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Korrespondenzadresse:
mir.s.ducic@gmail.com

[1]) University of Novi Sad, Faculty of Agriculture, Department of Veterinary Medicine, 21000 Novi Sad, Trg Dositeja Obradovića 8, Serbia; [2]) University of Novi Sad, Faculty of Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

Review:
Overview of treatments for improving the microbial safety of dry fermented sausages

Übersichtsarbeit:
Überblick über Behandlungen zur Verbesserung der mikrobiellen Sicherheit von trockenfermentierten Würsten

Miroslav S. Dučić[1]), Siniša L. Markov[2])

Summary

Dry fermented sausages are a valuable food with complex composition and processing, resulting in the great diversity of these products. The microbial safety of industrially produced sausages is based on fast acidification and drying, with simultaneous additional effects of other factors. Epidemiological evidence between consumption of these products and foodborne disease outbreaks induced more stringent safety criteria in many countries. Approaches to risk reduction of the main biohazards should consider the following aspects: starter cultures, meat batter composition, preparation conditions, processing parameters; and application of heat treatments, high pressure processing or irradiation. These additional measures enhance the safety, but the application of only one measure, while greatly reducing biohazards, can be followed by negative effects to the sensorial properties of the sausages. This review summarizes the literature on measures for risk reduction of the main bacterial foodborne pathogens in dry fermented sausages (pathogenic *Escherichia coli*, *Salmonella enterica*, *Listeria monocytogenes*).

Keywords: dry fermented sausages, safety, *Escherichia coli, Salmonella, Listeria monocytogenes*

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Introduction

In modern industrial production, the safety of dry fermented sausages (DFS) is primarily based on fast acidification (pH <5.3) and drying, with additional effects from nitrites, sodium chloride and spices. Simultaneous application of several mild antimicrobial factors (hurdle technology) induces negative effects at different levels inside the microorganisms (membranes, enzymes, genetic material, and energy consumption), leading to their metabolic exhaustion and disturbed homeostasis (Leistner, 2000). The drop in pH is achieved by lactic acid bacteria growth and/or by acidulant activity, while the fast decrease of water activity (aw) is ensured by drying in special air-conditioned rooms. However, with this approach, complete pathogen suppression is not ensured. Hence, if the raw materials are of poor hygienic status and/or deviations occur during the production process, pathogens can survive in final product and be present at levels sufficient to cause foodborne disease in consumers (Ducic et al., 2016). During the last decades, several outbreaks involving DFS occurred worldwide. In Canada, an outbreak of *Escherichia coli* O157:H7 in 1999, with 143 cases including 6 with severe symptoms, was associated with salami (MacDonald et al., 2004). In Norway, an outbreak in 2006 caused by *E. coli* O103:H25 affected 17 people, mostly young children, one of whom died. Investigation showed that the source was DFS with mutton as an ingredient (Sekse et al., 2009). Gieraltowski et al. (2012) investigated the epidemiological link of *Salmonella* Montevideo with 272 cases in the USA, during the 2009/2010 season and found a significant association with consumption of Italian style salami. Also, Kuhn et al. (2011) investigated an outbreak of *Salmonella* Typhimurium which involved 20 cases, including children aged ten years or younger, and found a strong link between illness and eating particular salami products containing pork and venison.

Epidemiological links between consumption of DFS and foodborne outbreaks led to changes of attitudes regarding the safety of these products and consequential introduction of more stringent control measures in some countries. The US Food Safety and Inspection Service (FSIS) and Canadian Food Inspection Agency introduced measures to ensure a 6.5 log unit (hereafter called log) reduction of *Salmonella* and a 5 log reduction of verotoxigenic *E. coli* (O157 serotype primarily) during production and storage of DFS made from pork and beef, respectively (Anonymous, 2000; Anonymous, 2001).

The application of more stringent safety criteria in DFS production and distribution has prompted research on the methods of achieving desired reduction levels, and different conceptual approaches were investigated (Porto-Fett et al., 2010; Holck et al., 2011; Kim et al., 2012). Hence, the aim of this review is to present treatments that have potential as additional measures in the risk reduction of the main bacterial foodborne pathogens in DFS (pathogenic *Escherichia coli*, *Salmonella* and *Listeria monocytogenes*), and also the possible impacts of the measures on sensory quality of products.

Approaches for improving the safety of DFS

The conventional methods of DFS industrial production reduce VTEC (primarily *E. coli* O157:H7) in the range of 1 to 4 logs, depending on the serotype of this bacteria (Getty, 2005; Holck et al., 2011; Rode et al., 2012; Ducic et al., 2016). The level of *Salmonella* reduction in DFS is slightly higher, i.e. 1.3 to 4.6 logs (Ihnot et al., 1998; Nissen and Holck, 1998; Nightingale et al., 2006; Porto-Fett et al., 2008; Porto-Fett et al., 2010; Ducic et al., 2016). On the other hand, *L. monocytogenes* is significantly more resistant than the other two pathogens, with average reduction of approximately 1 log or less during fermentation and drying (Lahti et al., 2001; Pidcock et al., 2002; Thevenot et al., 2005; Nightingale et al., 2006; Porto-Fett et al., 2008; Porto-Fett et al., 2010; Ducic et al., 2016). However, some studies found greater reductions of *L. monocytogenes*, irrespective of sausage production being with or without the use of starter cultures (Buncic et al., 1991; Farber et al., 1993).

