10 different positions for each product using a pH meter (Basic 20, Crison Instruments, Spain). The pH values were measured from the product directly by inserting a pH meter probe into the sample.

2.5. Total Volatile Basic Nitrogen (TVB-N) measurements

155 The total volatile basic nitrogen (TVB-N) was evaluated by the method proposed by Pearson
156 (1973).
157
158 *2.6. Colour measurements*
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160 The colour was measured using a Minolta Chromameter CR-200 and the CIE Lab system. After
161 calibration with standard white tiles, the Chromameter was positioned perpendicular to the patty
162 surface, and 10 different positions were evaluated for each sample immediately after the package
163 was opened. The evaluated parameters were L*, a* and b*. L* describes the white intensity or
164 brightness, with values ranging from 0 (black) to 100 (white). The a* value describes the redness
165 (a* > 0), and b* describes the yellowness (b* > 0). The final value was expressed as the respective
166 average of ten measurements.
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168 *2.7. Sensory analysis*
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170 Sensory analyses were performed by 12 non-professional panellists. Cooked burgers from 4 lots,
171 containing each 10 packages, were evaluated. The panellists were asked to identify the products in
172 descending order from the best to the worst, taking into account the following parameters: odour
173 (fermented, rancid), taste (sweet, sour, fresh, pungent, meat-taste, rancid) and flavour (ammonia,
174 sweet, sour, bitter) (Vàlkovà et al., 2007; Baublis, et al., 2005).
175
176 *2.8. Statistical analysis*
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178 The values of the various parameters were compared using a one-way analysis of variance. The
179 averages were compared with the Tukey's honest significant test using the StatGraphics software
180 package from Statistical Graphics (Rockville, Maryland).

3. Results and discussion

The results of the microbiological analyses and the pH determination of the different batches are shown Table 1.

The inoculation level of LAB (lot 2, 3 and 4) was at T0 between 4.82 and 5.01 log CFU/g. These level rapidly increased, regardless of the presence of 70% O_2 in the MAP, reaching after 6 days loads between 6.16 and 7.65 Log CFU/g and after 9 and 12 days respectively loads between 7.68 and 8.88 Log CFU/g and loads between 8.05 and 8.90 Log CFU/g, respectively.

Considering the control lot 1, the initial LAB was almost 4 log CFU/g, in agreement with those obtained by previous authors (Paleari, et al., 2004). However, the concentration of LAB increased. Considering TVC, at T0, lot 1 (5.90±0.15 Log CFU/g) and lot 2 (5.90±0.55 Log CFU/g) resulted to have significantly lower loads if compared to lot 3 (6.60±0.11 Log CFU/g) and lot 4 (7.00±0.05 Log CFU/g). The higher TVC found in lot 3 and 4 is not related to the starter inoculation, as this was added to the meat at 5 log CFU/g but probably to the variability of the raw material. These values differed from those obtained by other authors for minced meat marketed in Italy: Paleari et al., (2004) observed TVC values lower than 5 log CFU/g in ground meat, increasing up to 8 and 9 log CFU/g, despite the presence of CO_2 in MAP at the end of the storage period. The same results were obtained by Andritsos et al., (2012) in minced pork prepared at retail stores in Greece: in that case, the psychrotrophic microorganisms, i.e., *B. thermosphacta* and *Pseudomonas* spp. mainly composed the initial microflora. *Pseudomonas* spp., generally have an advantageous growth rate in the presence of an aerobic atmosphere and became the main spoilage microorganisms at the refrigerated temperature. Their growth is ususally followed by *B. thermosphacta* and LAB growth, and together, these microorganisms represent the main species responsible for the reduced shelf life of minced meat in aerobic conditions (Kammenou et al., 2004; Koutsoumanis et al., 2012).

B. thermosphacta, the typical psychotropic microorganism responsible for the spoilage of meat products and refrigerated meat products grew in all the hamburgers. In the control products (lot 1)

this microorganism grew constantly from 2.40 Log CFU/g at the beginning of the trial reaching the level of 4.47 at T12. In lot 2 and 3 a significantly lower increase was revealed, if compare to lot 1 attesting an increase from T0 to T12 of 0.68 and 1.37 log CFU/g respectively; in any case the loads never overcame the level of 4 Log CFU/g for the whole period. Considering lot 4, a very limited increased was observe from T0 till the end of the trial (0.38 Log CFU/g). As a matter of fact, the bio-protective cultures partially inhibited the growth of *B. thermosphacta*. Similar results have also been obtained in different products by other authors (Andritsos et al., 2012; Papadopoulou et al., 2012).

