



Assessment of microbiological quality and safety of marinated pork products from German retail during shelf life



Rohtraud Pichner^{a,*}, Clien Schönheit^a, Jan Kabisch^b, Christina Böhnlein^a, Wolfgang Rabsch^c, Lothar Beutin^d, Manfred Gareis^e

^a Department of Microbiology and Biotechnology, Max Rubner-Institute, Kulmbach, Germany

^b Department of Microbiology and Biotechnology, Max Rubner-Institute, Kiel, Germany

^c National Reference Centre for Salmonella and Other Bacterial Enterics, Division 11 Enteropathogenic Bacteria and Legionella, Robert Koch Institute, Wernigerode, Germany

^d National Reference Centre for *E. coli*, Federal Institute for Risk Assessment, Berlin, Germany

^e Chair of Food Safety, Veterinary Faculty, Ludwig Maximilians-University, Munich, Germany

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ABSTRACT

Foodborne diseases are of major concern for public health. Here we assess the microbiological quality and safety of marinated pork steaks ($n = 300$) and marinades ($n = 30$) which were used for the production of marinated steaks by analyzing quantitative microbiological parameters and foodborne pathogens. *Salmonella* spp. and *Listeria monocytogenes* were isolated from about 2%, *Staphylococcus aureus* from 8% and *Bacillus cereus* from 21% of the steaks. One steak was MRSA-positive and one contained EHEC/STEC. *B. cereus* was the only pathogen detected in the marinades. Similar toxin patterns of *B. cereus* strains from meat and marinades suggested that a contamination of meat with *B. cereus* occurred via marinades.

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1. Introduction

Foodborne infections are of major concern for public health and cause substantial costs (Hoffmann, Batz, & Morris, 2012), despite of reinforced actions by governments and food industry for better food hygiene. Changing lifestyles might contribute to this situation, such as the rise of single-households possibly accompanied by a decreasing knowledge of proper food handling and a growing demand for convenience food (Brunner, van der Horst, & Siegrist, 2010).

Amongst the category of convenience food, pre-packed marinated meat products such as steaks, spare ribs and filets are becoming increasingly popular (O'Donnell, 2004; ZMP, 2007). In Germany, about 400,000 tons of barbecue products from pork, beef and chicken were sold during the barbecue season in 2007. Within this section of meat products, marinated pork neck steaks are the

most popular (ZMP, 2007). In Germany, marinated pork neck steaks are produced usually from frozen pork necks with industrial marinade. Minimum shelf life, as assigned by producers, ranges from 13 to 18 days.

A small, focused microbiological and sensory study, published by the German consumer's magazine "Stiftung Warentest" in 2008, indicated in part poor food hygienic quality for marinated, pre-packed meat products (Anonymous, 2008). Consequently, a possible obscuring of poor hygienic conditions by marinating was discussed in the public. Currently, there are no convincing data about the microbiological quality and safety of marinated pork steaks in Germany. Producers have to comply with the rules of Reg. (EC) No. 2073/2005. Additionally, the German Society for Hygiene and Microbiology (DGHM) has published indicative and warning values for several food categories, in particular for raw and unseasoned pork (DGHM, 2013). These criteria have to be regarded as recommendations based on scientific studies, observations by food control authorities and standards of food industry. It cannot be ascertained whether the recommendations of the DGHM are transferable to marinated pork products, where an introduction of

* Corresponding author. Tel.: +49 9221803220; fax: +49 9229803331.
E-mail address: rohtraud.pichner@mri.bund.de (R. Pichner).

pathogens might also occur via marinades. Producers of marinated pork steaks assign different values for minimum shelf life to similar products, which further complicates an assessment of product safety.

The aim of our work was to evaluate the microbiological quality and safety of marinated pork products. Therefore, we analyzed vacuum packed, marinated pork neck steaks from German retail for microbiological parameters and foodborne pathogens. To assess the impact of marinades on the microbiological safety of the steaks, marinades used for the production of marinated pork steaks were also investigated.

2. Material and methods

2.1. Sampling

2.1.1. Meat samples

A total of 300 vacuum-packed, marinated pork steak-products were purchased in two periods from self-service counters of retail stores in Bavaria, Germany: 150 samples from June to October 2008 and 150 samples from March to June 2009. Samples originated from 13 different producers (Table 1). Producers and product categories were identical in the two sampling periods. Due to the wide variety of marinated pork steaks offered in the German retail, samples were classified – based on the main type of flavor or seasonings – as products with mustard-/beer-marinate, paprika-marinate and herbs-/garlic-marinate according to Frey (1999). Dispersions on water–oil-basis with thickening ingredients were classified as mustard/beer-marinades. Paprika-marinades contained pure seasoning oils, emulsion- or dispersion-marinades or

mixtures. Oil–spices-mixtures without water were categorized as herbs-/garlic marinades. One hundred products were sampled from each category during sampling periods. Two pork steaks with equal minimum shelf life were purchased from the same producer at different days. After collection, samples were transported to the laboratory under cooling conditions and stored at 4 °C. Microbiological analyses were carried out 3 days after purchase and at the end of minimum shelf life (± 1 day).

