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1 **Decontamination of incoming beef trimmings with hot lactic acid solution to**
2 **improve microbial safety of resulting dry fermented sausages – a pilot study**

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20 **Abstract**

21 In the study, beef trimmings intended for sausage production were subjected to different
22 decontamination treatments based on lactic acid-hot water combination, with aim to eliminate or
23 reduce foodborne pathogens *E. coli* O157, *Salmonella* Typhimurium and *Listeria monocytogenes*
24 in resultant dry, fermented sausages. In finished sausages, produced from untreated trimmings,
25 “natural” reductions of inoculated *E. coli* O157 and *Salmonella* Typhimurium were on average
26 1.9 logs, *L. monocytogenes* count remained unchanged, and no detectable concentrations of
27 biogenic amines were found. The same type of sausages were also produced by using beef
28 trimmings which was pathogen-inoculated and then decontaminated by: hot, 4% lactic acid in
29 water solution (90°C for 10 s; treatment HLA1); or hot, 4% lactic acid in water solution (85°C
30 for 20 s; treatment HLA2), or hot, 4% lactic acid in water solution (80°C for 30 s; treatment
31 HLA3). The use of HLA-decontaminated beef trimmings resulted in total *E. coli* O157
32 reductions of at least 3.9 logs and in total *Salmonella* Typhimurium reductions of at least 3.6
33 logs, whilst biogenic amines were not detected in finished sausages. The overall sensorial
34 acceptability of finished sausages produced with HLA-decontaminated beef trimmings was
35 somewhat diminished. Further work is required to optimise the HLA-based incoming beef
36 treatments.

37
38 Keywords: Fermented beef sausages; *Escherichia coli* O157; *Salmonella* Typhimurium; *Listeria*
39 *monocytogenes*; biogenic amine; starter culture.

40 1. Introduction

41 The production process of fermented sausages is conducted at relatively high temperatures,
42 which vary with sausage types roughly between 20°C and 40°C, and involves two distinct stages.
43 During the first 2-3 days, the fermentation takes place, which is associated with production of
44 lactic acid (pH drop) by fast-multiplying, microaerophilic lactic acid bacteria (LAB) “replacing”
45 the initial aerobic, psychrotrophic meat microbiota. Subsequently, the maturation, commonly
46 including drying, takes place for time periods varying with sausage type (whether spreadable,
47 semidry or dry) between a few days to a few weeks. The microbial safety and storage stability
48 (shelf-life) of fermented sausages depend on combined effects of multiple antimicrobial factors
49 acting in the sausage matrix: acidity (low pH), low water activity (a_w) due to added salt and
50 drying, nitrites, and LAB due to their competitiveness and production of inhibitory compounds
51 such as bacteriocins (Lücke, 2000). Generally, in raw, fermented sausages produced under
52 proper and controlled conditions, microbial foodborne pathogens can survive but their counts are
53 reduced and they usually do not multiply. Microbial safety concerns associated with fermented
54 sausages relate to: a) primarily, bacterial foodborne pathogens originating from raw materials
55 used for sausage production (i.e. incoming meat); and b) to a lesser extent, toxic biogenic amines
56 (BA) such as histamine and tyramine produced by background microbiota.

57 A number of outbreaks of toxicoinfections by verocytotoxigenic *Escherichia coli*
58 (VTEC) associated with consumption of fermented sausages (mainly containing beef, but also
59 lamb) have been reported in different countries, as reviewed by Holck et al. (2011). Also,
60 consumption of fermented sausages has been associated or epidemiologically linked with other
61 foodborne illnesses caused by *Staphylococcus aureus* (Bacus, 1986), *Salmonella* (Sauer,
62 Majkowski, Green & Eckel, 1997) or *Listeria monocytogenes* (WHO/FAO, 2004). Literature

63 data clearly indicate that fermented sausages pose an increased potential risk with respect to high
64 content of the main, toxic biogenic amines (BA) histamine and tyramine, and they also can
65 contain other biogenic amines (e.g. putrescine and cadaverine) which are less toxic themselves
66 but can potentiate the toxic effects of the former two (Ruiz-Campilas & Jimenez-Comlenero,
67 2004). BA accumulation is usually related to the decarboxylase activity of contaminant bacteria
68 (e.g. enterobacteria, pseudomonads), but technological bacteria such as LAB may also contribute
69 significantly to aminogenesis (Bover-Cid, Izquierdo-Pulido & Vidal-Carou, 2001; Bover-Cid,
70 Hernández-Jover, Miguélez-Arrizado & Vidal-Carou, 2003). Hence, the BA accumulation in
71 fermented sausages can be reduced by using non-BA-forming LAB starter cultures (Bover-Cid et
72 al., 2001). Although no cases of histamine or tyramine intoxications have implicated fermented
73 sausages as vehicles to date, the products may occasionally contain these BA at levels generally
74 considered as toxic (EFSA, 2011), and the lack of the published intoxications may be due to
75 underreporting.

