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Quality aspects of a spreadable raw sausage product manufactured from wild boar meat

Qualitätseigenschaften einer Modell-Mettwurst aus Wildschweinfleisch

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Summary

A model soft raw sausage was produced from 60 % shoulder muscle of wild boar and 40 % backfat from domestic pig, with addition of nitrite curing salt (27 g/kg), spices and commercial starter cultures. Batter was vacuum-packaged in 90 µm PA/PE film. Chemical composition was fulfilling the requirements of the austrian food codex. The microflora and concentrations of biogenic amines were tested at the day of manufacture and after two and seven days storage at 18 °C. Microflora developed as expected. The selection of the starter culture was found critical for biogenic amine formation and sensory preference. The batch with a starter culture containing *Lactobacillus curvatus, Staphylococcus xylosus, Debaryomyces hansenii* was characterized by significantly lowest histamine and putrescine concentrations (averages of 10 and 30 mg/kg at day seven, respectively) and also significantly preferred by a trained tester panel. Concentrations of cadaverine and of the polyamines spermidine and spermine remained virtually constant, whereas tyramine concentrations increased to >50 mg/kg fresh matter at day 7. Only a slight increase was observed for artificially inoculated *Listeria monocytogenes* (NCTC 11994). Initial inocula were 3.53.9 log cfu/g and concentrations at day seven were 3.94.1 log cfu/g. With respect to the tested parameters, safety and quality issues of this model fermented sausage from wild boar meat were comparable to those expected for a spreadable raw sausage from pork from slaughter pigs.

Keywords: Biogenic amines, *Listeria monocytogenes,* wild boar meat, „Mettwurst“

Zusammenfassung

Aus 60 % Schulterfleisch vom Wildschwein und 40 % Bauchfett vom Hausschwein wurde unter Zusatz von Nitritpökelsalz (27 g/kg), Gewürzen und kommerziellen Starterkulturen eine streichfähige Modellrohwurst hergestellt, wobei die Wurstmasse in eine 90 µm dicke PE/PA Folie vakuumverpackt wurde. Die chemische Zusammensetzung entsprach den Anforderungen des Österreichischen Lebensmittelbuches. Am Tag der Herstellung und nach zwei und sieben Tagen Lagerung bei 18 °C wurde die Mikroflora und die Konzentration der biogenen Amine bestimmt. Dabei verhielt sich die Entwicklung der Mikroflora wie man es für streichfähige Rohwürste erwarten würde. Die Auswahl der Starterkulturen hatte eien Einfluss auf die Bildung biogener Amine und sensorische Eigenschaften. Die Charge mit einer Starterkultur aus *Lactobacillus curvatus, Staphylococcus xylosus* und *Debaryomyces hansenii* zeigte signifikant geringste Histamin- und Putrescinkonzentrationen (durchschnittlich 10 bzw. 30 mg/kg am Tag sieben) und wurde zusätzlich von einem geschulten Kollektiv als sensorisch signifikant besser eingestuft. Die Cadaverinkonzentration und die der Polyamine Spermidin und Spermin blieben nahezu konstant, während die Tyraminkonzentration auf >50 mg/kg (Frischmasse) am Tag sieben anstieg. Die Konzentrationen von künstlich zugesetzten *Listeria monocytogenes* (NCTC 11994) stiegen nur geringfügig an, von 3.5 bis 3.9 log cfu/g am Tag der Herstellung auf 3.9-4.1 log cfu/g am siebenten Tag. Hinsichtlich der geprüften Parameter, Sicherheits- und Qualitätseigenschaften war die Modellrohwurst aus Wildschweinfleisch mit jenen, die man bei streichfähigen Rohwürsten vom Hausschwein erwarten würde, vergleichbar.

Schlüsselwörter: biogene Amine, *Listeria monocytogenes,* Wildschweinfleisch, Mettwurst

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Introduction

The production of game meat receives increasing attention. Favourable sensory and nutritional properties make meat from game and venison attractive for the modern consumer (Hoffman and Wiklund, 2006). Not all of the meat can be marketed as "fresh meat". In fact, the percentage of high quality meat cuts suitable for frying and grilling will be about 55 to 65 %, depending on species, age and shot lesions. The remaining muscle cuts can be processed into comminuted meats, as goulash, or minced meat. From data presented by Kujawski (2007) it can be estimated that for wild boar about 33 % of the muscle tissue have to be processed to comminuted meats (goulash etc.) and about 11 % to minced meat. Similarly, a survey recently conducted in a major Lower Austrian game-handling establishment showed that wild boars with an average carcass weight of 44 kg (skin-on, n=3600) would yield 23 kg muscle tissue, of which 3 kg (about 13 %) are comminuted meats which are subsequently processed into sausages or paté (Winkelmayer and Paulsen, 2008).

