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 Original

 Availability:

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 Publisher:

 Published

 DOI:10.1016/j.lwt.2015.02.022

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| 1 | Use of bio-protective cultures to improve the shelf-life and the sensorial characteristics of |
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| 2 | commercial hamburgers |
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| 4 | Giuseppe Comia, Erica Tirlonib, Debbie Adyantoa, Marisa Manzanoa, Lucilla Iacumina* |
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| 6 | ^a Department of Food Science, University of Udine, Via Sondrio 2/A, 33100 Udine, Italy. |
| 7 | bDepartment of Health, Animal Science and Food Safety, Università degli Studi di Milano, via |
| 8 | Celoria 10, 20133, Milano, Italy |
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| 15 | *Corresponding author: |
| 16 | Lucilla Iacumin |
| 17 | Dipartimento di Scienze degli Alimenti, Università degli Studi di Udine |
| 18 | Via Sondrio 2/A |
| 19 | 33100 Udine, Italy |
| 20 | e-mail: lucilla.iacumin@uniud.it |
| 21 | Phone: +39 0432 558126 |
| 22 | Fax: +39 0432 558130. |
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- 26 Abstract

| 28 | The use of mixtures of bio-protective cultures, like Lactobacillus sakei subsp. |
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| 29 | carnosus/Lactobacillus sakei + Staphylococcus xylosus (1/1 ratio), Lactococcus lactis spp. |
| 30 | lactis/Lactobacillus sakei + Staphylococcus xylosus (1/1 ratio), and Lactobacillus sakei subsp. |
| 31 | carnosus/Lactobacillus sakei + Staphylococcus xylosus (1/2 ratio), inoculated in beef hamburger |
| 32 | packaged in modified atmosphere and stored at 4 \pm 2 °C, determined a better microbiological and |
| 33 | chemical-physical quality of the products. In particular, they inhibited the growth of <i>B</i> . |
| 34 | thermosphacta resulting in no white slime on the products as well as they determined a low |
| 35 | concentration of total volatile basic nitrogen (TVB-N). Moreover, the bio-protective cultures |
| 36 | influenced the flavour and the odour of the hamburgers. For this reason, the shelf life of the |
| 37 | products added with starter cultures could be extended up to 12 days. |
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| 40 | Keywords: Hamburger, bio-protective cultures, shelf life. |
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1. Introduction

53 Muscle meat from healthy animals (Nychas et al., 2008) is usually free of microorganisms but is 54 susceptible to microbial contamination by both pathogenic and spoilage bacteria, even up to the 55 moment of cooking and consumption (Andritsos et al., 2012; Papadopoulouet al., 2011; 56 Papadopoulou et al., 2012). The potential source of contamination depends on the condition of 57 the animals before, during and after slaughter and the transportation, by marketing and 58 consumer handling of the meat. Microorganisms such as *Pseudomonas* spp., *Brochothrix* 59 thermosphacta, Shewanella putrefaciens, coagulase-negative cocci and Enterobacteriaceae can 60 cause spoilage (Papadopoulou et al., 2012; Nychas et al., 2008; Xu et al., 2010; Russo et al., 61 2006). Contamination can also be caused by psychrotrophic and pathogenic species such as 62 Staphylococcus aureus, Listeria monocytogenes, Clostridium perfringens, Campylobacter jejuni 63 and Yersinia enterocolitica and by enteropathogenic bacteria such as Escherichia coli and 64 Salmonella spp. (Nastasijevic et al., 2009; Cloak et al., 2001; Stock & Stolle, 2001). Minced 65 meat used for hamburger production, in particular, is a potentially hazardous substrates for 66 bacterial growth and has a very short shelf life (Andritsos et al., 2012). The storage temperature 67 and the packaging may influence the microbial quality of minced meat (von Holy & Holzapfel, 68 1988), as well as the effect of the type of retail outlet sampled and the season of analysis 69 (Andritsos et al., 2012). It is well documented that *Pseudomonas* spp. dominates the microbial 70 population of meat stored under aerobic conditions, while *B. thermosphacta* becomes the main 71 spoilage microorganism for meat packaged in modified atmosphere (MAP) (Russo et al., 2006). 72 Enterobacteriaceae and lactic acid bacteria (LAB) also contribute with B. thermosphacta to the 73 spoilage of minced meat packaged in air, under vacuum or in MAP (Papadopoulou et al., 2012; 74 Xu et al., 2010). The use of MAP, which contains oxygen and carbon dioxide, is intended to 75 preserve and increase the shelf life of hamburgers. During refrigerated storage, the presence of 76 high concentrations of oxygen (40-80%) causes the transformation of myoglobin into 77 oxymyoglobin, a process that results in the bright red colour of meat (Lambert et al., 1991). The