Given the complex composition and long production process of DFS, various conceptual approaches for improvement of their safety were studied. In the following text, such innovative treatments are presented in correspondence with production steps.

Starter cultures

The effectiveness of starter cultures in pathogen suppression is largely dependent on their adaptation to the environment. Thus, starter cultures adapted to one type of sausage are not necessarily adapted to the environmental conditions prevailing in other sausage types (Leroy et al., 2006). Therefore, it is important that the proper temperature during fermentation, as well as other production parameters, enable the starter cultures to rapidly grow from initial 10^6 to 10^8 or even 10^9 CFU/g in the final product. As a consequence of growth and metabolic activity of starter cultures, pH drops in a period of 2–3 days after the sausage stuffing is pressed into casings, which leads to growth inhibition and reduction of any pathogens present. Concurrent application of different cultures to produce significant, additional reduction of enterohaemorrhagic *E. coli* and other pathogens has been studied (Table 1).

In Hungarian salami, Pidcock et al. (2002) investigated the effects of commercial starter culture (*Pediococcus pentosaceus* and *Staphylococcus xylosus*) and 15 strains of lactic acid bacteria (*P. pentosaceus, Lactobacillus acidophilus, Lactobacillus paracasei, Lactobacillus casei Immunitas, Lactobacillus reuteri, Bifidobacterium lactis* and *Bifidobacterium longum*) originating from dairy products and human intestines, previously cultured in anaerobic conditions at 37 °C during 48 h. After seven days of fermentation, most lactic acid bacteria strains in conjunction with starter induce reductions of *E. coli* O111 and *L. monocytogenes* greater than 2.5 logs; this is more than 1 log greater reduction than is achieved using only the commercial starter culture.

Muthukumarasamy and Holley (2007) added *Lb. reuteri* to a commercial starter culture (*P. pentosaceus* and *Staphylococcus carnosus*) which led to 3 log reduction of *E. coli* O157:H7, compared to 1.7 log reduction in the control sausages, while addition of *Bifidobacterium longum* did not cause any significant reduction of this pathogen compared to the control sausages. In addition, protection of *Lb. reuteri* by microencapsulation increased its survival but reduced its inhibitory action against *E. coli* O157:H7.

Erkkila et al. (2000) investigated the bio-protective properties of probiotic strains of *Lactobacillus rhamnosus* and found similar *E. coli* O157:H7 reductions (2.5–3 logs) in both sausages with probiotics and control sausages with mixed commercial starter culture (*P. pentosaceus* and *S. xylosus*).

TABLE 1: *The effects of starter cultures on verotoxigenic E. coli in dry fermented sausages.*

Type of product	Investigated microbiota	Overall pathogen reduction	Reference
Salami (beef)	*Pediococcus acidilactici* with $MnSO_4$ and/or Oxyrase	*P. acidilactici* reduced *E. coli* O157:H7 by 2.2 – 3.3 logs, stimulated by presence of Mn ion and/or enzyme	Kang & Fung (1999)
DFS (pork and beef)	Commercial starter (*Pediococcus pentosaceus*) or *Lactobacillus rhamnosus* (3 separately used strains)	No significant difference in reduction of *E. coli* O157:H7 (2.5 – 3 logs) between starter and starter+probiotic strains	Erkkila et al. (2000)
Hungarian salami	Commercial starter (*Pediococcus pentosaceus* + *Staphylococcus xylosus*) with *Pediococcus pentosaceus* (2 strains) or *Lactobacillus acidophilus* (2) or *Lactobacillus paracasei* (5) or *Lactobacillus casei* Immunitas or *Lactobacillus reuteri* or *Bifidobacterium lactis* or *Bifidobacterium longum*	- 9 culture combinations reduced *E. coli* O111 by more than 2.5 logs - 10 culture combinations reduced *L. monocytogenes* by more than 2.5 logs	Pidcock et al. (2002)
DFS (pork and beef)	Commercial starter (*Pediococcus pentosaceus* + *Staphylococcus carnosus*) with *Lactobacillus reuteri* and/or *Bifidobacterium longum*	Starter with *Lb. reuteri* reduced *E. coli* O157:H7 by 3 logs	Muthukumarasamy & Holley (2007)

Kang and Fung (2000, 1999a) examined the possibilities of enhancing the competitive properties of usual starter cultures against pathogens. They found that adding $MnSO_4$ into salami stuffing, independently or in combination with biologically degradable enzyme (Oxyrase™), stimulates *Pediococcus acidilactici* growth and, thus, enhances its inhibitory effects on *E. coli* O157:H7 and *L. monocytogenes* – the reductions were between 1 to almost 1.5 logs greater than in control sausages without these substances added.

These findings demonstrate that the approach of adding probiotic bacteria and preparing sausages according to formulations that positively influence the growth of useful microbiota produces up to 1.5 log greater reductions of enterohaemorrhagic strains of *E. coli* and *L. monocytogenes* than in control sausages. However, the effects on the sensory properties of DFS have not been investigated.