The initial yeast concentration was between 1.7 and 2.5 log CFU/g. Lot 3 appeared initially to be the less contaminated by yeast but increased 1 log CFU/g by day 12, becoming the most contaminated; in any case it never overcame the level of 3.1 Log CFU/g. The yeast counts for lots 1, 2, and 4 remained constant over 12 days.

Clostridium H_2S producers, *Staphylococcus aureus* and *Escherichia coli* were below the threshold limit of the detection method (1 log CFU/g), while classical meat pathogens such as *Salmonella* spp., *Listeria monocytogenes, Campylobacter* spp. and *Yersinia enterocolitica* were absent in 25 g foer the whole period. The absence of *L. monocytogenes* and *Salmonella* spp. seems unexpected because it is estimated that at least 10% of fresh meat is contaminated with *L. monocytogenes* and approximately 6-20% of meat is contaminated with *Salmonella* spp. despite the application of strict microbiological hygienic controls (Cloak et al., 2001; Stock & Stolle, 2001). In fact, our data were different from those obtained by other authors: Marino et al., (1995), found that the presence of *Escherichia coli* exceeded the limit imposed by the current EEC Regulation 2073/2005 (Anonymous, 2005) in some of the analysed samples. The same results were obtained by Adritsos et al., (2012). However, the absence of *S. aureus* and *Clostridium* H_2S+ producers observed, was in agreement with the findings by Marino et al., (1995); *S. aureus,* in particular is often associated with human contamination due to poor hygienic conditions during handling of the product (Adritsos et al., 2012).

The results of the pH and total volatile basic nitrogen (TVB-N) are reported in Table 1. Lots inoculated with bio-protective cultures showed a lower starting pH if compared to lot 1 and demonstrated a constant decrease of the pH during the whole period.

Considering the TVB-N, in lot 1 significantly higher increase over time was observed, overcoming the limit of 30 mg nitrogen/100 g, suggested for fishery products by Commission Decision 95/149/EC from 8 March 1995, from T9. Lots 2, 3, and 4 never overcame this limit up to 12 days of storage. It is plausible that competition caused by the bio-protective starters slowed and/or inhibited the spoilage and consequently reduced the production of TVB-N.

Table 2 shows the results of the colour evaluation using the L*, a* and b* parameters at days 0, 6, 9 and 12. No significant differences were observed between lot 1 and the other lots. Until day 12, the L*, a* and b* parameters were similar between the lots. As expected, there were no significant colour changes in the hamburgers from all lots after 12 days of storage. During this time, the hamburgers discoloured due to the oxidation of myoglobin caused by the presence of oxygen in the MAP. However, visual analysis determined that the colour of the hamburgers in lots 3 and 4 was more attractive than that of lots 1 and 2 at day 12. Table 4 describes the hamburgers colours at day 0 and 12.

Considering microbial and TVB-N results, hamburgers were acceptable for up to 12 days of storage at 4 ± 2 ºC.

The sensory analysis supported this conclusion. Table 3 shows that the bio-protective cultures improved the sensory attributes of the hamburgers. Hamburgers with bio-protective cultures did not present odours, flavours or sticky white slime that are indicative of spoilage. In contrast, a sticky white slime was observed in some hamburgers from lots 1 and 2. In lot 2, this may have been due to the rapid growth of the bio-protective cultures, as the concentration of LAB (9 log CFU/g) was significantly higher than in the other lots ($p < 0.05$). The panellists preferred the taste of the hamburgers from lots 3 and 4, which contained bio-protective inoculations of *Lactococcus lactis* spp. *lactis*/*Lactobacillus sakei* + *Staphylococcus xylosus* (ratio of 1/1) and *Lactobacillus sakei*

subsp. *carnosus*/*Lactobacillus sakei* + *Staphylococcus xylosus* (ratio of 1/2), respectively. These microorganisms seemed to have improved the sensory quality of the hamburgers and inhibited the growth of autochthonous bacteria (Table 1).

Oxidation of meat pigments was not observed in the four lots of hamburgers regardless of the presence of bio-protective cultures. However, these findings are beneficial for the elimination of slimes, discolouration and browning caused by autochthonous LAB.

4. Conclusions

Bio-protective cultures, used as mixed cultures of *Lactobacillus sakei* subsp. *carnosus*/*Lactobacillus sakei* + *Staphylococcus xylosus* (1/1 ratio), *Lactococcus lactis* spp. *lactis*/*Lactobacillus sakei* + *Staphylococcus xylosus* (1/1 ratio), and *Lactobacillus sakei* subsp. *carnosus*/*Lactobacillus sakei* + *Staphylococcus xylosus* (1/2 ratio), could be employed as bio-protective cultures for beef hamburger. These cultures inhibited the growth of *Brochothrix thermosphacta*, determining an improvement of the microbial and organoleptic qualities of the meat. Bio-protective cultures, which inhibited the spoilage bacteria, were able to reduce the TVB-N to values below 30 mg nitrogen/100 g. The sensory traits of the hamburgers were positively influenced by the presence of the bio-protective cultures, as the odours, flavours, and the sticky white slime, that are indicative of deterioration, were not observed in the inoculated samples. The bio-protective cultures evaluated in this study can potentially extend the shelf life up to 12 days and improve the sensory properties of hamburger meat.

