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1 **Protective cultures against foodborne pathogens in a nitrite reduced fermented meat**
2 **product**

3

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17

18 **Abstract**

19 In the present work, a combined hurdle approach for fermented meat preservation was
20 investigated. Challenge tests were performed in *Chorizo* sausage model using the maximum
21 allowed NaNO₂ amount (150mg/kg), a reduced amount (75 mg/kg) and no nitrite, with and
22 without protective cultures inoculation. Cocktail strains of *L. monocytogenes* and *Salmonella*
23 spp. were used as indicator strains. In a nitrite reduced sausage model, *L. monocytogenes*
24 growing trend did not significantly change (p>0.05) when compared with that containing
25 higher nitrite concentration (150 mg/kg NaNO₂). The addition of *L. plantarum* PSC20
26 significantly lowered *L. monocytogenes* growth when compared with control batches without
27 PCS20 (p<0.05), obtaining 3.84 log cfu/g and 2.62 log cfu/g lower counts in the batches with
28 150mg/kg NaNO₂ and 75mg/kg NaNO₂ respectively. None of the protective cultures
29 demonstrated *in situ* antagonistic activity against *Salmonella* spp.

30 This work pointed out that the reduction of nitrites with the combined use of a protective
31 culture could be a feasible approach to control *L. monocytogenes* growth in fermented meat
32 foods.

33

34 **Keywords:** Protective cultures; nitrite reduction; *Listeria monocytogenes*; *Salmonella* spp.;
35 fermented pork meat

36

37 1. Introduction

38 In the era where demand for ready to eat and preservative free products is constantly
39 growing, the microbiological food safety has to be guaranteed, proportionally with this
40 ongoing trend. In the recently published European Food Safety Authority (EFSA) foodborne
41 outbreak report, referred to 2016, *Salmonella* spp. human infections had the same high level
42 of the previous year (94.530 confirmed cases), whereas human listeriosis, caused mainly by
43 *Listeria monocytogenes*, showed a 9.3% increase (2.536 confirmed cases) (EFSA, 2017).
44 Despite the relatively low incidence of listeriosis, compared with the number of
45 campylobacteriosis and salmonellosis cases, its importance is due to the severity of the
46 disease and the higher case-fatality rate (Baffoni et al. 2017; D'Ostuni et al., 2016; EFSA,
47 2017).

48 Curing with nitrite is the most used approach to control foodborne pathogens in the meat
49 (Honikel, 2008). Nitrites have additional functions in the meat, as they help to prevent lipid
50 oxidation and rancidity, guarantee a bright red color and a typical “cured” flavor (Sebranek &
51 Bacus, 2007). Although nitrites are widely used in the meat industry, they are classified by
52 International Agency for Cancer Research as potentially carcinogenic agents (IARC, 2010),
53 due to their ability to react with amines in the gastrointestinal tract, resulting in N-
54 nitrosamines formation. Nitrites, hitherto, are the most effective solution against *C. botulinum*
55 growth in meat products (EFSA 2003; Hospital, Hierro & Fernández, 2014; Hospital, Hierro,
56 Stringer & Fernández, 2016). Therefore, 150 mg/kg NaNO₂ and 300 mg/kg NaNO₃ were
57 authorized as maximum added levels in meat in Europe until May 2018 (EFSA, 2003;
58 European Commission, 2011). Starting from May 2018, a new regulation, proposed by the
59 Danish authorities in 2015, was approved and the maximum accepted nitrite level in
60 fermented salami is now 100 mg/kg (European Commission, 2018). Additionally, the EC
61 Regulation N° 889/2008 for organic meat products, establishes 80 mg/kg for added nitrite and

62 50 mg/kg for residual nitrite (European Commission, 2008). The U.S. FDA accepts a
63 maximum level of 200 mg/kg NaNO₂ and 500 mg/kg NaNO₃ in meat finished products
64 (CFR, 2018). Although outbreaks regarding food poisoning by nitrite derived from meat
65 products are not described in the literature, unintentional poisoning has been reported upon
66 eating homemade sausages (Cvetković, Živković, Lukić, & Nikolić, 2018).

67 Therefore, meat industries are challenged to employ healthier and safer approaches for meat
68 preservation. In the attempt of finding alternatives to nitrites for fermented food preservation,
69 several authors suggested the use of lower nitrite levels in combination with other compounds
70 or processing technologies, in a way that antimicrobial properties against the common
71 foodborne pathogens could be guaranteed without alteration of sensory qualities (Alahakoon,
72 Jayasena, Ramachandra & Jo, 2015; Cavalheiro et al., 2015). Lactic acid bacteria (LAB) with
73 demonstrated *in vitro* antimicrobial activity against a wide spectrum of foodborne pathogens
74 (Leroy, Geyzen, Janssens, De Vuyst & Scholliers, 2013) as well as the addition of natural
75 extracts or phytochemicals are the mostly studied approaches for the development of
76 innovative processed meat products (Alahakoon et al., 2015; Gaggia, Di Gioia, Baffoni &
77 Biavati, 2011; Oliveira, Ferreira, Magalhães & Teixeira, 2018). However, several natural
78 extracts may contain even more than the allowed nitrate amount, thus the nitrosamine
79 formation is questioned (Bedale, Sindelar, & Milkowski, 2016). **LAB strains with**
80 **demonstrated sensorial or health promoting properties are approved by FDA as Generally**
81 **Recognized as Safe (GRAS) and by EFSA with the Qualified Presumption of Safety (QPS)**
82 **status (EFSA, 2018; FDA, 2018).**

83 In the present work, we studied the effectiveness of a combined hurdle approach, *i.e.* a 50%
84 reduction of nitrites plus the addition of previously characterized *Lactobacillus* strains
85 (*Lactobacillus plantarum* PCS20 or *Lactobacillus delbrueckii* DSM 20074), against common
86 foodborne pathogens in *Chorizo*, a dry fermented sausage produced in Spain.

87 **2. Material and methods**

88 *2.1 Bacterial strains*

89 *L. plantarum* PCS20 (MSCL P977) and *L. delbrueckii* DSM 20074 were used as protective
90 cultures for their demonstrated anti-microbial activity against several pathogens (Di Gioia et
91 al., 2016; Savino et al., 2011). They were grown in de Man Rogosa Sharpe medium (MRS,
92 Oxoid Ltd., Basingstoke, England) in anaerobic conditions (Anaerogen, AN0025A, Oxoid),
93 at 37 °C for 48 h.

94 A cocktail of *Listeria monocytogenes* strains has been used: *L. monocytogenes* CECT 5366
95 (serovar 4b, source: human), CECT 934 (serovar 4a, source: brain of sheep with circling
96 disease), CECT 4032 (serovar 4b, source: associated with case of meningitis after eating soft
97 cheese) and LTA0020 (isolated from poultry minced meat in Burgos, Spain), already used in
98 similar studies (Melero, Diez, Rajkovic, Jaime, & Rovira, 2012; Melero, Vinuesa, Diez,
99 Jaime, & Rovira, 2013). The strains were grown at 37°C in Brain Heart Infusion Broth (BHI,
100 Oxoid). For evaluation of viable cell population Chromogenic *Listeria* agar (Oxoid)
101 supplemented with OCLA (ISO) Selective Supplement (SR 0226E, Oxoid) and Brilliance
102 *Listeria* Differential Supplement (SR 0228E, Oxoid) was used.

103 Four *Salmonella* strains were also employed in the challenge tests. All strains were isolated
104 from meat and cheese products in Burgos. Bacterial strains were grown at 37°C in BHI.
105 Brilliance *Salmonella* agar (Oxoid) supplemented with *Salmonella* Selective Supplement (SR
106 0194, Oxoid) was used for the evaluation of viable cell population.