Bacteriocins from lactic acid bacteria

Antimicrobial effects of lactic acid bacteria in DFS are primarily based on production of lactic acid and other substances (acetic acid, carbon dioxide, ethanol, hydrogen peroxide, diacetyl, etc.) that acidify the sausage content. However, some lactic acid bacteria strains that are important in meat fermentation produce antimicrobial substances – bacteriocins – that are primarily effective against Gram positive bacteria (De Vuyst and Leroy, 2007). Bacteriocins are ribosomally synthesized antimicrobial peptides or proteins that either inhibit the growth of or destroy some competitive bacteria, pathogenic or not (Cotter et al., 2005). All bacteriocins express an antimicrobial effect by inducing pores to form on the target cell membrane that disrupt the concentration gradient of the proton pump, leading to permeabilisation, consequent energy depletion and cell death (Lücke, 2000; Koch, 2004). They are mostly effective against microorganisms with which the producer bacteria compete for nutrients. Therefore, research on the antimicrobial efficacy of bacteriocins was mostly directed to *L. monocytogenes* due to the phylogenetic relatedness of this species with lactic acid bacteria.

Bacteriocins are applied directly in purified form or indirectly through bacteriocin-producing cultures. Bacteriocin-producing lactic acid bacteria originating from DFS (e.g. *Lactobacillus sakei*, *Lactobacillus curvatus*, *Lactobacillus plantarum, P. acidilactici* or *Enterococcus faecium*) are considered as the most appropriate against *L. monocytogenes* (Rocourt and Buchrieser, 2007; Vignolo et al., 2010). Numerous studies were conducted (Table 2) to examine the efficacy of bacteriocin-producing starter cultures, acting independently or in combination with other bacteriocin-negative starter cultures. These reveal that it is

TABLE 2: *The effectiveness of bacteriocinogenic microbiota in dry fermented sausages.*

Type of product	Investigated microbiota	Overall pathogen reduction	Reference
Spanish style DFS	*Enterococcus faecium* CCM 4231 or *E. faecium* RZSC 13	*Listeria innocua* decreased 3.3 logs by each starter	Callewaert et al., (2000)
DFS (pork and beef)	Starter A: *Staphylococcus xylosus* DD-34 with *Pediococcus acidilactici* PA-2 and *Lactobacillus bavaricus* MI-401 Starter B: *S. carnosus* MIII with *Lb. curvatus* Lb3	*E. coli* O157:H7decreased < 1 log by starter A and 1.5 – 2 log by starter B *L. monocytogenes* decreased < 1 log by starter B and 2 - 2.5 logs by starter A	Lahti et al., (2001)
Pork fermented sausages	*Lactobacillus sakei* CTC 494 with black pepper or $MnSO_4$	*L. monocytogenes* decreased ~ 3.5 logs	Hugas et al., (2002a)
DFS not specified	Commercial starter + *Lactococcus lactis* sub. *lactis* LMG 21206 or *Lactobacillus curvatus* LBPE	*L. monocytogenes* decreased by a maximum 1.5 log in both combinations	Benkerroum et al., (2005)
Cacciatore salami	Commercial starter + *Lactobacillus sakei* CTC 494	*L. monocytogenes* decreased 1.8 logs	Ravyts et al., (2008)
Sucuk	*Pediococcus acidilactici* 13	*L. monocytogenes* decreased 3.3 logs	Cosansu et al., (2010)

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possible to achieve up to 3 logs of additional reduction of *L. monocytogenes*, compared to sausage production either using starter cultures with no marked anti-listerial effect or without starter cultures at all (Callewaert et al., 2000; Lahti et al., 2001; Hugas et al., 2002a; Koch, 2004; Benkerroum et al., 2005; Ravyts et al., 2008; Cosansu et al., 2010).

However, the use of purified or semi-purified bacteriocins is less efficient. The reasons include their poor solubility, binding to other food ingredients and their degradation by enzymes originating from muscle and fat tissues. Furthermore, when purified bacteriocin is used, there is a higher probability the pathogens will acquire resistance during the production process; therefore, starter cultures that produce bacteriocins in sufficient quantities during the whole sausage production process are favoured (Koch, 2004; Vignolo and Fadda, 2007; Drosinos et al., 2009).

Studies into the use of bacteriocin-producing starter cultures show this approach can achieve a maximum 3 logs of additional reduction of *L. monocytogenes* in DFS. On the other hand, sensory properties were evaluated only in a few studies and significant differences were not determined.

Antimicrobial substances

The use of natural antimicrobial substances in DFS production is related to the pronounced trend of consumers towards desiring minimally processed foods (Garriga and Aymerich, 2009). Diacetyl is important in dairy product flavour, but it also has antimicrobial properties; it interferes with arginine binding enzymes, and inhibits the growth of some Gram negative bacteria (Jay et al., 2005). The investigation of diacetyl use in DFS production revealed that a concentration of 300 ppm reduces *E. coli* O157 and *S.* Typhimurium by one log more than in control sausages, with no significant effect on the lactic acid bacteria population. However, the effect on the sausages' sensory quality has not been investigated (Kang and Fung, 1999b).