| 78 | carbon dioxide (20-30%) in MAP inhibits the growth of aerobic spoilage and pathogenic |
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| 79 | bacteria (Zakrys et al., 2009). The presence of oxygen maintains the attractive appearance of the |
| 80 | burgers for a few days (Paleari et al., 2004; Scanga et al., 2000), but the burger colour |
| 81 | eventually darkens due to the growth of aerobic bacteria (Zhao & Wells, 1994). Generally, high |
| 82 | concentrations of CO ₂ , used in MAP, inhibits the growth of microorganisms, but higher |
| 83 | concentrations are necessary to prevent the growth of aerobic spoilage bacteria (Paleari et al., |
| 84 | 2004). This however results in a corresponding reduction in O ₂ concentrations (< 60%) that |
| 85 | further may lead to a loss of the bright red colour of meat (Paleari et al., 2004). For these |
| 86 | reasons a proper balance of the two gasses is needed. Commercial hamburgers packaged in |
| 87 | MAP and stored at refrigeration temperature have a shelf life of 7 days, as based on the expiry |
| 88 | date assigned by the producers. Recently, combinations of hygienic quality control and |
| 89 | protective technology have been used to improve and extend the shelf life of meat and meat |
| 90 | products by limiting the growth of spoilage and pathogenic bacteria. Among the main protective |
| 91 | technologies, bio-protective cultures are of particular concern (Comi et al., 2011; Vasilopoulos |
| 92 | et al., 2010). Aim of this study was the evaluation of different mixtures of bio-protective |
| 93 | cultures to improve the microbiological quality, the physical-chemical parameters and sensory |
| 94 | attributes of beef hamburgers in order to extend their shelf life in MAP. |
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| 96 | 2. Materials and Methods |
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| 98 | 2.1. Sample preparation, storage condition and sampling methods |
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| 100 | Meat cut from different anatomical parts of adult cattle were ground, mixed and divided into 4 |
| 101 | batches of 50 kg each. The first batch, representing lot 1, was formed into patties, directly packaged |
| 102 | and used as control. The other batches were inoculated with a mixture of LAB and coagulase- |

103 negative, catalase-positive cocci (CNCPC) at a final concentration of 105 CFU/g of product before

| 104 | being formed into patties. Lot 2 was inoculated with a mix of Lactobacillus sakei subsp. |
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| 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% O2 and 30% CO2, and |
| 109 | placed inside 15 x 10 x 3 cm rectangular trays of 200 μ m 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 °C 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. |
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| 115 | 2.2. Bacterial strains, preparation and inoculation of hamburgers |
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| 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 107 CFU/ml was used to directly inoculate the ground meat (hamburgers), and the |
| 125 | final bacterial cell concentration was approximately 105 CFU/g hamburger. |
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| 127 | 2.3. Microbiological analysis |

