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

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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. *Bacillus 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.

Keywords: marinated meat; consumer protection; microbiological criteria; virulence factor; toxigenicity; pathogen;

1. Introduction

Food borne infections are of major concern for public health and cause substantial costs (Hoffmann et al., 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 et al., 2010).

Amongst the category of convenience food, pre-packed marinated meat products such as steaks, spare ribs and filets are becoming increasingly popular (O´Donell, 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 (Anon., 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 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**

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-marinade, paprika-marinade and herbs-/garlic-marinade 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 ℃. Microbiological

analyses were carried out 3 days after purchase and at the end of minimum shelf life (+/- 1 day).

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-marinade 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 grams 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 Shiga toxin producing *E. 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 µL 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 µL 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.

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, Figure 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 to 2 log during minimum storage life to values up to 8 or 9 log cfu g^{-1} (Figure 1). Plate counts correlated with numbers of lactic acid bacteria, which were analyzed in the second sampling period (Table 4). Our results is consistent with report of Schirmer et al. (2009). As in fresh packed meat, lactic acid bacteria developed in marinated pork products to the dominant bacterial population after 3 - 4 weeks of cold storage with cell counts up to 8 log cfu g^{-1}. This resulted in lowered pH-values and increased aerobic mesophilic plate counts (Blixt & Borch, 2002; Holley et al., 2004). Aerobic plate counts in meat products higher than log 6 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 (Figure 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.

147 According to Holley et al. (2004), *Enterobacteriaceae* present on stored vacuum-packed pork
148 are useful indicators of efficacy of plant sanitation. Although *Enterobacteriaceae* occurred in
149 most steak samples (Table 4), the proportion of *Enterobacteriaceae* was in the most samples
150 of our study (266/300) below 5 % of the microbiological population. Generally, this could be
151 considered as a reflection of acceptable sanitation practice and hygiene (Holley et al., 2004).
152 However, in 11 % of the samples (34/300) *Enterobacteriaceae* counts of more than log 5 cfu
153 g^{-1} were found (Table 4). These steak samples originated from 12 producers (data not
154 shown). In one product with paprika marinade, 8 products with mustard marinade and 20
155 samples with herbs-/garlic marinade the proportion of *Enterobacteriaceae* was more than 5
156 % of the total bacterial population (Table 4). In Germany, marinated pork neck steaks are
157 produced usually from frozen pork necks. Therefore, producers should verify their incoming
158 quality control of the raw meat.
159 *Salmonella* were detected only during the second sampling period in 2 % of the steaks
160 (Table 4). These results are in accordance with recent studies, which reported a presence of
161 *Salmonella* spp. in pork within 2 and 6 % of samples (Rabsch et al 2013; Hauser et al. 2010;
162 Meyer et al., 2010; Prendergast et al., 2008). *Salmonella* positive steak samples were not
163 related to those with high *Enterobacteriaceae* counts (> 5 log cfu g^{-1}).
164 Serotyping of *Salmonella* isolates resulted in serotype Typhimurium (Table 5), which is one
165 of the most common serotypes in pork products (Friedrich et al., 2010). Our analyses
166 showed persistence of this serotype in 6 steaks with paprika marinades (Table 4). These
167 products originated from the same producer and isolates were classified as monophasic
168 variants of the serotype O4,12 phage type DT 193 and biphasic variants of the serotype O
169 4,5,12 phage type DT 120 (Table 5). Therefore, *Salmonella* could survive in these products
170 over a period of 18 days at 4 °C.

171 One steak contained STEC, and further differentiation resulted in serotype ONT:H19 and
172 virulence factor Stx2e (Table 5). STEC producing Stx2e are associated with edema disease
173 in pigs. *stx2e*-positive STEC-isolates were frequently detected in pork with percentage of
174 more than 50 (Beutin et al., 2007), but not in patients with severe STEC-caused diseases
175 (Beutin et al., 2008). Only mild diarrhea was described after STEC-infection with Shigatoxin
176 2e-producing STEC (Friedrich et al., 2002). Therefore, marinated pork steaks might not to be
177 a common source for highly pathogenic EHEC strains.

178 *L. monocytogenes* were detected in 5 meat samples (1.7 %) with herbs-/garlic-marinade with
179 counts ranging within 1 and 2 log cfu g^{-1}. Earlier studies documented an occurrence of
180 *L. monocytogenes* in pork within 1 and 36 % (Yeh et al., 2005; Netschajew et al., 2009). In
181 our analyses *L. monocytogenes* were mainly isolated at the 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 *St. aureus* with counts up to 2.53 log 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 food borne intoxication with *Staphylococcus* enterotoxins, an amount of 0.1 to 1 µg per kg body weight is necessary. A production of toxic dose demands *St. aureus* counts of more than 4 log 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 et al., 2001; Normanno et al., 2007; Nitzsche 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) analysed 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 et al., 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).