107 *2.2 Study design*

108 Two Challenge tests in sausage prototypes were designed, referred to as 1 and 2. Challenge
109 test 1 aimed at studying the effect of *L. plantarum* PCS20 against *L. monocytogenes* and
110 *Salmonella* spp. in fermented sausages, both without nitrite addition and with 150 mg/kg of
111 nitrite. Challenge test 2 was focused on the effects of two protective cultures, *L. plantarum*

112 PCS20 and *L. delbrueckii* DSM 20074, against *L. monocytogenes* strains in pork meat batters
113 treated with 75 mg/kg and 150 mg/kg of nitrite. Challenge test protocols are detailed below
114 (2.3 and 2.4).

115 2.3 Inocula preparation

116 2.3.1 Pathogen strains

117 Each *L. monocytogenes* and *Salmonella* spp. strain was grown at 37°C overnight in BHI
118 broth up to 9 log cfu/ml. Cells were washed and suspended in sterile Ringer solution (Oxoid).
119 For Challenge test 1, dilutions were performed in order to obtain a final concentration of 4.5
120 log cfu/g in the meat batter (Figure 1), whereas for Challenge test 2, meat batter was
121 inoculated with *L. monocytogenes* cocktail strains in order to obtain the final concentration of
122 3 log cfu/g (Figure 2).

123 2.3.2 Protective cultures

124 *L. plantarum* PCS20 and *L. delbrueckii* DSM 20074 were grown at 37°C overnight in MRS
125 broth up to 9.5-10 log cfu/ml. Cells were washed and suspended to a final concentration of 6-
126 7 log cfu/g (Figure 2).

127 2.4 Challenge tests

128 The batter was composed of ground pork meat and fat (70% and 30%, respectively) supplied
129 by a meat processing company in Burgos (Spain). Spices were not used not to interfere with
130 the results obtained.

131 For Challenge test 1, the ground meat (4 kg) was divided in 2 trays, each containing 2 kg. In
132 one tray, 2% NaCl was added whereas, in the other tray, meat was supplemented with 2%
133 NaCl plus 150 mg/kg NaNO₂ (Figure 1). After homogenization in a vacuum mixer, each 2 kg
134 portion was splitted in two: 1 kg was inoculated with *L. plantarum* PCS20 whereas the other
135 kg was not inoculated with any protective culture. Subsequently, each kg was divided in 3
136 batches (333 g), one inoculated with the cocktail of *Salmonella* strains, the second one with

137 the *L. monocytogenes* strains and the last one was not inoculated with any pathogen (control).
138 The 12 treatments and the relative acronyms are shown in Fig. 1.
139 For Challenge test 2, the ground meat (4 kg) was divided in 2 trays of 2 kg meat each. 2 kg
140 were amended with 2% of NaCl, 0.5% dextrose and 75 mg/kg NaNO₂ and 2 kg with 2% of
141 NaCl, 0.5% dextrose and 150 mg/kg NaNO₂. Each tray was divided in two (1 kg each): one
142 kg was inoculated with *L. monocytogenes* and the other kg was not inoculated with *L.*
143 *monocytogenes*. Then each kg of meat was divided in three batches (333 g each) and
144 submitted to different treatments: inoculated with PCS 20, with DSM 20074 and not
145 inoculated with protective cultures. The 12 treatments and the relative acronyms are shown in
146 Fig. 2. Each batch containing 333 g of meat batter was used to produce two sausages (two
147 replicates per treatment). Sausages were then stuffed in collagen casings (45 mm diameter)
148 (Viscofan, Navarra, Spain). For Challenge test 1, the fermentation was performed for 2 days
149 at 23°C, 95% humidity, followed by a short ripening of 6 days at 15°C and lower humidity
150 (80-75%). pH evaluation and microbiological analyses were performed at the following days:
151 D0, D1, D2, D4, D6 and D8. Differently, for Challenge test 2, the fermentation was studied
152 for 2 days followed by 5 days of short ripening in the same conditions as for the Challenge
153 test 1. pH evaluation and microbiological analyses were performed at the following days: D0,
154 D3, D5 and D7.

155 2.5 pH analysis

156 pH was measured with a pin electrode of a pHmeter (micropH2001, Crison, Barcelona,
157 Spain) inserted directly 3 times into the sample.

158 2.6 Microbiological Analysis

159 Meat samples (10 g per sampling point) were aseptically removed from each *Chorizo* (two
160 sausages per treatment) and homogenized in 90 ml of Buffered Peptone Water (BPW; AES
161 Laboratoire, Combourg, France) for 2 min in a sterile plastic bag using a Smasher (AES

162 Laboratoire). For cell counts, decimal dilutions (1:10 in BPW) of the meat homogenate were
163 prepared and aliquot of 100 µl were inoculated onto selective solid agar plates for, both, lactic
164 acid bacteria and for pathogens growth. The counts were performed in triplicate. Lactic acid
165 bacteria were counted on MRS agar plates, incubated anaerobically for 48 h at 37°C.
166 Randomly picked colonies were subjected to morphological and PCR analysis with LAB
167 specific primers (data not shown). Previously described selective solid medias were used for
168 *L. monocytogenes* and *Salmonella* spp. counts determination. Then, plates were incubated for
169 24h and for 48h, respectively, at 37°C.
170 ISO protocols were used for the detection of natural contamination in not artificially
171 inoculated batches: ISO 11290–1:1996 (ISO, 1996) and ISO 6579:2002 (ISO, 2002) for *L.*
172 *monocytogenes* and *Salmonella* spp., respectively.

173 2.7 Statistical analysis

174 The results of microbiological analysis, for each sampling point, were obtained from two
175 chorizo replicates per treatment; for each replicate counts were performed in triplicate. Data
176 were subjected to one-way ANOVA analysis. Differences among means were tested by
177 Duncan's multiple range test (significance $p < 0.05$). All the analyses obtained from the
178 Challenge tests were performed using the Statistica 8.0 (StatSoftInc., USA). Results of
179 statistical analysis are presented as mean value \pm standard deviation.

180

181 3. Results

182 3.1 Challenge test 1

183 3.1.1 pH analysis

184 No differences in pH were observed during the fermentation and short ripening process (data
185 not shown). Considering the slight decrease of pH observed, 0.5% of dextrose was added in

186 pork meat batter in Challenge test 2 with the aim of stimulating the *Lactobacillus* growth and
187 acidification.

188 3.1.2 Microbiological analysis

189 The growing trend of *L. monocytogenes* and *Salmonella* spp. in Challenge test 1 is shown in
190 Figure 3. Both pathogens demonstrated ability to survive and colonize the pork meat in the
191 sausage model.

192 Regarding *L. monocytogenes* growth, a significantly lower counts ($p < 0.05$) of 0.95 and 2.78
193 log cfu/g, were observed at day 4 and 6, respectively, in the batch with 150 mg/kg NaNO₂
194 and PCS20 (NLP) when compared with the batch containing nitrite but without PCS20 (NL)
195 (Figure 3A). Moreover, considering the initial inoculum, in the NL batch, an increase of 3.55
196 log cfu/g of *L. monocytogenes* counts was observed, whereas this increase was of 1.96 log
197 cfu/g in the NL+ batch P (Figure 3A) at the last sampling time (D8). Comparing control
198 batches without nitrate addition, P+L and L, significantly ($p < 0.05$) lower *L. monocytogenes*
199 counts of 0.60 and 0.52 log cfu/g, were observed at day 4 and 6, respectively, whereas no
200 significant differences were observed at D8.

201 Lower *L. monocytogenes* growth was observed in batches where NaNO₂ was added
202 (NL/NL+P) in comparison with batches without additives (L/P+L). At the last sampling day
203 (D8), significant ($p < 0.05$) decrease of *L. monocytogenes* counts of 2.37 log cfu/g was
204 observed when comparing NL+P and P+L batches, whereas significant ($p < 0.05$) decrease of
205 0.58 log cfu/g was observed when comparing batches NL Ctr and L.