| 129 | The Total Viable Count (TVC) was enumerated onto Plate Count Agar (Oxoid, Italy) that was |
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| 130 | incubated at 30°C for 48-72 h; LABs were grown in De Man Rogosa Sharpe (MRS) agar (Oxoid, |
| 131 | Italy), incubated in microaerophilic conditions at 42°C for 48 h; yeasts and moulds were grown on |
| 132 | Malt Agar (MA) (Oxoid, Italy), incubated at 25°C for 72-96 h; Escherichia coli was grown in Violet |
| 133 | Red Bile Agar (VRBGA) (Oxoid, Italy), incubated at 44°C for 24 h; Coagulase positive |
| 134 | staphylococci were grown on Baird-Parker agar medium (BP) (Oxoid, Italy), supplemented with |
| 135 | egg yolk tellurite emulsion (Oxoid, Italy) and incubated at 35°C for 24-48 h after confirmation with |
| 136 | a coagulase test. Brochothrix thermosphacta was enumerated in streptomycin-sulfate-thallous |
| 137 | acetate-cycloheximide agar (SSTAA, Oxoid, Italy) with selective supplement SR 151 (Oxoid, |
| 138 | Italy), following incubation at 22°C for 48-96 h. Sulphite-reducing clostridia were quantified in |
| 139 | Differential Reinforced Clostridia Medium (DRCM) (VWR, USA), incubated at 37°C for 24-48 h |
| 140 | in an anaerobic jar with an anaerobic kit (gas pack anaerobic system, BBL, Becton Dickinson, |
| 141 | USA). Campylobacter jejuni, Campylobacter coli (ISO 10272), Salmonella spp. (ISO 6579), |
| 142 | Listeria monocytogenes (ISO 11290-1), and Yersinia enterocolitica (ISO 10273) were detected |
| 143 | according to the recommended methods for the microbiological analysis of foods (Lombardy |
| 144 | Region – Official Bulletin of the Lombardy Region, 4th Suppl. Extraordinary No. 24, June 17th |
| 145 | 1995 and methods OM 7/12/93). |
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147 2.4. pH measurements

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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.

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153 2.5. Total Volatile Basic Nitrogen (TVB-N) measurements

155 The total volatile basic nitrogen (TVB-N) was evaluated by the method proposed by Pearson156 (1973).

157

158 2.6. Colour measurements

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160The colour was measured using a Minolta Chromameter CR-200 and the CIE Lab system. After161calibration with standard white tiles, the Chromameter was positioned perpendicular to the patty162surface, and 10 different positions were evaluated for each sample immediately after the package163was opened. The evaluated parameters were L*, a* and b*. L* describes the white intensity or164brightness, with values ranging from 0 (black) to 100 (white). The a* value describes the redness165(a* > 0), and b* describes the yellowness (b* > 0). The final value was expressed as the respective166average of ten measurements.

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Sensory analyses were performed by 12 non-professional panellists. Cooked burgers from 4 lots,
containing each 10 packages, were evaluated. The panellists were asked to identify the products in
descending order from the best to the worst, taking into account the following parameters: odour
(fermented, rancid), taste (sweet, sour, fresh, pungent, meat-taste, rancid) and flavour (ammonia,
sweet, sour, bitter) (Vàlkovà et al., 2007; Baublis, et al., 2005).

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176 2.8. Statistical analysis

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The values of the various parameters were compared using a one-way analysis of variance. The averages were compared with the Tukey's honest significant test using the StatGraphics software package from Statistical Graphics (Rockville, Maryland).