76 In respect to the pathogen of particular concern in beef/lamb fermented sausages, VTEC,
77 it is known that it survives the standard fermented sausage production process and a 5-log
78 reduction, required by regulation in the USA (Reed, 1995) but not in the EU, cannot be
79 consistently achieved solely through manipulations of product pH, salt/nitrites and a_w levels
80 (Glass, Loeffelholz, Ford & Doyle, 1992). Consequently, various heat treatment regimes, applied
81 to fermented sausages either at the post-fermentation or post-maturation stages of production
82 process and aimed at VTEC-reduction, have been evaluated, as reviewed by Holck et al. (2011).
83 The heat treatment of fermented sausages is often used in practice (and accepted by consumers)
84 in the USA, but is used very rarely, if at all, in Europe.

85 An alternative, more preventative approach to controlling microbial hazards in beef
86 fermented sausages could be decontamination of incoming raw beef; however, no such studies
87 have been published to date. Hence, the main objective of the present study was to evaluate the
88 potential of decontaminating the incoming beef trimmings, based on combined effects of heat
89 and lactic acid, to control the main microbial pathogens and BA in fermented sausages produced
90 from these trimmings.

91

92 **2. Materials and methods**

93 2.1 Inoculation of incoming raw beef

94 Strains of pathogens *E. coli* O157 (four strains: ATCC 35150 and three strains from our own
95 collection, isolated from bovine intestines), *S. Typhimurium* (two strains: ATCC 14028 and
96 ATCC 13311) and *L. monocytogenes* (five strains: ATCC 19112, ATCC 19114, ATCC 19115
97 and two strains from our own collection, isolated from beef sausage and from smoked rib eye
98 steak). Multiple strains per pathogen were used to accommodate for well-known between-strain
99 diversity in respect to both growth potential and inactivation dynamics. Each strain was
100 individually grown in mEC+Novobiocin selective enrichment broth (Merck, Germany),
101 Rappaport Vassiliadis Broth with soya broth (Merck) and L-PALCAM broth (Merck) at 37°C,
102 41.5°C and 30°C, respectively for 12 hours. Appropriate volumes of each strain were added to
103 distilled water so to obtain multi-pathogen, multi-strain suspension containing approximately 4
104 logs CFU of each pathogen per ml of water. Beef trimmings (pieces roughly 300 g each, chilled
105 at approximately 2°C) were surface inoculated by submersion for 10 s in the suspension of
106 pathogens, and drained for 10-15 minutes.

107

108 2.2 Decontamination of inoculated beef trimmings

109 Inoculated beef trimmings were divided into groups, and each group subjected to one of the
110 following surface decontamination treatments:

111 (a) hot lactic acid treatment 1 (HLA1): beef trimmings were submerged in hot 4% lactic acid
112 water solution (90°C) for 10 s;

113 (b) hot lactic acid treatment 2 (HLA2): beef trimmings were submerged in hot 4% lactic acid
114 water solution (85°C) for 20 s; and

115 (c) hot lactic acid treatment 3 (HLA3): beef trimmings were submerged in hot 4% lactic acid in
116 water solution (80°C) for 30 s.

117 Fatty tissue used for sausage production was subjected neither to inoculation nor to
118 decontamination treatments described above, because hot water melts the fat and makes the
119 treated fatty tissue unsuitable for production of fermented sausages.

120

121 2.3 Preparation of raw, fermented, dried beef sausages (“Sudzuk” type)

122 Four groups of sausages were prepared: a) by using inoculated but not decontaminated beef
123 trimmings (control for treatments); b) with inoculated and HLA1-decontaminated beef; c) with
124 inoculated and HLA2-decontaminated beef; and d) with inoculated and HLA3-decontaminated
125 beef. In addition, within each of groups b), c) and d), some sausages were not inoculated with
126 pathogens, but were subjected to corresponding decontamination treatment (destined for sensory
127 panel examination, see below). Apart from differences in the status of incoming beef, as
128 indicated above, the formulation and production process for all the sausages were the identical.
129 Beef trimmings from leg (45%), beef trimmings from shoulder (45%) and beef fat (10%) were
130 chopped in meat mincer (La Minerva A/E22, Italy) and transferred to kneader mixer (Mainca

131 RM20R, Spain). During meat mixing, 2% of commercially-supplied salt for curing (containing
132 sodium nitrite), 0.25% dextrose and 0.8% spices (powdered garlic, black pepper and paprika)
133 were added to the batter in the kneader mixer. Then, a starter culture comprising LAB strains
134 *Lactobacillus sakei* CTC 41, *L. sakei* CTC 287, *L. sakei* CTC 6469 and *L. sakei* CTC 6626 (all
135 identified as not producing biogenic amines, obtained from Dr. Sara Bover-Cid, Institute for
136 Food and Agricultural Research and Technology-IRTA, Spain) was added at level 4×10^5 of each
137 strain per gram of sausage batter. Subsequently, the sausage batter was stuffed into collagen
138 casings (Koko $\varnothing 35$, Viscofan, Serbia) and sausages were placed in a controlled-environment
139 chamber (Stagionello STG 100 MTF, Italy) and subjected to a fermentation and
140 maturation/drying process for 20 days in total, under the following temperature/relative air
141 humidity conditions: 1st day - 20°C/92%; 2nd day - 19°C/86%; 3rd day - 18°C/80%; 4th day -
142 17°C/72%; 5th to 20th day - 16°C/65%). There were three sausages sampling points during the
143 process: at day 0 (immediately after stuffing into casings), at day 3 (on completion of
144 fermentation) and at day 20 (finished product).