2.1.2. Marinades

Thirty marinades were delivered by five different producers from spice industry in customary packing during August 2008 and August 2009 and stored at 4 °C until analyses. The marinades, commonly used for production of marinated pork steaks in Germany, were also classified into the three categories mustard-/beer-marinades, paprika-marinades and herbs-/garlic-marinate as described above (Table 1).

2.2. Physicochemical and microbiological analyses of marinated pork samples and marinades

Analyses were carried out on the basis of the German official collection of methods according to § 64 of German Food and Feed Legislation (LFGB, 2013). Each sample was tested twice.

Measurement of pH-value and water activity: pH-values were measured in the cores of the steaks or in marinades (pH 537, WTW, Weilheim, Germany). Water activity measurements of marinades were done at 25 °C using Novasina a_w Sprint TH 500 (Axair Ltd., Switzerland).

Microbiological analyses: For detection and enumeration of aerobic mesophilic bacteria, Enterobacteriaceae, coagulase+staphylococci, *Bacillus cereus*, sulfite reducing clostridia and *Listeria monocytogenes*, 20 g of steaks or marinades were weighed into sterile stomacher bags. After homogenization for 2 min in 180 ml sterile physiological sodium chloride solution (0.85% NaCl) in a Coolworth Stomacher 400 (Seward, England) and serial dilution in sterile physiological sodium chloride solution (method L.06.00-16; LFGB, 2013), samples were plated onto appropriate agars and incubated as shown in Table 2.

For testing the presence of *Salmonella* spp. and Shigatoxin producing *Escherichia coli* 25 g of the samples were enriched in an appropriate selective enrichment broth (Table 2).

Identification and further characterization of isolated pathogen strains were performed by biochemical, molecular biological and/or immunological methods as listed in Table 3.

Sample preparation for PCR assays of enriched samples were done according to De Medici et al. (2003). Briefly, 1 ml of sample enrichments were centrifuged at 10,000 g for 5 min, supernatant was discarded and the sediment was resuspended in phosphate buffered saline (PBS) (pH 7.4) and centrifuged again at 10,000 g for 5 min. This procedure was repeated two times. The pellet was resuspended in 300 μ L PBS, heated at 100 °C in a thermoblock (Eppendorf AG, Germany) for 10 min, immediately cooled on ice and centrifuged for 1 min at 10,000 g. The supernatant was used as PCR template.

For strain confirmation, single colonies were taken from the respective agars, re-suspended in 500 μ L PBS, heated for 10 min at 100 °C and centrifuged at 10,000 g for 1 min. Supernatants were used for the PCR assays.

2.3. Statistical analyses

Median, mean values and standard deviations were calculated by Microsoft Excel 2010. Box plot diagrams were compiled by Statistica® version 7.1.

Table 1

Types and numbers of analyzed pork steaks and marinades: Steak samples were purchased during two sample periods in 2008 and 2009 (M = Products with mustard-/beer-marinate, P = Products with paprika-marinate, H = Products with herbs-/garlic-marinate). Marinades were purchased during August 2008 and August 2009. (M* = mustard-/beer-marinate, P* = paprika-marinate, H* = herbs-/garlic-marinate).

Marinated pork steaks						
Producer-code	Number of analyzed samples				Minimum shelf life	Use of frozen meat
	M	P	H	Total		
A	–	12	12	24	17 days	no
B	12	4	8	24	15 days	yes
C	–	20	8	28	14 days	yes
D	16	12	24	52	15 days	yes
E	4	8	12	24	14 days	yes
F	–	8	8	16	16 days	yes
G	–	4	4	8	18 days	yes
H	16	–	–	16	No information	no
I	36	4	4	44	18 days	no
J	–	8	8	16	No information	yes
K	–	4	4	8	18 days	yes
L	8	16	8	32	13 days	yes
M	8	–	–	8	13 days	no
Total	100	100	100	300		
Marinades						
Producer-code	Number of analyzed samples				Minimum shelf life	Use of frozen meat
	M*	P*	H*	Total		
N	1	1	–	2	No information	
O	2	–	–	2	No information	
P	5	5	6	16	No information	
Q	2	1	1	4	No information	
R	–	3	3	6	No information	
Total	10	10	10	30	No information	

Table 2
Growth media and growth conditions for microbiological analyses of pre-packed marinated pork steaks and marinades.