206 *Salmonella* spp. counts within the study period are shown in Figure 3B. *L. plantarum* PCS20
207 did not show antimicrobial activity against *Salmonella* spp. growth. However, nitrites
208 demonstrated a significant decrease ($p < 0.05$) of *Salmonella* spp. growth (1.23 log cfu/g) in
209 N+S batch in comparison with batch S at D8.

210 Initial counts of LAB in the meat without protective culture were between 3-4.5 log cfu/g.
211 The level of PCS20 inoculum was 5.6-5.9 log cfu/g. After 3 days, when the fermentation
212 conditions were settled, LAB counts increased in all batches of 2.5-3.5 log cfu/g, reaching
213 values in the range 7-9 log cfu/g in batches with protective culture and 7-8 log cfu/g in
214 uninoculated batches, at the end of the study (data not shown).

215 3.2 Challenge test 2

216 3.2.1 pH analysis

217 pH trend in the meat subjected to different treatments is shown in Table 1. As expected, the
218 addition of 0.5% dextrose caused a significant pH reduction at D7 (from 5.80 to 5.05;
219 $p < 0.05$), in all batches where *L. plantarum* PCS20 was inoculated. Differently, the addition
220 of *L. delbrueckii* DSM 20074 did not lead to a significant pH reduction ($p > 0.05$).

221 3.2.2 Microbiological analysis

222 Figure 4 shows the trend of *L. monocytogenes* inoculated at 3 log cfu/g in all batches.

223 Comparing batches containing 75 mg/kg NaNO₂, with and without PSC20 (batches ½NL+P
224 and ½NL Ctr, respectively, Fig. 4A), a significantly lower counts ($p < 0.05$) of 2.20 and 2.62
225 log cfu/g of the inoculated *L. monocytogenes* were observed at day 3 and 5, respectively, in
226 the batch where PCS20 was inoculated (½NL+P); this reduction was maintained until D7.

227 Interestingly, considering the initial inoculum, the pathogen counts increase of only 1.61 log
228 cfu/g in the batch ½NL+P compared with a 3.99 log cfu/g increase in the batch ½NL Ctr, at
229 D7. On the other hand, in batches with higher nitrites concentration a significantly lower
230 counts of *L. monocytogenes* of 3.93 log cfu/g were observed at D5, in batch containing
231 PCS20 as protective culture (NL+P) in comparison with batch without PCS20 (NL Ctr), with
232 a final decrease of *L. monocytogenes* of 3.84 log cfu/g at D7. In summary, at the end of the
233 study, the pathogen growing trend was not statistically different ($p > 0.05$) when compared
234 batches with 75 or 150 mg/kg of nitrites (½NL Ctr and NL Ctr), while, in batches with

235 PCS20, *L. monocytogenes* counts were higher in ½NL+P compared with NL+P (difference of
236 1.49 log cfu/g).

237 Figure 4B shows the *L. monocytogenes* growth in pork meat batter with 150 mg/kg or 75
238 mg/kg NaNO₂ with or without *L. delbrueckii* DSM 20074 inoculum. At the end of the study,
239 no significant differences in *L. monocytogenes* growth were observed among batches.

240 Counts of LAB growth were under the detection limit (<2 log cfu/g) in the control batches

241 without protective culture inoculum at D0; whereas LAB counts were in the range 6-7 log

242 cfu/g in the batches inoculated with PCS20 at D0 (Table 2). At the end of the study, LAB

243 counts reached 7-8 log cfu/g in batches without PCS20, and 8-9.2 log cfu/g in batches with

244 PCS20. Batches inoculated with DSM 20074 did not reach the same LAB count level as

245 PCS20. In particular, 5.89 log cfu/g were obtained in the control batch with 150 mg/kg

246 NaNO₂ and 6.36 log cfu/g in that with 75mg/kg NaNO₂, at D7. These counts are almost 3 log

247 lower than those obtained for PCS20.

248 Similarly to the previous experiment, significant differences (p<0.05) were observed between

249 D1 and D3, i.e. in the final part of the fermentation period (3rd day). At the end of the short

250 ripening period, LAB reached counts in the range 7-9 log cfu/g.

251

252 **4. Discussion**

253 The aim of the present work was to evaluate the possibility of using protective cultures to

254 eliminate or reduce nitrite amount in fermented meat products. For this purpose, the

255 biopreservative activity of previously characterized LAB strains, *L. plantarum* PCS20 and *L.*

256 *delbrueckii* DSM 20074, was studied against *L. monocytogenes* and *Salmonella* spp. in a dry

257 fermented sausage model without nitrite, with half (75 mg/kg) and maximum (150 mg/kg)

258 allowed nitrite amount considering the maximum amounts allowed in Europe Until May

259 2018.

260 The results showed that the addition of *L. plantarum* PCS20 as protective culture in nitrite-
261 free sausages, artificially contaminated with pathogen, is capable of significantly reducing the
262 pathogen load after 4 and 6 days from the beginning of the fermentation, although the same
263 effect was not observed at D8. On the contrary, the antimicrobial activity of PCS20 was not
264 observed against the cocktail of *Salmonella* strains, whereas their growth was significantly
265 ($p < 0.05$) reduced in the presence of 150 mg/kg nitrites. Interestingly, Hospital et al. (2014)
266 obtained complete *Salmonella* inactivation using a halved nitrite amount (75 mg/kg) in
267 fermented sausages at the end of the storage period. Other works showed the ineffectiveness
268 of commercial protective cultures, as well as of meat-isolated *Lactobacillus* strains, against
269 *Salmonella* spp., when inoculated in different meat models (Dias, Duarte, Ramos, Martins
270 Santos & Schwan, 2013; Kotzekidou & Bloukas, 1998). The outcomes of this study support
271 the Hugas (1998) consideration on the hurdle effect strategy.

272 Our study also shows that it is possible to reduce *Listeria* counts by inoculating the meat with
273 *L. plantarum* PCS20 and a halved amount of nitrite (75 mg/kg). This result is particularly
274 important considering the EC decision of adopting more stringent criteria for potential
275 carcinogenic additives. Therefore, the combination of a protective culture with a reduced
276 nitrite amount is an effective hurdle approach in fermented sausage production that may
277 allow both to reduce pathogen load and to have the known positive effects of nitrites, such as
278 the bright color.

279 The anti-*Listeria* activity observed is in agreement with a recent work (Giello, La Stora, De
280 Filippis, Ercolini & Villani, 2018) that showed the effectiveness of the bacteriocin-producing
281 *Lactobacillus curvatus* 54M16 strain in fermented sausages. Several authors pointed out that
282 bacteriocin action can be hindered *in carnis* by bacteriocin binding to food matrixes or
283 degradation by proteases or their production can be prevented by nitrites (Galvez, Abriouel,
284 Lopez, & Ben, 2007; Kouakou et al., 2009). Therefore, non-bacteriocin producing strains

285 showing anti-listerial activity can be of great importance in fermented meat production, in
286 particular in the presence of nitrites. This is the case of *L. plantarum* PCS20 strain, that does
287 not produce bacteriocins (Cho, G.S., Huch, M., Hanak, A., Holzapfel, W.H., & Franz,
288 C.M.A.P. 2010) and exerts anti-microbial activity in the presence of a reduced amount of
289 nitrites. Its anti-microbial activity against *L. monocytogenes* can be attributed to cell-to-cell
290 contact mechanisms or the production of organic acidic metabolites. An additional strength of
291 our study is the use of four different *L. monocytogenes* strains, belonging to different serovars
292 (Lianou & Koutsoumanis 2013; Scott et al. 2005).

