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

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Abstract

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

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

1. Introduction

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

A number of outbreaks of toxicoinfections by verocytotoxigenic *Escherichia coli* (VTEC) associated with consumption of fermented sausages (mainly containing beef, but also lamb) have been reported in different countries, as reviewed by Holck et al. (2011). Also, consumption of fermented sausages has been associated or epidemiologically linked with other foodborne illnesses caused by *Staphylococcus aureus* (Bacus, 1986), *Salmonella* (Sauer, 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 todate, 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.

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

2. Materials and methods

2.1 Inoculation of incoming raw beef

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

2.2 Decontamination of inoculated beef trimmings

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

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

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

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

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

2.3 Preparation of raw, fermented, dried beef sausages ("Sudzuk" type)

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

RM20R, Spain). During meat mixing, 2% of commercially-supplied salt for curing (containing sodium nitrite), 0.25% dextrose and 0.8% spices (powdered garlic, black pepper and paprika) were added to the batter in the kneader mixer. Then, a starter culture comprising LAB strains *Lactobacillus sakei* CTC 41, *L. sakei* CTC 287, *L. sakei* CTC 6469 and *L. sakei* CTC 6626 (all identified as not producing biogenic amines, obtained from Dr. Sara Bover-Cid, Institute for Food and Agricultural Research and Technology-IRTA, Spain) was added at level $4x10^5$of each strain per gram of sausage batter. Subsequently, the sausage batter was stuffed into collagen casings (Koko ø35, Viscofan, Serbia) and sausages were placed in a controlled-environment chamber (Stagionello STG 100 MTF, Italy) and subjected to a fermentation and maturation/drying process for 20 days in total, under the following temperature/relative air humidity conditions: 1st day - 20°C/92%; 2nd day - 19°C/86%; 3rd day - 18°C/80%; 4th day - 17°C/72%; 5th to 20th day - 16°C/65%). There were three sausages sampling points during the process: at day 0 (immediately after stuffing into casings), at day 3 (on completion of fermentation) and at day 20 (finished product).

2.4 Sampling and sample homogenates preparation

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

2.5 Determination of *Escherichia coli* O157

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

2.6 Determination of *Salmonella* Typhimurium

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

2.7 Determination of *Listeria monocytogenes*

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

2.8 Determination of lactic acid bacteria count

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

2.9 Determination of physicochemical parameters in the sausages

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

2.10 Determination of biogenic amines (BA)

Each of four BA standards (putrescine dihydrochloride, cadaverine dihydrochloride, histamine dihydrochloride, tyramine hydrochloride; Sigma-Aldrich, Germany) was separately diluted in distilled water to obtain standard BA solutions with concentrations of 100µg/ml. Internal standard was obtained by dissolving 50 mg of 1.7-diaminoheptan (Sigma-Aldrich) in 50 ml of water. Dansyl chloride solution was prepared by dissolving 20 mg of dansyl chloride (Sigma-Aldrich) in 2 ml of acetone. Extraction and derivatization of sausage samples (2 g) was conducted according to Eerola, Hinkkanen, Lindfors & Hirvi (1993), and BA content was determined by High Performance Liquid Chromatography (Agilent 1200 series; Agilent Technologies, USA) according to Tasic et al. (2012).

2.11 Sensory analysis

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

2.12 Analysis of the results

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

3. Results and discussion

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

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

In control sausages produced from inoculated but non-decontaminated beef, *E. coli* O157 survived throughout the production process, although the initial level was reduced, on average, by 1.9 logs in the finished product (Table 1). However, the *E. coli* O157 reduction rates in sausages produced from inoculated and decontaminated beef were comparably much higher (Table 1). In sausages produced by using HLA3-treated beef, no *E. coli* O157 was found in finished product either by direct plating or by enrichment methods, which indicates that the total pathogen reduction was likely higher than 3.9 logs (the initial concentration in control sausages). In sausages produced by using HLA1-treated beef, no *E. coli* O157 was found in finished product by direct plating, but it was detected in two sausages (out of 6) by enrichment, which indicates that the total *E. coli* O157 reduction rate achieved was probably around 3.9 logs (the initial concentration in control sausages). In sausages produced by using HLA2-treated beef, 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^{o}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.
302
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).

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

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

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

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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** mean log CFU/g ±SD	***E. coli* O157** mean log CFU/g±SD (number of enrichment-method positive sausages out of 6 tested)**	***S.* Typhimurium** mean log CFU/g±SD (number of enrichment-method positive sausages out of 6 tested)**	***L. monocytogenes*** mean log CFU/g±SD	**Water activity (a_w)** mean ±SD	**pH** mean ±SD
0 (immediately after stuffing in casings)	Control (untreated; n=6)	6.7±0.1	3.9±0.2	3.6±0.4	2.2±0.1	0.978± 0.001	5.59± 0.09
	HLA1 (90°C/10 s; n=6)	6.2±0.2	1.2±0.1(6)	0.5±0.5(6)	1.9±0.2	0.979± 0.003	5.26± 0.07
	HLA2 (85°C/20 s; n=6)	6.5±0.3	0.1±0.2(6)	0.1±0.3(5)	2.0±0.3	0.979± 0.001	5.21± 0.06
	HLA3 (80°C/30 s; n=6)	5.9±0.5	0.7±0.5(6)	0.1±0.1(5)	1.9±0.2	0.978± 0.001	5.24± 0.03
3 (end of	Control	8.5±0.1	3.5±0.1	2.4±0.2	2.0±0.1	0.970±	5.05±

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