Al-Nabulsi and Holley (2007) exploited the antimicrobial potential of milk lactoferrin during DFS fermentation and ripening to reduce *E. coli* O157 by 3 logs (1.8 logs more than in sausages without this substance) and without negative effects on starter culture population. However, lactoferrin use is limited to its narrow spectrum of activity – it is only effective against some *E. coli* O157 strains and mostly has only bacteriostatic effects. The sensorial quality of the sausages was not examined.

Chacon et al. (2006) examined *E. coli* O157:H7 reductions in DFS that contained different levels of allyl isothiocyanate (a substance present in mustard, horseradish and some other plants from the Brassicaceae family). Allyl isothiocyanate at 500 ppm, and 28 days after stuffing into casings, reduced the pathogen level by 4.75 logs while the sausages' sensorial properties were assessed as acceptable. Furthermore, as allyl isothiocyanate has a very pungent odour and bitter taste, the possibility of substituting it with ground heat-treated white or brown mustard has been investigated. However, to produce a 5 log reduction of *E. coli* O157:H7, deodorized mustard has to be applied at a level that diminishes the sensory properties of the final product due to other components (Graumann and Holley, 2008; Luciano et al., 2011; Li et al., 2013).

Summarizing, the application of antimicrobial substances other than bacteriocins can induce up to 3 log reductions of some strains of *E. coli* O157 over reductions achieved in control sausages. In the case of mustard or its purified compounds, almost 5 log reductions of *E. coli* O157 can be achieved, but the sensory properties of the final sausages are diminished.

Temperature change during preparation of DFS

Treatment of incoming raw meat. In a study by Blagojevic et al. (2015), beef trimmings intended for production of Sudzuk type DFS were submerged into 4% lactic acid in water solution and heated to 80 to 90 ºC for 10 to 30 seconds.

These treatments reduced previously inoculated *E. coli* O157 and *S.* Typhimurium in the trimmings by 3.6 and 3.9 logs, respectively (while average reductions during the production process in control sausages were 1.9 logs, for both pathogens). On the other hand, the heat treatments reduced *L. monocytogenes* by only 0.3 to 0.5 logs (the production process in control sausages did not reduce levels of this pathogen). It is noteworthy that the DFS produced with heat treated meat had moderately to significantly diminished sensorial qualities, depending on the temperature/time combination of heat treatment.

The effect of stuffing temperature. Faith et al. (1998) investigated the effects of stuffing temperature on *E. coli* O157:H7 reduction. The stuffing inoculated with this pathogen at a level of 7.5 or 7.7 log CFU/g was subjected to: a) common refrigeration temperature of 4 °C -control; b) freezing (–20 °C) and thawing (4 °C) – first combination, and; c) temperature of 13 °C, with subsequent freezing (–20 °C) and thawing (4 °C) – second combination. The outlined treatments led to *E. coli* O157:H7 reductions in finished sausages of 1.1 log, 1.6 logs and 2.1 logs, respectively.

From these findings, it can be concluded that the second combination of stuffing temperatures produces up to 1 log additional reduction of *E. coli* O157, compared to control sausages. The effect on sensory quality has not been investigated.

The effect of production parameters and formulations

Comprehensive investigations were conducted (Table 3) to determine the effects of production parameters and/or ingredients in reducing *E. coli* O157:H7 and other pathogenic bacteria in different types of DFS.

Riordan et al. (1998) investigated variation of final pH, and NaCl (2.5–4.8 %) and $NaNO_2$ (100–400 ppm) contents on *E. coli* O157:H7 in pepperoni sausages. Fermentation was conducted at 38 ºC and the pH of the finished products ranged from 4.4 to 5.6. Pathogen reductions ranged from 0.7 to 4.8 logs, while for standard conditions a reduction of 0.8 logs was observed. Greater reductions were achieved with a decrease in pH and increase in NaCl and $NaNO_2$ content.

Casey and Condon (2000) examined pathogenic *E. coli* O157:H45 reduction in DFS fermented at 37 ºC and with pH of 4.7 and concentrations of NaNO2 ranging from 0 to 300 ppm. They found that reductions are 2 logs greater when the concentration of additive was at the maximum.

Duffy and Vanderlinde (2000) studied fermented salami at different temperatures (26, 31, 35, 42 ºC) and recorded an increase of pathogen reduction roughly from 1 log to 4.5 logs due to increase of temperature.

Naim et al. (2003) studied DFS at 27 or 37 ºC, then dried to a_w 0.91 or 0.79, achieving pathogen reductions of around 2 logs and from 4 to 5 logs, respectively.