This means that the manufacture of meat products from game meat can be a significant branch of game meat marketing. Among those meat products spreadable raw sausages – despite the ease in manufacturing – have received considerably low attention.

While there are numerous studies on how quality factors of meat products from pork can be controlled or optimized, comparably few studies explore meat products from wild boar (e.g. Soriano et al., 2006; Bauer et al., 2007; Zochowska-Kujawska et al., 2007; Paulsen et al., submitted).

Hence, we studied food quality aspects of a simple model spreadable raw sausage produced from wild boar meat, with special attention to selection of starter cultures.

Material and methods

3 kg of lean wild boar meat (shoulder muscles) and 2 kg backfat from domestic pigs were cut into cubes and cooled to –1 °C. Meat and fat were then ground to 5 mm, mixed well with nitrite curing salt (27 g/kg NaCl with 0.6 % $NaNO_2$; Aula, Austria), spices (6.5 g/kg Campania 379, "Frische Rohwurst", Wiberg, Germany). Five different batches were prepared by addition of different starter cultures (see Tab. 1.).

The sausage batter was divided into 100 g portions, which were formed to cylinders of 2 cm diameter, vacuum packaged (PA/PE bag 13 x 21 cm, thickness 90 µm; Felzmann, Austria; residual pressure 0.1 bar). Sausages were stored at 18 °C for up to seven days. At days 0, two and seven, three to five sausages were taken. pH was measured and samples were subject to microbiological examination and determination of biogenic amines and polyamines (Tab. 2.).

Proximate chemical composition was determined from meat batter at day 0. Moisture, protein, fat, ash and collagen were determined according to German official methods (§64 LFBG methods L 06.00-3, 2004; L 06.00-7, 2007; L 06.00-6, 1980; L 06.00-8, 1980, respectively). Results are reported for fresh matter. Unless stated otherwise, reagents were obtained from Merck, Germany.

Experiments were done in triplicate (series S1 to S3). In S3, sausage batches were additionally subject to sensory testing by 22 semitrained panelists [ranking method L00.90-4 (1999) of german official methods collection acc. to §64 LFBG].

Biogenic amines concentrations and pH were tested by ANOVA (Statgraphics 3.0, Statistical Graphics Corp., USA) considering series, batch (starter culture) and sampling day. Fisher's LSD was used to discriminate among means, significance was established at $p < 0.05$.

Additionally, three series S4-S6 were prepared as S1-S3, but batches were challenged with pathogenic bacteria. In brief, S4 was inoculated during the manufacture process with *Listeria monocytogenes* NCTC 11994 (about 4 log cfu/g). In S5 and S6 *Listeria* was combined with *Escherichia coli* ATCC 25922 (4 log cfu/g) and *Staphylococcus aureus* ATCC 25923 (5 log cfu/g), respectively. Inocula were prepared by growing the respective bacterial strains in BHI overnight at 30 °C and adjusting cell concentration via comparing the optical density against McFarland standards. Listeria monocytogenes counts were assessed on ALOA agar (Tab. 2); counts of *E. coli* and *Staph. aureus* were also determined, but data are not presented in this contribution.

TABLE 2: *Examination scheme for spreadable raw model sausages (days 0,2, and 7).*

Parameter	Method
Total aerobic count	Colony counting on plate count agar (ISO 4833:2003)
Lactic acid bacteria	Colony counting on Lb agar (Oxoid), incubation 37 °C, 48h
Pseudomonas sp.	Colony counting on GSP agar (Merck), incubation 25 °C, 72h
Enterobacteriaceae	ISO 21528-2:2004 (VRBG agar; BioMerieux), but only oxidase reaction for colony confirmation
Listeria monocytogenes	Enrichment of 25 g sample in half and full Fraser broth (ISO 11290-1:1996, Amd.1:2004) ALAO Agar (Biolife), incubation 37 °C, 48 h; or direct enumeration on ALOA agar
Lecithinase + *Staph. aureus*	KRANEP agar (Merck), incubation 37°C, 48h
pH	pH electrode
Biogenic amines and polyamines	HPLC method (Paulsen et al., 1997)