293 Moreover, our work confirmed that dextrose is an important pH lowering agent, allowing to
294 reach pH values between 4.5 and 5.5, a range in which nitrite is mainly in the undissociated
295 state, possessing the greatest antibacterial activity. Moreover, a rapid pH drop below 5.1 is
296 considered as a desirable acidification rate for protective cultures in fermented meat products
297 (Ammor and Mayo 2007). On the other hand, the inability of *L. delbrueckii* DSM 20074
298 strain to demonstrate a significant pH lowering, resulted in an antagonistic failure against *L.*
299 *monocytogenes* at the end of the study, even when 150 mg/kg of NaNO₂ were added.

300 Our study supports the outcomes of a recent survey (Hung et al. 2016), in which meat
301 industry stakeholders expressed interest in the development of innovative and healthier
302 processed meat products but asked the scientific community to provide additional evidences
303 of the microbiological safety of developed approaches. Consumers are important players in
304 industrial innovation shaping, thus the taste and the microbiological safety are the most
305 important criteria for the novel food formulations (Bedale et al. 2016, Hung et al. 2016).

306

307 **5. Conclusions**

308 This work pointed out that a combined approach based on half of the allowed nitrite amount
309 and of protective culture may be effective in a dry-fermented meat product (*chorizo*) to

310 reduce the growth of *L. monocytogenes*, a pathogen with high case fatality incidence and
311 causing severe diseases. This study has also shown that the effectiveness of nitrites against
312 this pathogen is not related to their amount; the inoculation with lactic acid bacteria
313 contributing to pH lowering and to reach the effective dissociation state of nitrite is probably
314 a crucial factor for their effectiveness. However, further studies aimed at better elucidating
315 the anti-microbial mechanisms against pathogens in food matrix need to be pursued.
316 In conclusion, the results obtained from this study will provide additional scientific evidence
317 in the evaluation of microbiological and preservative risks/benefits in fermented meat
318 products. The proposed combined hurdle approach (a reduced amount of nitrite plus the
319 inoculation of a protective culture) is promising for innovative fermented meat products
320 development.

321

322 **Conflict of interest**

323 The authors declare that they have no conflict of interest.

324

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328

329 **References**

330 Alahakoon, A. U., Jayasena, D. D., Ramachandra, S., & Jo, C. (2015). Alternatives to nitrite
331 in processed meat: Up to date. *Trends Food Science and Technology*, *45(1)*, 37–49.

332 Ammor, M. S., & Mayo, B. (2007). Selection criteria for lactic acid bacteria to be used as
333 functional starter cultures in dry sausage production: an update. *Meat Science*, *76(1)*, 138–

334 146.

335 Baffoni, L., Gaggia, F., Garofolo, G., Di Serafino, G., Buglione, E., Di Giannatale, E., & Di
336 Gioia, D. (2017). Evidence of *Campylobacter jejuni* reduction in broilers with early synbiotic
337 administration. *International Journal of Food Microbiology*, 251, 41–47.

338 Bedale, W., Sindelar J. J., & Milkowski A. L. (2016) Dietary nitrate and nitrite: Benefits,
339 risks, and evolving perceptions. *Meat Science*, 120, 85-92.

340 Cavaleiro, P. C., Ruiz-Capillas, C., Herrero, A. M., Jimenez-Colmenero, F., De Menezes, R.
341 C., & Fries, M. L. L. (2015). Application of probiotic delivery systems in meat products.
342 *Trends Food Science and Technology*, 46(1), 120–131.

343 CFR-Code of Federal Regulations Title 21. (2018). Retrieved from [https:// www. accessdata.
344 fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=172.175](https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=172.175).

345 Cvetković, D., Živković, V., Lukić, V., & Nikolić, S. (2018). Sodium nitrite food poisoning
346 in one family. *Forensic Science, Medicine and Pathology*

347 Cho, G. S., Huch, M., Hanak, A., Holzapfel, W.H., & Franz, C.M.A.P. (2010). Genetic
348 analysis of the plantaricin EFI locus of *Lactobacillus plantarum* PCS 20 reveals an unusual
349 plantaricin E gene sequence as a result of mutation. *International Journal of Food
350 Microbiology*, 141, 117–124.

351 Di Gioia, D., Mazzola, G., Nikodinoska, I., Aloisio, I., Langerholc, T., Rossi, M., Raiomondi,
352 S., Melero, B. & Rovira, J. (2016). Lactic acid bacteria as protective cultures in fermented
353 pork meat to prevent *Clostridium* spp. growth. *International Journal of Food Microbiology*,
354 235, 53–59.

355 Dias, F. S., Duarte, W. F., Ramos, E. M., Martins Santos, M. R. R., & Schwan, R. F. (2013).
356 Screening of *Lactobacillus* isolated from pork sausages for potential probiotic use and
357 evaluation of the microbiological safety of fermented products. *Journal of Food Protection*,
358 76(6), 991–998.

359 Dimitrovski, D., Cencic, A., Winkelhausen, E., & Langerholc, T. (2014). *Lactobacillus*
360 *plantarum* extracellular metabolites: in vitro assessment of probiotic effects on normal and
361 cancerogenic human cells. *International Dairy Journal*, 39(2), 293–300.

362 D'Ostuni, V., Tristezza, M., De Giorgi, M. G., Rampino, P., Grieco, F., & Perrotta, C. (2016).
363 Occurrence of *Listeria monocytogenes* and *Salmonella* spp. in meat processed products from
364 industrial plants in Southern Italy. *Food Control*, 62, 104–109.

365 EFSA. (2003). The effects of nitrites/nitrates on the microbiological safety of meat products.
366 *EFSA Journal*, 14, 1–31.

367 EFSA, & ECDC. (2017). The European Union summary report on trends and sources of
368 zoonoses, zoonotic agents and food-borne outbreaks in 2016. European Food Safety
369 Authority and European Centre for Disease Prevention and Control. *EFSA Journal*, 14, 231.

370 EFSA. (2018). Update of the list of QPS-recommended biological agents intentionally added
371 to food or feed as notified to EFSA 7: suitability of taxonomic units notified to EFSA until
372 September 2017. *EFSA Journal*, 16, 5131.

373 European Commission. (2008). Commission Regulation (EC) n 889/2008 of 5 September
374 2008 laying down detailed rules for the implementation of Council Regulation (EC) n
375 834/2007 on organic production and labeling of organic products with regard to organic
376 production, labeling and control. *Official Journal of the European Union*, L295, 1–177.

377 European Commission. (2011). Commission Regulation (EU) n 1129/2011 of 11 November
378 2011 amending Annex II to Regulation (EC) n 1333/2008 of the European Parliament and of
379 the Council by establishing a Union list of food additives. *Official Journal of the European*
380 *Union*, L295, 1–177.

381 European Commission. (2015). Commission decision (EU) 2015/826 of 22 May 2015
382 concerning national provisions notified by Denmark on the addition of nitrite to certain meat
383 products. *Official Journal of the European Union*, L130, 10–18.

384 European Commission. (2018). Commission Decision (EU) 2018/702 of 8 May 2018
385 concerning national provisions notified by Denmark on the addition of nitrite to certain meat
386 products. *Official Journal of the European Union*, L118, 7-15.

387 FDA. (2018). Microorganisms & Microbial-Derived Ingredients Used in Food (Partial List)
388 [https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/MicroorganismsMicrobialD](https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/MicroorganismsMicrobialDerivedIngredients/default.htm)
389 [erivedIngredients/default.htm](https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/MicroorganismsMicrobialDerivedIngredients/default.htm)

390 Gaggia, F., Di Gioia, D., Baffoni, L., & Biavati, B. (2011). The role of protective and
391 probiotic cultures in food and feed and their impact in food safety. *Trends Food Science and*
392 *Technology*, 22, 58–66.

