Quantifying the effect of natural microflora on growth of salmonellae in fresh pork

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Abstract
This study was undertaken to provide predictive models to help prevent health problems in relation to salmonellae in fresh pork. The models consider different time and temperature of storage as well as microbial interaction with the natural microflora in the meat. At six temperatures between 4 and 20°C, duplicate growth curves of Salmonella Typhimurium DT104 and Salmonella Derby were established in both sterile (irradiated) minced pork as well as in minced pork with a natural microflora. The inoculated meat was incubated in aerobic atmosphere. Each growth curve was fitted to the exponential growth model to obtain estimates of maximum specific growth rate, μmax. The effect of storage temperature on μmax was modelled using a square-root type model. Faster growth of both Salmonella serovars was observed in sterile meat at 8 to 16°C as compared to meat with a natural microflora. Around 20°C, growth was, however, independent on type of meat. Besides testing of Salmonella, appearance and odour of all meat samples, having a natural microflora, was evaluated throughout the incubation periods. These observations determined for how long the meat was acceptable for consumption at different temperatures. Above 10°C, both Salmonella serovars started to grow before the meat was rejected for consumption. This indicated that safety, rather than spoilage, could be the shelf-life limiting factor of fresh pork at temperatures from 12 to 20°C. This interaction between Salmonella and the natural microflora should be included in risk assessment models regarding salmonellae in fresh pork.

Introduction
Mathematical models that predict growth of pathogens in food are usually developed in laboratory broth (McClure et al., 1994). However, these models do not always provide relevant predictions of pathogen growth in non-sterile and non-homogeneous food (Ross, 1996), because interaction or competition between the pathogen and the background flora may occur. Thus, there is a need to develop growth models for pathogens in food with the natural microflora present (Oscar, 2007). The strategy of the present work was to build scenarios for baseline temperature abuse as well as for the presence of a natural microflora in fresh pork in order to predict the growth potential of Salmonella in fresh minced pork.

Material and Methods
Meat. Packages of approx. 500 g modified atmosphere packaged minced lean pork meat were obtained from local retailers. The packages used for preparing sterile meat were mixed manually in a sterile bag for 10 min. Portions of 100 ± 3 g were vacuum packaged and frozen at -18°C. Sterilization of volumes of 5 times 100 g was done by irradiation at a dose of 5 kGy for 523 min, followed by freezing at -18°C. In the beginning of every new test round meat packages were defrosted in water at a temperature of approx. 40°C for 30 min. Packages of meat with a natural microflora were obtained from local retailers one day before each test round and divided into 100 g portions at the following day.

Preparation of Salmonella cultures. A cocktail of Salmonella Typhimurium DT104, carrying resistance to ampicillin, chloramphenicol, florfenicol, streptomycin and sulfa, and Salmonella Derby, carrying resistance to gentamycin, streptomycin, sulfa and spectinomycin, were used. Both Salmonella serovars had been isolated from pigs. One loop of a stock culture (–80°C) of each isolate was cultured separately in 10 ml LB-broth by overnight shaking at 37°C. Subsequently, the tubes were stored at 5°C for 3 days. Prior to inoculation, the cultures were diluted in phosphate buffered saline to obtain a concentration of approx. 106 CFU ml-1 and equal volumes of each was mixed and used as the inoculation cocktail.

Storage experiments. Each 100-g-meat sample was aseptically transferred to separate sterile stomacher bags and inoculated with 1 ml of the Salmonella cocktail to a final concentration 104 CFU g-1. To ensure even distribution of the cocktail in the whole meat sample, it was mixed by stomaching for 2 times 1 min. The inoculated samples were stored in normal atmosphere at selected temperatures between 4°C and 20°C. At appropriate time intervals, the whole meat sample was removed from the incubator and mixed in a stomacher for 2 times 1 min. Subsequently, 5 g meat was sampled aseptically and transferred to a sterile filter bag and the remaining meat sample was returned to the incubator within 5 min. The samples were diluted in 45 ml of buffered peptone water and mixed in a stomacher for 2 min. For bacterial enumeration, further 10-fold dilutions were performed using isotonic saline solution and appropriate dilutions were drop-plated (10 µl) onto XLD+ampicillin and XLD+gentamycin agars to enumerate S. Typhimurium DT104 and S. Derby, respectively. The plates were incubated at 37°C for 16 to 24 h.