145

146 2.4 Sampling and sample homogenates preparation

147 Sausage samples (100 g) were placed in homogeniser bags with filters (6x9", Nasco Whirl-Pak,
148 USA), 100 ml of maximum recovery diluent (MRD; Oxoid, UK) was added, and each bag was
149 repeatedly squeezed manually for 5 minutes and further decimal dilutions were made in MRD
150 (ISO method 6887-1:1999). Sample homogenates or their appropriate dilutions were used for
151 microbiological analysis as described below.

152

153 2.5 Determination of *Escherichia coli* O157

154 For detection of *E. coli* O157, 25 ml of each sample homogenate was transferred to 225 ml of
155 enrichment medium (mEC+Novobiocin selective enrichment broth; Merck), and incubated at
156 37°C for 18 h, followed by detection of *E. coli* O157 by using an immunochromatographic rapid
157 test (Singlepath® *E. coli* O157; Merck). Positive samples were confirmed by streaking onto CT-
158 SMAC (Merck) and incubating at 37°C for 24 h. For direct enumeration of *E. coli* O157, 1 ml
159 volumes from appropriate sample dilutions were spread onto CT-SMAC (Merck), incubated at
160 37°C for 24 h and the typical colonies were counted.

161

162 2.6 Determination of *Salmonella* Typhimurium

163 For detection of *S. Typhimurium*, 25 ml of each sample homogenate was transferred to 225 ml of
164 buffered peptone water (BPW; Merck), and incubated at 37°C for 24 h. After incubation, 0.1 ml
165 of enrichment medium was transferred into 10 ml of Rappaport Vassiliadis Broth with soya
166 (RVS; Oxoid) and incubated at 41.5°C for 24 hours, followed by detection of *Salmonella* by
167 using an immunochromatographic rapid test (Singlepath® *Salmonella*; Merck). Positive samples
168 were confirmed by streaking onto Xylose Lysine Deoxycholate Agar (XLDA; Oxoid) and/or
169 *Salmonella* Chromogenic Agar (SCA; Oxoid) and/or Brilliant Green Agar (BGA; Oxoid) and
170 incubating at 37°C for 24 hours. For direct enumeration of *Salmonella* Typhimurium, 1 ml
171 volumes from appropriate sample dilutions were spread onto Xylose Lysine Deoxycholate Agar
172 (XLDA; Oxoid) and/or *Salmonella* Chromogenic Agar (SCA; Oxoid) and/or Brilliant Green
173 Agar (BGA; Oxoid), incubated at 37°C for 24 hours and the typical colonies were counted.

174

175 2.7 Determination of *Listeria monocytogenes*

176 For detection of *L. monocytogenes*, 25 ml of each sample homogenate was transferred to 225 ml
177 of enrichment medium (L-PALCAM broth; Merck), and incubated at 30°C for 24 h. After
178 incubation, 0.1 ml of enrichment medium was transferred into 10 ml of L-PALCAM broth and
179 incubated on 30°C for 24 hours, followed by detection of *L. monocytogenes* by using an
180 immunochromatographic rapid test (Singlepath® *Listeria monocytogenes*; Merck). Positive
181 samples were confirmed by streaking onto PALCAM Listeria Selective Agar (Merck) and
182 incubating at 30°C for 24 hours. For direct enumeration of *L. monocytogenes*, 1 ml volumes from
183 appropriate sample dilutions were spread onto PALCAM Listeria Selective Agar (Merck)
184 incubated at 30°C and the typical colonies were counted after 24 - 48 h.

185

186 2.8 Determination of lactic acid bacteria count

187 From the sample homogenate, decimal dilutions were made in MRS broth (Biokar Diagnostics,
188 France) and 1 ml volumes from appropriate sample dilutions were spread onto Aerobic Count
189 Plate Petrifilms (3M Health Care, St. Paul, USA). Inoculated Petrifilms and one bag of
190 Anaerocult® C (Merck) were placed into anaerobic jars (Merck) and incubated at 30°C for 48 h,
191 after which the colonies were counted.

192

193 2.9 Determination of physicochemical parameters in the sausages

194 Water activity was determined by using LabSwift-aw set Euro-plug & BAT (Novasina,
195 Switzerland) and pH was determined using a portable pH meter - Testo 205 (Testo AG,
196 Germany).

197

198 2.10 Determination of biogenic amines (BA)

199 Each of four BA standards (putrescine dihydrochloride, cadaverine dihydrochloride, histamine
200 dihydrochloride, tyramine hydrochloride; Sigma-Aldrich, Germany) was separately diluted in
201 distilled water to obtain standard BA solutions with concentrations of 100µg/ml. Internal
202 standard was obtained by dissolving 50 mg of 1,7-diaminoheptan (Sigma-Aldrich) in 50 ml of
203 water. Dansyl chloride solution was prepared by dissolving 20 mg of dansyl chloride (Sigma-
204 Aldrich) in 2 ml of acetone. Extraction and derivatization of sausage samples (2 g) was
205 conducted according to Eerola, Hinkkanen, Lindfors & Hirvi (1993), and BA content was
206 determined by High Performance Liquid Chromatography (Agilent 1200 series; Agilent
207 Technologies, USA) according to Tasic et al. (2012).