Apaydin et al. (2009) investigated different concentrations of $NaNO_2$ (0-400 ppm) and drying time for sudžuk fermented at 18 ºC. They found greater reductions with drying

TABLE 3: *Effects of production parameters on pathogen reduction in dry fermented sausages.*

Type of product	Ingredients and conditions	Reductions of pathogen	Reference
Pepperoni	NaCl (2.5 – 4.8%), $NaNO_2$ (100; 200; 300; 400 ppm), dextrose (0.1%, 0.63%, 2.5%)	*E. coli* O157:H7 deceased maximally by 4.8 logs in combination: NaCl 3.3%, $NaNO_2$ 300 ppm, 0.1% dextrose)	Riordan et al., (1998)
Laboratory fermented sausage	$NaNO_2$ (0; 50; 100; 200; 300 ppm)	*E. coli* O157:H45 decreased maximally by 3.5 logs ($NaNO_2$ 300 ppm)	Casey & Condon, (2000)
Salami	Fermentation temperature: 26 °C; 31 °C; 35 °C; 42 °C	*E. coli* O157:H7 decreased maximally by 4.65 logs (42 °C)	Duffy & Vanderlinde, (2000)
DFS (pork and beef)	a) temperature: 37 °C (24 h) or 24 °C (48 h), b) ± pre-drying phase (5 days) c) drying: 7 – 9 or 18 – 20 days	*E. coli* O157:H7 decreased maximally by 5 logs in combination : fermentation 37 °C (24 h) + pre-drying phase + drying (18 – 20 days)	Naim et al., (2003)
Sucuk	Drying time: 14; 21; 28 days KNO_3 (0; 200; 400 ppm)	*E. coli* O157:H7 decreased by 1.5 log (day 14), 3.3 logs (day 21) and ≥ 4 logs (day 28) Nitrate level has no effect	Apaydin et al., (2009)
Soudjouk	Dextrose (0.25%; 0.5%; 0.7%) a_w (0.92; 0.89; 0.86)	Reduction: *E. coli* O157:H7 up to 4.4 logs *L. monocytogenes* up to 0.9 log *S.* Typhimurium up to 4.6 logs at lowest pH and a_w values	Hwang et al., (2009)
Morr (pork, mutton, beef) Salami (pork, beef)	NaCl (3.6%; 4.5%; 5%) $NaNO_2$ (100; 300; 500 ppm) Glucose (0.5%; 1.25%) Temperature: 20 °C; 30 °C Sausage diameter: 43 mm; 70 mm	*E. coli* O103:H25 decreased by 3 logs in combination with high levels of ingredients and temperature Diameter increase: lower reduction rate	Heir et al., (2010)
Salami	Fat content: 18.46%; 9.67% Diameter: 32; 55; 80 mm	*E. coli* O157:H7 decreased 5 logs after 32, 46 and 53 days of ripening for diameter 32, 55 and 88 mm, respectively.	De Souza et al., (2018)

TABLE 4: *The effectiveness of storage conditions on safety of dry fermented sausages.*

Type of vacuum packed product	Conditions of storage	Pathogen reduction	Reference
Pepperoni slices	Duration: up to 90 days T: - 20 °C; 4 °C; 21 °C Atmosphere: vacuum; air; CO_2	*E. coli* O157:H7 fastest decreased up to 4.5 logs after 28 days of storage under air at 21 °C	Faith et al. (1997)
Pepperoni	Duration: 56 days T: 4 °C; 21 °C	*S.* Typhimurium DT104 decreased: a) 1.7 log at 4 °C and b) 3.7 logs at 21 °C	Ihnot et al. (1998)
Norwegian DFS (mutton)	Duration: 5 months T: 4 °C, 20 °C	a) *L. monocytogenes* decreased negligible at 4°C and ~ 2.5 logs at 20 °C b) *E. coli* O157:H7 decreased ~ 2.5 logs at 4°C and > 3 logs at 20 °C c) *S.* Kentucky decreased at least 1.5 log at both temperatures	Nisen & Holck (1998)
Soudjouk	Products fermented to pH 5.3 or 4.8 and stored at 4 °C; 10 °C; 21 °C one month	Level of decrease: a) up to 1.8 log for *L. monocytogenes* b) up to 3.7 logs for *S.* Typhimurium c) up to 3.2 logs for *E. coli* O157:H7	Porto-Fett et al. (2008)
Sliced pepperoni	Duration up to 180 days T: 4 °C; 12 °C; 25 °C	*L. monocytogenes* decreased up to 3.5 logs at day 60	Byelashov et al. (2009)
Salami (pork and beef)	a) duration: 1 or 2 months T: 20 °C; 16 °C; 4°C b) precondition (freezing 24 h; -18 °C) + storage (duration of 1 month; T – same as in a)	Shiga toxigenic strains of *E. coli* decreased: a) up to 3.9 logs b) up to 4.4 logs	Rode et al., (2012)

time (1.6 logs on day 13; 3.3 logs on day 21), but not with $NaNO_2$ level.

Hwang et al. (2009) manipulated pH and a_w in sudžuk, and concluded that reductions of pathogens (0–4.4 logs) are induced by a decrease in sausage pH (5.2 to 4.6).

Heir et al. (2010) concluded that reductions of the pathogen *E. coli* O103:H25 in salami and morr sausages are significantly increased with increases in temperature, salt and $NaNO_2$ concentrations and with a decrease in sausage pH. On the other hand, pathogen reduction was less associated with increase of casing diameter or with the fat content.

De Souza et al. (2018) investigated the conditions for achieving 5 log reductions of *E. coli* O157:H7 in salami with different diameters and fat contents. Fat content does not affect the level of pathogen reduction, while the larger diameter requires an extension of drying time.