TABLE 1: *Starter cultures used in a model spreadable raw sausage (inoculum size: 6.5–7 log cfu/g).*

Code	Composition	Commercial name and supplier
A	–	–
B	*Lact. curvatus, Staph. xylosus, Debaryomyces hansenii*	Biobak Classic (Wiberg)
C	*Lact. sake, Pediococcus pentosaceus, Staph. xylosus, Staph. carnosus*	Biobak K (Wiberg)
D	*Staph. xylosus, P. pentosaceus*	FloraCarn-FF (Chr.Hansen's)
E	*Staph. carnosus, P. pentosaceus*	FloraCarn-SP (Chr. Hansen's)

Results and discussion

Autochtonous microflora of the wild boar meat

Wild boar meat cuts used for sausage production were characterized by a total aerobic count of 5.7±0.7 log cfu/g (range 4.3 to 7.0) and Enterobacteriaceae and Pseudomonas counts of 3.9±0.7 log cfu/g (range 3.0 to 5.4) and 4.9±1.0 log cfu/g (range 4.0 to 7.2), respectively.

Similar results for total aerobic counts of commercial wild boar meat cuts in Germany have been reported in a recent study (Türck, 2009) with a range of 2.6–8.2 log cfu/g. In our study concentrations of Enterobacteriaceae and Pseudomonas were, however, higher than those obtained by other authors (Boers et al., 1994; Türck, 2009). E.g. Türck (2009) reported, for commercial meat cuts, ranges of 1.05.8 and 1.03.9 log cfu/g, respectively. This indicates that the raw material used for sausage manufacture in this study was not of optimum quality. Principally, it should be possible to produce meat cuts from wild boar in a microbiological quality comparable to that of slaughter pigs (Gill, 2007) as the microbiological contamination of carcasses of freshly shot wild boar is comparable to that of slaughter pigs (Atanassova et al., 2008). It is well known that deficiencies in the hygienic quality of raw material can be detrimental to the quality of the finished fermented sausage (Wirth et al., 1990); in particular, higher concentrations of biogenic amines can be expected (Maijala et al., 1995; Paulsen and Bauer, 1997; Bover-Cid et al., 2000a; Bauer and Paulsen, 2001; Suzzi and Gardini, 2003).

Sensory characteristics, pH and proximate chemical composition

Visual appearance and smell of the sausages were generally not objectionable. Testing of the series S3 by a sensory panel (n=22), with the testing criterion "flavour", resulted in the following ranking (rank sums in parentheses): B (34), D (62), E (72), C (80), A (83). The critical range was 43–67 (α=95 %). Thus, the batch with a rank sum below this range ("B") was significantly preferred to those above this range (i.e. "E", "C", and "A").

Initial pH of the sausage batters was 5.69±0.05. For batches produced without addition of starter cultures, pH values were 5.64±0.03 at day two and 5.11±0.12 at day seven. For batches produced with addition of starter cultures, corresponding results were somewhat lower, with 5.42±0.20 and 5.02±0.20 at day two and seven, respectively. These pH values were in range as could be expected for a sausage fermented at about 20 °C, i. e. 5.6–5.2 for days two to four and 5.2–4.8 for the following days (Wirth et al., 1990). Generally, pH decline tended to be fastest in batches "D". This was, however, not statistically significant ($p>0.05$). As could be expected, in batches produced with addition of starter cultures containing lactic acid bacteria (batches "B" to "E"), the pH decline was faster than in the spontaneously fermented batch "A", the main underlying mechanism being the formation of lactic acid from glucose by facultatively homofermentative lactic acid bacteria (Hammes and Knauf, 1992).

Chemical composition of the sausages was as follows: moisture: 52.6±1.5 g/100 g; crude protein: 15.3±0.5 g/100 g; crude fat: 28.3±2.0 g/100 g; collagen-free protein: 14.0±1.0 g/100 g. Ratio of moisture:protein and fat:protein was 3.4 and 1.9, respectively. Percentage of collagen related to crude protein was 10.2. These results did not exeed the maximum tolerable limits (3.6; 3.7 and 20, respectively) for "Mettwurst" laid down in the Austrian Codex Alimentarius (2005).