Bacillus cereus were isolated from 64 samples (21 %) of marinated pork steaks. Highest contamination rates were found in samples with herbs-/garlic-marinade, (< 3 log 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 log 5 - 6 cfu g^{-1} (Ehling-Schulz et al., 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 (Schulz, 2004; Altayar & Sutherland, 2006; Kreuzberger et al., 2008; Rau et al., 2009). 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 grow and produce cereulide at temperatures below 10 ℃ (Carlin et al., 2006).

About 98 % of analyzed *B. cereus* isolates possessed genes coding for diarrhoea 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 (Guinebretriere et al., 2002; Moravek et al., 2006; Molva et al., 2009). 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 (Figure 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 et al., 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 diarrhoea 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 et al., 2003; Psomas et al., 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).

Conclusions

Our data demonstrate the occurrence of food borne 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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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-marinade, P = Products with paprika-marinade, H = Products with herbs-/garlic-marinade). Marinades were purchased during August 2008 and August 2009. (M* = mustard-/beer-marinade, P* = paprika-marinade, H* = herbs-/garlic-marinade).

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**				
	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* (=DIN EN ISO 4833:2003) Surface-plating	Standard I Agar (Merck, 107881)	48 h, 30 ℃
Enterobacteriaceae	DIN 10164-1:1986-08** Surface plating	Desoxycholat-Hydrogen-Sulfide-Lactose- (DHL-)Agar; (Merck, Darmstadt, Germany; Art. 114350,)	48 h, 30 ℃
Sulfite-reducing clostridia	Angelotti et al., (1962); Eisgruber & Reuter, (1995)	Sulfite-Polymyxin-Sulfadiazine- (SPS-) Agar (Merck, 110235)	48 h, 37 ℃
Bacillus cereus	L 00.00-25*	Mannitol Egg Yolk Polymyxin (MYP-) Agar	24 h, 37 ℃
Listeria spp. and *Listeria monocytogenes*	DIN EN ISO 11290-2 Detection and enumeration	Oxoid-Chromogen-Listeria-Agar (OCLA) [Oxoid; Art.Nr. PO5165A]	48 h, 37 ℃
Staphylococcus aureus	L.00.00-55*	Baird-Parker-Agar; [OXOID; CM1127]	48 h, 37 ℃;
Salmonella spp.	DIN EN ISO 6579:2003**	Buffered water (OXOID; CM0509)	16 to 20 h, 37 ℃
		First enrichment	
		Rappaport-Vassiliadis Soja Peptone broth; (Oxoid; CM 0866)	24 h, 42 ℃
		Second selective enrichment	
		Selenite-Cystine-enrichment-broth (Merck, 107709)	24 h, 37 ℃
		Second selective enrichment	
		Desoxycholat-Hydrogen-sulfid-Lactose-Agar (DHL); [Merck; 114350]	24 h, 37 ℃
		Xylose-Lysine-Tergitol-4-(XLT4)Agar; [Oxoid; CM 1061, SR 0237]	24 h, 37 ℃
Shigatoxin-producing *Escherichia coli*	According to method L 00.00-92* DIN 10118:2004	Modified Tryptic Soy Broth (m-TSB) (Merck, 105459, 104054, 105104)	16 h, 37 ℃, 180 rpm
		Sorbitol MacConkey (SMAC) Agar (Sifin, TN 1220)	24 h, 37 ℃

* Amtliche Sammlung von Untersuchungsverfahren nach § 64 LFGB = German official collection of methods according to § 64 of German Food and Feed legislation (LFGB)
** DIN = Deutsches Institut für Normung e.V.

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

Pathogen	Method		Reference
Salmonella spp.	*invA*-gene-detection		(Rahn et al., 1992)
	Serotyping/ phage typing		(Rabsch, 2007)
L. monocytogenes	rhamnose-/xylose fermentation hemolysis		(Groves et al., 1977)
	serotyping (multiplex-PCR)		(Doumith et al., 2004)
B. cereus	*gyrB*-gene-detection		(Yamada et al., 1999)
	Detection of toxigenecity		
	Emetic toxin cereulid	*cesB*	(Ehling-Schulz et al., 2004a and 2006)
	Diarrhetic toxin	*hblA*	(Mäntynen et al., 1998)
		hblC	(Moravek et al., 2004)
		hblD	(Ryan et al., 1997)
		nheA	(Moravek et al., 2004)
		nheBC	(Dietrich et al., 2005)
STEC	*stx1*		(Rüssmann et al., 1995)
	stx2		(Cebula et al., 1995)
	eae		(Schmidt et al., 1993)
	hlyA		(Schmidt et al., 1995)
	Serotyping and subtypes of *stx2*		(Beutin et al., 2007)
St. aureus	Latexagglutination-assay		(Zschöck et al., 2005)
	Assay for enterotoxins (ELISA)		(Park et al., 1996)
	MRSA-screening (Cefoxitin-agar diffusion assay)		(Boubaker et al., 2004)
	(confirmation of MRSA by PCR)		(Mehrotra et al., 2000)