208

209 2.11 Sensory analysis

210 Sensory quality parameters (external appearance, cross-cut surface appearance, colour, texture,
211 juiciness, odour, flavour, and overall acceptability) of finished sausages produced from
212 pathogen-uninoculated but HLA1/2/3-treated beef were scored by a trained, eight-member
213 sensory panel using a 1-7 point scale.

214

215 2.12 Analysis of the results

216 Each sausage group at each sampling point was tested in six replicas. The microbial counts were
217 converted to log CFU per g to normalize the data before further analyses. The means and
218 standard deviations were calculated using MS Excel 2007.

219

220 **3. Results and discussion**

221 No significant differences in dynamics of development/levels of the LAB population,
222 resultant pH drop, or water activity were observed between sausages produced using HLA1-,
223 HLA2- or HLA3-treated beef and control sausages (Table 1). For all the groups of sausages,
224 values obtained for these parameters (Table 1) can be considered as typical for this type of
225 fermented sausage. This also indicates that the multi-strain LAB starter culture functioned well
226 under all the experimental conditions used in this study. When considering the findings from the
227 study outlined below, one should keep in mind that the results are based on six replicas per
228 sampling point, but one batch per group of sausages, so the results do not show whether and how
229 large between-batch variability would have existed.

230

231 3.1 The effects of incoming beef decontamination on *E. coli* O157 in sausages

232 In control sausages produced from inoculated but non-decontaminated beef, *E. coli* O157
233 survived throughout the production process, although the initial level was reduced, on average,
234 by 1.9 logs in the finished product (Table 1). However, the *E. coli* O157 reduction rates in
235 sausages produced from inoculated and decontaminated beef were comparably much higher
236 (Table 1). In sausages produced by using HLA3-treated beef, no *E. coli* O157 was found in
237 finished product either by direct plating or by enrichment methods, which indicates that the total
238 pathogen reduction was likely higher than 3.9 logs (the initial concentration in control sausages).
239 In sausages produced by using HLA1-treated beef, no *E. coli* O157 was found in finished
240 product by direct plating, but it was detected in two sausages (out of 6) by enrichment, which
241 indicates that the total *E. coli* O157 reduction rate achieved was probably around 3.9 logs (the
242 initial concentration in control sausages). In sausages produced by using HLA2-treated beef,
243 only 0.1 log of *E. coli* O157 was found in finished product by direct plating, and the pathogen

244 was detected in only one sausage (out of 6) by enrichment, which indicates that the total *E. coli*
245 O157 reduction rate achieved was probably only slightly lower than 3.9 logs (the initial
246 concentration in control sausages). No other studies on the use of hot water-lactic acid
247 decontamination treatments of incoming raw beef trimmings to control *E. coli* O157 in
248 fermented sausages have been published to date. On the other hand, treatments of raw meat cuts
249 or trimmings unrelated to fermented sausage production, based on using 2% to 5% lactic acid
250 solutions at temperatures of up to 55°C applied either by spraying or misting, resulted in
251 STEC/VTEC reductions varying between 0.1 and 2.3 log CFU (Echeverry, Brooks, Miller,
252 Collins, Loneragan & Brashears, 2009; Heller et al., 2007; Calicioglu, Kaspar, Buege &
253 Luchansky, 2002; Harris, Miller, Loneragan & Brashears, 2006). However, the decontamination-
254 related STEC/VTEC reduction rates achieved on beef cuts/trimmings only and those obtained in
255 finished fermented sausages produced from decontaminated beef trimmings cannot be compared
256 directly, at least for two reasons. Firstly, the latter also includes the effects of antimicrobial
257 factors other than decontamination. Secondly, when temperatures of meat decontaminating
258 solutions are above 72°C (as in the present study), the heat applied is a decontaminating
259 procedure in itself (EFSA, 2010).

260

261 3.2 The effects of incoming beef decontamination on *Salmonella* Typhimurium in sausages

262 In control sausages produced from inoculated but non-decontaminated beef, *Salmonella* survived
263 throughout the production process, but the final count of this pathogen in finished sausages was
264 on average 1.9 logs lower than the initial count (Table 1). However, in sausages produced by
265 using any of the HLA1, HLA2 and HLA3 treatments, no *Salmonella* was found in finished
266 product either by direct plating or by enrichment methods, which indicates that the *Salmonella*

267 reduction rates achieved were probably higher than 3.6 logs (the initial concentration in control
268 sausages). No other studies on the use of hot water-lactic acid decontamination treatments of
269 incoming raw beef trimmings to control *Salmonella* in fermented sausages have been published
270 to date. Treatments of raw meat cuts or trimmings unrelated to fermented sausage production,
271 based on using 2% to 5% lactic acid solutions at temperatures of up to 55°C applied either by
272 spraying or misting, resulted in *Salmonella* reductions varying between 0.2 and 1.6 log CFU
273 (Echeverry et al., 2009; Harris et al., 2006). However, these results cannot be directly compared
274 with those from the present study, for the same reasons indicated above for VTEC/STEC
275 reductions.