Polyamines and biogenic amines

No statistically significant changes were observed between series and storage times for concentrations of the polyamines spermidine (3.0±0.8 mg/kg) and spermine (13.0±2.2 mg/kg), see Table 3. This is in accordance with a number of studies on spreadable as well as dry fermented sausages, where the concentrations related to dry mass of spermidine and spermine undergo no or only minor changes (Wortberg and Woller, 1982; Rogowski and Döhla, 1984; Treviño-Treviño, 1993; Paulsen, 1994; Bover-Cid et al., 1999, 2000b; Latorre-Moratalla et al., 2007). As we used a virtually moisture-impermeable packaging, it was not necessary to correct for changes in moisture during fermention/ripening process.

TABLE 3: *Concentrations of the polyamines spermine and spermidine (sampling days 0,2 and 7 pooled) in a model spreadable raw sausage from wild boar meat (mean ± std. dev., n = 36).*

Series	Spermidine	Spermine
S1	2.7 ± 0.8	12.9 ± 1.8
S2	4.0 ± 1.0	16.3 ± 3.0
S3	2.5 ± 0.7	12.4 ± 1.8

Average concentrations of histamine, tyramine, cadaverine and putrescine at day 0 were <5 mg/kg. At day two, tyramine and histamine concentrations in batch "B" were significantly higher than at day 0. Expectedly (Suzzi and Gardini, 2003), cadaverine concentrations remained at a low level, whereas average tyramine concentrations increased to >50 mg/kg and were significantly higher in batch "C" (>150 mg/kg) than in the other batches (Fig. 1.).

An increase of putrescine was observed in all batches, with significantly lowest concentrations in batch "B" (<40 mg/kg), and highest concentrations in batches "A" (i. e. without starter culture) and "C" in the range of 100 mg/kg (Fig. 2.). The same observation was made in a previous study with identical starter cultures in a "Feine Mettwurst" type sausage from pork (Paulsen, 1994). It can be hypothesized that the selection of the starter culture is critical. On a first view, the main difference between the starter culture "B" and "C", "D", "E" is the inclusion of a yeast (which has unfavourable growth conditions under oxygen-reduced atmospheres, as is the case in the vacuum-package used in this study), but intra-species variability in lactobacilli and staphylococci could account for observed differences (Hammes and Knauf, 1994).

Histamine concentrations at day seven were consistently and significantly lowest in batch "B" (average about 10 mg/kg) and about two times higher in other batches produced with starter cultures and significantly highest in batches without starter cultures ("A"), see Figure 3. A number of studies report that the use of starter cultures in production of fermented sausages is usually associated with lower concentrations of biogenic amines (Buncic et al., 1993; Maijala et al., 1995; Hernandez-Jover et al., 1997; Bover-Cid et al., 2000a,b, 2001a,b,c; Latorre-Moratalla et al., 2007; Gardini et al., 2000; Komprda et al., 2001).

Development of the microflora

Autochtonous pathogenic bacteria

For the three series S1–S3, *Listeria monocytogenes* could not be detected in a 25 g aliquot of the sausage batter at the day of manufacture. However, wild boar meat should not be neglected as a source of *Listeria monocytogenes,* e. g. Atanassova et al. (2008) isolated this pathogen from 7 of 127 (5.5 %) fresh carcasses, and other authors report prevalences from about 5 to 25 % (Kanai et al., 1997; Jaksic et al., 2003; Wacheck et al., in press). Counts of lecithi-

