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Meat Science xxx (2013) xxx

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Highlights

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Effects of post-processing treatments on sensory quality and Shiga toxigenic *Escherichia coli* reductions in dry-fermented sausages

E. Heir [a,*], A.L. Holck [a], M.K. Omer [b], O. Alvseike [b], I. Måge [a], M. Høy [a], T.M. Rode [a], M.S. Sidhu [b], L. Axelsson [a]

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► Fermented sausages represent potential microbiological risk products ► Post-process treatments provide high quality fermented sausages with enhanced safety ► The sensory quality of the sausages were minimally affected by the treatments ► The treatments should be applicable for implementation in industrial production

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http://dx.doi.org/10.1016/j.meatsci.2012.12.020

Meat Science xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Meat Science

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Effects of post-processing treatments on sensory quality and Shiga toxigenic *Escherichia coli* reductions in dry-fermented sausages

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ARTICLE INFO

Article history:
Received 2 July 2012
Received in revised form 21 December 2012
Accepted 29 December 2012
Available online xxxx

Keywords:
Microbiology
Shiga toxigenic *E. coli*
EHEC
Food safety
Dry-fermented sausage
Sensory characteristics

ABSTRACT

The effects of post-processing treatments on sensory quality and reduction of Shiga toxigenic *Escherichia coli* (STEC) in three formulations of two types of dry-fermented sausage (DFS; salami and morr) were evaluated. Tested interventions provided only marginal changes in sensory preference and characteristics. Total STEC reductions in heat treated DFS (32 °C, 6 days or 43 °C, 24 h) were from 3.5 to >5.5 log from production start. Storing of sausages (20 °C, 1 month) gave >1 log additional STEC reduction. Freezing and thawing of sausages in combination with storage (4 °C, 1 month) gave an additional 0.7 to 3.0 log reduction in STEC. Overall >5.5 log STEC reductions were obtained after storage and freezing/thawing of DFS with increased levels of glucose and salt. This study suggests that combined formulation optimisation and post-process strategies should be applicable for implementation in DFS production to obtain DFS with enhanced microbial safety and high sensory acceptance and quality.

1. Introduction

Dry-fermented sausages (DFS) encompass a wide diversity of products and the manufacturers of DFS range from large companies to small producers. Common to most DFS are their main ingredients being raw, ground meat preserved by fermentation and drying in the production process. No specific bactericidal treatments or true critical control points are usually applied in the production process. This means that the microbial safety of these types of products mainly depends on the collective action of acidic pH, lactate produced, reduced water activity and presence of sodium chloride (NaCl) and curing salts ($NaNO_2$ or $NaNO_3$) in the products. Various types of DFS such as salami, Norwegian "Morr" and organic beef sausage, have been implicated in several foodborne outbreaks (Ammon, Petersen, & Karch, 1999; Ethelberg et al., 2009; MacDonald et al., 2004; Paton et al., 1996; Sartz et al., 2008; Schimmer et al., 2008). The causative agents in many of these outbreaks have been enterohaemorrhagic *Escherichia coli* (EHEC), a subgroup of Shiga toxigenic *E. coli* (STEC). EHEC can cause severe human illness. Other foodborne pathogens, e.g. *Salmonella*, have also been implicated as causative agents in DFS outbreaks (Bremer et al., 2004; Emberland et al., 2006; Kuhn, Torpdahl, Frank, Sigsgaard, & Ethelberg, 2011). This means that many DFS production processes do not adequately maintain the microbial food safety and DFS products in general should be regarded as risk products if no interventions are applied to ensure microbial food safety.

The potential low infectious dose of EHEC (Tilden et al., 1996) demands strategies that not only inhibit growth but also eliminate the bacteria. Various intervention strategies including thermal treatments or validated production strategies have been introduced in e.g. USA (Anonymous, 2001), Canada (Anonymous, 2000) and Australia (Anonymous, 2002) to ensure microbial safe DFS. Strategies should be effective in eliminating STEC and also be easily and cost-effectively implemented while maintaining or if possible enhancing the sensory qualities of the product.

A previous study showed the complexity, options and limitations in obtaining robust interventions for STEC reductions during the DFS production process (Heir et al., 2010). The study showed that optimisation of formulation and production processes may provide an approximate 3 log kill of *E. coli* during the production process compared to 1.5 log reduction obtained in a standard process. No significant negative effects on sensory acceptance of the sausage were recorded. The study showed that additional interventions are required to ensure the microbial safety of DFS before they are placed on the market. To achieve the desired 5 log STEC reductions according to requirements and recommendations in USA (Reed, 1995) and Canada (Anonymous, 2000), respectively, manufacturers of DFS request documented STEC

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0309-1740/$ – see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.meatsci.2012.12.020

elimination strategies that can easily be implemented in industrial production. The effects of heating on STEC reductions are well documented (Calicioglu, Faith, Buege, & Luchansky, 1997; D.C.R. Riordan et al., 2000; Duffy et al., 1999; Hinkens et al., 1996; Rode, Holck, Axelsson, Høy, & Heir, 2012). However, less is known on how other temperature treatments suitable for industrial DFS processing affect both STEC elimination and the sensory properties of DFS. In this study, interventions were selected according to various criteria: i) to be effective with regard to STEC reductions, ii) to have no or minimal negative effects on sensory qualities, iii) to provide high potential for practical implementation in commercial sausage production. The object of the present study was to determine how various post-process thermal treatments of DFS including storage at various temperatures, freezing/thawing of DFS and short term heating affect sensory DFS characteristics and survival of STEC in DFS. A wide variety of salami sausages exist. This study investigates popular products, a Norwegian salami type of DFS, and "Morr" sausages which were the source of the EHEC outbreak in Norway in 2006 (Schimmer et al., 2008).