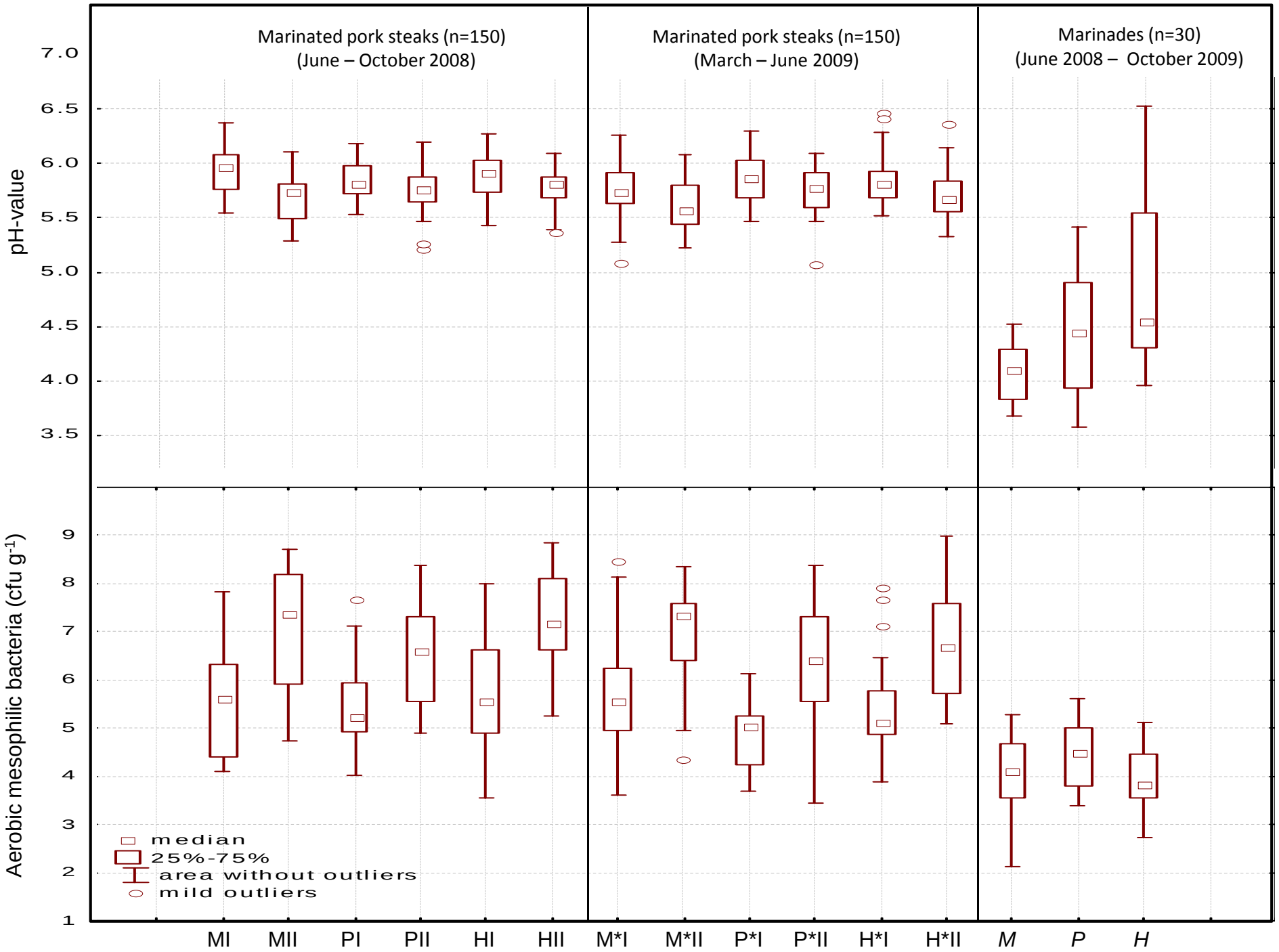
Table 4: Microbiological parameters of vacuum packed marinated pork steaks from self service areas of German retail (n=300). Cat.: Steak samples were categorized into three groups. M = products with mustard-/beer-marinade (n=100), P = products with paprika-marinade (n=100), H = products with herbs/garlic-marinade (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. ↑: Increase of bacterial counts ≥ 1 log cfu g^{-1} and percentage >10 %; →: no change of cell counts (< 1 log cfu g^{-1} and <10 %,); ↓: Reduction of cell counts ≥ 1 log cfu g^{-1} and percentage of positive samples >10 %;. * Results were rounded to one decimal place. ** qualitative detection after sample enrichment; n.a. = not applicable; - = not detected.

