PS8.07 Selection for spoilage microbiota during the production and storage of artisan-type cooked ham packed under modified atmosphere 118.00

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***Abstract*—a Belgian, artisan-type, modified-atmosphere-packaged (MAP) cooked ham was found to be a highly-perishable product, sensitive to microbial spoilage, due to its low content in salt and absence of preservatives. Its microbiota was analyzed through selective plating, followed by $(GTG)_5$-PCR-fingerprinting of isolates, of samples obtained throughout the processing chain. Whereas the raw tumbled meat was characterized by the presence of a versatile microbiota consisting of around 5 log(cfu g^{-1}), pasteurisation reduced bacterial counts below 2 log(cfu g^{-1}) and selected mainly for leuconostocs, carnobacteria, *Brochothrix thermosphacta,* and several *Enterobacteriaceae*. Next, intermediate manipulation, handling, storage, and post-pasteurisation procedures further selected for specific *Enterobacteriaceae* and lactic acid bacteria. After slicing and packaging under modified atmosphere, the refrigerated end-product was characterised by a microbiota consisting of leuconostocs, carnobacteria, enterococci, and *Br. thermosphacta*. The chill temperature of the end-product determined the relative composition of the latter bacteria and their metabolite production, the extent of which was responsible for spoilage.**

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***Index Terms*—cooked ham, microbial spoilage, rep-PCR, modified atmosphere**

I. INTRODUCTION

HIGH quality, artisan-type, modified-atmosphere-packaged (MAP) cooked ham has a *de facto* shorter shelf life than more conventional cooked meat products, mainly due to its intrinsic parameters such as lower salt concentrations than usually applied and absence of preservatives (Vasilopoulos *et al.,* 2008).

In general, MAP in combination with low temperatures will select for a specific fraction of microorganisms that will eventually dominate and spoil the product due to their metabolic action [5, 6, 8]. Whereas Gram-positive bacteria are favored by these environmental conditions, certain Gram-negative ones, such as *Enterobacteriaceae,* have the potential to grow to spoilage levels, mainly due to their psychrophilic character and ability to grow in microaerophilic conditions [10]. As a result, the microbiota of packed meat products generally consists of several lactic acid bacteria, *Brochothrix thermosphacta*, and *Enterobacteriaceae* [2].

During processing of brined raw ham logs, cooking plays inarguably the largest role in bacterial selection. However, deviations from proper cooking practices frequently occur and other processing and manipulation effects seem to influence the ham microbiota [7]. Consequently, it is important to characterize "weak spots" throughout the production chain of MAP cooked ham that can serve as a niche to spoilage-associated microbiota. Also, efforts are needed for improved mapping of the ham microbiota throughout the production chain. Hence, the aim of this study was to link the microbiota of the raw material, its handling processes, and the spoilage of the final stored product.

II. MATERIALS AND METHODS

A. Origin of the samples

All samples were obtained from a commercial facility for the production of sliced, MAP, artisan-type, cooked ham. Raw deboned meat was injected with brine and tumbled. The raw tumbled meat was shaped into ham logs, packed, and subjected to a pasteurisation process (F value of 200 min). Subsequently, the logs were cooled, unpacked, washed, and left to dry for a period of two days in an intermediate room ("high-care" area). After that, logs were repacked with pasteurisation bags and subjected to a second, milder pasteurisation treatment (post-pasteurisation). After a storage period of three weeks at 0-2°C, the final post-pasteurised product was sliced and packed under modified atmosphere containing 70% N_2 and 30% CO_2. As microbial numbers were generally too low to determine the bacterial composition through culture-dependent molecular methods (see below), spoilage-induced tests were conducted, allowing the bacterial groups present to reach detectable numbers. Thus, the dominating psychrotrophic microbiota at each processing step was determined by storing the samples at a constant

temperature of 7°C for a period of four weeks. At the end of this spoilage induction period, samples were taken for further analysis.

B. Microbiological analysis

For microbiological analysis, 10 g of meat were taken and were diluted ten times in saline solution (0.85%, [w/v], NaCl), followed by homogenization. Spread plates were prepared for the enumeration of total aerobic viable bacteria on Plate Count Agar (PCA; VWR International, Darmstadt, Germany) and lactic acid bacteria on modified de Man-Rogosa-Sharpe agar (mMRS; MRS without sodium acetate).