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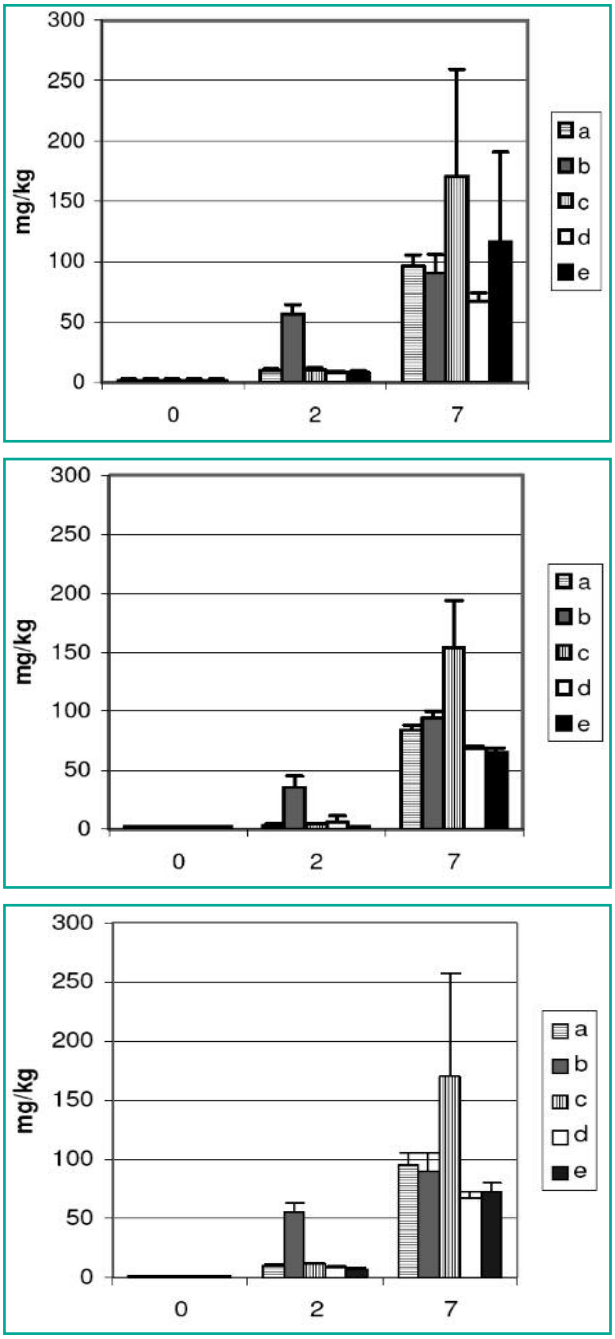

FIGURE 1: *Concentration of tyramine (mean ± std. dev.) in model spreadable raw sausages made from wild boar meat (a: without starter culture, b to e: commercial starter cultures); top to bottom: series S1 to S3.*

nasepositive *Staph. aureus* were consistently below 3 log cfu/g for all series, at days 0, two and seven.

Other Gram negative bacteria

Average Enterobacteriaceae counts were 3.6–3.8, 5.1–5.5 and 3.0–3.3 log cfu/g for series S1, S2 and S3 at day 0 and decreased to 2.3–2.8, 2.0–3.5; <2 log cfu/g at day seven in batches manufactured without addition of starter cultures. Similarly, *Pseudomonas* concentrations ranged from 3.0, 3.9–4.5 and 4.0 at day 0 to 2.4, 4.1 and 2.0–2.3 log cfu/g at day seven. A reduction in numbers of Gram negative bacteria concurrent with pH decline can be expected and is also associated with a reduction in biogenic amine formation, and in particular, lower histamine accumulation (Hernandez-Jover et al., 1997; Bover-Cid et al., 1999, 2000a, 2001a).

Other Gram positive bacteria: Lactic acid bacteria and lecithinasenegative staphylococci

In batches manufactured without addition of starter cultures, average lactic acid bacteria counts were 3.7–3.8 log cfu/g for series at day 0 and increased to finally (i. e. day seven) 8.3–8.7; 7.0–7.3 and 8.7–8.8. Concentrations of lecithinasenegative Staphylococci were 3.3–3.8 log cfu/g at day 0 and, at day seven, 3.1–3.3, 3.7–4.0 and 4.6 log cfu/g in series S1, S2 and S3, respectively. In the batches manufactured with starter cultures "B" to "E" concentrations of lactic acid bacteria increased to >7.0 log cfu/g. Only minor changes were observed for average counts of lecithinasenegative staphylococci from day 0 to day seven with a maximum increse of about 1 log cfu/g in S3.