Almost none of the antimicrobial factors was found to independently fulfil the intended level of DFS safety, i.e. 5 log reductions of pathogenic *E. coli* serotypes. Joint implementation of several factors can ensure the desired level of microbiological safety; however, there are negative effects on the quality and overall acceptability of the final sausages (Holck et al., 2011).

Pathogen reduction during DFS storage

After reaching the desired level of dryness, DFS are packaged and placed in storage to give them a shelf-life of several months. During this storage period, the effects of antimicrobial factors that reduce pathogens continue. In Table 4, important results on reducing pathogens during the storage period are summarized.

Rode et al. (2012) investigated salami of pork and beef origin and found the reduction of enterohaemorrhagic *E. coli* is enhanced with increased storage temperature (20 > 16 > 4 °C) and with storage duration (2 > 1 month). This study also showed that freezing sausages to –18 °C for one day before storage enhances reduction by an additional 0.9 logs while the best reduction of roughly 4 logs (not accounting for the previous reduction during fermentation and ripening) was obtained after freezing for 24 hours followed by storage for 1 month at 20 °C. It is noteworthy that sensorial properties of sausages after storage were not investigated in this study.

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In an earlier study, Faith et al. (1997) found the storage temperature affects the level of *E. coli* O157:H7 reduction more than does the packaging type. Pathogen reduction after storage at 21 ºC for two weeks was 3.8 and 2 logs in air and vacuum packed sliced DFS, respectively, while the reduction was 4 to 5 logs after four weeks of storage in both types of package. On the other hand, in sausages stored for three months at 4 or –20 ºC, *E. coli* O157:H7 reduction was about 2 logs, no matter whether the products were kept under air, vacuum or CO_2 atmosphere. The authors noted that storage at ambient temperature produces undesirable changes in sausage appearance.

Nissen and Holck (1998) found roughly 1 log better survival of *E. coli* O157:H7 in vacuum packed Norwegian lamb DFS stored at 4 ºC than at 20 ºC; sausages were sampled 46 days and 5.5 months after the beginning of production. In sausages stored at 4 ºC, the overall reduction of *E. coli* O157:H7 after 5.5 months was around 4 logs, while in sausages kept at 20 ºC the reduction was 5 logs. Similarly, the overall reduction of *Salmonella* Kentucky in sausages stored at 4 ºC after 46 days was roughly 2 logs, while in sausages kept at 20 ºC the reduction was about 3 logs. At 5.5 months after the beginning of production, S. Kentucky was not detectable in DFS stored at either temperature. On the other hand, *L. monocytogenes* reductions at both temperatures were negligible and not significantly different between the two storage temperatures at 46 days after the beginning of production. However, at 5.5 months after the beginning of production, the *L. monocytogenes* level in sausages stored at 20 ºC was below the limit of detection (150 CFU/g), while *L. monocytogenes* in sausages stored at 4 ºC remained practically unchanged, i.e. the reduction was only 0.2 logs.

In a study by Ihnot et al. (1998) on pepperoni DFS that were vacuum-packed and stored for 56 days at 4 ºC, *S.* Typhimurium was reduced by 1.7 logs, while the reduction was 3.7 logs when the sausages were stored at 20 ºC (in both cases, reductions obtained during the production process were not included).

Porto-Fett et al. (2008) investigated reductions of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* that were inoculated into the stuffing of soudjouk-type sausages with lower (4.8) and higher (5.3) pH levels during the fermentation phase. The sausages were vacuum-packed and stored at three different temperatures (4 ºC, 10 ºC and 21 ºC) for 30 days. In general, reductions of all three pathogens were better with the highest storage temperature and in sausages with low pH. *L. monocytogenes* was the most resistant (2.5 log reduction at pH 4.8/ 21 ºC/30 days' storage), *E. coli* O157:H7 was somewhat less resistant (3.5 log reduction) while *S.* Typhimurium was the most sensitive (5 log reduction with same parameters). Furthermore, to simulate post-process contamination, this study investigated the pathogens' survival after surface contamination of sliced sausages. Reductions of all three pathogens were smaller when sausage surfaces were contaminated compared with inoculation into the stuffing.

In a study by Byelashov et al. (2009), three cocktails of ten *L. monocytogenes* strains, previously sub-cultured as non-acid adapted, acid adapted or pepperoni extract habituated, were separately inoculated onto the surface of sliced pepperoni sausage to obtain a level of 3–4 log/cm^2. The slices were subsequently vacuum-packed and stored at 4, 12 or 25 ºC for 180 days. The *L. monocytogenes* cocktail of acid adapted strains expressed the fastest death rate while non-acid adapted strains had the slowest death rate. With regard to the storage temperature, *L. monocytogenes* reductions were smaller at 4 ºC than at 12 ºC or 25 ºC (reductions were similar at those two temperatures). After 60 days of storage, the pathogen levels were under the limit of detection, regardless of differences in the storage temperatures or strain adaptation. However, sensory evaluation of the fermented sausages was not investigated in this research, as is the case in the three previously mentioned studies (Ihnot et al., 1998; Porto-Fett et al., 2008; Byelashov et al., 2009).

