8   SUMMARY

Composition of the psychrotrophic microflora of fresh minced meat with microecological and hygienic evaluation of their main components

Due to the large surface, minced meat is exposed to particular microbial load. Spoilage bacteria and pathogenic bacteria can be transmitted by contact with equipment and personnel. "Psychrotrophs" are micro-organisms, which are able to grow at temperatures below 5°C. There is barely any quantitative information about the distribution of individual bacteria groups and species within the microflora of minced meat because of the high laboratory effort for the identification of the heterogeneous psychrotrophic microflora. Commercial clinical test systems for the identification of isolates are often not helpful to identify foodborne species.

The aim of this study was to analyse the entire psychrotrophic microflora of four different commercially available types of fresh minced meat, namely minced beef, minced beef with low content of fat and connective tissue (special minced beef), minced pork and mixed minced meat of beef and pork qualitatively as well as quantitatively on the basis of classical culture methods and suitable phenotypical reactions. In addition, a genotypic method was applied for the identification of the *Acinetobacter*-isolates. The intention was to assess the ecological situation of psychrotrophics as well as the role of minced meat as a vehicle for opportunistic pathogenic psychrotrophic bacteria.

35 charges of "industrially" processed minced meat, each consisting of 5 samples in parallel, were analysed according to Annex 2a of the German Meat Hygiene Regulation (FlHV) dating from June 29th, 2001, namely: 8 charges of minced beef, 10 charges of special minced beef, 8 charges of minced pork and 9 charges of mixed minced meat of beef and pork, in total 175 samples. The samples were taken by the manufacturer during the nightly production process and stored at 2±2°C until further preparation for bacteriological analysis 1 to 4 hours later. The preparation of the samples was performed according to L 06.00-16-standard (official collection of investigation methods according to §35 LMBG of food hygiene law). After incubation at 25±1°C for 48 hours morphologically differing colonies representing mixed

microflora were subcultured from the plate-count agar, registering the quantities as well. At least 8 up to 18 isolates were taken from each investigated charge. The growth of the isolates was proved on ISO-agar and in ISO-bouillon at 4±0.5°C for 7 to 10 days. Further identification was performed by classical methods using suitable test reactions according to current literature. For the *Acinetobacter* spp. a molecular-biological testing followed by analysing a partial highly variable 16S rDNA sequence based on a computer supported system (RIDOM: Ribosomal Differentiation of Medical Microorganisms) developed in the Institute of Hygiene and Microbiology of the University of Würzburg.

From 419 minced meat isolates 404 could be identified. 14 of 17 of the *Acinetobacter* isolates were identified to the species level. The psychrotrophic total cell counts (pcfu) ranged between 4.24 and 6.47 lg cfu/g with a median of 5.22 lg cfu/g. Comparing the types of minced meat no remarkable differences were found. The exceeding of the official limits of counts according to FlHV (more than 2 samples of one charge ranged between the index value of 6.18 lg cfu/g and the limit value of 6.7 lg cfu/g) occurred in one charge of mixed minced meat.

The Gram-positive bacteria dominated in minced beef with low content of fat and connective tissue. However, the differences between the Gram-positives and Gram-negatives in minced beef, minced pork and mixed minced meat were only small. The Gram-positives were essentially determined by the lactic acid bacteria and *Brochothrix thermosphacta* with parts of 21.0% to 64.0% of total psychrotrophic bacterial counts and with quantities, calculated over the charges (x_C), of 3.71 to 4.96 lg cfu/g.

In all types of minced meat the Gram-negatives were represented by *Pseudomonas* spp. with parts of the total psychrotrophic counts from 21.6% to 43.4% and with mean bacterial counts from 4.33 to 4.79 lg cfu/g (x_C). *Ps. fragi* and *Ps. fluorescens* were the predominant species. Psychrotrophic *Enterobacteriaceae* occurred irregularly with a ranging from 2.3% to 9.6% of total psychrotrophic count (pcfu). In minced meat of pork the data were 15.6% of pcfu with x_C of 2.99 lg cfu/g. *Serratia liquefaciens* occurred most often. *Aeromonas hydrophila* was present in one charge of minced beef with low content of fat and connective tissue with a mean of 2.88 lg cfu/g. *Acinetobacter*-strains ranged from 5.5% to 17.4% of pcfu with x_C from 2.81 to 3.32 lg cfu/g. The RIDOM-system yielded similarities for the *Acinetobacter*-

isolates from 94.01 to 99.88% to the reference strains available from the database. *A. lwoffii* occurred most often and reached 4.74 lg cfu/g in one sample. *A. baumanii* and genospecies 3, which represent the most important pathogens of nosocomial infections according to literature, could not be identified.

In conclusion the composition of the psychrotrophic microflora in minced meat is therefore manifold even in the course of "industrial" processing, i.e. processing in special production facilities under optimal technical conditions and strict observance of the chilling conditions. The total load with psychrotrophic micro-organisms of all four types of minced meat is approximately equal in quantity. The Gram-positive psychrotrophic minced meat microflora was substantially determined by the spoilage bacteria *Brochothrix thermosphacta* as well as by the group of lactic acid bacteria. The Gram-negative microflora was dominated by *Ps. fragi* and *Ps. fluorescens*. "Industrially" processed minced meats of these types do usually not harbour any health hazard for the consumer caused by psychrotrophic bacteria. Single charges with extended bacterial counts will be identified by provided microbial processing control and retrospectively eliminated. The potential of minced meat as vehicle of psychrotrophic pathogens is obviously out of question.

Rapid identification of the Gram-negative psychrotrophic microflora of minced meat is now as ever difficult. Classical methods are labour-intensive procedures and not reliable in all cases. Selective culture media as well as rapid identification systems seem to be an improvement. The identification of the *Acinetobacter* spp. from minced meat with the RIDOM-system does not make sense in every case because reference strains available up to date are predominantly of human clinical origin. Whether other molecular based methods are indicated for the identification of the psychrotrophic minced meat microflora cannot be answered at this point. The cost-benefit-effect cannot be verified on the basis of this study. Thus, for the time being, the assessment of the minced meat microflora will still have to be based on the testing with classical methods more or less comprehensive due to hygienic requirement.