2. Materials and methods

2.1. Production of dry-fermented sausages

For DFS subjected to heat treatments, two types of sausages (salami and morr) with STEC were produced as previously described (Heir et al., 2010). The salami batters contained meat from beef and pork (37.8% each) and lard from pork (20%), whereas the Morr batters contained meat from pork (37.6%), mutton (31.3%) and heart meat from pork and beef (15.3% each). Standard formulations of both sausage types (see below), were made and fermented at 20 °C. For DFS subjected to storage and freeze/thaw treatments, three defined formulations termed Standard (SR), Moderate (MR) and High (HR) were made for both salami and morr. The formulations differed in added levels of NaCl (3.6, 4.5 and 5.0%, respectively), $NaNO_2$ (100, 300, 500 ppm, respectively) and glucose (0.5, 1.25, 1.25%, respectively) which were added to the batters in accordance with estimated final levels of each ingredient in the water phase of the sausage batter (Heir et al., 2010, Experiment 3 (Table 1)). Fermentations were performed at both 20 and 30 °C before being ripened until finished at day 23. Finished sausages were subjected to microbial and physicochemical analyses as described below. Prior to post-process interventions, sausages were vacuum packed and stored at 4 °C for a maximum one week before performing post-process treatments. Salami and morr for sensory analyses were obtained freshly made from two commercial suppliers.

2.2. Preparation of STEC and starter culture

Two STEC outbreak isolates linked to DFS were used: A human case *E. coli* O103:H25, *stx*2+ isolate from a Norwegian STEC outbreak in 2006 (Schimmer et al., 2008) and an *E. coli* O157:H7, *stx*2+ isolate from an outbreak in Sweden in 2002 (Sartz et al., 2008). Rifampicin resistant (Rif^R) derivatives of both strains were prepared and used as inoculum (10^7 CFU/g sausage batter) as previously described (Heir et al., 2010). Starter culture LS-25 (*Lactobacillus sakei* and *Staphylococcus carnosus*; Gewürzmüller, GmbH, Germany) was prepared in dH_2O and added to the batters (10^6 CFU/g).

2.3. Post-process treatments of dry-fermented sausages

Post-process treatments (heating, storage and freezing/thawing) were performed on vacuum-packed DFS with STEC. Also, commercial brands of salami and morr without STEC were vacuum-packed and subjected to the same processes (if not otherwise specified) in parallel experiments with subsequent sensory analyses.

Table 1
Mean score values of two sensory tests ("Overall acceptance" and "Just about right" (JAR)) of salami and after different heat treatments (1), (2) or (4) and non-treated controls.[a]

	Treatment		Overall test[b]	JAR test[c]			
			Acceptance	Colour	Salty taste	Fatty taste	Texture
Salami	Before storage	Control	4.6	2.9	3.1	3.4	2.7
		1	4.4	2.7	3.4	3.4	3.1
		2	3.9**	2.7	3.5*	3.1*	3.3***
		4	3.9*	2.2***	3.7**	3.3	3.3***
	After storage	Control	4.3	2.8	3.3	3.6	2.6
		1	4.4	2.6*	3.5	3.9	2.6
		2	4.5	2.7	3.4	3.6	2.8
		4	4.6	2.4***	3.5	3.6	2.9
Morr	Before storage	Control	4.6	2.7	3.0	3.5	2.8
		1	4.8	3.1	3.3	3.2	2.9
		2	4.5	2.5	3.6***	3.2	2.8
		4	4.2	2.7	3.6***	3.2	3.0
	After storage	Control	3.9	2.6	2.8	3.4	2.9
		1	4.9***	3.0***	3.1	3.4	2.8
		2	4.6**	2.6	3.3	3.4	3.1
		4	4.4	3.1***	3.4	3.2*	3.1

[a] Each sensory test was performed just after heat treatment of freshly made sausages (before storage) and after 6 weeks of storage at 4 °C subsequent to heat treatments. The number of respondents were, Before storage: salami = 39, morr = 43, After storage, salami = 68, morr = 71.
[b] Overall acceptance shown by mean preference score values on a 7-point scale (1 = very bad; 7 = very good). Significant differences from the control are indicated (significance limits: * 10%, **5%, ***1%).
[c] Mean score values of four sensory attributes in a Just about right (JAR) test. Each attribute was ranked on a 5-grade scale from having too little (score = 1) to having too much (score = 5) with optimal value 3. Significant differences from the control are indicated (significance limits: * 10%, **5%, ***1%).

2.3.1. Heat treatments

A total of 7 heat treatments were initially selected. The treatments were selected based on published guidelines by Health Canada to obtain 5 log reductions of STEC during the production process (Anonymous, 2000) and on the ability of DFS producers to implement the treatments in commercial production. The 7 treatments included: (1) 32 °C, 6 days; (2) 43 °C, 24 h; (3) 43 °C, 4 days; (4) 43 °C 1 h + 53 °C 6 h; (5) 60 °C, 12 min; (6) 50 °C, 30 min (7) and 65 °C, 30 min. Heat treatments (1)–(3) were conducted in incubation chambers (Termaks, Norway) while heat treatments (4)–(7) where performed in water baths to increase heat transfer. Heat treatments with STEC were performed on vacuum packed uniform sized pieces (30–40 g) of DFS. After heat treatments, the sausage pieces were immediately cooled in an ice-water bath before microbial analyses. The internal sausage temperature was measured by an automatic temperature logging device (Termometerfabriken, Viking AB, Eskilstuna, Sweden). Control sausages were stored at 4 °C without heat treatment.