Sensory evaluation. Throughout the incubation periods, appearance and odour of all meat samples, having a natural microflora, was evaluated by a four-member expert panel. Besides describing appearance and odour, the panel was also asked to evaluate whether they found the meat acceptable for consumption.

Data analysis. The growth model described by Baranyi and Roberts (1994) was fitted to the experimental growth curves and estimates of the maximum specific growth rate, µmax, were obtained for each growth curve using the freeware DMFit web edition. For the description of the effect of temperature on µmax, a square-root-type model (Eq. 1) was applied separately for meat with and without a natural microflora.

$$(\mu_{max})^{1/2} = b \bullet (T - T_{min}) \qquad \text{(Eq. 1)}$$

where b is a constant to be estimated, T is the temperature in °C and where the estimated value of Tmin is the intercept between the model and the temperature axis.

Results and discussion

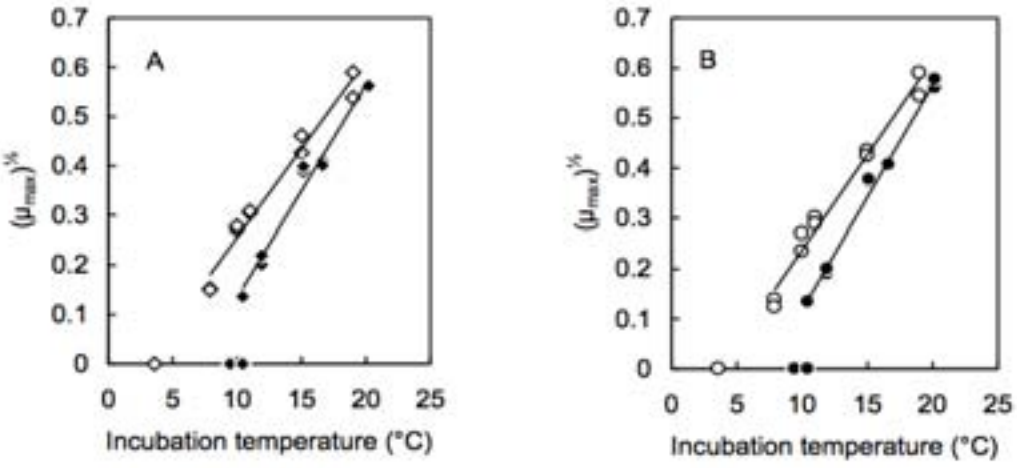

Figure 1. Influence of storage temperature (°C) on growth rates (μ_{max}, 1/h) of A) Salmonella Derby and B) Salmonella Typhimurium DT104 in sterile minced pork (open symbols) and minced pork with a natural microflora (closed symbols).

Faster growth of both S. Typhimurium DT104 and S. Derby was observed in the sterile meat at the temperatures below 20°C (Figure 1). The growth rate for each growth curve was estimated by fitting the Baranyi & Roberts growth model (1994) to observed data. The results confirmed the ability of competitive microflora to suppress growth of Salmonella during incubation of food samples in isolation broths as reported by Beckers et al. (1987). Oscar (2006) detected a similar effect when studying growth of S. Typhimurium DT104 in ground chicken breast with a competitive microflora. Figure 1 compares the effects of storage temperature and natural microflora in fresh minced pork on the growth rate of S. Typhimurium DT104 and S. Derby. The growth rates in minced pork could not be predicted satisfactory from literature models developed in chicken meat (Oscar, 2006; Oscar 2007), and it was necessary to develop new predictive models