393 Galvez, A., Abriouel, H., Lopez, R. L., & Ben, O. N. (2007). Bacteriocin-based strategies for
394 food biopreservation. *International Journal of Food Microbiology*, 120(1-2), 51-70

395 Giello, M., La Stora, A., De Filippis, F., Ercolini, D., & Villani, F. (2018). Impact of
396 *Lactobacillus curvatus* 54M16 on microbiota composition and growth of *Listeria*
397 *monocytogenes* in fermented sausages. *Food Microbiology*, 72, 1-15.

398 Honikel, K. O. (2008). The use and control of nitrate and nitrite for the processing of meat
399 products. *Meat Science*, 78(1-2), 68–76.

400 Hospital, X. F., Hierro, E., & Fernández, M. (2014). Effect of reducing nitrate and nitrite
401 added to dry fermented sausages on the survival of *Salmonella typhimurium*. *Food Research*
402 *International*, 62, 410–415.

403 Hospital, X. F., Hierro, E., Stringer, S., & Fernández, M. (2016). A study on the toxigenesis
404 by *Clostridium botulinum* in nitrate and nitrite-reduced dry fermented sausages. *International*
405 *Journal of Food Microbiology*, 218, 66–70.

406 Hung, Y., Verbeke, W., & de Kok, T. M. (2016b). Stakeholder and consumer reactions
407 towards innovative processed meat products: Insights from a qualitative study about nitrite
408 reduction and phytochemical addition. *Food Control*, 60, 690–698.

409 Hugas, M. (1998). Bacteriocinogenic lactic acid bacteria for the biopreservation of meat and
410 meat products. *Meat Science*, 49, 139–150.

411 IARC. (2010). IARC monographs on the evaluation of carcinogenic risks to humans.
412 <http://monographs.iarc.fr/ENG/Monographs/vol96/index.php>.

413 ISO 11290–1:1996. (1996). Microbiology of food and animal feeding stuffs -- Horizontal
414 method for the detection and enumeration of *Listeria monocytogenes* -- Part 1: Detection
415 method.

416 ISO 11290-2:1998. (1998). Microbiology of food and animal feeding stuffs -- Horizontal
417 method for the detection and enumeration of *Listeria monocytogenes* -- Part 2: Enumeration
418 method

419 ISO 6579:2002. (2002). Microbiology of food and animal feeding stuffs -- Horizontal method
420 for the detection of *Salmonella* spp.

421 Kotzekidou, P., & Bloukas, J. G. (1998). Microbial and sensory changes in vacuum-packed
422 frankfurter-type sausage *Lactobacillus alimentarius* and fate of inoculated *Salmonella*
423 *enteritidis*. *Food Microbiology*, 15, 101–111.

424 Kouakou, P., Ghalfi, H., Destain, J., Dubois-Dauphin, R., Evrard, P., & Thonart, P. (2009).
425 Effects of curing sodium nitrite additive and natural meat fat on growth control of *Listeria*

426 *monocytogenes* by the bacteriocin-producing *Lactobacillus curvatus* strain CWBI-B28. *Food*
427 *Microbiology*, 26(6), 623-628.

428 Larsen, M. H., Dalmasso, M., Ingmer, H., Langsrud, S., Malakauskas, M., Mader, A.,
429 Møretrø, T., Mozina, S. S., Rychli, K., Wagner, M., Wallace, R. J., Zentek, J., & Jordan, K.
430 (2014). Persistence of foodborne pathogens and their control in primary and secondary food
431 production chains. *Food Control*, 44, 92–109.

432 Leroy, F., Geyzen, A., Janssens, M., De Vuyst, L., & Scholliers, P. (2013). Meat
433 fermentation at the crossroads of innovation and tradition: a historical outlook. *Trends Food*
434 *Science and Technology*, 31(2), 130–137.

435 Lianou, A., & Koutsoumanis, K. P. (2013). Strain variability of the behavior of foodborne
436 pathogens: A review. *International Journal of Food Microbiology*, 167(3), 310–321.

437 Melero, B., Diez, A. M., Rajkovic, A., Jaime, I., & Rovira, J. (2012). Behaviour of non-
438 stressed and stressed *Listeria monocytogenes* and *Campylobacter jejuni* cells on fresh
439 chicken burger meat packaged under modified atmosphere and inoculated with protective
440 culture. *International Journal of Food Microbiology*, 158(2), 107–112.

441 Melero, B., Vinuesa, R., Diez, A. M., Jaime, I., & Rovira, J. (2013). Application of protective
442 cultures against *Listeria monocytogenes* and *Campylobacter jejuni* in chicken products
443 packaged under modified atmosphere. *Poultry Science*, 92(4), 1108–1116.

444 Nissen, L., Chingwaru, W., Sgorbati, B., Biavati, B., & Cencic, A. (2009). Gut health
445 promoting activity of new putative probiotic/protective *Lactobacillus* spp. strains: a
446 functional study in the small intestinal cell model. *International Journal of Food*
447 *Microbiology*, 135(3), 288–294.

448 Oliveira, M., Ferreira, V., Magalhães, R., Paula Teixeira, P. (2018). Biocontrol strategies for
449 Mediterranean-style fermented sausages. *Food Research International*, 103, 438–449.

450 Santini, C., Baffoni, L., Gaggia, F., Granata, M., Gasbarri, R., Di Gioia, D., & Biavati, B.
451 (2010). Characterization of probiotic strains: an application as feed additives in poultry
452 against *Campylobacter jejuni*. *International Journal of Food Microbiology*, *141*, 98–108.

453 Savino, F., Cordisco, L., Tarasco, V., Locatelli, E., Di Gioia, D., Oggero, R., & Matteuzzi, D.
454 (2011). Antagonistic effect of *Lactobacillus* strains against gas-producing coliforms isolated
455 from colicky infants. *BMC Microbiology*, *11*, 157.

456 Scott, V. N., Swanson, K. M. J., Freier, T. A., Pruett Jr., W. P., Sveum, W. H., Hall, P. A.,
457 Smoot, L. A., & Brown, D. G. (2005). Guidelines for conducting *Listeria monocytogenes*
458 challenge testing of foods. *Food Protection Trends*, *25*, 818–825.

459 Sebranek, J. G., & Bacus, J. N. (2007). Cured meat products without direct addition of nitrate
460 or nitrite: what are the issues? *Meat Science*, *77(1)*, 136–147.

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463

464 **Figure Captions**

465

466 **Figure 1 Study design of the 12 treatments related to Challenge test 1.** Legend: **Ctrl**=meat batter;
467 **Ctrl-N**=150 mg/kg NaNO₂ added; **Ctrl-P**=PCS20 added; **Ctrl-NP**=PCS20+150 mg/kg NaNO₂ added;
468 **L**=*L. monocytogenes* added; **N+L**=150 mg/kg NaNO₂+ *L. monocytogenes* added; **P+L**=PCS20+*L.*
469 *monocytogenes* added; **NP+L**=150 mg/kg NaNO₂+ PCS20+*L. monocytogenes* added; **S**=*Salmonella*
470 *spp.* added; **N+S**=150 mg/kg NaNO₂ + *Salmonella spp.* added; **P+S**=PCS20+*Salmonella spp.* added;
471 **NP+S**=150 mg/kg NaNO₂ +PCS20+*Salmonella spp.* added. For each condition two sausages were
472 prepared and processed.