2.3.2. Storage

Vacuum packed sausages with and without added STEC were stored at 4, 16 and 20 °C in the dark for one and two months.

2.3.3. Combined freezing/thawing

DFS were subjected to two freezing/thawing treatments FT1 and FT2. The freeze/thaw parameters were FT1: −20 °C for 17 h and thawing at 20 °C for 7 h; FT2: 4 repetitive cycles of treatment FT1. Freeze/thaw treated DFS and untreated control sausages were stored for 1 month at 4 °C before microbial and sensory analyses of DFS with and without added STEC, respectively.

2.4. Sensory analyses

2.4.1. Heat treated DFS

The sensory tests included a preference "overall acceptance" test followed by a "Just about right" (JAR) test on salami and morr.

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Evaluations were performed by 38 consumers after heat treatments (1, 2 and 4) and by 68 consumers on the same sausages after 6 weeks storage at 4 °C. Not heat treated DFS stored at 4 °C were blind controls. Approximately 0.5 cm slices of sample DFS were served at room temperature on white plastic dishes identified by random three-digit numbers. The sausages were randomly presented to the consumers. Overall acceptability of the DFS was ranked on a 7-point scale (1 = very bad; 7 = very good). In the JAR test, specific characteristics linked to the overall liking of the sausages were scored. The selected DFS properties colour, salty taste, perception of fatty taste and texture were evaluated by the panellists ranking the sausages on a 5-grade scale; from having too little (1) to having too much (5) with regard to the specific property. Optimal quality had value 3.

2.4.2. DFS stored at various temperatures or subjected to freeze/thaw treatments

Identical descriptive sensory tests (ISO 6564:1985E), but performed on separate occasions were performed on sausages subjected to storage for two months (4 °C (control), 16 °C and 20 °C) and freeze/thaw treatments (FT1 and FT2), respectively. The descriptive sensory tests were performed by a trained sensory panel of 12. Approximately 0.5 cm slices of salami and morr were served the panellists at room temperature on white plastic dishes identified by random three-digit numbers. Evaluations were performed in individual booths under white fluorescent lighting. Three repeated evaluations were performed by each panellist in randomized trials. Salami and morr were evaluated for 22 common characteristics of smell, colour, taste and texture and included: smell (smell of pork/cattle meat; sourish; metal; spice; rancidity; maturity), colour (tone; strength; whiteness), taste (taste of pork/cattle meat; sourness; salt; sweetness; bitterness; metal; spice; rancidity; maturity), texture (hardness; tenderness; greasy; juicy). In addition, smell and taste of mutton were evaluated for morr. For each sample, panellists scored the sensory characteristics on a 9 point scale where 1 indicated no intensity and 9 significant intensity. Water and unsalted crackers were served to the panellists to clean their palates between samples.

2.5. Microbial and physicochemical analyses

For microbiological analyses, sausage samples (10 g) were added to 90 ml of peptone water and homogenized for 1 min in a stomacher (AES Smasher, AES Chemunex, Bruz, France). STEC were quantified (CFU/g) by serial plating, using a Whitley Automatic Spiral Plater (Don Whitley Scientific Ltd., West Yorkshire, UK), on tryptic soy agar (TSA, 24 h incubation, 37 °C) with rifampicin (200 μg/ml). Lactobacilli were determined by plating on deMan Rogosa Sharpe agar (MRS agar, 48 h incubation, 30 °C). The detection limit for STEC was 20 CFU/g sausage. The pH of the meat batters and sausages was measured on the stomacher homogenized solution. Water activity (a_w) of the sausages was measured at 25 °C (AquaLab, series 3TE, Decagon Devices, Inc., Washington, USA). At least three replicate samples were used in the analyses.

2.6. Experimental designs and statistical analyses

The full factorial designed experiment of DFS with STEC included three formulations (Standard, Moderate, High), two sausage types (salami and morr), fermented at two temperatures (20 and 30 °C). Four replicates provided a total of 48 DFS. STEC log reductions during production were calculated: log (*E. coli* CFU/g from sausage batter at production day (day 0)) − log (*E. coli* CFU/g from DFS (day 23)). *Escherichia coli* log reductions during post-process interventions were calculated: log (*E. coli* CFU/g from DFS (day 23)) − log (*E. coli* CFU/g after post-processing). Analysis of variance (ANOVA) was used to determine statistically significant effects of the post-process interventions and their interactions with formulation and fermenting temperature (Minitab® 16 Statistical software, State College, PA: Minitab, Inc.,www.minitab.com). The consumer sensory test on heat treated DFS were also analysed using Minitab® 16 Statistical software, and a Bonferroni test was used to compare each treatment with the control. The sensory preference tests on storage and freeze/thaw treatments were analysed using ANOVA (SAS version 9.2, SAS Institute, Cary, NC, USA). Tukey's test was used in conjunction with the ANOVA to determine significant differences ($p<0.05$) between the groups for each sensory characteristic.

3. Results

3.1. Effects of mild heat treatments of DFS

3.1.1. Sensory characteristics

After preliminary sensory evaluations of 7 DFS heat treatments, 3 treatments (1; 32 °C, 6 days), (2; 43 °C, 24 h) and (4; 43 °C 1 h + 53 °C 6 h) were selected for studying the effects on the sensory quality of salami and morr. The preference test showed only small differences between heat treated DFS and control DFS (Table 1). A small, though statistically significant ($p \leq 0.05$), reduced overall acceptability of salami sausages subjected to treatment (2) were obtained. Interestingly, these overall acceptance differences were not obtained after 6 weeks storage (4 °C) of the heat treated salami. For morr, no significant differences were obtained on the overall acceptability of heat treated or control sausages (Heat treatments (1), $p = 1.000$, (2), $p = 1.000$ and (4), $p = 0.218$). Significantly improved overall acceptability scores were obtained after 6 weeks storage of morr subjected to treatments (1; $p = 0.0003$) and (2; $p = 0.0083$) compared to control. For salami, treatment (4) had a small though statistically significant negative effect on perception of colour while the opposite colour effects were observed for morr subjected to treatments (1) and (2) and stored for 6 weeks. For the other sensory attributes tested (salty taste, fatty taste, texture) only minor differences between control sausages and heat treated sausages were observed.