C. Bacterial DNA extraction and $(GTG)_5$-PCR fingerprinting

Bacterial DNA of isolates obtained from the spread plates was extracted, based on enzymatic lysis of the cells and subsequent use of phenol-chloroform-isoamyl alcohol [4]. The obtained DNA served as a template for further PCR analyses. Using the oligonucleotide primer $(GTG)_5$ (5'-GTGGTGGTGGTGGTG-3'), PCR-fingerprinting was applied to discriminate between the bacterial isolates [4]. Image analysis of the obtained $(GTG)_5$ patterns was carried out using BioNumerics Version 5.1 software (Applied Maths, Sint-Martens-Latem, Belgium) as described elsewhere [11]. From each cluster obtained through numerical analysis of the $(GTG)_5$ fingerprints, assignment of each group of bacteria was performed by comparing with an in-house library. For further identification and verification of the assigned groups, sequencing of the 16S rRNA gene of representative isolates was performed [3]. Sequencing was performed in a commercial facility (VIB, Antwerp, Belgium) and the results were evaluated performing a BLAST analysis based on the NCBI database.

D. Volatile analysis

Volatile compounds were determined by gas chromatography coupled to mass spectrometry, using static headspace (SH-GC-MS) and solid phase microextraction (SPME-GC-MS) analysis. Analyses were performed with an Agilent 6890 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) coupled to an Agilent 5973N mass spectrometer (Agilent Technologies), equipped with an MPS2 Gerstel autosampler (Gerstel GmbH & Co. KG, Mülheim-an-der-Ruhr, Germany) as described elsewhere [6]. The capillary column used was a DB-WAXetr (Agilent Technologies). For SPME analyses, a carboxen-polydimetylsiloxane (CAR-PDMS) fiber (Supelco, Bellefonte, PA, USA) was used. Identification of the peaks obtained was done by comparison with pure standard compounds and library data (NIST98, http://www.nist.gov; [9]). The main metabolites detected were expressed in arbitrary units (AU), calculated as [peak area of compound x g IS (peak area IS x g meat sample)$^{-1}$ x 10^6] and plotted as a function of time. The peak areas of the compounds examined were considered significant only in cases where the peak heights exceeded three times the baseline signal.

Production of bacterial metabolites was linked to bacterial growth through modelling. Bacterial growth in the sliced MAP ham (X, in cfu g^{-1}) was modelled with the logistic growth equation, which is suitable for the modelling of lactic acid bacteria growth. The production of bacterial volatiles was modelled with a Pirt-type equation that takes into account both a concentration increase, due to active cell growth, as well as maintenance-based production by the biomass already present.

III. RESULTS AND DISCUSSION

Besides looking at the entire production process (Fig. 1), the microbial composition of the sliced, MAP end-products of a Belgian artisan-type cooked ham was assessed in detail. Some degree of variability was detected (presence or absence of enterococci, *Leuconostoc mesenteroides* and *Br. thermosphacta*), which may be due to batch variations and differences in storage temperature [11]. Nevertheless, *Carnobacterium* spp. and *Leuconostoc carnosum* were largely predominant in the sliced MAP end-products, which is in agreement with earlier observations [1, 8]. The storage temperature of the sliced, MAP end-products influenced both the bacterial population growth and the composition of the microbiota. *Carnobacterium divergens* and *Leuc. carnosum* were dominant lactic acid bacteria at all temperatures investigated (4, 7, 12, and 26°C). *Enterococcus faecalis* did not develop at low temperatures (4 and 7°C), but was present at 12°C, and it became the most dominant lactic acid bacterium population at 26°C. The production of lactic acid paralleled the growth of these bacteria. However, spoilage development was not only due to growth-associated acidification but also to the production of volatiles [6]. The end-products were characterized by the presence of several volatile compounds, mostly due to heating effects and oxidation. Maillard-derived compounds, leading to the formation of furans, probably originated from endogenous reactions during cooking. Several aliphatic compounds probably originated from fatty acid oxidation reactions, including thermal fat degradation, chemical auto-oxidation, and enzymatic β-oxidation. The compounds 3-methyl butanol and ethanol, however, were related with bacterial cell growth and storage temperature, as could be shown through modelling. *Br. thermosphacta, Carnobacterium* spp., and *Leuconostoc* spp. likely form 3-methyl butanol [2]. Other volatiles that were possibly originating from

bacterial metabolism were acetoin, acetate, 2-methyl propanol, benzaldehyde, acetaldehyde, and methanethiol.