473

474 **Figure 2 Study design Challenge test 2.** Legend: **N Ctrl**=meat batter added with 150mg/kg NaNO₂;
475 **½N Ctrl**=75 mg/kg NaNO₂ added; **N+P**=150mg/kg NaNO₂+PCS20 added; **½N+P**=75mg/kg
476 NaNO₂+PCS20 added; **N+D**=150mg/kg NaNO₂+DSM 20074 added; **½N+D**=75mg/kg NaNO₂+DSM
477 20074 added; **NL Ctrl**=150mg/kg NaNO₂+*L. monocytogenes* added; **½NL Ctrl**=75mg/kg NaNO₂+*L.*
478 *monocytogenes* added; **NL+P**=150mg/kg NaNO₂+*L. monocytogenes*+PCS20 added; **NL+P**=75mg/kg
479 NaNO₂+*L. monocytogenes*+ PCS20 added; **NL+D**=150mg/kg NaNO₂+*L. monocytogenes*+DSM
480 20074 added; **½NL+D**=75mg/kg NaNO₂+DSM 20074 added. For each condition two sausages were
481 prepared and processed.

482

483 **Figure 3 Antimicrobial activity of *L. plantarum* PCS20 against *L. monocytogenes* and *Salmonella***
484 *spp.* in dry fermented sausage with and without 150 mg/kg NaNO₂.

485 A) *L. monocytogenes* counts within the ripening period. **L**=*L. monocytogenes* added; **N+L**=150 mg/kg
486 NaNO₂+ *L. monocytogenes* added; **P+L**=PCS20+*L. monocytogenes* added; **NP+L**=150 mg/kg
487 NaNO₂+ PCS20+*L. monocytogenes* added; B) *Salmonella spp.* counts within the ripening period.
488 **S**=*Salmonella spp.* added; **N+S**=150 mg/kg NaNO₂ + *Salmonella spp.* added;
489 **P+S**=PCS20+*Salmonella spp.* added; **NP+S**=150 mg/kg NaNO₂ +PCS20+*Salmonella spp.* added.

490

491 **Figure 4** Antimicrobial activity of *L. plantarum* PCS20 (A) and *L. delbrueckii* DSM20074 (B) against
492 *L. monocytogenes* in dry fermented sausage added with 75 or 150 mg/kg NaNO₂. A) *L.*
493 *monocytogenes* counts in batches inoculated with or without *L. plantarum* PCS20. Legend: **NL**
494 **Ctr**=150mg/kg NaNO₂+*L. monocytogenes* added; $\frac{1}{2}$ **NL Ctr**=75mg/kg NaNO₂+*L. monocytogenes*
495 added; **NL+P**=150mg/kg NaNO₂+*L. monocytogenes*+PCS20 added; $\frac{1}{2}$ **NL +P**=75mg/kg NaNO₂+*L.*
496 *monocytogenes*+PCS20 added. B) *L. monocytogenes* counts in batches inoculated with *L. delbrueckii*
497 DSM 20074. Legend: **N+D**=150mg/kg NaNO₂+DSM 20074 added; $\frac{1}{2}$ **N+D**=75mg/kg NaNO₂+DSM
498 20074 added; **NL Ctr**=150mg/kg NaNO₂+*L. monocytogenes* added; $\frac{1}{2}$ **NL Ctr**=75mg/kg NaNO₂+*L.*
499 *monocytogenes* added; **NL+P**=150mg/kg NaNO₂+*L. monocytogenes*+PCS20 added; **NL+P**=75mg/kg
500 NaNO₂+*L. monocytogenes*+PCS20 added; **NL+D**=150mg/kg NaNO₂+*L. monocytogenes* +DSM
501 20074 added; $\frac{1}{2}$ **NL+D**=75mg/kg NaNO₂+*L. monocytogenes*+DSM 20074 added.

Table 1. Challenge test 2. The trend of pH during the fermentation and ripening period

Batches**	Days *			
	0	3	5	7
N Ctr	5.96 ±0.03 ^B	5.78 ±0.03 ^C	6.12 ±0.06 ^A	6.03 ±0.06 ^B
½N Ctr	5.90 ±0.09 ^B	5.99 ±0.03 ^B	6.11 ±0.04 ^A	5.90 ±0.01 ^B
½NL Ctr	5.77 ±0.08 ^B	5.89 ±0.02 ^A	5.92 ±0.02 ^A	5.91 ±0.02 ^A
NL Ctr	5.85 ±0.03 ^B	5.99 ±0.01 ^A	6.10 ±0.04 ^A	5.85 ±0.02 ^B
N+P	5.86 ±0.06 ^A	5.44 ±0.02 ^B	5.23 ±0.02 ^C	5.02 ±0.04 ^D
½N+P	5.85 ±0.07 ^A	5.28 ±0.06 ^B	5.21 ±0.02 ^B	5.05 ±0.02 ^C
NL+P	5.77 ±0.06 ^A	5.31 ±0.01 ^B	5.14 ±0.04 ^C	5.09 ±0.04 ^C
½NL+P	5.83 ±0.01 ^A	5.30 ±0.01 ^B	5.16 ±0.01 ^C	5.02 ±0.01 ^D
N+D	5.87 ±0.03 ^A	5.93 ±0.04 ^A	5.93 ±0.05 ^A	5.89 ±0.04 ^A
½N+D	5.80 ±0.04 ^B	5.93 ±0.02 ^A	5.93 ±0.02 ^A	5.89 ±0.05 ^A
NL+D	6.05 ±0.01 ^A	5.94 ±0.03 ^B	6.04 ±0.05 ^A	5.97 ±0.03 ^B
½NL+D	6.17 ±0.04 ^A	5.91 ±0.01 ^B	5.94 ±0.04 ^B	5.80 ±0.04 ^C

* Data are expressed as mean of n=3 measurements.

** Batch : **N Ctr**=meat batter added with 150mg/kg NaNO₂; **½N Ctr**=75 mg/kg NaNO₂ added; **½NL Ctr**=75mg/kg NaNO₂+*L.monocytogenes* added; **NL Ctr**=150mg/kg NaNO₂+*L.monocytogenes* added; **N+P**=150mg/kg NaNO₂+PCS20 added; **½N+P**=75mg/kg NaNO₂+PCS20 added; **NL+P**=150mg/kg NaNO₂+*L.monocytogenes*+PCS20 added; **½NL+P**=75mg/kg NaNO₂+*L.monocytogenes*+ PCS20 added; **N+D**=150mg/kg NaNO₂+DSM 20074 added; **½N+D**=75mg/kg NaNO₂+DSM 20074 added; **NL+D**=150mg/kg NaNO₂+*L.monocytogenes*+DSM 20074 added; **½NL+D**=75mg/kg NaNO₂+DSM 20074 added.

***^{A,B,C}: Mean values in the same row (corresponding to the same batch) differ significantly (p < 0.05).

Table 2. Challenge test 2. LAB counts (log cfu/g) within the 7 days of fermentation and ripening period

