

Spontaneous acidification of fermented sausages is no guarantee for bacterial contribution to the flavour profile

Janssens M.¹, Myter N., De Vuyst L.¹ and Leroy F.¹

¹ Research Group of Industrial Microbiology and Food Biotechnology, Vrije Universiteit Brussel, Brussels, Belgium

Abstract— The effect of spontaneous acidification of fermented sausages on the meat microbiota and its metabolite production was investigated in two sausage types (Belgian-style salami and Boulogne). The sausages were prepared without added lactic acid bacteria (LAB) but a *Staphylococcus carnosus* starter culture was used for colour formation. On the raw materials, a large species diversity was found for the coagulase-negative staphylococci, a group of bacteria that has the potential to contribute to enhanced flavour and colour properties. Nevertheless, this diversity was suppressed during fermentation by the added *S. carnosus* culture. With respect to the LAB, a restricted species diversity was found on the raw materials but during fermentation *Lactobacillus sakei* was by far the most dominant species. Despite a certain degree of species diversity at the onset of fermentation, spontaneous acidification led to dominance of this restricted microbiota, which did not result in a pronounced production of bacterial metabolites.

Keywords — fermented sausage, *Staphylococcus carnosus*, *Lactobacillus sakei*

spontaneously fermented sausages, a number of CNS species are commonly present too. *Staphylococcus xylosus*, *S. saprophyticus*, and *S. equorum* frequently occur, sometimes accompanied by *S. warneri*, *S. pasteurii*, *S. vitulinus*, *S. epidermidis*, *S. sciuri*, *S. carnosus*, or *S. succinus* [3-10].

Several questions about the competitiveness of LAB and CNS species during spontaneous fermentation remain and their effect on sausage quality is not always well understood. Indeed, artisan fermented sausages are often perceived as superior to industrial variants, partially because of the specific microbiota that is established due to spontaneous acidification. Therefore, the influence of spontaneous acidification on the species diversity, community dynamics, and metabolite production kinetics of the microbiota of Belgian spontaneously fermented sausages was investigated. This should indicate whether the absence of an added LAB starter culture allows the development of a more diverse microbiota, matching artisan processes.

I. INTRODUCTION

Meat fermentation is driven by microbial activity, mainly from lactic acid bacteria (LAB) and coagulase-negative staphylococci (CNS), whether or not used as starter culture [1]. Industrialization of the production of fermented sausages has led to the use of starter cultures [2]. In the absence of a starter culture, the indigenous microbiota that is present on the raw materials and in the manufacturing environment (the so-called house microbiota) is responsible for fermentation. The most common LAB species in spontaneously fermented sausages is *Lactobacillus sakei*, but also other species such as *Lb. curvatus*, *Lb. pentosus*, *Lb. plantarum*, *Lb. brevis*, *Pediococcus acidilactici*, and *P. pentosaceus* may be found [1,2]. In

II. MATERIALS AND METHODS

Two kinds of spontaneously acidified Belgian-style sausages were prepared without added LAB but with addition of a *S. carnosus* starter culture (Texel M72, Danisco, France). The latter culture was added for reasons of colour development. A first sausage was made from pork and beef (Belgian-style salami), whereas a second one contained pork and horse meat (Boulogne sausage). A multiphasic approach, using both culture-dependent (plating on selective agar media and cell count determination as well as (GTG)₅-PCR-fingerprinting of picked up colonies) and culture-independent techniques (16S rRNA-PCR-DGGE) was used to determine the bacterial community dynamics and species diversity [11]. Targeted metabolites

evolving during the sausage fermentations were studied, using chromatography (HPAEC-PAD for carbohydrates and HPAEC-CIS for organic acids) and mass spectrometry (GC-MS for volatile analysis) [11].

III. RESULTS

On the raw materials, mostly LAB were present, in an amount of 4.0 log (cfu/g). The dominant species was *Lb. sakei*, albeit with different fingerprint types. The case for Belgian-style salami is presented in Fig. 1. An exception was found for the horse meat that was used for the production of Boulogne sausage, which contained mainly CNS and few LAB. However, this did not affect the LAB population during fermentation of the Boulogne sausage, which was in both sausage types largely dominated by *Lb. sakei*. Due to the absence of a LAB starter culture, acidification was slow and LAB counts only reached a maximum of 9.0 log (cfu/g) and a pH of 4.8 after 5 days of fermentation. In contrast to the LAB species diversity, the CNS species diversity on the raw materials was large, as is represented for the Belgian-style salami in Fig. 2. The beef and pork meat contained mainly *S. xylosus*, *S. equorum*, *S. saprophyticus*, and *S. pasteurii*. The horse meat was again atypical and contained *S. carnosus*, *S. vitulinus/flueretti*, and *S. epidermidis*. Nevertheless, the CNS species diversity of the raw materials was annihilated during fermentation by the added *S. carnosus* culture, which suppressed the background CNS (Fig. 2). This was the case for both sausage types. Other microorganisms, such as enterococci and yeasts, were of no importance during the production process but remained present in the

background, while *Enterobacteriaceae* vanished during the first week of the fermentation (data not shown).

The volatiles fraction in both sausage types was mainly composed of aldehydes that originated from lipid oxidation and spices-derived compounds. With the exception of acetoin in the Belgian-type salami, no metabolites of pyruvate metabolism were present. Aromatic compounds that are typically associated to CNS activity, such as end-products from the catabolism of branched-chain amino acids, were also absent in the Belgian-style salami and only marginally present in the Boulogne sausage (3-methylbutanal and 2-methylpropanol).