specifically for Salmonella in fresh pork with a natural microflora in order to improve accuracy of predictions. Therefore, secondary predictive models, following a square-root-type equation, were developed for describing the effect of temperature on the maximum specific growth rates, μ_{max} found in this study (Table 1). The T_{min} estimates of the fitted models suggested that the minimum growth temperature for both Salmonella serovars was lower in sterile meat as compared to meat with a natural microflora. Predictions from the models also showed that below 15°C, the natural microflora of fresh pork meat slowed down the generation time of both Salmonella serovars with more than 2-fold. When evaluating the consumer risk from Salmonella in pork these observations suggest that different growth models have to be considered in situations where where the background flora has been inactivated by decontamination as opposed to traditionally slaughtered pork. The observed differences between growth rates of S. Derby and S. Typhimurium DT104 were of minor importance, in this respect.

Table 1. Predictive models describing the effect of temperature on growth rates (μ_{max}, 1/h) of Salmonella Derby and Salmonella Typhimurium DT104 in minced pork with and without a natural microflora present.

Salmonella	Pork type	Secondary model	R^2
S. Derby	Sterile (irradiated)	$(\mu_{max})^{1/2} = 0.0357 \cdot (T - 2.84)$	0.974
	With a natural microflora	$(\mu_{max})^{1/2} = 0.0429 \cdot (T - 6.86)$	0.978
S. Typhimurium DT104	Sterile (irradiated)	$(\mu_{max})^{1/2} = 0.0377 \cdot (T - 3.70)$	0.979
	With a natural microflora	$(\mu_{max})^{1/2} = 0.0446 \cdot (T - 7.34)$	0.990

Besides the quantification of Salmonella, appearance and odour of the meat samples, having a natural microflora, were assessed throughout the incubation periods. These observations determined for how long the meat was acceptable for consumption at different storage temperatures. As shown in Figure 2, the shelf-life of minced pork was found to be 4 days at 9.5°C and below 4 hours at 20°C. At all storage temperatures above 10°C, both S. Typhimurium DT104 as well as S. Derby started to grow before the meat was rejected for consumption (Figure 2). Growth was most pronounced around 15°C, where an increase of more than 1 log-unit was found, but also at 12°C and 20°C Salmonella was observed to initiate growth before the meat was spoiled (Figure 2). This indicated that safety, rather than spoilage, could be the shelf-life limiting factor of fresh pork at temperatures from 12 to 20°C.

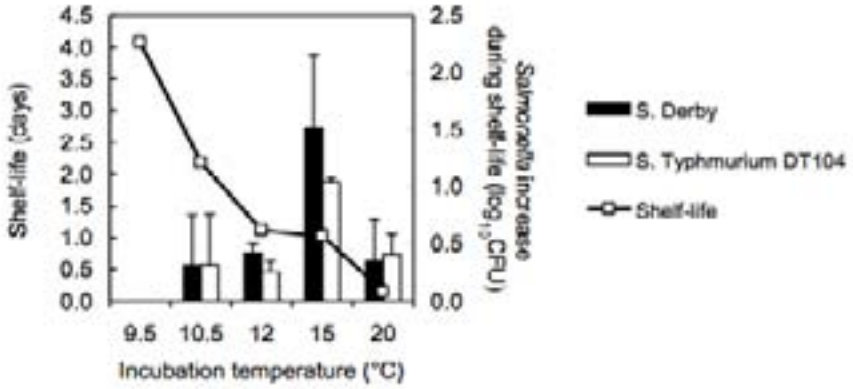

Figure 2. Average increase (log10CFU) of Salmonella Derby and Salmonella Typhimurium DT104 in minced pork with a natural microflora measured on the final day of shelf-life.

Conclusion

The present study found that the natural background flora in pork slowed down growth of salmonellae considerably at temperatures below 20°C. Risk assessment models have to consider this. It was also observed that temperature abuse, even in the chilled temperature area, may induce critical Salmonella growth before spoilage occur. This is important in relation to the setting of critical limits in the cold chain, i.e. for temperature shifts during handling.

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