3.1.2. STEC reductions

Heat treatments (1) and (2) were investigated for evaluations of STEC reductions during heat treatments of salami and morr (Fig. 1). Treatment (2) showed higher STEC reductions (log 2.4–>3.8) than treatment (1; log 1.8–2.1). STEC reductions in salami were higher than in morr for both tested treatments. STEC were reduced to below the detection limit in regime (2) treated salami.

3.2. Storage of DFS at various temperatures

3.2.1. Sensory characteristics

The flavour profiles of commercial brands of salami and morr stored for two months at 20, 16 and 4 °C (control) were very similar. Results for salami are shown in Fig. 2. Small though statistically significant differences were found between salami stored at 16 °C versus 4 °C (respective mean value intensity scores in parenthesis) for only three characteristics: odour of pork/beef meat (4.33 versus 4.60), metallic flavour (3.71 versus 3.96), whiteness (4.33 versus 4.60). For morr, no significant differences in the tested attributes were obtained for the tested storage conditions.

3.2.2. STEC reductions

Sausages of three formulations (SR, MR and HR) of both salami and morr were stored at 20, 16 and 4 °C for 1 and 2 months to study storage effects on STEC reduction. In general, higher STEC reductions were obtained with increasing storage time (2 months versus 1 month) and higher temperatures (20 and 16 °C versus 4 °C; Fig. 3). The STEC reductions obtained during storage were in addition to the previously reported reductions during the 23 day

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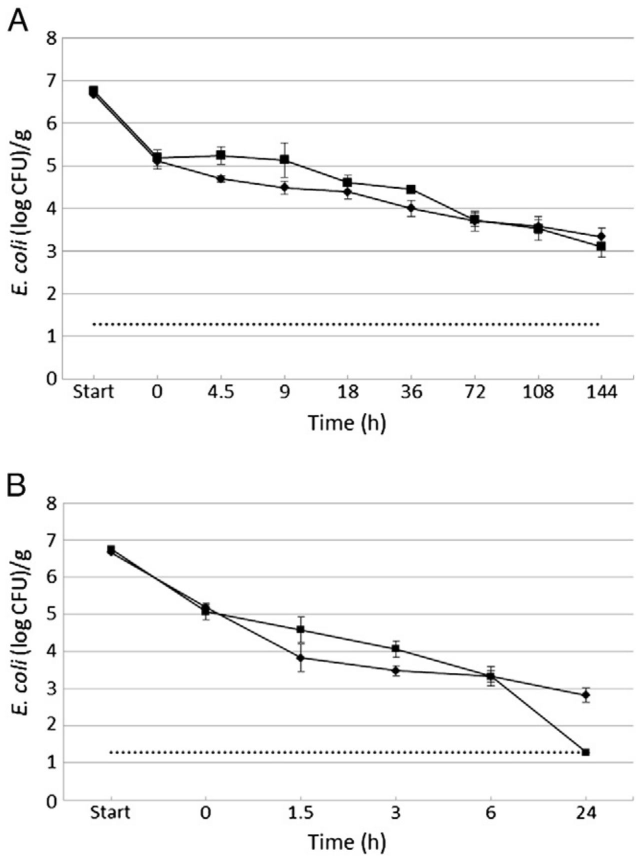

Fig. 1. Survival of STEC during post-process heat treatments of salami (■) and morr (♦). A: Heat treatment (1); 32 °C for 6 days. B: Heat treatment (2); 43 °C for 24 h. Start, indicates inoculation level at sausage production. Time 0, indicates level in mature sausages before heat treatment. The abscissa indicates hours of heat treatment. Note the different time scales. Detection limit shown as dotted line. Data are mean values of three replicates.

production period being between log 1.39–2.92 and log 1.6–3.27 for salami and morr, respectively (Heir et al., 2010). Highest reductions were obtained at 20 °C storage. STEC reductions were >1 log in all sausages both using SR, MR or HR formulations stored at 20 °C for 1 month. In three morr sausages and two salami sausages, STEC colony counts were reduced to levels below the detection limit (log 1.3) at this condition. After two months storage at 20 °C, STEC numbers in salami and morr samples with MR or HR formulations were reduced to below the detection limit. In general, storage at 4 °C provided <1 log STEC reductions after both 1 and 2 month storage regardless of formulation and fermentation temperature.

Sausage formulation and fermentation temperature during processing also influenced STEC reductions during storage. For both salami and morr, higher STEC reductions during storage were obtained for DFS with the HR and MR formulations (with higher levels of salt and glucose) compared to the standard formulations (SR; Fig. 3). For salami, high fermentation temperature (30 °C) provided more STEC reductions during storage than salami fermented at 20 °C. No significant influence of fermentation temperature on STEC reductions in morr was observed. The reductions of STEC within each formulation, fermentation temperature and storage condition may vary considerably. This is evident from the distribution plot after storage of salami and morr for two months (Fig. 4). STEC numbers were reduced to below the detection limit showing >5.5 log total reductions in 3 process/storage temp. combinations for salami (HR 30 °C/stored at 16 or 20 °C, MR 30 °C/stored at 20 °C) and in 6 combinations for morr (HR 20 °C/stored at 20 °C, HR 30 °C/stored at 16 or 20 °C, MR 20 °C/stored at 20 °C and MR 30 °C/stored at 16 or 20 °C).