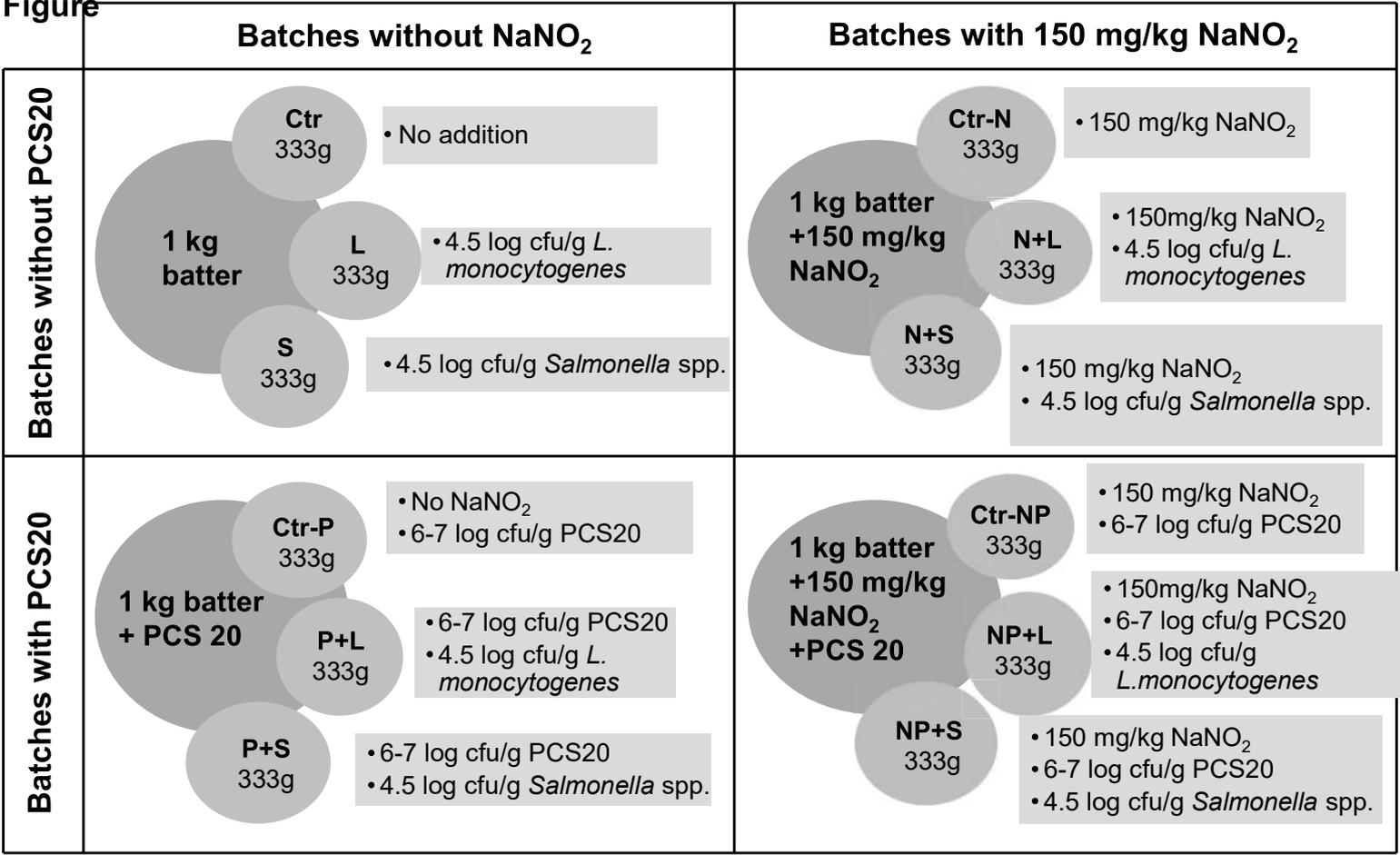
Batches**	Days *			
	0	3	5	7
N Ctr	<2 ±0.00 ^D	6.63 ±0.25 ^C	7.82 ±0.12 ^B	7.89 ±0.12 ^A
½N Ctr	<2 ±0.00 ^B	7.18 ±0.46 ^A	7.60 ±0.17 ^A	7.09 ±0.34 ^A
NL Ctr	<2 ±0.00 ^D	6.59 ±0.28 ^C	8.15 ±0.07 ^A	7.38 ±0.29 ^B
½NL Ctr	<2 ±0.00 ^B	6.89 ±0.27 ^A	7.15 ±0.51 ^A	7.43 ±0.28 ^A
N+P	6.34 ±0.15 ^C	8.87 ±0.20 ^B	9.09 ±0.12 ^{AB}	9.13 ±0.10 ^A
½N+P	6.34 ±0.13 ^D	9.06 ±0.13 ^B	9.26 ±0.06 ^A	8.03 ±0.13 ^C
NL+P	6.59 ±0.17 ^C	8.86 ±0.12 ^B	9.10 ±0.07 ^A	9.14 ±0.09 ^A
½NL+P	6.61 ±0.13 ^B	9.04 ±0.07 ^A	9.04 ±0.08 ^A	9.17 ±0.08 ^A
N+D	5.71 ±0.26 ^C	6.49 ±0.22 ^B	7.29 ±0.38 ^A	5.89 ±0.37 ^C
½N+D	5.84 ±0.11 ^D	6.98 ±0.19 ^B	7.44 ±0.15 ^A	6.36 ±0.13 ^C
NL+D	5.98 ±0.11 ^D	6.52 ±0.12 ^C	7.69 ±0.28 ^A	7.19 ±0.08 ^B
½NL+D	6.09 ±0.15 ^C	6.35 ±0.04 ^B	7.44 ±0.15 ^A	7.58 ±0.13 ^A

* Data are expressed as mean of n=3 measurements.

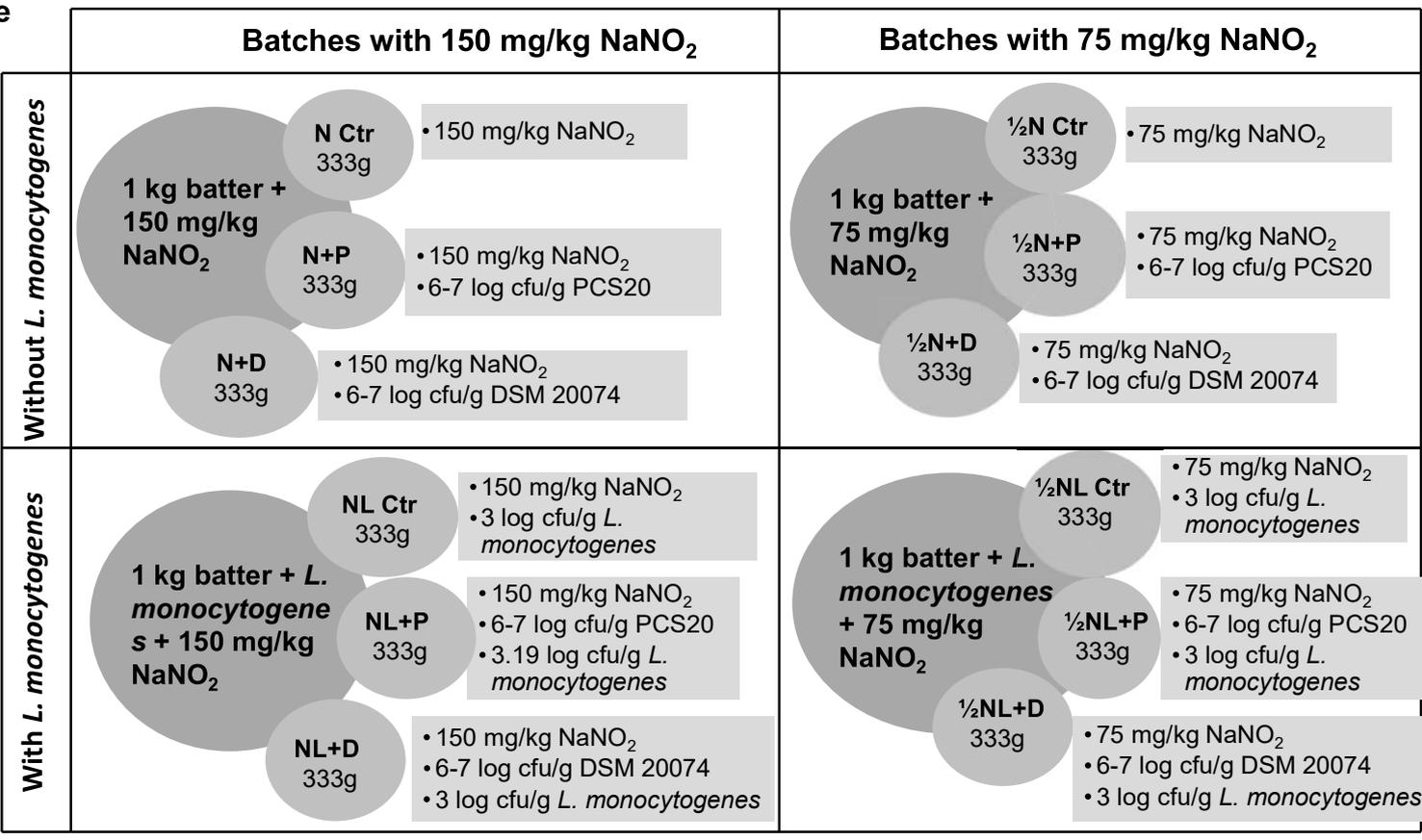
** Batch N Ctr=meat batter added with 150mg/kg NaNO₂; ½N Ctr=75 mg/kg NaNO₂ added; N+P=150mg/kg NaNO₂+PCS20 added; ½N+P=75mg/kg NaNO₂+PCS20 added; N+D=150mg/kg NaNO₂+DSM 20074 added; ½N+D=75mg/kg NaNO₂+DSM 20074 added; NL Ctr=150mg/kg NaNO₂+*L.monocytogenes* added; ½NL Ctr=75mg/kg NaNO₂+*L.monocytogenes* added; NL+P=150mg/kg NaNO₂+*L.monocytogenes*+PCS20 added; NL+P=75mg/kg NaNO₂+*L.monocytogenes*+ PCS20 added; NL+D=150mg/kg NaNO₂+*L.monocytogenes*+DSM 20074 added; ½NL+D=75mg/kg NaNO₂+DSM 20074 added.