^{168 2.7.} Sensory analysis

181 **3. Results and discussion**

182

- 183 The results of the microbiological analyses and the pH determination of the different batches are184 shown Table 1.
- 185 The inoculation level of LAB (lot 2, 3 and 4) was at T0 between 4.82 and 5.01 log CFU/g. These
- 186 level rapidly increased, regardless of the presence of 70% O₂ in the MAP, reaching after 6 days
- 187 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.
- 189 Considering the control lot 1, the initial LAB was almost 4 log CFU/g, in agreement with those
- 190 obtained by previous authors (Paleari, et al., 2004). However, the concentration of LAB increased.
- 191 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
- 193 Log CFU/g). The higher TVC found in lot 3 and 4 is not related to the starter inoculation, as this
- 194 was added to the meat at 5 log CFU/g but probably to the variability of the raw material. These

195 values differed from those obtained by other authors for minced meat marketed in Italy: Paleari et

196 al., (2004) observed TVC values lower than 5 log CFU/g in ground meat, increasing up to 8 and 9

197 log CFU/g, despite the presence of CO₂ in MAP at the end of the storage period. The same results

198 were obtained by Andritsos et al., (2012) in minced pork prepared at retail stores in Greece: in that

199 case, the psychrotrophic microorganisms, i.e., *B. thermosphacta* and *Pseudomonas* spp. mainly

200 composed the initial microflora. *Pseudomonas* spp., generally have an advantageous growth rate in

201 the presence of an aerobic atmosphere and became the main spoilage microorganisms at the

202 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).
- 205 *B. thermosphacta*, the typical psychotropic microorganism responsible for the spoilage of meat
- 206 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 207 208 level of 4.47 at T12. In lot 2 and 3 a significantly lower increase was revealed, if compare to lot 1 209 attesting an increase from T0 to T12 of 0.68 and 1.37 log CFU/g respectively; in any case the loads 210 never overcame the level of 4 Log CFU/g for the whole period. Considering lot 4, a very limited 211 increased was observe from T0 till the end of the trial (0.38 Log CFU/g). As a matter of fact, the 212 bio-protective cultures partially inhibited the growth of *B. thermosphacta*. Similar results have also 213 been obtained in different products by other authors (Andritsos et al., 2012; Papadopoulou et al., 214 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.

219 Clostridium H2S producers, Staphylococcus aureus and Escherichia coli were below the threshold 220 limit of the detection method (1 log CFU/g), while classical meat pathogens such as Salmonella 221 spp., Listeria monocytogenes, Campylobacter spp. and Yersinia enterocolitica were absent in 25 g 222 foer the whole period. The absence of L. monocytogenes and Salmonella spp. seems unexpected 223 because it is estimated that at least 10% of fresh meat is contaminated with L. monocytogenes and 224 approximately 6-20% of meat is contaminated with Salmonella spp. despite the application of strict 225 microbiological hygienic controls (Cloak et al., 2001; Stock & Stolle, 2001). In fact, our data were 226 different from those obtained by other authors: Marino et al., (1995), found that the presence of 227 Escherichia coli exceeded the limit imposed by the current EEC Regulation 2073/2005 228 (Anonymous, 2005) in some of the analysed samples. The same results were obtained by Adritsos 229 et al., (2012). However, the absence of S. aureus and Clostridium H₂S+ producers observed, was in agreement with the findings by Marino et al., (1995); S. aureus, in particular is often associated 230 231 with human contamination due to poor hygienic conditions during handling of the product (Adritsos 232 et al., 2012).

233 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

235 demonstrated a constant decrease of the pH during the whole period.

236 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

238 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 inhibitedthe 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

246 MAP. However, visual analysis determined that the colour of the hamburgers in lots 3 and 4 was

247 more attractive than that of lots 1 and 2 at day 12. Table 4 describes the hamburgers colours at day

248 0 and 12.

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

251 The sensory analysis supported this conclusion. Table 3 shows that the bio-protective cultures 252 improved the sensory attributes of the hamburgers. Hamburgers with bio-protective cultures did not 253 present odours, flavours or sticky white slime that are indicative of spoilage. In contrast, a sticky 254 white slime was observed in some hamburgers from lots 1 and 2. In lot 2, this may have been due to 255 the rapid growth of the bio-protective cultures, as the concentration of LAB (9 log CFU/g) was 256 significantly higher than in the other lots (p < 0.05). The panellists preferred the taste of the 257 hamburgers from lots 3 and 4, which contained bio-protective inoculations of Lactococcus lactis 258 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.