3.3. Combined freezing/thawing of DFS

3.3.1. Sensory characteristics

Commercial brands of salami and morr were subjected to 1 (FT1) or 4 (FT2) freeze/thaw cycles and stored at 4 °C for 1 month as described in Materials and methods. For the commercial salami brand, the flavour profiles of FT1 and FT2 treated sausages were very similar to the control salami, though statistically significant differences were obtained (Fig. 5). FT2 treated salami had significantly lower intensity of the attributes odour of meat, sour odour, colour intensity, whiteness and sour flavour. Significantly higher intensity scores were obtained for the FT2 treated salami compared to the control salami for the attributes odour of spices and mature flavour. Sensory score values for FT1 treated salami were neither highest nor lowest for any of the tested attributes. No significant differences in any of the sensory characteristics were obtained for freeze/thaw treated commercial brand of morr (FT1 or FT2) compared to control morr stored at 4 °C (data not shown).

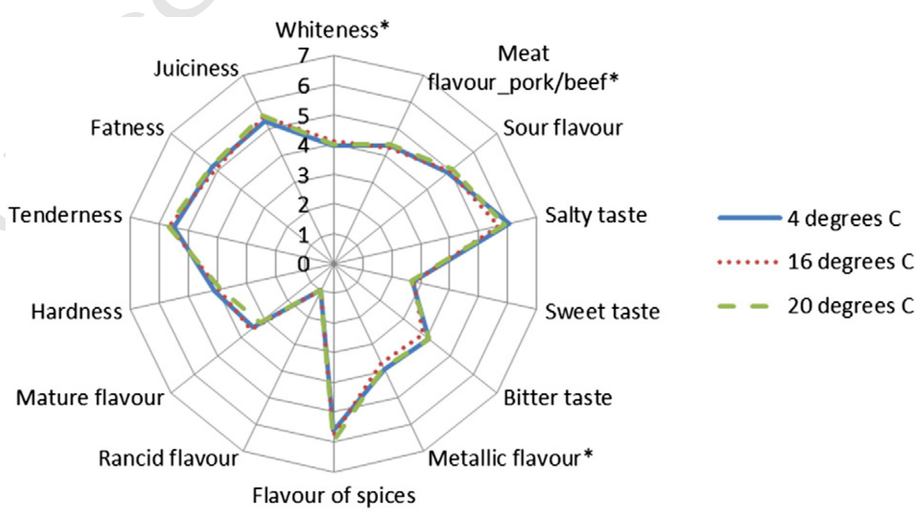

Fig. 2. Sensory profiles of salami stored for two months at 4 (control), 16 and 20 °C. Significant differences ($p \leq 0.05$) in sensory characteristics of the treated sausages indicated (*). Data are mean values of 3 replicates using 11 assessors.

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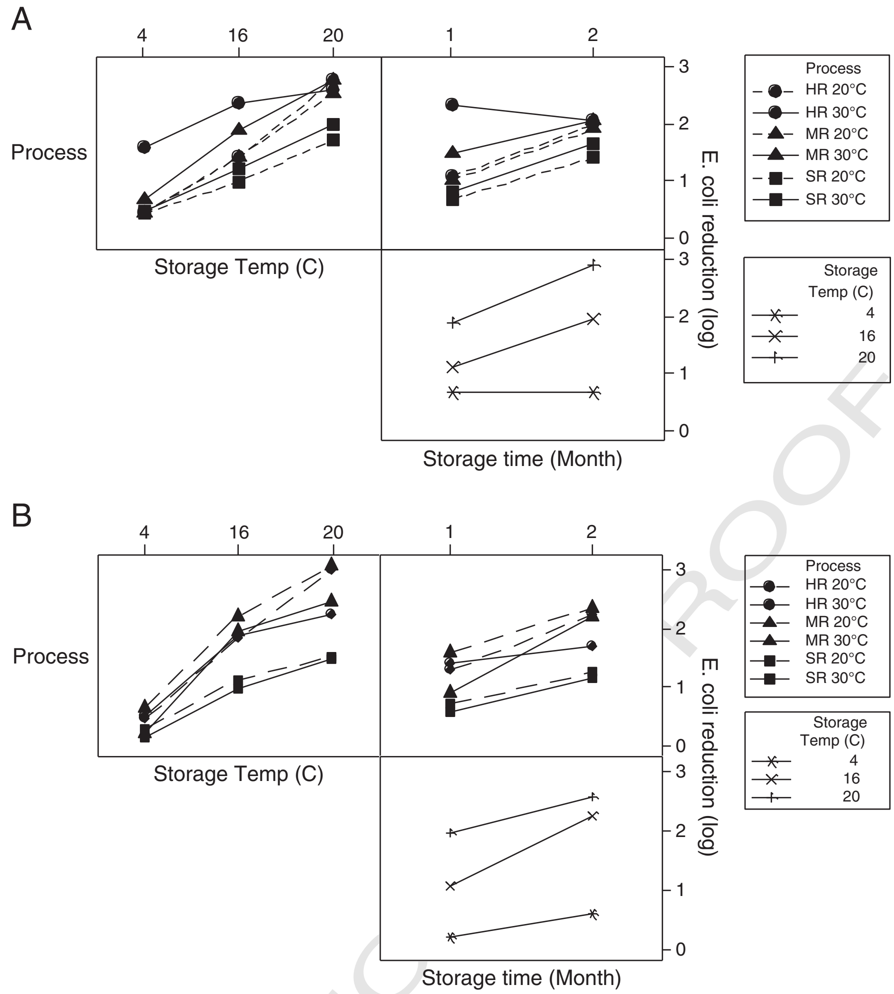

Fig. 3. The effects of storage temperature (4, 16, 20 °C) and storage time (1 and 2 months) on three formulations (SR, 3.6% NaCl, 100 ppm nitrite, 0.5% glucose, MR, 4.5% NaCl, 300 ppm nitrite, 1.25% glucose and HR, 5% NaCl, 500 ppm nitrite, 1.25% glucose) of salami (A) and morr (B) fermented at two temperatures (20 °C or 30 °C). STEC reductions due to storage are shown (not including STEC reduction during sausage production). Data are mean values of four replicates.