Extending the storage period of DFS together with increasing their storage temperature can successfully reduce all three main foodborne pathogens. However, a consequence such treatments could be negative effects on the sensorial properties of the sausages.

Irradiation

The potential of irradiation in food safety has been studied for several decades, but wider application in the food industry is still lacking, mostly due to consumer resistance since the irradiated food has to be declared (Sofos, 2014; Chen et al., 2012). Several studies found that an ionizing radiation dose of ≤ 2 kGy, needed to achieve the desired level of microbiological safety, does not significantly alter the sensorial properties of DFS. The negative effects that can be seen if higher doses are applied (i.e. ≥ 4 kGy) include inhibition of technologically useful microorganisms, oxidation changes with production of free radicals, and protein degradation. The sum of these effects leads to changes in sensorial properties, quality and shelf-life of DFS (Kim et al., 2012; Cabeza et al., 2009; Chouliara et al., 2006; Samelis et al., 2005; Johnson et al., 2000).

Irradiation is a possible approach for improving the microbiological safety of DFS, but with the limitation of low consumer acceptance.

High pressure processing (HPP)

HPP (50–1000 MPa; Table 5) enables food safety improvements without the application of high temperatures and guarantees better preservation of the original characteristics and quality of foods than when high temperature is used (Hugas et al., 2002b). HPP technology is environmentally friendly and well accepted by consumers. Furthermore, it enables the creation of innovative meat products, and hence, its wider application is expected in the near future. The antimicrobial effect of high pressure is achieved by matrix compression that leads to different disorders in microorganisms' cells, particularly in the spatial distribution of large molecules and the function of the cytoplasm membrane. HPP effects are dependent on the characteristics of the microorganisms, their physiological state, the intensity and length of the compressive force, and on food composition, pH, a_w and temperature. Pathogen reduction under HPP is, thus, less successful in products that are rich in nutrients (proteins, lipids, sugars, vitamins) or with lower water contents (Campus, 2010; Considine et al., 2008), as is the case with DFS. HPP is applied only as an additional decontamination step to finished DFS, and a pressure of least 400 MPa is needed to achieve the desired level of safety. One of the advantages of HPP is that it does not distort sensorial qualities of sausages. Moreover, some studies (Alfaia et al., 2015; Marcos et al. 2007) even found that HPP improves the texture of sausages due to better cohesiveness, firmness and chewiness of the treated products. Negative effects include changes in colour, although these are rare, not very pronounced and are most-

TABLE 5: *The effects of high pressure processing in different types of dry fermented sausage.*

Type of product	Form of treated product/ Production phase	HPP treatment	Reduction of pathogens/spoilage microbiota	Sensorial changes	Reference
Fuet Chorizo	Whole sausages/ after stuffing	300 MPa – 10 min.	*Salmonella* Derby/London/Schwarzengrund cocktail: about1.5 log; *L. monocytogenes*: 1 log after HPP but then increase 1 log after ripening;	Whitening effect (increase in brightness)	Marcos et al. (2005)
Fuet Chorizo	Whole sausages/ after drying	400 MPa – 10 min.	*Enterobacteriaceae*/*Enterococci*: Fuet – 1 log/ no reduction; Chorizo: 3.8 logs / 2 logs;	Slight decrease in colour intensity	Marcos et al. (2007)
Fuet	Whole sausages/ after drying	400 MPa – 10 min.	*Salmonella* London: 2 logs; *L. monocytogenes*: 0.6 log; *Staphylococcus aureus*: ≤ 0.3 log; Additionally, HPP induce long term inhibitory effect during storage (except for *S. aureus*);	ND	Jofre et al. (2009)
Fuet	Whole sausages/ after drying	600 MPa – 5 min.	*L. monocytogenes*: 1 log; *S. aureus*: 1 log; *Enterobacteriaceae*: ≥ 0.3 log	ND	Rubio et al. (2013)
Morr Salami	Slices/ after drying	600 MPa; 6 min.	*Escherichia coli* O103:25 (enterohaemorrhagic) – about 3 logs for both types of products;	No	Omer et al. (2010)
Morr Salami	Meat trimmings	600 MPa – 6 min.	*Enterobacteriaceae*: up to 1.5 log	Lower colour, aroma, taste, texture and overall appreciation	Omer at al. (2015)
Genoa salami	Whole sausages/ after drying	483 MPa – 1-5 min.; 600 MPa – 5-12 min.	*E. coli* O157:H7: ≥4.7 ≥ 5.8 logs; *S.* Typhimurium: ≥ 1.9 ≥ 2.4 logs; *L. monocytogenes*: 1.6 ≥5 logs;	ND	Porto-Fett et al. (2010)
DFS laboratory model	Slices - QDS® (Quick Dry Slice technology process)	600 MPa – 5 min.	*L. monocytogenes*: no reduction;	ND	Marcos et al. (2013)
Chouriço	Whole sausages/ after drying	202 MPa up to 600 MPa – 2.6 min. up to 30 min.	*Enterobacteriaceae*: ≥ 3.5 logs	Improve colour, cohesion and firmness	Alfaia et al. (2015)

ND – Not determined

ly expressed after DFS are treated with high pressure at the beginning of their production process. The studies on HPP effects on the main foodborne pathogens and spoilage microbiota in different DFS types are summarized in Table 5.