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Table 1: Microbiological analysis results (log CFU/g), pH, and TVB-N (mg N/100) in hamburgers of the different lots.

Days of storage	Parameter	Lot 1 Control	Lot 2 L.c./L.s./S.x.	Lot 3 Lac./L.s./S.x	Lot 4 L.c./L.s./S.x.
0	TVC	5.90 ± 0.15a	5.90 ± 0.55a	6.60 ± 0.11b	7.00 ± 0.05c
	Yeast	2.30 ± 0.22a	2.53 ± 0.32a	1.70 ± 0.25b	2.10 ± 0.14a
	LAB	3.63± 0.26a	4.90 ± 0.55b	4.82 ± 0.26b	5.01 ± 0.40b
	B. thermosphacta	2.40 ± 0.15a	2.90 ± 0.05b	1.70 ± 0.12c	2.00 ± 0.21d
	pH	6.16 ± 0.75a	5.93 ± 0.40b	5.81 ± 0.11c	5.70 ± 0.21c
	TVB-N	16.9 ± 1.5a	15.4 ± 2.5a	15.4 ± 1.3a	15.3 ± 3.3a
6	TVC	7.43 ± 0.05a	7.40 ± 0.20a	8.05 ± 0.21b	8.14 ± 0.21b
	Yeast	2.36 ± 0.22a	2.55 ± 0.50a	1.90 ± 0.30a	2.37 ± 0.10a
	LAB	4.44 ± 0.20a	7.65 ± 0.50b	6.97 ± 0.10c	6.16 ± 0.22d
	B. thermosphacta	3.30 ± 0.11a	3.39 ± 0.30a	2.86 ± 0.11b	3.39 ± 0.12a
	pH	5.68 ± 0.35a	5.59 ± 0.40a	5.47 ± 0.32a	5.31 ± 0.25a
	TVB-N	24.5 ± 3.3a	21.5 ± 2.2a	19.2 ± 3.2a	20.2 ± 3.6a
9	TVC	8.80 ± 0.15a	8.40 ± 0.22b	8.85 ± 0.50ab	8.37 ± 0.11b
	Yeast	2.67 ± 0.17a	2.61 ± 0.78ab	1.97 ± 0.23b	2.47 ± 0.30a
	LAB	6.53 ± 0.30a	7.68 ± 0.11b	8.88 ± 0.15c	7.90 ± 0.35a
	B. thermosphacta	4.60 ± 0.51a	3.50 ± 0.40b	2.89 ± 0.30c	2.44 ± 0.45c
	pH	5.88 ± 0.50a	5.98 ± 0.30a	5.64 ± 0.50a	5.64 ± 0.45a
	TVB-N	35.3 ± 4.2a	25.4 ± 3.4b	21.4 ± 3.5b	22.5 ± 3.3b
12	TVC	8.97 ± 0.11a	8.59 ± 0.51ab	8.63 ± 0.33ab	8.54 ± 0.15b
	Yeast	2.50 ± 0.40ab	2.68 ± 0.15a	3.09 ± 0.25b	2.45 ± 0.45a
	LAB	7.37 ± 0.15a	8.90 ± 0.40b	8.05 ± 0.17c	8.36 ± 0.30bc
	B. thermosphacta	4.47 ± 0.22a	3.58 ± 0.26b	3.07 ± 0.11c	2.38 ± 0.13d
	pH	5.98 ± 0.40a	5.78 ± 0.10a	5.46 ± 0.25a	5.47 ± 0.25a
	TVBN	43.2 ± 5.1a	28.4 ± 4.3b	24.3 ± 6.3b	25.1 ± 6.3b

Legend: TVC: Total viable count; LAB: Lactic acid bacteria. TVB-N: Total Volatile Basic Nitrogen. Control (Lot 1): no starter; L.c/L.s./S.x (Lot 2): *Lactobacillus sakei subsp. carnosus*/*Lactobacillus sakei* + *Staphylococcus xylosus* (1/1 ratio); Lac./L.s./S.x. (Lot 3): *Lactococcus lacts ssp. lactis*/*Lactobacillus sakei* + *Staphylococcus xylosus* (1/1 ratio); Lc./L.s./S.x. (Lot 4): *Lactobacillus sakei subsp. carnosus*/*Lactobacillus sakei* + *Staphylococcus xylosus* (1/2 ratio). Data represent the means ± standard deviations of the total samples; Mean with the same letters within a row (following the values) are not significantly differently ($p < 0.05$)

Table 2: Color analysis results of hamburgers treated with bio-protective culture.