276

277 3.3 The effects of incoming beef decontamination on *L. monocytogenes* in sausages

278 In control sausages produced from inoculated but non-decontaminated beef, *L. monocytogenes*
279 survived throughout the production process, and the final count of this pathogen in finished
280 sausages was very similar to the initial 2.2 log concentration (Table 1). The results reflect the
281 marked resistance of *L. monocytogenes* against antimicrobial factors such as low pH, higher salt
282 and lower a_w in general (Buncic & Avery, 2004). Furthermore, the marked survival ability of this
283 pathogen in the same beef fermented sausages (“Sudzuk”) was also reported in another study
284 where *L. monocytogenes* showed either practically no reduction (0.1 log) or only up to 0.9 log
285 (Hwang, Porto-Fett, Juneja, Ingham, Ingham & Luchansky, 2009). In finished sausages produced
286 from decontaminated beef, only small reduction rates (around 0.5 log) were observed (Table 1),
287 and they did not differ significantly between the HLA1, HLA2 and HLA3 treatments. Overall,
288 the efficacy of the three HLA treatments applied to incoming beef in reducing *L. monocytogenes*
289 in the finished sausages can be considered as unsatisfactory. Nevertheless, considering that *L.*

290 *monocytogenes* was unable to grow in the fermented sausages (Table 1), and that it is widely
291 accepted that levels of up to 100 CFU/g (1 log) are considered as tolerable in ready-to-eat foods
292 where it cannot grow (Buncic & Avery, 2004; WHO/FAO, 2004; EC, 2005), total *L.*
293 *monocytogenes* elimination from fermented sausages is not an absolute requirement as long as
294 low initial counts and growth prevention are reliably ensured. No other studies on the use of hot
295 water-lactic acid decontamination treatments of incoming beef trimmings to control *L.*
296 *monocytogenes* in fermented sausages have been published to date. On the other hand, hot lactic
297 acid treatments (2% LA, 55°C, 30 sec) of raw beef slices unrelated to fermented sausage
298 production resulted in *L. monocytogenes* reductions varying from 1.4 logs to 2.6 logs
299 (Koutsoumanis et al., 2004; Ikeda, Samelis, Kendall, Smith & Sofos, 2003). However, these
300 results cannot be directly compared with those from the present study, due to large between-
301 studies differences in the experimental design and conditions.

303 3.4 The effects of incoming beef decontamination on biogenic amines content in sausages

304 In the present study, only relatively low concentrations of tyramine, but no histamine, were
305 found in control sausages (with non-decontaminated beef) during the first stage of the production
306 process (Table 2). However, neither tyramine nor histamine were found in finished sausages,
307 regardless of whether they were control sausages (produced with non-decontaminated beef) or
308 were produced with beef treated by any of the three HLA treatments. During the first stage of the
309 production process, relatively low concentrations of cadaverine and putrescine were initially
310 found in control sausages, and only traces were detected in sausages produced with
311 decontaminated beef (Table 2). It is considered that the main reason for the absence of BA in the
312 finished sausages was the use of the starter culture which was comprised of LAB strains

313 previously proven as non-BA-producers; such an effect was also reported previously (Bover-Cid
314 et al., 2001). The LAB starter strains were obviously very competitive and grew very well in the
315 sausages (Table 1), which probably resulted in suppression of growth and/or amine production of
316 wild microbiota in control (untreated) sausages. In sausages produced with decontaminated beef,
317 this effect was additionally enhanced by elimination of a significant proportion of the wild
318 microbiota, which would be expected to be potential BA producers. Hence, because BA
319 formation was successfully controlled by our non-BA-producing LAB starter even in the control
320 sausages, any effect of HLA treatment on BA in these sausages could not be measured by
321 comparing HLA treated sausages with control sausages. No other studies on the use of hot water-
322 lactic acid decontamination treatments of incoming meat to control BA in fermented sausages
323 have been published.

324

325 3.5 The effects of incoming beef decontamination on sensory acceptability of sausages

326 The overall sensorial acceptability of finished control (non-decontaminated beef) sausages
327 produced under the influence of both wild microbiota and the LAB starter was very good -
328 average score 6.2 out of 7 (Table 3). However, the overall acceptability of finished sausages
329 produced with beef decontaminated by any HLA treatment was lower than the control sausages.
330 Those produced with HLA1-treated beef had best overall acceptability (1.6 points lower than the
331 control, but still within the acceptable range for this point scale), followed by HLA2-treated beef
332 sausages (1.8 points lower than the control), whilst HLA3-treated beef sausages had the worst
333 overall acceptability (3.7 points lower than the control). Generally, it seems that the duration of
334 HLA treatment was more critical for the sensorial acceptability of sausages than the temperature
335 – the shorter the HLA treatment, the better the sensorial acceptability (Table 3).