3.3.2. STEC reductions

Combined freezing/thawing and storage at 4 °C of DFS produced using the three formulations SR, MR and HR for both salami and morr provided additional STEC reductions compared to 4 °C storage only (Fig. 6). STEC reductions obtained using a single freeze/thaw event combined with 1 month storage at 4 °C (FT1) were in the range 0.7 to >2.6 log (stdev in the range 0.1–0.7 within the four replicates of each formulations and sausage type). Using four sequential freeze/thaw cycles (FT2) provided further reductions (1.03 to >2.98 log; stdev in the range 0.1–0.4). Highest reductions at both FT treatments were obtained in sausages with increased levels of glucose and salt (MR and HR formulations) compared to standard formulations. STEC reductions in FT1 and FT2 treated salami were higher in sausages fermented at 30 °C compared to sausages fermented at 20 °C. No clear associations between fermentation temperature and STEC reductions obtained during FT treatments of morr were observed (Fig. 6).

4. Discussion

Several foodborne outbreaks linked to DFS contaminated with bacterial pathogens have revealed that DFS must be regarded as potential microbiological risk products. This has emphasized the need for strategies for obtaining improved microbiological safety of DFS. To be of relevance to DFS manufacturers, intervention strategies should be easily implemented in the production process and be effective in providing enhanced food safety. Of outmost importance, interventions should not provide negative sensory effects but must maintain or improve the sensory quality of the final products. Relevant post-process treatments to fulfil criteria regarding effects on STEC reductions and on sensory attributes and with potential for easy implementation in industrial DFS production were tested.

Reductions of potential harmful microorganisms in DFS can be obtained through strategies in the production chain including raw material decontamination and control (Buckenhuskes & Fischer, 2001; Faith et al., 1998; Samelis, Kakouri, Savvaidis, Riganakos, & Kontominas, 2005), formulation and process optimisation (Al-Nabulsi & Holley, 2007; Casey & Condon, 2000; Chacon, Muthukumarasamy, & Holley, 2006; Chikthimmah, Anantheswaran, Roberts, Mills, & Knabel, 2001; D.C. Riordan et al., 1998; Heir et al., 2010) and post process treatments (Badr, 2005; Byelashov et al., 2009; Gill & Ramaswamy, 2008; Glass et al., 2012; Omer et al., 2010; Porto-Fett et al., 2010). It was shown previously that approximately 3 log STEC reductions could be obtained by optimizing formulation (levels of salt, glucose, nitrite) and production process parameters (fermentation temperature) compared to 1.5 logs reduction by standard formulation and process (Heir et al., 2010). The potential of relevant post process treatments (mild heat treatment, storage and freezing-thawing) for STEC reductions in standard salami for different STEC serogroups and strains was recently shown (Rode et al., 2012). Q2

The present study shows that the selected post process treatments in addition to providing DFS with enhanced microbiological safety

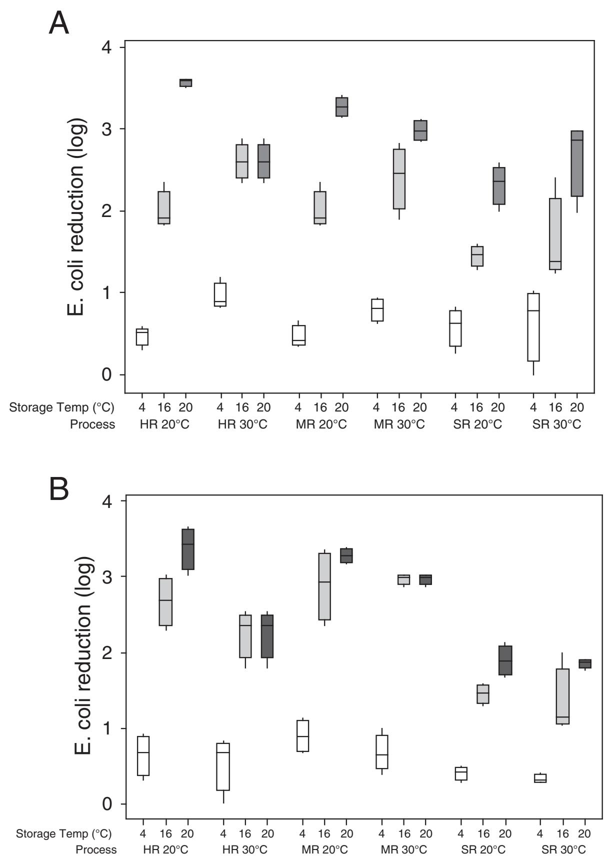

Fig. 4. Box plot showing distributions in reductions of STEC in three formulations (SR, 3.6% NaCl, 100 ppm nitrite, 0.5% glucose, MR, 4.5% NaCl, 300 ppm nitrite, 1.25% glucose and HR, 5% NaCl, 500 ppm nitrite, 1.25% glucose) of salami (A) and morr (B) after two month storage at 4, 16 and 20 °C. The bottom and top of each box represent the first and third quartile of the data values, respectively. The horizontal line within the boxes represents the 50th percentile (median value). The upper and lower whiskers extend to the highest and lowest data value, respectively. Boxes: white, 4 °C, grey, 16 °C, dark grey, 20 °C. Data are mean values of four replicates.

also provide high sensory qualities to different types of DFS, salami and morr. Very similar sensory attributes compared to non-treated control DFS were obtained for both sausage types. Additionally, potential interaction effects between formulation parameters and post-process treatments on STEC reduction in salami and morr were determined.