Target organism	Method	Media	Incubation
Aerobic mesophilic bacteria	L00.00-18 ^a (=DIN EN ISO 4833:2003) Surface-plating	Standard I Agar (Merck, 107881)	48 h, 30 °C
<i>Enterobacteriaceae</i>	DIN 10164-1:1986-08 ^b Surface plating	Desoxycholate-Hydrogen-Sulfide-Lactose- (DHL-) Agar; (Merck, Darmstadt, Germany; Art. 114350)	48 h, 30 °C
Sulfite-reducing clostridia	Angelotti, Hall, Lewis, and Foter (1962), Eisgruber and Reuter (1995)	Sulfite-Polymyxin-Sulfadiazine- (SPS-) Agar (Merck, 110235)	48 h, 37 °C
<i>Bacillus cereus</i>	L 00.00-25 ^a	Mannitol Egg Yolk Polymyxin (MYP-) Agar	24 h, 37 °C
<i>Listeria</i> spp. and <i>Listeria monocytogenes</i>	DIN EN ISO 11290-2 Detection and enumeration	Oxoid-Chromogen-Listeria-Agar (OCLA) [Oxoid; Art.Nr. PO5165A]	48 h, 37 °C
<i>Staphylococcus aureus</i>	L.00.00-55 ^a	Baird-Parker-Agar; [OXOID; CM1127]	48 h, 37 °C;
<i>Salmonella</i> spp.	DIN EN ISO 6579:2003 ^b	Buffered water (OXOID; CM0509) First enrichment Rappaport-Vassiliadis Soja Peptone broth; (Oxoid; CM 0866) Second selective enrichment Selenite-Cystine-enrichment-broth (Merck, 107709) Second selective enrichment Desoxycholate-Hydrogen-sulfide-Lactose-Agar (DHL); [Merck; 114350] Xylose-Lysine-Tergitol-4-(XLT4)Agar; [Oxoid; CM 1061, SR 0237]	16–20 h, 37 °C
Shigatoxin-producing <i>Escherichia coli</i>	According to method L 00.00-92 ^a DIN 10118:2004	Modified Tryptic Soy Broth (m-TSB) (Merck, 105459, 104054, 105104) Sorbitol MacConkey (SMAC) Agar (Sifin, TN 1220)	16 h, 37 °C, 180 rpm 24 h, 37 °C

^a Amtliche Sammlung von Untersuchungsverfahren nach § 64 LFGB = German official collection of methods according to § 64 of German Food and Feed legislation (LFGB).

^b DIN = Deutsches Institut für Normung e.V.

3. Results and discussion

3.1. Marinated pork steaks

This study presents data on microbiological quality and safety of marinated pork steaks from self service areas of local German supermarkets from two sampling periods. Varieties and number of samples from 13 German producers were equal for the two sampling periods (Table 1, Fig. 1). Therefore, a direct comparison of the data and an estimation of potential seasonal effects can be derived. Because of large product diversity, steak samples were divided into

categories of products with mustard-/beer-marinade, paprika- or herbs-/garlic-marinade based on their main flavors, according to Frey (1999).

Until the end of minimum shelf life at 4 °C, pH-values of marinated pork steaks decreased by about 0.45 units. Concurrently, aerobic plate counts rose by about 1–2 log during minimum storage life to values up to 8 or 9 log cfu g⁻¹ (Fig. 1). Plate counts correlated with numbers of lactic acid bacteria, which were analyzed in the second sampling period (Table 4). Our results are consistent with report of Schirmer, Heir, and Langsrud (2009). As in fresh packed meat, lactic acid bacteria developed in marinated pork

Table 3
Methods for confirmation and characterization of isolates of foodborne pathogens in vacuum packed marinated pork neck steaks.