336 The diminished sensory qualities of the finished fermented sausages due to use of HLA-
337 treated beef was probably multifactorial, but at least two potential factors played important role.
338 The first factor was that the heat treatment associated with HLA decontamination changes the
339 surface meat colour from red (fresh meat) to grey-brown (cooked meat), and the latter is
340 unacceptable if it occurs in finished sausages. However, although a change of beef colour
341 occurred immediately after HLA treatments, this was transferred to some extent – but not to the
342 full extent – through the production process into the finished sausages. Because only a thin
343 surface layer of the meat changed colour, this undesirable colour was “diluted” by the normal
344 and acceptable red colour of deeper, unaffected parts of meat during the meat chopping in
345 mincing/cutter machines. Also, it is possible that the grey-brown surface layers of treated meat
346 recovered their red colour to some extent due to increased oxygen pressure, when air was forced
347 into the batter during the meat mincing/cutting operations in machines that operated without
348 vacuum. The second factor was the probable elimination of a proportion (but not complete
349 elimination) of the wild microbiota from incoming beef by HLA; wild microbiota plays some
350 role in the development of desirable sensory qualities (Lücke, 2000). The composition of wild
351 microbiota is normally very complex, as is its contribution to the desirable sensory qualities of
352 fermented meats. However, it seems that, in the present study, our multi-strain LAB starter
353 culture played a beneficial role in somewhat nullifying those potentially negative consequences
354 of reduced wild microbiota in incoming beef on the quality of the fermented sausages.

355 Overall, the use of hot lactic acid decontamination treatments of incoming beef
356 significantly reduced *E. coli* O157 and *Salmonella* counts (but not *L. monocytogenes*) and
357 prevented accumulation of toxic BA in resultant raw, fermented, dried sausages. Fatty tissue
358 added to sausage batter (10%) could not be HLA-decontaminated for technological reasons, and

359 it is not known whether nor how much it contributed to initial contamination of the sausage
360 batter, or if its contaminating microbiota diminished the pathogen-reduction effects of the HLA-
361 based beef treatments as observed in finished sausages. The overall sensorial quality of finished
362 sausages produced with HLA-decontaminated beef was somewhat reduced, but, importantly,
363 remained in the acceptable category as long as the shorter duration HLA-treatment was used.
364 Further work is required to optimize HLA-based beef decontamination treatments so to improve
365 sensorial acceptability whilst maintaining the marked pathogen reduction in resultant fermented
366 sausages.

367

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373

374 **5. References**

375 Bacus, J. N. (1986). Fermented meat and poultry products. In A. M. Pearson & T. R. Dutson
376 (Eds.), *Advances in Meat Research*. Vol. 2, *Meat and Poultry Microbiology*, (pp. 123-
377 164). Westport, CT: AVI Publishing.

378 Bover-Cid, S., Izquierdo-Pulido, M., & Vidal-Carou, M.C. (2001). Effectiveness of a
379 *Lactobacillus sakei* starter culture in the reduction of biogenic amine accumulation as a
380 function of the raw material quality. *Journal of Food Protection*, 64, 367-373.

- 381 Bover-Cid, S., Hernández-Jover, T., Miguélez-Arrizado, M.J., & Vidal-Carou, M.C. (2003).
382 Contribution of contaminant enterobacteria and lactic acid bacteria to biogenic amine
383 accumulation in spontaneous fermentation of pork sausages. *European Food Research
384 and Technology*, 216, 477-482.
- 385 Calicioglu, M., Kaspar, C.W., Buege, D.R., & Luchansky, J.B. (2002). Effectiveness of spraying
386 with Tween 20 and lactic acid in decontaminating inoculated *Escherichia coli* O157:H7
387 and indigenous *Escherichia coli* biotype I on beef. *Journal of Food Protection*, 65, 26-32.
- 388 EC (2005). European Commission Regulation 2073/2005 of 15 November 2005 on
389 microbiological criteria for foodstuffs. *Official Journal of the European Union*, L338, 1-
390 26.
- 391 Echeverry, A., Brooks, J.C., Miller, M.F., Collins, J.A., Loneragan, G.H., & Brashears, M.M.
392 (2009). Validation of intervention strategies to control *Escherichia coli* O157:H7 and
393 *Salmonella Typhimurium* DT 104 in mechanically tenderized and brine-enhanced beef.
394 *Journal of Food Protection*, 72, 1616-1623.
- 395 Eerola, S., Hinkkanen, R., Lindfors, E., & Hirvi, T. (1993). Liquid chromatographic
396 determination of biogenic amines in dry sausages. *Journal of AOAC International*, 76,
397 575-577.
- 398 EFSA (2011). Scientific Opinion on risk based control of biogenic amine formation in fermented
399 foods. *EFSA Journal*, 9:2393, 1-93.
- 400 EFSA (2010). Scientific Opinion on the safety and efficacy of using recycled hot water as a
401 decontamination technique for meat carcasses. *EFSA Journal*, 8:1827, 1-69.