Day	Parameter	Lot 1 Control	Lot 2 L.c./L.s./S.x.	Lot 3 Lac./L.s./S.x	Lot 4 L.c./L.s./S.x.
0	L*	43.20 ± 1.20a	44.12 ±4.63a	41.89± 5.19a	44.99 ±5.70a
	a*	14.12 ±1.74a	15.46 ± 1.62a	15.19 ± 3.33a	16.11 ± 0.81a
	b*	5.13±1.97a	5.74 ±1.35a	6.57 ±0.34a	5.90 ±0.28a
6	L*	43.80 ±0.06a	42.33 ±1.96a	44.38 ±0.80a	43.98 ±0.09a
	a*	16.46 ±0.57a	14.25 ±0.12b	16.09 ± 0.87a	15.53 ±0.85a
	b*	5.46 ±0.57a	5.25 ±0.12b	6.09 ± 0.87a	5.53 ±0.85a
9	L*	45.84 ±114a	44.00 ±1.48a	45.52 ±0.56a	45.23 ±1.73a
	a*	16.08 ±4.86a	16.26 ± 3.54a	16.88 ±0.69a	16.00 ±2.65a
	b*	5.77 ±0.70a	5.73±0.77a	5.71± 1.09a	5.97 ±0.99a
12	L*	42.78 ±2.83a	41.82 ±0.95a	40.41 ±4.02a	43.57 ±4.02a
	a*	16.40 ±2.50a	16.22 ±1.41a	17.37 ±1.51a	16.67 ±1.19a
	b*	5.80 ± 1.30a	6.08 ±1.42a	6.40 ±0.19a	5.58 ±0.83a

Legend: Index L*, lightness; a*, redness; b*, yellowness; Control (Lot 1): no starter; L.c/L.s./S.x (Lot 2): *Lactobacillus sakei subsp. carnosus*/*Lactobacillus sakei* + *Staphylococcus xylosus* (1/1 ratio); Lac./L.s./S.x. (Lot 3): *Lactococcus lacts ssp. lactis*/*Lactobacillus sakei* + *Staphylococcus xylosus* (1/1 ratio); Lc./L.s./S.x. (Lot 4): *Lactobacillus sakei subsp. carnosus*/*Lactobacillus sakei* + *Staphylococcus xylosus* (1/2 ratio). Microbial data log CFU/g. Data represent the means ± standard deviations of the total samples; Mean with the same letters within a row (following the values) are not significantly differently ($p < 0.05$).

Table 3: The sensory panel scores of cooked hamburgers.

Sensory attribute	Lot 1 Control	Lot 2 L.c./L.s./S.x.	Lot 3 Lac./L.s./S.x	Lot 4 L.c./L.s./S.x.
Fermentation	10/12	3/12	4/12	4/12
Rancid	5/12	4/12	3/12	3/12
Sweet	2/12	5/12	5/12	4/12
Pungent	10/12	5/12	5/12	5/12
Meat	3/12	6/12	6/12	9/12
Sour	6/12	6/12	7/12	7/12
Bitter	9/12	6/12	3/12	5/12
Ammonia	12/12	6/12	3/12	4/12
Slimes	7/12	7/12	4/12	5/12
Final scores*	**4**	**3**	**1**	**2**

*Final scores: the panellists requested to ranked the products within the scale from 1 (excellent) to 4 (worst).
Control (Lot 1): no starter; L.c/L.s./S.x (Lot 2): *Lactobacillus sakei subsp. carnosus*/*Lactobacillus sakei* + *Staphylococcus xylosus* (1/1 ratio); Lac./L.s./S.x. (Lot 3): *Lactococcus lacts ssp. lactis*/*Lactobacillus sakei* + *Staphylococcus xylosus* (1/1 ratio); Lc./L.s./S.x. (Lot 4): *Lactobacillus sakei subsp. carnosus*/*Lactobacillus sakei* + *Staphylococcus xylosus* (1/2 ratio).

Table 4. Pictures of the hamburgers at 0 and 12 days of storage.

Day	Control (no starter)	L.c/L.s./S.x ratio 1/1 (Lot 2)	Lac./L.s./S.x ratio 1/1 (Lot 3)	Lc./L.s./S.x. ratio 1/2 (Lot 4)
0				
12