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# Technology-induced selection towards the spoilage microbiota of artisan-type cooked ham packed under modified atmosphere

Charalampos Vasilopoulos [a], Hannelore De Maere [b], Eveline De Mey [b], Hubert Paelinck [b], Luc De Vuyst [a], Frédéric Leroy [a,*]

[a] Research Group of Industrial Microbiology and Food Biotechnology (IMDO), Faculty of Sciences and Bio-engineering Sciences, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium

[b] Research Group for Technology and Quality of Animal Products, Department of Industrial Engineering, KaHo Sint-Lieven, Gebroeders Desmetstraat 1, B-9000 Ghent, Belgium

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ABSTRACT

The microbiota associated with a highly-perishable Belgian artisan-type cooked ham was analyzed through plating and $(GTG)_5$-fingerprinting of isolates throughout its processing chain. The raw tumbled meat was characterized by the presence of a versatile microbiota around 4.8 log(cfu $g^{-1}$), consisting of lactic acid bacteria, staphylococci, *Brochothrix thermosphacta*, Gram-negative bacteria, and yeasts. Pasteurisation of the ham logs reduced bacterial counts below 2 log(cfu $g^{-1}$) and subsequent manipulations selected for leuconostocs and carnobacteria. Also, *B. thermosphacta* and several Enterobacteriaceae were found at this stage. During storage in an intermediate high-care area for 2 days, a selection towards certain Enterobacteriaceae (*Hafnia alvei, Enterobacter* spp., and *Pantoea agglomerans*) and lactic acid bacteria (mainly vagococci and *Streptococcus parauberis*) was observed. *B. thermosphacta*, *Leuconostoc carnosum* and carnobacteria were also detected, but only after allowing bacterial outgrowth by incubating the meat logs at 7 °C for four weeks. After a mild post-pasteurisation process and subsequent handling, incubation of the meat logs at 7 °C for four weeks led to outgrowth of Enterobacteriaceae (mainly *Enterobacter* spp. and Serratia spp.). *B. thermosphacta*, and lactic acid bacteria (*Enterococcus faecalis*, *Leuc. carnosum*, and *Carnobacterium maltaromaticum*) were also found. After slicing and packaging under modified atmosphere, the microbiota of the refrigerated end-product consisted of leuconostocs, carnobacteria, and *B. thermosphacta*.



## 1. Introduction

Microbial growth and metabolism contribute to the limitation of the shelf-life of cooked meat products. The manifestation of the metabolic action is perceived by the consumer as spoilage, resulting from the combined effect of off-flavours, discolouration, and/or slime formation on the surface of the product (Gram et al., 2002; Bruhn et al., 2004; Holley et al., 2004). Modified-atmosphere-packaging (MAP), in combination with chilling, is one of the most widespread methods to delay spoilage in cooked meat products. Not only does packaging act as a barrier against contaminants, it also plays a crucial role in the selection of spoilage microorganisms due to its effect on oxygen availability (Labadie, 1999; McMillin, 2008; Nychas et al., 2008). From the large group of microorganisms that initially colonise the raw meat ecosystem, lactic acid bacteria and *Brochothrix thermosphacta* mostly prevail in the cooked end-product, thereby outcompeting most Gram-negative bacteria such as Pseudomonadaceae and Enterobacteriaceae (Borch et al., 1996; Rattanasomboon et al., 1999; Blixt and Borch, 2002; Gram et al., 2002). Lactic acid bacteria that are most commonly encountered are *Lactobacillus* spp., *Carnobacterium* spp., *Leuconostoc* spp., and *Enterococcus* spp. (Björkroth and Korkeala, 1997; Metaxopoulos et al., 2002; Peirson et al., 2003b; Vasilopoulos et al., 2008).