IV. DISCUSSION

Although a final pH of 4.8, typical for Northern-European type sausages, was reached, acidification was rather slow. This was due to its spontaneous character relying on the background LAB. The fact that *Lb. sakei* dominated the process is in accordance with previous observations that underline the very competitive behaviour of this species during sausage fermentation [5,6,12,13]. In contrast, CNS, are usually less competitive during meat fermentation due to their acid sensitivity and are outcompeted in industrial sausages prepared with LAB starter cultures. Although the slow acidification process in this study had the potential to allow for the development of background CNS, this was not the case. The encountered CNS species on the raw materials, such as *S. xylosus*, *S. saprophyticus*, *S. pasteurii*, and *S. equorum*, have been reported as typical for artisan fermented sausages

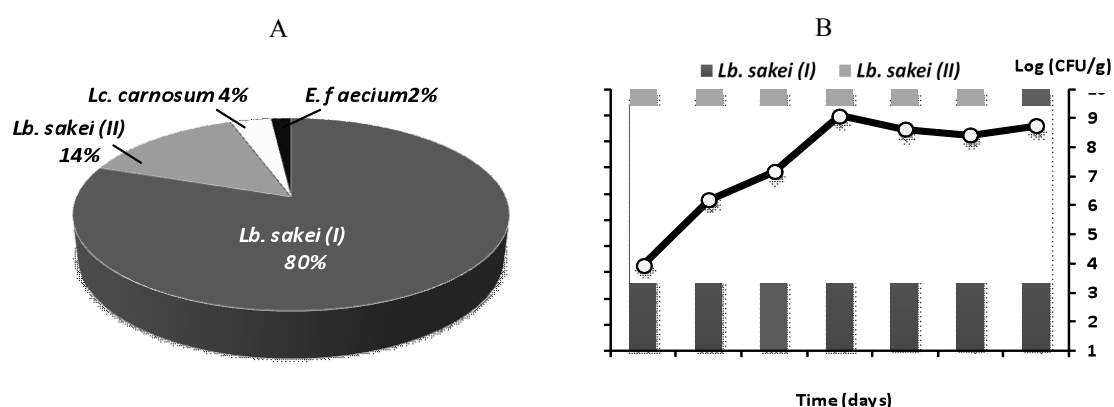


Fig. 1 (A) Diversity of LAB species (*Lb.*, *Lactobacillus*; *Lc.*, *Leuconostoc*; and *E.*, *Enterococcus*), isolated from MRS agar, of the different raw materials (beef, pork meat, and pork fat) used for Belgian-style salami. (B) Temporal evolution of different LAB species, isolated (bars) from and counted (curve) on MRS agar, in spontaneously acidified Belgian-style salami. Isolates were classified and identified by GTG₅-PCR-fingerprinting.

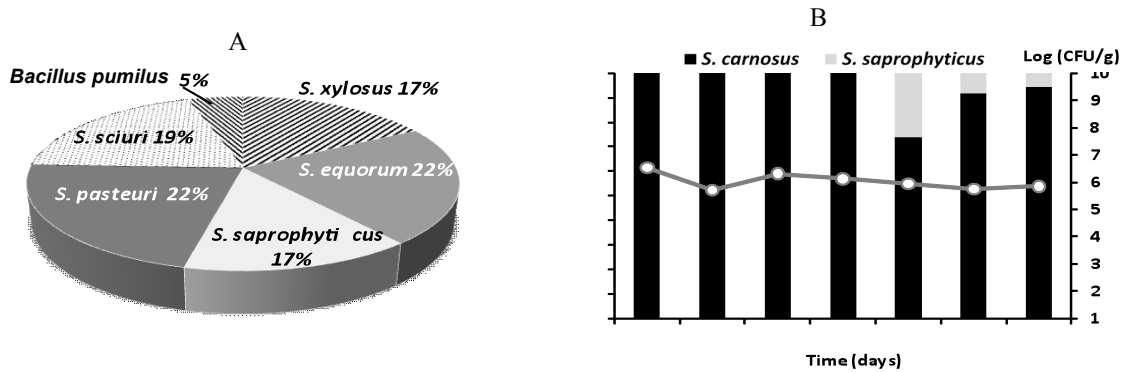


Fig. 2 (A) Diversity of CNS species (*S.*, *Staphylococcus*), isolated from MSA, of the different raw materials (beef, pork meat, and pork fat) used for Belgian-style salami. (B) Temporal evolution of different CNS species, isolated (bars) from and counted (curve) on MSA agar, in spontaneously acidified Belgian-style salami. Isolates were classified and identified by GTG₅-PCR-fingerprinting.

[1,2,14,15]. Nevertheless, the added *S. carnosus* starter culture did not allow the development of these background CNS. Moreover, *S. carnosus* cell counts remained constant during the production process, despite the low final pH of 4.8. It has been shown before that *S. carnosus* is generally more tolerant to acidity than other CNS, such as *S. xylosus*, *S. sciuri*, and *S. succinus* [11,16]. These observations are of importance since staphylococci may improve flavour by producing volatiles from amino acid catabolism and pyruvate metabolism, as well as methylketones from β -oxidation of fatty acids [16]. Unfortunately, *S. carnosus* seems less prone to produce high amounts of such volatiles [11,17]. In addition, the metabolic impact of *Lb. sakei* on volatile flavour formation is also limited [17,18].

V. CONCLUSION

Spontaneous acidification of Belgian-type fermented sausages leads to a natural dominance of *Lb. sakei*. Although the development of *Lb. sakei* is much slower than in industrial products prepared with starter cultures, it does not allow CNS from the background microbiota to develop in the presence of a starter culture of *S. carnosus*. As such, spontaneous acidification only is no guarantee for bacterial contribution to the flavour profile.

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