Pathogen	Method	Reference
<i>Salmonella</i> spp.	<i>invA</i> -gene-detection Serotyping/phage typing	Rahn et al. (1992) Rabsch, (2007)
<i>L. monocytogenes</i>	rhamnose-/xylose fermentation hemolysis serotyping (multiplex-PCR)	Groves and Welshimer (1977) Doumith, Buchrieser, Glaser, Jacquet, and Martin (2004)
<i>B. cereus</i>	<i>gyrB</i> -gene-detection Detection of toxigenicity Emetic toxin cereulide Diarrhetic toxin	Yamada, Ohashi, Agata, & Venkateswaran (1999) Ehling-Schulz, Fricker, and Scherer (2004a) and Ehling-Schulz et al. (2006) Mäntynen and Lindström (1998) Moravek et al. (2004) Ryan, Macmillan, and Zilinskas (1997) Moravek et al. (2004) Dietrich, Moravek, Burk, Granum, and Märklbauer (2005)
STEC	<i>stx1</i> <i>stx2</i> <i>eae</i> <i>hlyA</i> Serotyping and subtypes of <i>stx2</i>	Rüssmann et al. (1995) Cebula, Payne, and Feng (1995) Schmidt, Rüssmann, and Karch (1993) Schmidt, Beutin, and Karch (1995) Beutin et al. (2007)
<i>St. aureus</i>	Latex agglutination-assay Assay for enterotoxins (ELISA) MRSA-screening (Cefoxitin-agar diffusion assay) (confirmation of MRSA by PCR)	Zschock, Nesseler, and Sudarwanto (2005) Park, Akhtar, and Rayman (1996) Boubaker et al. (2004) Mehrotra, Wang, and Johnson (2000)

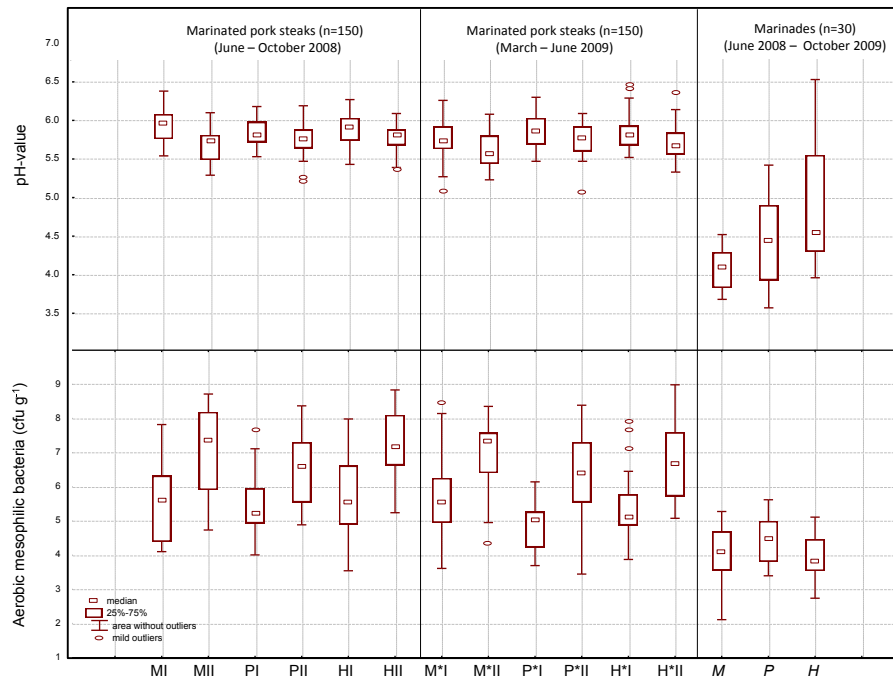


Fig. 1. Box-plot of pH-values and aerobic mesophilic plate counts (PC) of vacuum packed marinated pork steaks from self service areas of German retail and of marinades from German producers. Steak samples were categorized into three groups. M = products with mustard-/beer-marinade, P = products with paprika-marinades, H = products with herbs-marinades. M I, P I, H I: 25 samples were analyzed 3 days after purchasing for each category. M II, P II, H II: Analyses of 25 steak samples at the end of dedicated minimum shelf life for each category. *Steak samples were purchased and analyzed in 2009. M, P, H: Mustard-, paprika-, or herbs-/garlic-marinades, samples were analyzed between June 2008 and October 2009.

products to the dominant bacterial population after 3–4 weeks of cold storage with cell counts up to $8 \log \text{cfu g}^{-1}$. This resulted in lowered pH-values and increased aerobic mesophilic plate counts (Blixt & Borch, 2002; Holley, Peirson, Lam, & Tan, 2004). Aerobic plate counts in meat products higher than $\log 6 \text{cfu g}^{-1}$ are often accompanied by first signs of spoilage (Blixt & Borch, 2002; Holley et al., 2004). Therefore, our findings at the second examination time suggest that possible spoilage indications may be obscured by marinades.

We did not observe significant differences between the results of the two sampling periods (Fig. 1). Consequently, no noticeable seasonal effects of varying climatic conditions or different consumer demands were observed. This might be an indication of consistent conditions during production and distribution of marinated pork steaks, e.g. adequate cooling temperatures.