HPP as a novel non thermal technology has good consumer acceptance and can improve the safety of DFS, although some changes to the sensorial quality of sausages are recorded.

Heat treatments

It is generally accepted that the application of heat is the most efficient method of pathogen reduction. However, it is also associated with a certain distortion of original sensory characteristics and quality of fermented foods (Rode et al., 2012; McQuestin et al., 2009; Chacon et al., 2006). Regarding DFS, heat treatments can be applied after fermentation or to the finished sausages.

Heat treatment after fermentation. The US Food Safety and Inspection Service (FSIS) consolidated the various studies into the reduction of *E. coli* O157 and *Salmonella* and gave recommendations on different time/temperature treatments of all semi-dry and dry fermented sausage types for producers (Anonymous, 2001).

Riordan et al. (2000) investigated the effects of heat treatments of DFS (pepperoni) after fermentation and found that, at the same temperature (between 58.3 °C and 61 °C), a longer time is needed to achieve 5 log reduction of *E. coli* O157:H7 compared to the durations recommended by the FSIS. As an explanation of the discrepancies in the results, the authors stated differences in the sausage composition, production method, and enumeration protocols.

L. monocytogenes inactivation by heat treatments of pepperoni sausages were examined by Glass and Doyle (1989). They found a 1 log reduction requires heating of the sausages, after fermentation, to 51.7 ºC for 4 hours.

Heat treatment after drying. Glass & Doyle (1989) heated pepperoni sausages that were dried for 26 days and found treatment at 51.7 ºC for 4 hours reduced *L. monocytogenes* by 2 logs. Shay and Souness (1995) investigated *Salmonella* reduction by heat treatment of salami at the end of the production process. By heating finished sausages to an internal temperature of 55 ºC in a chamber (air temperature of 65 ºC and 90% relative humidity), the pathogen was reduced by 5 logs, without compromising the sensory properties of the product (moreover, sensorial properties of the heat treated sausages were, in general, assessed as better than the control sausages). However, these results were only for sausages that had, by application of starter cultures, a pH of <5 at 24 hours after the beginning of fermentation. On the other hand, Johnson et al. (2000) found that pepperoni subjected to heating to an internal temperature of 60 ºC in a chamber (air temperature of 70 ºC and 85 % relative humidity) had significantly altered texture and colour when compared to the control sausages. Heir et al. (2013) and Rode et al. (2012) found that keeping DFS at 43 ºC for 24 hours reduced enterohaemorrhagic *E. coli* by 2 to 4 logs, whereby the sensorial properties of the sausages were only slightly distorted or were not distorted at all. The same authors no-

ted that total sensory acceptability of the heat treated and vacuum packed sausages actually improved after six weeks of storage at 4 °C. In a study by Ducic et al. (2016), *S.* Typhimurium and *E. coli* O157 in finished DFS made from pork and beef, respectively, were successfully reduced (5 logs for *E. coli* O157 and 6.5 logs for *S.* Typhimurium) by applying mild heat treatments, while sensorial acceptability of the treated sausages was not diminished. For *Salmonella* reduction, pork sausages were treated at temperatures from 47 ºC to 53 ºC, and for *E. coli* O157 reduction, higher temperatures were applied (53 ºC to 59 ºC), given that in preliminary investigations, *E. coli* O157 expressed higher thermal resistance. This study also found that *L. monocytogenes* (in both types of sausages) was far more resistant to heat treatments than the other two pathogens – the desired (2 log) reduction required that DFS were treated at 66 ºC for 2 or more hours, which ultimately led to significant distortion of their sensorial properties.

Application of mild heat treatments after fermentation or after ripening induces successful reductions of *E. coli* and *S.* Typhimurium, but with sensorial changes to the sausages, which could have a either a positive or a negative influence on consumer acceptance. Mild heat treatment of finished DFS does not usefully reduce *L. monocytogenes.*

Conclusions

DFS are characterized by complex composition and manufacturing processes that result in a great diversity of these products. Many of them have a long tradition of production and great potential as export items. On the other hand, intensifying production and distribution of DFS and their links with food borne disease outbreaks has induced more stringent safety criteria. This has prompted research on various approaches to reduce the risks of the main foodborne pathogens occurring in DFS. The findings from these studies were largely positive, but also show that the application of only one measure to greatly reduce the hazards is usually followed by a negative effect on the sensorial properties of DFS.

On the other hand, the combination of additional, milder, pathogen reduction measures in different production steps lessens their negative consequences. Therefore, it is necessary to carefully select and apply treatments in specific steps during DFS production in order to both enhance the safety of these products and to preserve their desirable "natural" properties.

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Conflict of interest

The authors declare no conflict of interest.

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Adresse der korrespondierenden Autorin:
Miroslav S. Dučić
University of Novi Sad
Faculty of Agriculture
Department of Veterinary Medicine
21000 Novi Sad
Trg Dositeja Obradovića 8
Serbia
mir.s.ducic@gmail.com