***^{A,B,C}: Mean values in the same row (corresponding to the same batch) differ significantly (p < 0.05).

Figure

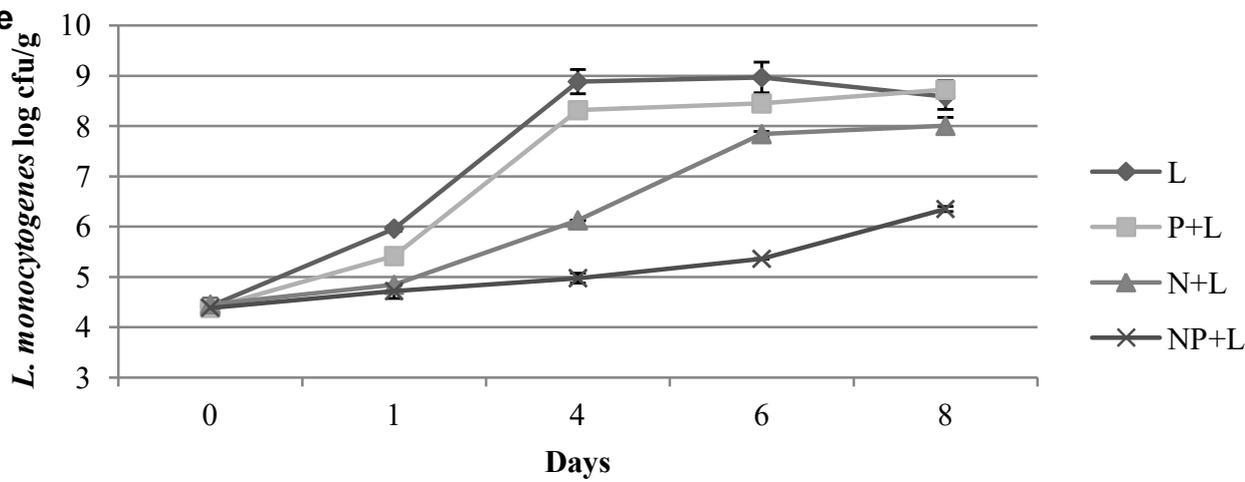


Figure



Figure

A)



B)

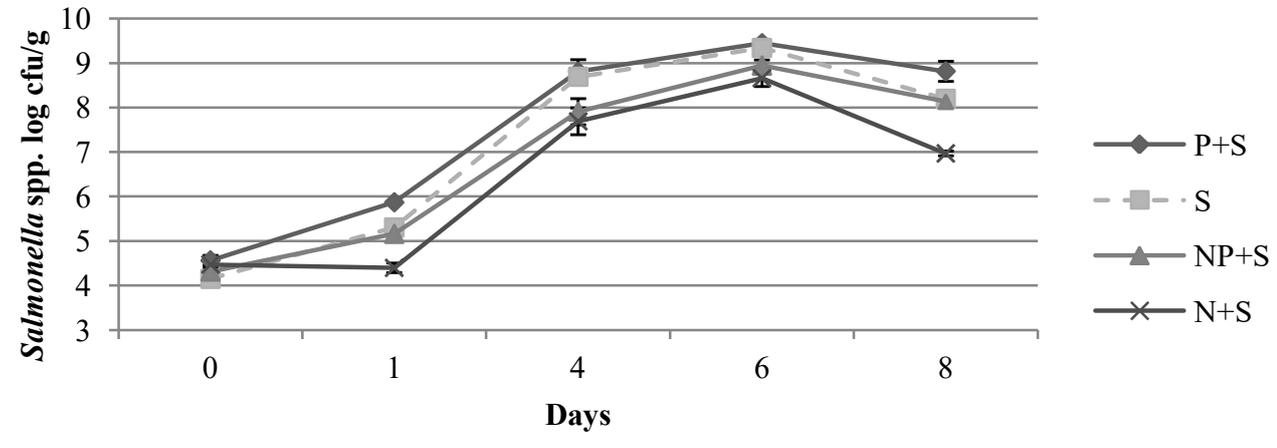
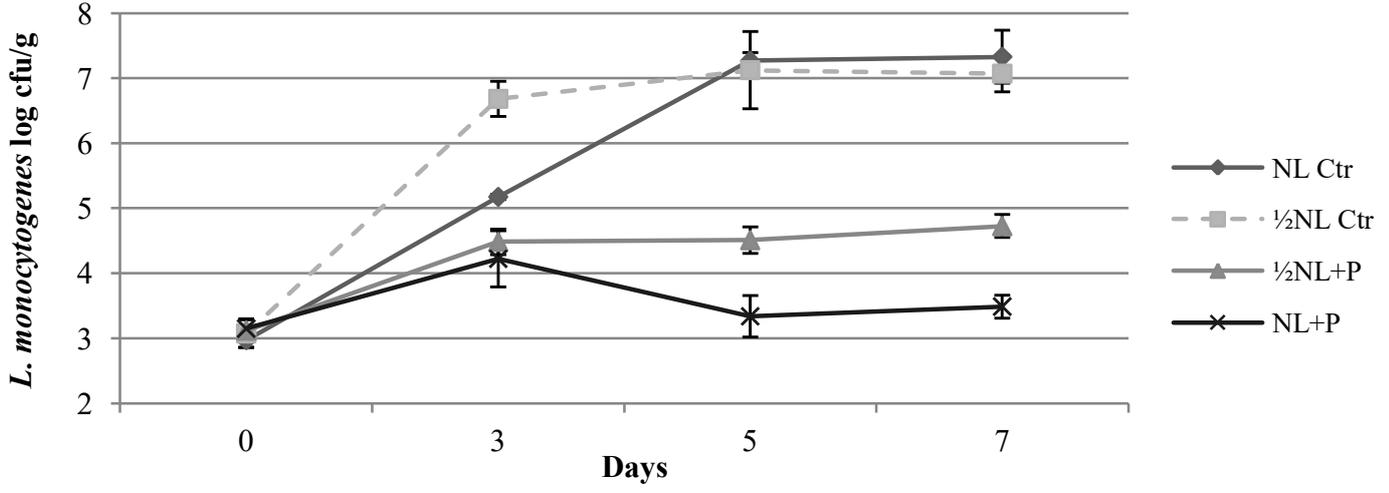


Figure A)



B)