According to Holley et al. (2004), Enterobacteriaceae present on stored vacuum-packed pork are useful indicators of efficacy of plant sanitation. Although Enterobacteriaceae occurred in most steak samples (Table 4), the proportion of Enterobacteriaceae was in the most samples of our study (266/300) below 5% of the microbiological population. Generally, this could be considered as a reflection of acceptable sanitation practice and hygiene (Holley et al., 2004).

However, in 11% of the samples (34/300) Enterobacteriaceae counts of more than $\log 5 \text{cfu g}^{-1}$ were found (Table 4). These steak samples originated from 12 producers (data not shown). In one product with paprika marinade, 8 products with mustard marinade and 20 samples with herbs-/garlic marinade the proportion of Enterobacteriaceae was more than 5% of the total bacterial population (Table 4). In Germany, marinated pork neck steaks are produced usually from frozen pork necks. Therefore, producers should verify their incoming quality control of the raw meat.

Salmonella were detected only during the second sampling period in 2% of the steaks (Table 4). These results are in accordance with recent studies, which reported a presence of *Salmonella* spp. in pork within 2 and 6% of samples (Hauser et al. 2010; Meyer, Thiel, Ullrich, & Stolle, 2010; Prendergast et al., 2008; Rabsch, Simon, & Humphrey, 2013). *Salmonella* positive steak samples were not related to those with high Enterobacteriaceae counts ($>5 \log \text{cfu g}^{-1}$).

Serotyping of *Salmonella* isolates resulted in serotype Typhimurium (Table 5), which is one of the most common serotypes in pork products (Friedrich et al., 2010). Our analyses showed persistence of this serotype in 6 steaks with paprika marinades (Table 4). These products originated from the same producer and isolates were classified as monophasic variants of the serotype O4,12 phage type DT 193 and biphasic variants of the serotype O4,5,12 phage type DT 120 (Table 5). Therefore, *Salmonella* could survive in these products over a period of 18 days at 4°C .

One steak contained STEC, and further differentiation resulted in serotype ONT:H19 and virulence factor Stx2e (Table 5). STEC producing Stx2e are associated with edema disease in pigs. stx2e-positive STEC-isolates were frequently detected in pork with percentage of more than 50 (Beutin et al., 2007), but not in patients with severe STEC-caused diseases (Beutin et al., 2008). Only mild diarrhea was described after STEC-infection with Shigatoxin 2e-producing STEC (Friedrich et al., 2002). Therefore, marinated pork steaks might not to be a common source for highly pathogenic EHEC strains.

L. monocytogenes were detected in 5 meat samples (1.7%) with herbs-/garlic-marinade with counts ranging within 1 and $2 \log \text{cfu g}^{-1}$. Earlier studies documented an occurrence of *L. monocytogenes* in pork within 1 and 36% (Netschajew, Fredriksson-Ahomaa, Sperner, & Stolle, 2009; Yeh, Chen, & Lin, 2005). In our analyses *L. monocytogenes* were mainly isolated at the

Table 4
Microbiological parameters of vacuum packed marinated pork steaks from self service areas of German retail ($n = 300$). Cat.: Steak samples were categorized into three groups. M = products with mustard-/beer-marinade ($n = 100$), P = products with paprika-marinade ($n = 100$), H = products with herbs/garlic-marinade ($n = 100$). Steak samples were purchased during two sampling periods in 2008 and 2009 and analyzed at date of purchase (I) and the end of dedicated minimum shelf life (II). PC = aerobic mesophilic plate count; LAB = counts of lactic acid bacteria. n = number of positive samples, N = number of analyzed steak samples. \uparrow : Increase of bacterial counts $\geq 1 \log \text{cfu g}^{-1}$ and percentage $>10\%$; \rightarrow : no change of cell counts ($<1 \log \text{cfu g}^{-1}$ and $<10\%$); \downarrow : Reduction of cell counts $\geq 1 \log \text{cfu g}^{-1}$ and percentage of positive samples $>10\%$.