265

266 4. Conclusions

267 Bio-protective cultures, used as mixed cultures of *Lactobacillus sakei* subsp.

268 *carnosus/Lactobacillus sakei* + *Staphylococcus xylosus* (1/1 ratio), *Lactococcus lactis* spp.

269 *lactis/Lactobacillus sakei* + *Staphylococcus xylosus* (1/1 ratio), and *Lactobacillus sakei* subsp.

270 carnosus/Lactobacillus sakei + Staphylococcus xylosus (1/2 ratio), could be employed as bio-

271 protective cultures for beef hamburger. These cultures inhibited the growth of *Brochothrix*

272 *thermosphacta*, determining an improvement of the microbial and organoleptic qualities of the

273 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 andimprove the sensory properties of hamburger meat.

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280 **References**

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

368 of the different lots.

| Days of | Parameter | Lot 1 | Lot 2 | Lot 3 | Lot 4 |
|---------|---------------|--------------------|--------------------|--------------------|-----------------------|
| storage | | Control | L.c./L.s./S.x. | Lac./L.s./S.x | L.c./L.s./S.x. |
| 0 | TVC | $5.90 \pm 0.15a$ | $5.90 \pm 0.55a$ | $6.60 \pm 0.11b$ | $7.00 \pm 0.05c$ |
| | Yeast | $2.30 \pm 0.22a$ | $2.53 \pm 0.32a$ | $1.70\pm0.25b$ | $2.10 \pm 0.14a$ |
| | LAB | $3.63 \pm 0.26a$ | $4.90\pm0.55b$ | $4.82\pm0.26b$ | $5.01\pm0.40b$ |
| | В. | $2.40 \pm 0.15a$ | $2.90\pm0.05b$ | $1.70 \pm 0.12c$ | $2.00\pm0.21d$ |
| | thermosphacta | | | | |
| | pH | $6.16 \pm 0.75a$ | $5.93 \pm 0.40 b$ | $5.81 \pm 0.11c$ | $5.70 \pm 0.21c$ |
| | TVB-N | $16.9 \pm 1.5a$ | $15.4 \pm 2.5a$ | $15.4 \pm 1.3a$ | $15.3 \pm 3.3a$ |
| 6 | TVC | $7.43 \pm 0.05a$ | $7.40 \pm 0.20a$ | $8.05 \pm 0.21b$ | $8.14\pm0.21\text{b}$ |
| | Yeast | $2.36 \pm 0.22a$ | $2.55\pm0.50a$ | $1.90 \pm 0.30a$ | $2.37 \pm 0.10a$ |
| | LAB | $4.44\pm0.20a$ | $7.65\pm0.50b$ | $6.97\pm0.10c$ | $6.16 \pm 0.22d$ |
| | В. | $3.30 \pm 0.11a$ | $3.39 \pm 0.30a$ | $2.86 \pm 0.11b$ | $3.39 \pm 0.12a$ |
| | thermosphacta | | | | |
| | pH | $5.68 \pm 0.35a$ | $5.59 \pm 0.40a$ | $5.47 \pm 0.32a$ | $5.31 \pm 0.25a$ |
| | TVB-N | $24.5\pm3.3a$ | 21.5 ± 2.2a | $19.2 \pm 3.2a$ | $20.2 \pm 3.6a$ |
| 9 | TVC | 8.80 ± 0.15a | $8.40 \pm 0.22b$ | $8.85 \pm 0.50 ab$ | $8.37 \pm 0.11b$ |
| | Yeast | $2.67 \pm 0.17a$ | $2.61 \pm 0.78 ab$ | $1.97\pm0.23b$ | $2.47 \pm 0.30a$ |
| | LAB | $6.53 \pm 0.30a$ | $7.68 \pm 0.11b$ | $8.88 \pm 0.15c$ | $7.90 \pm 0.35a$ |
| | В. | $4.60 \pm 0.51a$ | $3.50\pm0.40b$ | $2.89\pm0.30c$ | $2.44 \pm 0.45c$ |
| | thermosphacta | | | | |
| | pH | $5.88 \pm 0.50a$ | $5.98 \pm 0.30a$ | $5.64 \pm 0.50a$ | $5.64 \pm 0.45a$ |
| | TVB-N | 35.3 ± 4.2a | $25.4\pm3.4b$ | $21.4\pm3.5b$ | $22.5\pm3.3b$ |
| 12 | TVC | 8.97 ± 0.11a | 8.59 ± 0.51ab | 8.63 ± 0.33ab | $8.54 \pm 0.15b$ |
| | Yeast | 2.50 ± 0.40 ab | $2.68 \pm 0.15a$ | $3.09\pm0.25b$ | $2.45 \pm 0.45a$ |
| | LAB | $7.37 \pm 0.15a$ | $8.90 \pm 0.40b$ | $8.05 \pm 0.17c$ | $8.36 \pm 0.30 bc$ |
| | В. | $4.47 \pm 0.22a$ | $3.58 \pm 0.26b$ | $3.07 \pm 0.11c$ | $2.38 \pm 0.13d$ |
| | thermosphacta | | | | |
| | pH | $5.98 \pm 0.40a$ | $5.78 \pm 0.10a$ | $5.46 \pm 0.25a$ | $5.47 \pm 0.25a$ |
| | TVBN | $43.2 \pm 5.1a$ | $28.4 \pm 4.3b$ | $24.3 \pm 6.3b$ | $25.1 \pm 6.3b$ |