Cat.	Target	Microbiologcial 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 cfu g^{-1}	positive samples within x log cfu g^{-1} <1	1	2	3	4	5	6	7	8	Positive (n/N)	Range of cell counts log cfu g^{-1}	positive samples within x log cfu g^{-1} <1	1	2	3	4	5	6	7	8	
M	LAB*	25/25	1,6 – 6,6	-	3	2	6	4	7	3	-	-	25/25	3,0 – 7,1	-	-	-	3	4	3	13	2	-	↑
	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	-	→
	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	-	-	-	-	-	-	↓
	L. monocytogenes	0/50	-	-	-	-	-	-	-	-	-	-	0/50	-	-	-	-	-	-	-	-	-	-	
	B. cereus	5/50	<1 – 2,0	45	4	1	-	-	-	-	-	-	8/50	<1 – 2,0	42	6	2	-	-	-	-	-	-	→
	St. aureus	1/50	<1 – 1,9	49	1	-	-	-	-	-	-	-	1/50	<1 – 1,6	49	1	-	-	-	-	-	-	-	→
P	LAB*	25/25	1 – 5,4	-	3	7	8	4	3	-	-	-	25/25	2,5 – 9,0*	-	-	3	2	3	7	6	3	1	↑
	Enterobacteriaceae	46/50	<1 – 4,3	4	12	23	9	2	-	-	-	-	45/50	<1 – 7,1	5	13	16	11	3	-	1	1	-	→
	Salmonella	2/50**	n.a.	-	-	-	-	-	-	-	-	-	3/50**	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	-	-	-	-	-	-	↑
	L. monocytogenes	0/50	-	-	-	-	-	-	-	-	-	-	0/50	-	-	-	-	-	-	-	-	-	-	
	B. cereus	9/50	<1 – 2,0	41	8	1	-	-	-	-	-	-	14/50	<1 – 2,7	36	12	2	-	-	-	-	-	-	↑
	St. aureus	7/50	<1 – 1,8	43	7	-	-	-	-	-	-	-	5/50	<1 – 2,4	45	3	2	-	-	-	-	-	-	→
H	LAB*	25/25	1,8 – 8,0*	-	1	5	9	5	3	1	1	-	25/25	3,9 – 8,8	-	-	-	1	3	7	11	2	1	↑
	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	↑
	Salmonella	1/50**	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	-	-	-	-	-	-	↓
	L. monocytogenes	1/50	<1 – 1,3	49	1	-	-	-	-	-	-	-	4/50	<1 – 2,0*	46	4	-	-	-	-	-	-	-	
	B. cereus	13/50	<1 – 2,0	37	11	2	-	-	-	-	-	-	15/50	<1 – 2,2	35	12	3	-	-	-	-	-	-	↑
	St. aureus	7/50	<1 – 2,5	43	3	4	-	-	-	-	-	-	4/50	<1 – 2,2	46	3	1	-	-	-	-	-	-	→

Table 5: Numbers 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		
	n	(%)	n	n	(%)	
Salmonella						
Marinated pork	6	(2)	13	11	(85)	*S*. Typhimurium DT 193; O:4,12:i:-, monophasic
				2	(15)	*S*. Typhimurium DT 120; O:4,5,12:i:1,2, biphasic
Marinades	0					
STEC						
Marinated pork	1	(0.3)	4	4	(100)	Serotype ONT:H19, *stx2e*
Marinades	0					
L. monocytogenes						
Marinated pork	5	(2)	35	34	(97)	Serotype 1/2a
				1	(3)	Serotype 1/2c
Marinades	0					
St. aureus						
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					
B. cereus						
Marinated pork	64	(21)	127	3	(2)	Without toxin genes
				96	(76)	all parts of *hbl*- and *nhe*-operon
				121	(95.3)	*nhe*A
				123	(96.8)	*nhe*BC
				101	(79.5)	*hbl*A
				100	(78.7)	*hbl*C
				102	(80.3)	*hbl*D
				0	(0)	*cesB*, cereulide formation
Marinades	10	(33)	31	0	(0)	Without toxin genes
				96	(67.7)	all parts of *hbl*- and *nhe*-operon
				31	(100)	*nhe*A
				31	(100)	*nhe*BC
				22	(71)	*hbl*A
				24	(77.4)	*hbl*C
				25	(80.6)	*hbl*D
				0	(0)	*cesB*, cereulide formation

Figure 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.



Highlights

We analyzed 300 marinated pork steaks at beginning and end of shelf life.
Additionally 30 marinades, used for production of steaks were analyzed.
Food pathogens were detected in the steaks in a range from 0 to 21%.
Marinades with low pH-values showed higher bactericidal effects.