Cat.	Target	Microbiological results at date of purchase (I)										Microbiological results at the end of minimum shelf life (II)										Trend			
		Positive (n/N)	Range of cell counts ($\log \text{cfu g}^{-1}$)	Positive samples within $\times \log \text{cfu g}^{-1}$								Positive (n/N)	Range of cell counts ($\log \text{cfu g}^{-1}$)	Positive samples within $\times \log \text{cfu g}^{-1}$											
				<1	1	2	3	4	5	6	7			8	<1	1	2	3	4	5	6		7	8	
M	LAB ^a	25/25	1.6–6.6	–	3	2	6	4	7	3	–	–	25/25	3.0–7.1	–	–	–	3	4	3	13	2	–	\uparrow	
	Enterobacteriaceae	48/50	<1–7.1	2	11	18	8	4	2	4	1	–	44/50	<1–7.4	6	10	17	5	7	0	4	1	–	\rightarrow	
	Salmonella	0/50	–	–	–	–	–	–	–	–	–	–	0/50	–	–	–	–	–	–	–	–	–	–	–	
	STEC	0/50	–	–	–	–	–	–	–	–	–	–	0/50	–	–	–	–	–	–	–	–	–	–	–	
	Sulfite-red. clostridia	12/50	<1–2.4	38	7	5	–	–	–	–	–	–	5/50	<1–2.0	45	4	1	–	–	–	–	–	–	–	\downarrow
	<i>L. monocytogenes</i>	0/50	–	–	–	–	–	–	–	–	–	–	0/50	–	–	–	–	–	–	–	–	–	–	–	–
	<i>B. cereus</i>	5/50	<1–2.0	45	4	1	–	–	–	–	–	–	8/50	<1–2.0	42	6	2	–	–	–	–	–	–	–	\rightarrow
	<i>St. aureus</i>	1/50	<1–1.9	49	1	–	–	–	–	–	–	–	1/50	<1–1.6	49	1	–	–	–	–	–	–	–	–	\rightarrow
P	LAB ^a	25/25	1–5.4	–	3	7	8	4	3	–	–	–	25/25	2.5–9.0 ^a	–	–	3	2	3	7	6	3	1	\uparrow	
	Enterobacteriaceae	46/50	<1–4.3	4	12	23	9	2	–	–	–	–	45/50	<1–7.1	5	13	16	11	3	–	1	1	–	\rightarrow	
	Salmonella	2/50 ^b	n.a.	–	–	–	–	–	–	–	–	–	3/50 ^b	n.a.	–	–	–	–	–	–	–	–	–	–	–
	STEC	0/50	–	–	–	–	–	–	–	–	–	–	0/50	–	–	–	–	–	–	–	–	–	–	–	–
	Sulfite-red. clostridia	15/50	<1–2.1	35	13	2	–	–	–	–	–	–	13/50	<1–2.7	37	9	4	–	–	–	–	–	–	–	\uparrow
	<i>L. monocytogenes</i>	0/50	–	–	–	–	–	–	–	–	–	–	0/50	–	–	–	–	–	–	–	–	–	–	–	–
	<i>B. cereus</i>	9/50	<1–2.0	41	8	1	–	–	–	–	–	–	14/50	<1–2.7	36	12	2	–	–	–	–	–	–	–	\uparrow
	<i>St. aureus</i>	7/50	<1–1.8	43	7	–	–	–	–	–	–	–	5/50	<1–2.4	45	3	2	–	–	–	–	–	–	–	\rightarrow
H	LAB ^a	25/25	1.8–8.0 ^a	–	1	5	9	5	3	1	1	–	25/25	3.9–8.8	–	–	–	1	3	7	11	2	1	\uparrow	
	Enterobacteriaceae	47/50	<1–6.9	3	5	22	11	5	1	3	–	–	46/50	<1–8.6	4	7	11	7	3	3	7	6	2	\uparrow	
	Salmonella	1/50 ^b	n.a.	–	–	–	–	–	–	–	–	–	0/50	–	–	–	–	–	–	–	–	–	–	–	–
	STEC	1/50 ^{**}	n.a.	–	–	–	–	–	–	–	–	–	0/50	–	–	–	–	–	–	–	–	–	–	–	–
	Sulfite-red. clostridia	14/50	<1–2.1	36	13	1	–	–	–	–	–	–	13/50	<1–2.0	37	10	3	–	–	–	–	–	–	–	\downarrow
	<i>L. monocytogenes</i>	1/50	<1–1.3	49	1	–	–	–	–	–	–	–	4/50	<1–2.0 ^a	46	4	–	–	–	–	–	–	–	–	–
	<i>B. cereus</i>	13/50	<1–2.0	37	11	2	–	–	–	–	–	–	15/50	<1–2.2	35	12	3	–	–	–	–	–	–	–	\uparrow
	<i>St. aureus</i>	7/50	<1–2.5	43	3	4	–	–	–	–	–	–	4/50	<1–2.2	46	3	1	–	–	–	–	–	–	–	\rightarrow

n.a. = not applicable; – = not detected.

^a Results were rounded to one decimal place.