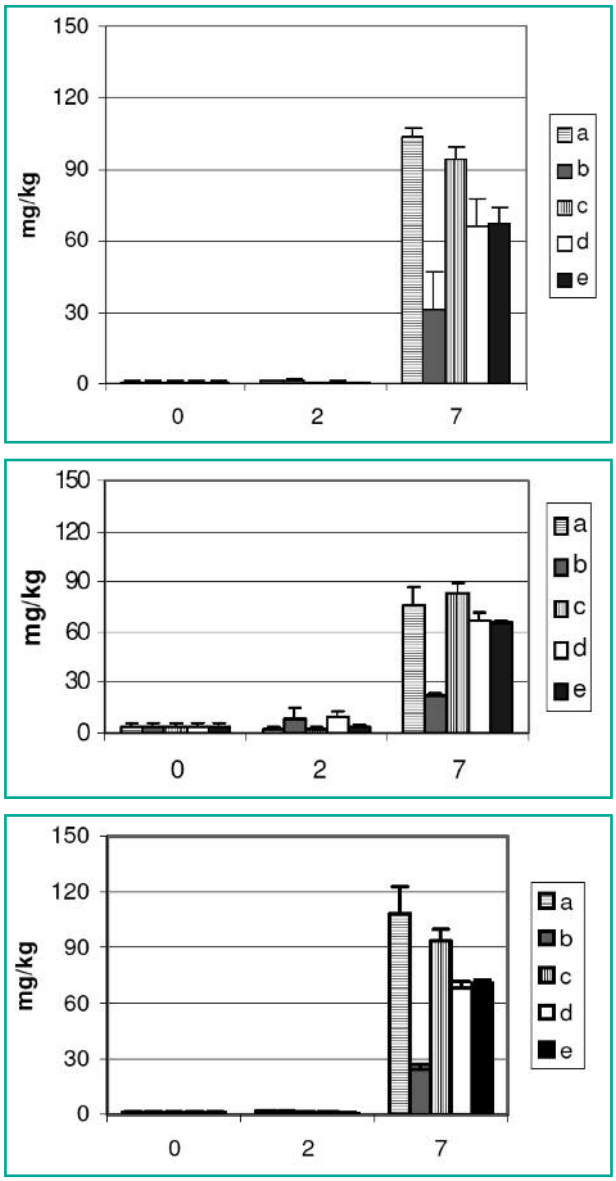

FIGURE 2: *Concentration of putrescine (mean ± std. dev.) in model spreadable raw sausages made from wild boar meat (a: without starter culture, b to e: commercial starter cultures); top to bottom: series S1 to S3.*