The bacteria encountered in the final product represented only a fraction of the high species variation within the bacterial population throughout the entire production chain (Fig. 1). Several other meat-associated bacteria were found only in the raw meat (pediococci, stahylococci) or were present on ham logs prior to post-pasteurisation (vagococci and *Streptococcus parauberis*). The pasteurisation process reduced overall bacterial counts from 5 log(cfu g^{-1}) on the raw meat to below 2 log(cfu g^{-1}) and selected mainly for leuconostocs, carnobacteria, *Br. thermosphacta,* and several *Enterobacteriaceae*, probably originating from the raw meat. Next, intermediate manipulation, handling, storage, and post-pasteurisation procedures further selected for specific lactic acid bacteria and *Enterobacteriaceae*.

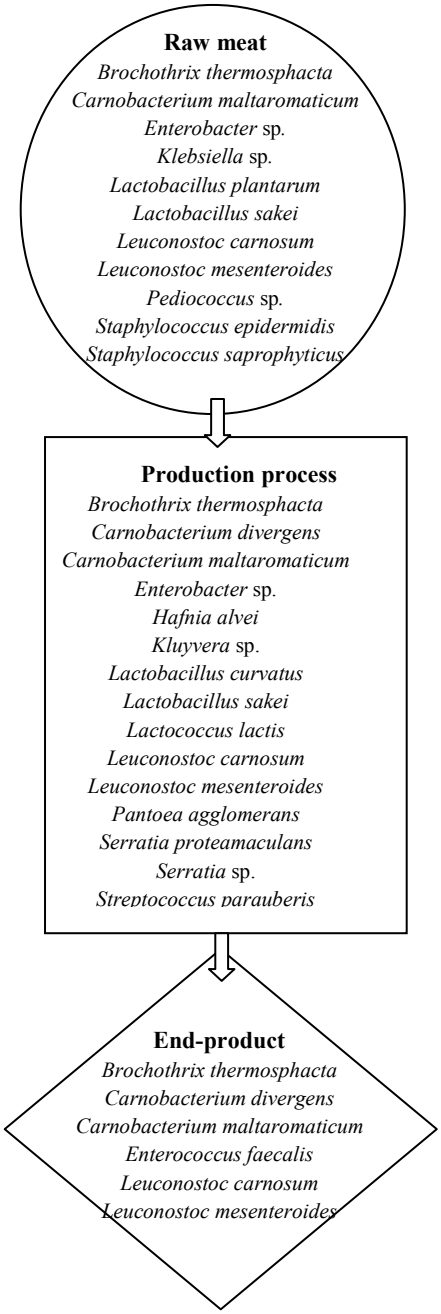

Fig 1. Overview of the ham microbiota throughout the production process line, as determined by $(GTG)_5$-PCR-fingerprinting.

Although *Enterobacteriaceae* were of little importance in the sliced, MAP end-products, they were omnipresent throughout the production chain (Fig. 1). This was especially the case in the production stages corresponding with "high-care" area treatments and after post-pasteurisation. *Hafnia alvei* was one of the most dominant contaminants of the processing area found after the pasteurisation, occurring mainly in the drying zone of the "high-care" area and onwards. *H. alvei* is one of the major spoilage enterobacteria found

in meat, in particular due to its psychrotolerant character, which gives it an adaptation advantage over other microbial members [2]. In addition to *H. alvei*, ham logs that were stored in the "high-care" area prior to post-pasteurisation were colonised within two days with *Enterobacter* spp. and *Pantoea agglomerans*. Besides the aforementioned species, *Serratia* spp. and *Kluyvera* spp. were among the enterobacteria commonly encountered before or after the thermal processes. It is known that within meat processing factories the levels of *Enterobacteriaceae* are higher than the ones found at retail level [10]. In general, this has been attributed to inadequate hygiene techniques, cross-contamination incidents and/or the psychrotrophic traits of these bacteria.

IV. CONCLUSION

Variations in conditions throughout the different stages of a Belgian artisan-type cooked ham production chain resulted in a diversity of meat-associated bacteria and an unwanted bacterial presence on the surface of the final MAP products. Despite this biodiversity, the MAP end-products were dominated by only a few bacterial groups that were highly competitive and were able to grow out from low contamination levels in the raw meat to large, spoilage-provoking populations. The latter populations outcompete *Enterobacteriaceae*, resulting mostly in lactic-type spoilage. Several of the detected volatile compounds during storage of the end-products could be ascribed to bacterial metabolism and their production was related to the storage temperature.

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