^b Qualitative detection after sample enrichment.

end of shelf life (after 18 d storage at 4 °C). Consequently, this pathogen survived in marinated pork samples. Most of the isolates belonged to serotype 1/2a (Table 5), which is detected commonly in pork products (Hellstrom et al., 2010). The other detected serotype 1/2c can often be found in food of animal origin and therefore sometimes in meat products (Hong et al., 2007).

Eight percent of the pork samples were positive for *Staphylococcus aureus* with counts up to 2.53 $\log \text{cfu g}^{-1}$. Cell numbers at the date of purchase were similar to those at end of shelf life (Table 4). Therefore, no growth of *St. aureus* was observed in marinated pork steaks. For a foodborne intoxication with *Staphylococcus* enterotoxins, an amount of 0.1–1 μg per kg body weight is necessary. A production of toxic dose demands *St. aureus* counts of more than 4 $\log \text{cfu g}^{-1}$ (Hennekinne et al., 2010). Therefore, there should be no impact for consumer's health, when the marinated pork steaks were stored before consumption at adequate cooling conditions.

Analysis of *St. aureus* strains for SE-production showed enterotoxin production in 65% of the strains (Table 5), which is slightly more than documented by other studies. They showed a prevalence of SE producing *St. aureus* in food of about 40–51% and of about 35–60% in raw meat (Atanassova, Meindl, & Ring, 2001; Nitzsche, Zweifel, & Stephan, 2007; Normanno et al., 2007; Pereira et al., 2009). The most common SE-type in our assays was SED (59%), followed by SEC (50%) and SEA (45%). These 3 toxin types occurred most frequently together in our *St. aureus* strains (Table 5). Our data are in accordance with results of Normanno et al. (2007). They detected SED (33.6%) as the most frequent enterotoxin type in milk and meat products, followed by SEA (18.4%) and SEC (15.2%). Conversely, Pereira et al. (2009) described SEA as the most frequent

enterotoxin in raw meat and fermented meat products. The difference to our data may be based on different methods, because we analyzed our *St. aureus* strains for toxin production by ELISA whereas Pereira et al. (2009) analyzed for genes encoding toxin production by molecular methods. In general, numbers of positive molecular biological results are higher than those for real toxin formation (Najera-Sanchez, Maldonado-Rodriguez, Olvera, & de la Garza, 2003).

One *St. aureus* strain was MRSA positive (Table 5). This is in accordance with other studies, which showed MRSA occurrence in pork in 0.3–10.7% of the samples (De Boer et al., 2009; Lim et al., 2010).

B. cereus were isolated from 64 samples (21%) of marinated pork steaks. Highest contamination rates were found in samples with herbs-/garlic-marinade, ($<3 \log \text{cfu g}^{-1}$, Table 4). For toxigenic *B. cereus* to cause implications for public health, an interruption of cold chain would be necessary with subsequent growth to numbers of up to $5\text{--}6 \log \text{cfu g}^{-1}$ (Ehling-Schulz, Fricker, & Scherer, 2004b). Marinated steaks are usually cooked thoroughly before consumption, which leads to inactivation of toxins of the diarrhea enterotoxin complex. A higher risk for consumer's health would exist, if *B. cereus* strains harbored the ability to form heat stable emetic toxin (Rajkovic et al., 2008). Our isolates possessed no genes for cereulide formation (Table 5). In food, an occurrence of cereulide forming *B. cereus* strains is likely within 0–13% of the samples (Altayar & Sutherland, 2006; Kreuzberger, Moravek, Dietrich, Scheurer, & Märtlbauer, 2008; Rau, Perz, Klittich, & Contzen, 2009; Schulz, 2004). Our results may be based on higher temperature optima of emetic *B. cereus* strains compared with non-emetic isolates, since emetic *B. cereus* strains cannot

Table 5Numbers of food pathogen positive samples and results of characterization of pathogenic isolates from marinated pork steaks ($n = 300$) and marinades ($n = 30$).