- 402 Glass, K.A., Loeffelholz, J.M., Ford, J.P., & Doyle, M.P. (1992). Fate of *Escherichia coli*
403 O157:H7 as affected by pH or sodium chloride and in fermented, dry sausage. *Applied*
404 *and Environmental Microbiology*, 58, 2513-2516.
- 405 Harris, K., Miller, M.F., Loneragan, G.H., & Brashears, M.M. (2006). Validation of the use of
406 organic acids and acidified sodium chlorite to reduce *Escherichia coli* O157 and
407 *Salmonella* Typhimurium in beef trim and ground beef in a simulated processing
408 environment. *Journal of Food Protection*, 69, 1802-1807.
- 409 Heller, C.E., Scanga, J.A., Sofos, J.N., Belk, K.E., Warren-Serna, W., Bellinger, G.R., Bacon,
410 R.T., Rossman, M.L., & Smith, G.C. (2007). Decontamination of beef subprimal cuts
411 intended for blade tenderization or moisture enhancement. *Journal of Food Protection*,
412 70, 1174-1180.
- 413 Holck, L.A., Axelsson, L., Mari Rode, T., Hoy, M., Mage, I., Alvseike, O., L'abée-Lund, T.M.,
414 Omer, M.K., Granum, P.E., & Heir, E. (2011). Reduction of verotoxigenic *Escherichia*
415 *coli* in production of fermented sausages: a review. *Meat Science*, 89, 286-295.
- 416 Hwang, C.A., Porto-Fett, A.C., Juneja, V.K., Ingham, S.C., Ingham, B.H., & Luchansky, J.B.
417 (2009). Modeling the survival of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and
418 *Salmonella* Typhimurium during fermentation, drying, and storage of soudjouk-style
419 fermented sausage. *International Journal of Food Microbiology*, 129, 244-252.
- 420 Ikeda, J.S., Samelis, J., Kendall, P.A., Smith, G.C., & Sofos, J.N. (2003). Acid adaptation does
421 not promote survival or growth of *Listeria monocytogenes* on fresh beef following acid
422 and nonacid decontamination treatments. *Journal of Food Protection*, 66, 985-992.
- 423 Koutsoumanis, K., Ashton, L., Geornaras, I., Belk, K.E., Scanga, J.A., Kendall, P.A., Smith,
424 G.C., & Sofos, J.N. (2004). Effect of single or sequential hot water and lactic acid

- 425 decontamination treatments on the survival and growth of *Listeria monocytogenes* and
426 spoilage microflora during aerobic storage of fresh beef at 4, 10, and 25°C. *Journal of*
427 *Food Protection*, 67, 2703–2711.
- 428 Lücke, F. K. (2000). Fermented meats. In B. M. Lund, T. C. Baird-Parker, & G.W. Gould (Eds.),
429 *The microbiological safety and quality of food*. Gaithersburg: Aspen Publishers Inc.
- 430 Reed, C.A. (1995). Approaches for ensuring the safety of dry and semi-dry fermented sausage
431 products. U.S. Department of Agriculture, Food Safety Inspection Service, Washington,
432 D.C., Letter to plant managers of August 21, 1995.
- 433 Ruiz-Capillas, C., & Jiménez-Colmenero, F. (2004). Biogenic amines in meat and meat products.
434 *Critical Reviews in Food Science and Nutrition*, 44, 489-499.
- 435 Sauer, C.J., Majkowski, J., Green, S., & Eckel, R. (1997). Foodborne illness outbreak associated
436 with a semi-dry fermented sausage product. *Journal of Food Protection*, 60, 1612-1617.
- 437 Tasic, T., Ikonic, P., Mandic, A., Jokanovic, M., Tomovic, V., Savatic, S., & Petrovic Lj. (2012).
438 Biogenic amines content in traditional dry fermented sausage Petrovská klobása as
439 possible indicator of good manufacturing practice. *Food Control*, 23, 107–112.
- 440 WHO/FAO (2004). Risk assessment of *Listeria monocytogenes* in ready-to-eat foods. Food and
441 Agriculture Organisation, Rome, Italy.

442 **Table 1. The effects of decontamination treatments of incoming beef on microbiological and physicochemical parameters of**
 443 **fermented sausages during the production process**

Day of the production process (stage)	Treatments of inoculated* raw meat for sausage production (by 4% hot lactic acid; HLA)	Lactic acid bacteria	<i>E. coli</i> O157	<i>S. Typhimurium</i>	<i>L. monocytogenes</i>	Water activity (a_w)	pH
		mean log CFU/g \pm SD	mean log CFU/g \pm SD (number of enrichment-method positive sausages out of 6 tested)**	mean log CFU/g \pm SD (number of enrichment-method positive sausages out of 6 tested)**	mean log CFU/g \pm SD	mean \pm SD	mean \pm SD
0 (immediately after stuffing in casings)	Control (untreated; n=6)	6.7 \pm 0.1	3.9 \pm 0.2	3.6 \pm 0.4	2.2 \pm 0.1	0.978 \pm 0.001	5.59 \pm 0.09
	HLA1 (90°C/10 s; n=6)	6.2 \pm 0.2	1.2 \pm 0.1(6)	0.5 \pm 0.5(6)	1.9 \pm 0.2	0.979 \pm 0.003	5.26 \pm 0.07
	HLA2 (85°C/20 s; n=6)	6.5 \pm 0.3	0.1 \pm 0.2(6)	0.1 \pm 0.3(5)	2.0 \pm 0.3	0.979 \pm 0.001	5.21 \pm 0.06
	HLA3 (80°C/30 s; n=6)	5.9 \pm 0.5	0.7 \pm 0.5(6)	0.1 \pm 0.1(5)	1.9 \pm 0.2	0.978 \pm 0.001	5.24 \pm 0.03
3 (end of production)	Control	8.5 \pm 0.1	3.5 \pm 0.1	2.4 \pm 0.2	2.0 \pm 0.1	0.970 \pm	5.05 \pm

