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## Meat quality and salt levels affect the microbiota during meat fermentation (#158)

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### Introduction

Quality development during meat fermentation relies on the metabolic activity of lactic acid bacteria (LAB; Ravyts et al., 2012) and catalase-positive, coagulase-negative cocci (CNC; Stavropoulou et al., 2018). Yet, certain deviations from standard practice, such as the use of dark-firm-dry meat (DFD; Adzitey & Nurul, 2011) or altered processing factors may not only affect the technologically beneficial microbiota but also allow the growth of undesirable microorganisms, including spoilage-inducing *Enterobacterales*. To that avail, this study aimed at the assessment of the impact of meat quality (DFD *versus* normal meat) and salt levels (0-4 %) on the spontaneous fermentation of pork mince batter. The dynamics of distinct microbial groups, namely LAB, CNC, and *Enterobacterales* were monitored.

### Methods

An experimental set-up with two meat batters that differed in quality (*i.e.*, normal meat with a pH of 5.74 ± 0.01 *versus* borderline DFD meat with a pH of 6.02 ± 0.06) was performed. The batters consisted of fresh pork mince (2 kg, 14 % fat fraction) that was cured with sodium nitrate (150 mg/kg), ascorbic acid (500 mg/kg), and sodium chloride (0.0, 1.0, 2.0, 3.0, and 4.0 %). Then, the mixture was fermented spontaneously at 23°C for 14 d. Samples were analyzed after 0, 3, 7, and 14 d of incubation and the pH was measured. Presumed LAB were enumerated on de Man-Rogosa-Sharpe (MRS) agar, CNC on mannitol-salt-phenol-red agar (MSA), and *Enterobacterales* on RAPID'Entero agar (RAPID). Bacterial colonies were subjected to DNA extraction and identified by $(GTG)_5$-PCR genomic fingerprinting. Species level identity was then obtained by sequencing the 16S rRNA, *rpoB*, and/or *tuf* and *rpoA* genes. Finally, a detrended correspondence analysis (DCA) was performed for all samples, using R software (version 3.4.2; R Core Team, 2018).

### Results

Upon fermentation, a divergence in pH course and community composition was found between the various samples, indicating a difference between the two meat quality levels used (Figure 1). In all cases, LAB governed the fermentation process, with final counts at about 8.0-9.0 log (CFU/g). *Lactobacillus sakei* wasthe main contenderduring fermentation of normal meat, whereas *Lactobacillus curvatus* prevailed in the case of DFD meat (Figure 2). The presence of high salt levels benefited the MSA counts, leading to final counts of approximately 5.0-6.0 log (CFU/g), with *Staphylococcus equorum*, *Staphylococcus saprophyticus*, and *Staphylococcus xylosus* as the prevailing species. Notably, during fermentation of normal meat, the presumptive enterobacterial counts were impaired by the addition of salt. In the case of DFD meat fermentation, however, they remained stable till the end of the fermentation (Figure 1).

### Conclusion

The present case study followed the bacterial dynamics of meat fermentation processes with respect to different meat qualities and salt levels. The results showed that differences in meat quality influenced the microbial community dynamics, while salt variation had less impact. Further, the study pinpointed the potential persistence of enterobacterial communities, especially in DFD meat fermentation with low salt concentration.

### Literature

Adzitey, F., & Nurul, H. (2011). Pale soft exudative (PSE) and dark firm dry (DFD) meats: causes and measures to reduce these incidences - a mini review. Int. Food Res. J., 18, 11-20. R Core Team (2018). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Ravyts, F., De Vuyst, L., & Leroy, F. (2012). Bacterial diversity and functionalities in food fermentations. Eng. Life Sci., 12, 356-367. Stavropoulou, D. A., Filippou, P., De Smet, S., De Vuyst, L., & Leroy, F., (2018). Effect of temperature and pH on the community dynamics of coagulase-negative staphylococci during spontaneous meat fermentation in a model system. Food Microbiol., 76, 180–188.

Notes

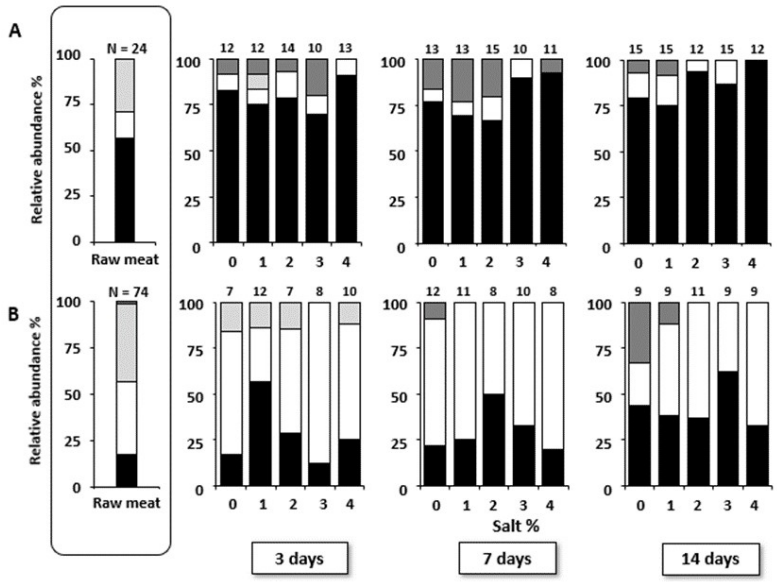


**Figure 2**
Temporal dynamics of species isolated from MRS agar related to the fermentation of (A) normal and (B) DFD meat, with Lactobacillus sakei (black), Lactobacillus curvatus (white), Carnobacterium spp. (light grey), and others (dark grey) reflecting the bacterial species diversity (displayed as relative abundances).

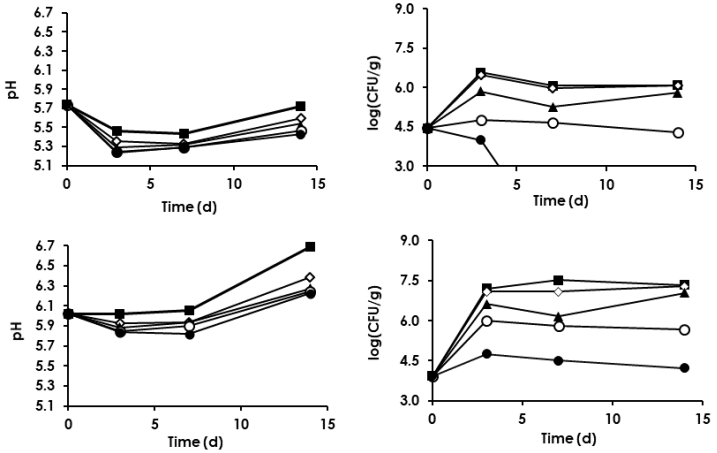


**Figure 1**
Community dynamics of the microbiota of spontaneously fermented (A) normal and (B) DFD meat, as well as the resulting pH course, at salt levels of 0 % (closed squares), 1 % (open diamonds), 2 % (closed
triangles), 3 % (open circles), and 4 % (closed circles). The bacterial counts on RAPID'Entero are expressed as log cfu/g.

Notes