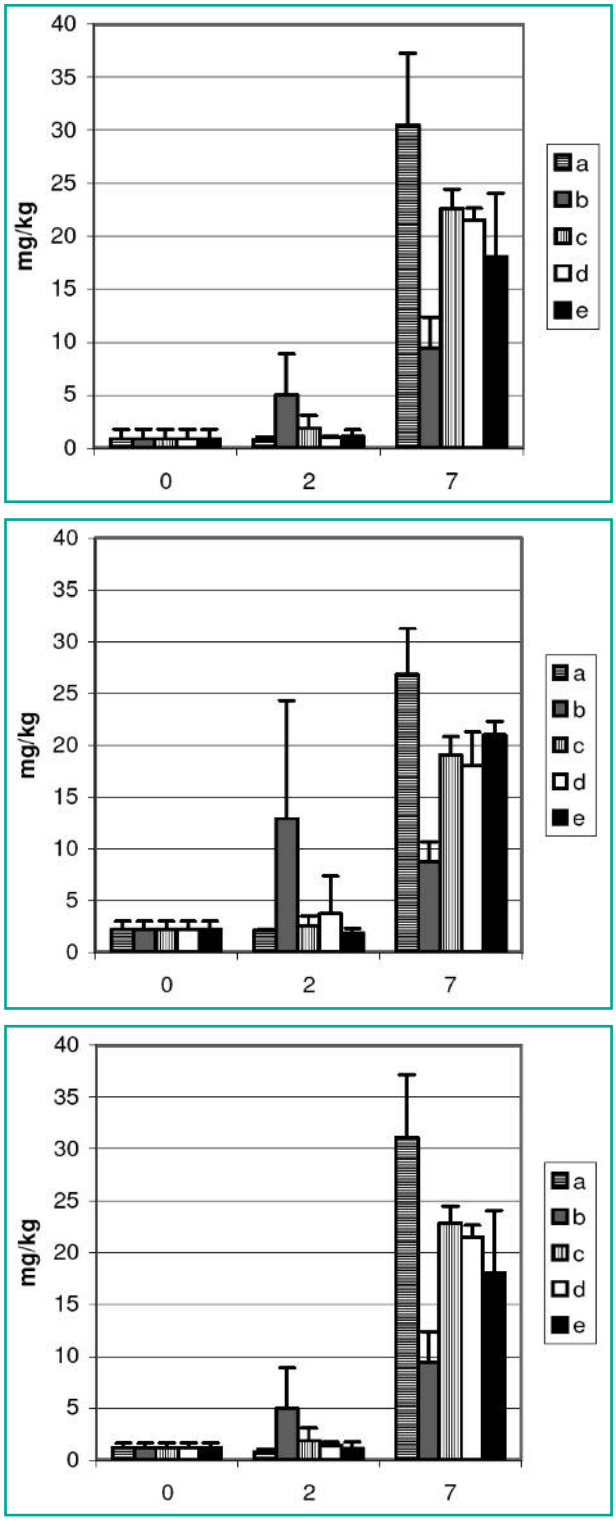

FIGURE 3: *Concentration of histamine (mean ± std. dev.) in model spreadable raw sausages made from wild boar meat (a: without starter culture, b to e: commercial starter cultures); top to bottom: series S1 to S3.*

In summary, the microflora was in the range as recommended by various expert groups (see Eisgruber and Bülte, 2006).

Challenge tests with Listeria monocytogenes

Average *Listeria monocytogenes* inocula were 3.5, 3.9 and 3.8 log cfu in series S4, S5 and S6, respectively. The maximum concentrations per single sample (both day two and day seven considered) were not significantly higher with 3.9, 4.1 and 3.9 log cfu/g. This observation is in accordance with numerous studies indicating that spreadable raw sausages do not constitute a favourable environment for growth and survival of *Listeria monocytogenes* in spreadable raw sausage (Dourous et al., 2009). Numbers will – under normal processing conditions – not significantly increase; they can be preserved (Albert, 2003; Albert et al., 2005; Gareis, 2004; Dourous et al., 2009) or decrease by more than 1 log unit (Buncic et al., 1991; Trüssel and Jemmi 1989); depending on processing technology (e. g. Encinas et al., 1999; Josupeit, 2006).

Relation of sensory testing to microbial and chemical characteristics

One of the experimental series (S3) was subject to a testing by trained panelists. Batch "B" was preferred to the other batches, with respect to the batches "C" and "E" and that without addition starter cultures ("A"). This difference was statistically significant. From a viewpoint of routine microbiology there was no difference between starter cultures as regards reduction of Gram-negative bacteria during the storage period.

Batch "B" was also associated with low contents of histamine and putrescine. There are reports that foods with high concentrations of amines, e. g. histamine, may give some sort of burning-bitter sensation in the mouth (Arnold and Brown 1978; Taylor, 1986; Beutling, 1996), but the concentrations in the present study seem to be too low for this effect. The sensory profile of fermented meats is a rather complex construct and is influenced by both protein and lipid degradation. With respect to fermented sausages from wild boar some preliminary studies have been presented (Soriano et al., 2006; Bauer et al., 2007) but further work would be necessary to elucidate this complex issue.

Food safety aspects

Biogenic amines, in particular, the vaosactive compounds histamine and tyramine have been implicated in food intoxication incidents (see Beutling, 1996; Lehane and Olley, 2000). In the present study average concentrations of ca. 30 mg/kg and 70–170 mg/kg were observed for histamine and tyramine, respectively. Considering standard serving sizes, histamine levels in all batches examined in the present study should be of no toxicological relevance for the average consumer (Rauscher-Gabernig et al., 2009). Likewise, no tyramine-related adverse effects should be expected for the average consumer with a no-effect-level of ca. 200 mg/person/day (McCabe-Sellers et al., 2006), whereas fermented sausages are generally a risk product for susceptible individuals (with a proposed no-effect-level of 5–6 mg/person/day; Boullata and Armenti, 2006; McCabe-Sellers et al., 2006), as demonstrated e. g. by Bover-Cid et al. (2008).

Listeria monocytogenes are not infrequently isolated from spreadable fermented sausage, e.g. Gareis (2004) reported a prevalence of ca. 5%, but mostly at levels < 2 log cfu/g and under normal conditions a multiplication of this pathogen for 1 or more log units is unlikely. Wild boar meat can clearly be source of this pathogen (Kanai et al., 1997; Jaksic et al., 2003; Atanassova et al., 2008; Wacheck et al., in press), but in sum there is no indication that this would pose a higher risk to the consumer than meat from domestic pigs.

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