Among the 7 heat treatments tested, 3 heat treatments ((1) 32 °C, 6 days; (2) 43 °C, 24 h; (4) 43 °C 1 h + 53 °C 6 h) were considered to be the most relevant with regard to sensory characteristics and potential for industrial implementation. The overall preference sensory analyses gave only marginal differences in preference between heat treated (all three treatments) and non-treated control salami and morr. As salami and morr are products with long shelf life, often being stored for several weeks prior to consumption, the sensory tests were performed both short time after heat treatments and after 6 weeks storage. Interestingly, the overall sensory scores were significantly higher after 6 weeks storage of heat treated morr (treatment 1 and 2) compared to non-treated morr. Heat treated salami also showed tendencies of higher overall preference scores after storage than before storage using the same heat treatment. A previous study on heat treated DFS reported visible negative quality effects of both short time (7 min) high temperature (60 °C) and longer time (360 h) low temperature (50 °C) treatments compared to 55 °C for 120 min (Duffy et al., 1999). Calicioglu also reported that heating to 63 °C resulted in a sensorially unacceptable product of soudjouk-style fermented sausage (Calicioglu, Faith, Buege, & Luchansky, 2002) The scoring of the tested sensory attributes together with obtained STEC reductions showed that the tested low temperature heat treatments provide a realistic and effective alternative for post process treatments of salami and morr.

Storage and freeze–thaw treatments of DFS had negligible sensory effects on treated salami and morr (Figs. 2 and 5). The sensory tests were performed after storage following the treatments to detect potential sensory attributes that could appear after a relevant storage period (2 months). Previous studies showed that storage of DFS at low temperatures (4 °C) provided limited reductions of STEC irrespective of type of formulation or fermentation temperature (Heir et al., 2010). In the present study, considerable reductions were obtained by increasing the storage temperature to 16 or 20 °C. For both salami and morr, lowest overall reductions were obtained in standard formulation (SR) sausages (low salt) while higher reductions were obtained in moderate salt formulation (MR) and high salt formulation sausages (HR). At 4 °C storage, neither the formulation (SR, MR, HR), fermentation temperature (20 °C or 30 °C) or storage time had significant effects on the STEC reductions obtained in salami

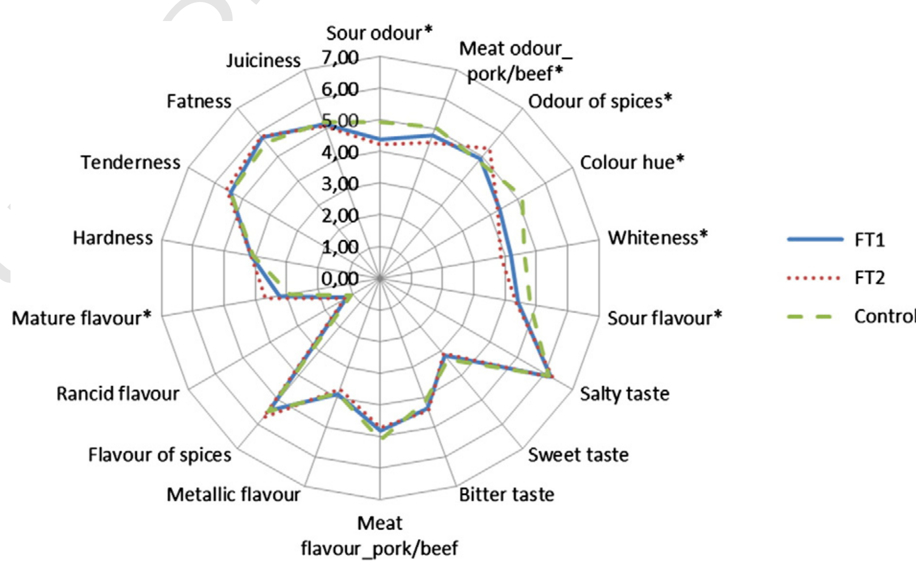

Fig. 5. Sensory profiles of salami after treatment by two freeze/thaw treatments (FT1 = 1 freeze/thaw cycle; FT2 = 4 freeze/thaw cycles) compared to untreated control. Significant differences (p ≤ 0.05) in sensory characteristics of the treated sausages are indicated (*). Data are mean values of two replicates using 12 assessors.