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 \pm 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.

| | | Lot 1 | Lot 2 | Lot 3 | Lot 4 |
|-----|-----------|-------------------|-------------------|-----------------|-------------------|
| Day | Parameter | Control | L.c./L.s./S.x. | Lac./L.s./S.x | L.c./L.s./S.x. |
| 0 | L* | $43.20 \pm 1.20a$ | 44.12 ±4.63a | 41.89± 5.19a | 44.99 ±5.70a |
| | a* | $14.12 \pm 1.74a$ | $15.46 \pm 1.62a$ | $15.19\pm3.33a$ | $16.11 \pm 0.81a$ |
| | 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 | $16.09\pm0.87a$ | 15.53 ±0.85a |
| | b* | 5.46 ±0.57a | 5.25 ±0.12b | $6.09\pm0.87a$ | 5.53 ±0.85a |
| 9 | L* | $45.84 \pm 114a$ | $44.00 \pm 1.48a$ | 45.52 ±0.56a | 45.23 ±1.73a |
| | a* | 16.08 ±4.86a | $16.26\pm3.54a$ | 16.88 ±0.69a | $16.00 \pm 2.65a$ |
| | 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 |

383Legend: 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).</th>

Table 3: The sensory panel scores of cooked hamburgers.

| | Lot 1 | Lot 2 | Lot 3 | Lot 4 |
|-------------------|---------|----------------|---------------|----------------|
| Sensory attribute | Control | L.c./L.s./S.x. | Lac./L.s./S.x | 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 |

401 *Final scores: the panellists requested to ranked the products within the scale from 1 (excellent) to 4 (worst).

402 Control (Lot 1): no starter; L.c/L.s./S.x (Lot 2): Lactobacillus sakei subsp. carnosus/Lactobacillus sakei +

403 Staphylococcus xylosus (1/1 ratio); Lac./L.s./S.x. (Lot 3): Lactococcus lacts ssp. lactis/Lactobacillus sakei +

404 Staphylococcus xylosus (1/1 ratio); Lc./L.s./S.x. (Lot 4): Lactobacillus sakei subsp. carnosus/Lactobacillus sakei +

- *Staphylococcus xylosus* (1/2 ratio).

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