Pathogen	No. of positive samples		No. of isolates	Results of characterization		
	<i>n</i>	(%)		<i>n</i>	(%)	
<i>Salmonella</i>						
Marinated pork	6	(2)	13	11	(85)	<i>S. Typhimurium</i> DT 193; O:4,12:i:-, Monophasic
Marinades	0			2	(15)	<i>S. Typhimurium</i> DT 120; O:4,5,12:i:1,2, Biphasic
STEC						
Marinated pork	1	(0.3)	4	4	(100)	Serotype ONT:H19, <i>stx2e</i>
Marinades	0					
<i>L. monocytogenes</i>						
Marinated pork	5	(2)	35	34	(97)	Serotype 1/2a
Marinades	0			1	(3)	Serotype 1/2c
<i>St. aureus</i>						
Marinated pork	25	(8)	212	74	(35)	No SE detectable (ELISA)
				138	(65)	SE positive
				97	(45.7)	SEA
				63	(29.7)	SEB
				106	(50)	SEC
				125	(59)	SED
				14	(6.8)	SEE
				1	(0.5)	MRSA
Marinades	0					
<i>B. cereus</i>						
Marinated pork	64	(21)	127	3	(2)	Without toxin genes
				96	(76)	all parts of <i>hbl</i> - and <i>nhe</i> -operon
				121	(95.3)	<i>nheA</i>
				123	(96.8)	<i>nheBC</i>
				101	(79.5)	<i>hblA</i>
				100	(78.7)	<i>hblC</i>
				102	(80.3)	<i>hblD</i>
				0	(0)	<i>cesB</i> , cereulide formation
Marinades	10	(33)	31	0	(0)	Without toxin genes
				96	(67.7)	all parts of <i>hbl</i> - and <i>nhe</i> -operon
				31	(100)	<i>nheA</i>
				31	(100)	<i>nheBC</i>
				22	(71)	<i>hblA</i>
				24	(77.4)	<i>hblC</i>
				25	(80.6)	<i>hblD</i>
				0	(0)	<i>cesB</i> , cereulide formation

grow and produce cereulide at temperatures below 10 °C (Carlin et al., 2006).

About 98% of analyzed *B. cereus* isolates possessed genes coding for diarrhea toxin complex (Table 5). Kreuzberger et al. (2008) also detected genes encoding this toxin complex in 99% of *B. cereus* strains. Most of our isolates (95.3%) harbored the three genes of the Nhe-complex (Table 5). Similar results were found in other studies (Guinebretiere, Broussolle, & Nguyen, 2002; Molva, Sudagidan, & Okuklu, 2009; Moravek et al., 2006). Remarkably, all isolates with all components of HBL-complex possessed also three gene components of Nhe-complex (Table 5). Therefore, these *B. cereus* isolates have the potential for production of both toxin components with maximal biologic activity (Hansen & Hendrikson, 2001).

3.2. Marinades

In the 30 analyzed marinades, usually used for production of marinated pork steaks, lower pH-values and plate counts were determined than in marinated steaks (Fig. 1). In addition, ranges of pH-values were larger in pure marinades than in marinated pork samples. These results clearly demonstrate the buffering capacity of meat. This fact might allow survival of bacteria as shown by our microbiological analyses: Enterobacteriaceae were detected in 92% of marinated meat samples but only in 6.7% of the marinades and

Enterobacteriaceae counts increased until the end of shelf life of marinated pork samples. This might also indicate that contamination with Enterobacteriaceae occurred during meat processing rather than by addition of marinades.

According to our results, marinades with low pH-values seemed to have higher bactericidal effects than marinades with high oil content and low water activity.

Microbiological analyses of marinades resulted in negative findings for *Salmonella* spp., STEC, *L. monocytogenes* and *St. aureus* (Table 4). Spices and herbs, which were used for production of marinades, might contain pathogens (e.g. *Salmonella* spp.), but antimicrobial substances in spices and herbs such as in garlic, onions or clove, are able to inhibit bacterial growth (Graubaum, Kleer, & Hildebrandt, 2005). This, in combination with low pH- and low water activity-values of marinades might explain negative results of vegetative pathogens in marinades.

Marinades contained spore forming bacteria such as *B. cereus* and sulfite reducing clostridia up to 2.36 log cfu g⁻¹ (Table 4). Molecular biological analyses of isolates from marinades for gene components of diarrhea and emetic toxin complex showed results similar to analyses of isolates from marinated pork steaks. Entry of toxigenic *B. cereus* into marinated pork products therefore appears to occur via marinades, particularly by herbs and spices (Little, Omotoye, & Mitchell, 2003; Psomas, Papantoniou, Petridis, & Panou, 2009). For information about genotypic relationships

within *B. cereus* isolates from steaks and marinades, further investigations are necessary, e.g. random amplification of polymorphic DNA (RAPD) or multi-locus sequence typing (MLST) (Ehling-Schulz et al., 2005; Guinebretiere et al., 2008).

4. Conclusions

Our data demonstrate the occurrence of foodborne infectious bacteria in marinated meat products. Marinating could not eliminate pathogens in pork steaks during shelf life period. However, the risk for consumer's health should be negligible, when the steaks are properly stored and prepared before consumption.

Based on the results of our work, the microbiological criteria of Reg. (EC) No. 2073/2005 and the recommendations of the DGHM (2013) the indicative and warning values for raw pork are transferable for pre-packed marinated pork neck steaks until the end of shelf life.

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