fermentation)	(untreated; n=6)					0.001	0.02
	HLA1					0.972±	4.99±
	(90°C/10 s; n=6)	8.5±0.1	0.4±0.6(6)	0.0(0)	2.0±0.2	0.001	0.04
	HLA2					0.971±	4.81±
	(85°C/20 s; n=6)	8.5±0.1	0.1±0.1(3)	0.0(4)	2.0±0.3	0.001	0.02
	HLA3					0.972±	4.80±
	(80°C/30 s; n=6)	8.5±0.1	0.2±0.4(4)	0.0(1)	1.8±0.4	0.001	0.01
20 (end of	Control					0.815±	5.35±
process -	(untreated; n=6)	8.5±0.1	2.0±0.5	1.7±0.2	2.2±0.1	0.008	0.02
finished	HLA1					0.790±	5.29±
sausages)	(90°C/10 s; n=6)	8.8±0.1	0.0(2)	0.0(0)	1.9±0.3	0.018	0.02
	HLA2					0.816±	5.17±
	(85°C/20 s; n=6)	8.7±0.1	0.1±0.1(1)	0.0(0)	1.9±0.3	0.018	0.03
	HLA3					0.798±	5.14±
	(80°C/30 s; n=6)	8.5±0.1	0.0(0)	0.0(0)	1.7±0.3	0.024	0.03

444 * Inoculated with a mixture of *E. coli* O157, *S. Typhimurium* and *L. monocytogenes* strains; ** Enrichment broths tested by the

445 GLISA rapid method

446 **Table 2. The effects of decontamination treatments of incoming beef on biogenic amine**
 447 **contents in fermented sausages during the production process**

Day of the production process (stage)	Treatments of inoculated* raw meat for sausage production (with 4% hot lactic; HLA)	Putrescine (mg/kg)	Cadaverine (mg/kg)	Histamine (mg/kg)	Tyramine (mg/kg)
3 (end of fermentation)	Control (untreated; n=3)	50.9±45.0	33.0±7.3	ND	24.8±21.8
	HLA1 (90°C/10 s; n=3)	ND	4.0±3.6	ND	ND
	HLA2 (85°C/20 s; n=3)	ND	1.9±1.5	ND	ND
	HLA3 (80°C/30 s; n=3)	1.7±3.0	3.2±1.7	ND	ND
20 (end of process - finished sausages)	Control (untreated; n=3)	ND	ND	ND	ND
	HLA1 (90°C/10 s; n=3)	ND	ND	ND	ND
	HLA2 (85°C/20 s; n=3)	ND	ND	ND	ND
	HLA3 (80°C/30 s; n=3)	ND	ND	ND	ND

448 ND – not detected; * Inoculated with a mixture of *E. coli* O157, *S. Typhimurium* and *L.*
 449 *monocytogenes* strains

450 **Table 3. The effects of decontamination treatments of incoming beef on sensory qualities of finished fermented sausages**

Treatments of raw meat for sausage production (by 4% hot lactic acid; HLA)	Sausage sensory qualities scores* (mean value±SD)							
	External appearance	Cross-cut surface appearance	Colour	Texture	Juiciness	Odour	Flavour	Overall acceptability
Control (untreated)	6.0±0.0	6.9±0.2	7.0±0.0	6.6±0.2	4.4±0.3	6.1±0.6	6.2±0.3	6.2±0.3
HLA1 (90°C/10 s)	5.0±0.0	6.3±0.3	6.9±0.2	6.2±0.3	3.2±0.5	6.2±0.6	4.3±0.8	4.6±0.8
HLA2 (85°C/20 s)	3.9±0.3	4.6±0.5	3.0±0.0	6.0±0.3	2.6±0.3	5.6±0.9	5.2±1.0	4.4±0.9
HLA3 (80°C/30 s)	3.2±0.4	3.9±0.6	2.0±0.0	6.0±0.3	2.2±0.3	4.7±0.7	3.0±1.3	2.5±0.3

451 * Using 1-7 scale; assessed by an eight-member trained sensory panel

Highlights

- Beef trimmings for fermented sausages were treated with hot, 4% lactic acid.
- *E. coli* O157 and *Salmonella* were markedly reduced in finished sausages.
- *L. monocytogenes* reduction in finished sausages was not significant.
- Biogenic amines were not detectable in finished sausages.
- Sensorial acceptability of finished sausages was somewhat diminished.