Please cite this article as: Heir, E., et al., Effects of post-processing treatments on sensory quality and Shiga toxigenic *Escherichia coli* reductions in dry-fermented sausages, *Meat Science* (2013), http://dx.doi.org/10.1016/j.meatsci.2012.12.020

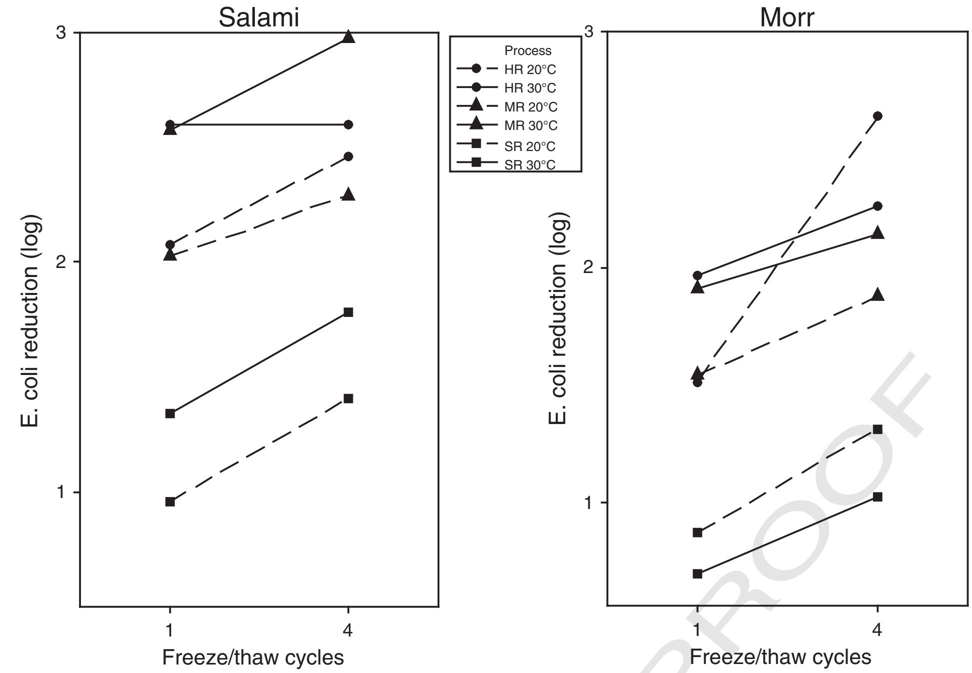

Fig. 6. STEC reductions in salami and morr after treatment by two freeze/thaw treatments (1 freeze/thaw cycle = FT1; 4 freeze/thaw cycles = FT2). For each sausage type, three formulations ((HR) high salt, (MR) moderate salt, (SR) standard salt), fermented at two temperatures, (20 or 30 °C) were included. Data are mean values of four replicates.

and morr during storage. One exception was the HR formulation salami fermented at 30 °C where STEC reductions at 4 °C were significantly higher compared to the other salamis. At low temperature storage (4 °C), limited effect of storage on STEC reductions can be expected irrespective of formulation optimisation. At higher temperatures (≥16 °C), STEC reductions obtained during storage is dependent on both storage time and temperature in addition to formulation and sausage parameters (e.g. final pH, a_w). Reductions of >5 log from production start to end of storage (20 °C for one or 2 months) were obtained for both salami and morr but not using standard formulation conditions. The data is in accordance with previous studies and also indicate that large variations in the effects of storage on STEC reductions occur between different sausages as reviewed by Holck et al. (2011). The main influence of temperature is in accordance with McQuestin et al. (2009) who performed a meta-analyses of 44 studies for the effect of temperature, pH and a_w on survival of *E. coli*.

STEC reductions obtained during the freeze–thaw treatments reflected in most cases reductions obtained during the 23 day production period, showing that formulation and production parameters affect post process treatment effects on STEC reductions. It was not possible to link this effect to specific parameters (e.g. final pH or a_w) of the DFS. However, overall higher effects on STEC reductions in freeze treated salami than morr were observed (Fig. 6).

This study shows that care must be exercised in inferring STEC reductions in DFS with different properties (e.g. salami and morr). One should also be aware of the possibilities for over-estimating STEC reductions due to various treatments. Sub-lethally damaged cells may not be able to grow on selective media used for STEC growth. Control experiments showed that the use of Rif^R STEC isolates and general plating media containing Rif made this source of error negligible in this study (data not shown). Improved STEC reduction effects of post process interventions could be obtained by other combinations of treatments or other treatments than tested here. Rode et al. (2012) reported that freezing of salami at −20 °C for 24 h and subsequent 1 month storage for 20 °C provided mean log reductions of 3.9, similar to reductions obtained by heat treatment of 43 °C for 24 h. However, effects on sensory properties were not performed and should be tested to determine the practical relevance of combinations of interventions. Other strategies reported are use of antimicrobial ingredients in DFS formulations (Al-Nabulsi & Holley, 2007; Chacon et al., 2006) as well as post process interventions including novel and traditional treatments (HPP (Omer et al., 2010), irradiation (Galan et al. 2011). However, there exist limitations for practical industrial use of many of these strategies including low effects on STEC reductions (Al-Nabulsi & Holley, 2007), significantly reduced sensory quality of treated sausages (Chacon, Muthukumarasamy & Holley, 2006; Galan, Selgas et al.; Kim, Lee, Kang et al.)) or investment costs, e.g. HPP.

The previous study showed only small differences in sensory attributes on salami and morr regardless of the formulation types SR, MR or HR (Heir et al., 2010). In conclusion, the present study including both sensory analyses and effects on STEC reductions of DFS suggests that combined formulation optimization and the tested post-process strategies could be considered for implementation in industrial DFS production as the tested interventions have significant effects on STEC reductions but only marginal effects on the sensory characteristics of the sausages.

Acknowledgements

The work was financially supported by The Research Council of Norway (project 178230/I10), Foundation on Levy on Foods, the Norwegian Research fees Fund for Agricultural Goods, Nortura SA, the Norwegian Independent Meat and Poultry Association and NHO Mat og Landbruk. We thank Birgitta Baardsen, Janina Berg, Tom Chr. Johannesen, Ahmed Abdelgani and Anette Wold Åsli for excellent technical assistance.

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