Despite the available knowledge on the nature of the microbial spoilage of MAP cooked meat products, little is reported on the source of the spoilage bacteria and the effect of the processing chain on the selection towards the final microbiota (Mäkelä and Korkeala, 1987; Björkroth and Korkeala, 1997; Borch et al., 1988; Dykes et al., 1991). Studies that deal with the processing chain focus mainly on food pathogens rather than on spoilage microorganisms (Berends et al., 1998; Chasseignaux et al., 2002; Aslam et al., 2004; Byrne et al., 2008). It is not fully clear to what extent microorganisms that are present in meat products originate from the meat itself or rather from handling-related operations in the processing

* Corresponding author. Tel.: +32 2 6293612; fax: +32 2 6292720.
*E-mail address:* fleroy@vub.ac.be (F. Leroy).

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line or from the environment (Björkroth et al., 1998; Samelis et al., 1998; Metaxopoulos et al., 2003; Sachindra et al., 2005; Chevallier et al., 2006; Vihavainen et al., 2006). Since bacterial communities that are present throughout the processing line can attach to various surfaces and thus serve as contaminants of the final product, it is of particular importance to determine these communities and their contribution to the shelf-life (Gill and McGinnis, 2004; Brightwell et al., 2006; Gounadaki et al., 2008).

During processing of brined raw ham logs, cooking plays inarguably the largest role in bacterial selection. The effect of cooking is often measured by monitoring the "core" temperature of the treated meat logs, which determines the application of appropriate temperature and time combinations. However, deviations from proper cooking practices frequently occur (Metaxopoulos et al., 2003). Moreover, sanitation of the processing line and cooking of the product, even intensive, is not always effective against handling-related post-contamination or presence of thermotolerant vegetative bacteria (Franz and von Holy, 1996a; Jessen and Lammert, 2003; Peirson et al., 2003a).

The present study deals with the characterisation of spoilage-associated microorganisms during the processing of a Belgian MAP artisan-type cooked ham. The latter product has *de facto* a 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. *Leuconostoc carnosum*, *Carnobacterium divergens*, and *B. thermosphacta* have been shown to dominate the microbiota of this sliced MAP end-product in the cold-chain at 4 and 7 °C (Vasilopoulos et al., 2008). Around room temperature (26 °C), however, enterococci dominate. Compounds related to the metabolism of the aforementioned bacteria are detected at the end of the shelf-life of this product, including lactic acid, acetic acid, acetoin, 3-methyl butanol, and hydrogen sulphide (Leroy et al., 2009). It is of utmost importance to understand in what way the cooked ham production process can serve as a source of handling-related bacterial contamination, resulting in specific species domination. Therefore, raw meat and intermediate products from different production stages of a Belgian artisan-type cooked ham were studied to monitor meat-associated microorganisms and their evolution and succession during processing.

## 2. Methods

### 2.1. Origin of the samples

All samples were obtained from a commercial facility for the production of sliced, MAP artisan-type cooked ham, starting from raw meat. During production, the raw meat is subjected to a number of technological treatments, finally resulting in the packaged and sliced MAP end-product (Fig. 1). Briefly, raw deboned meat is injected with brine and tumbled. Next, the raw tumbled meat is shaped into ham logs and subjected to a first pasteurisation process (the actual "cooking" process). An oxygen-impermeable pasteurisation bag is used (Krehalon Benelux NV, Turnhout, Belgium). Pasteurisation is done by submerging the ham logs into water according to the facility's standards (maximum water temperature of 72 °C; *F* value of 200 min). Subsequently, the logs are cooled, unpacked, washed, and left to dry for a period of 2 days in an intermediate room ("high-care" area). After that, logs are repacked with pasteurisation bags and subjected to a second, milder pasteurisation treatment (post-pasteurisation; *i.e.* a brief shower treatment with steamed water). After a storage period of three weeks at 0–2 °C, the final post-pasteurised product is sliced and packed under modified atmosphere containing 70% $N_2$ and 30% $CO_2$. The final product is packaged in two multilayer films. The upper film consists of a multilayer of polyethylene terephthalate/low density polyethylene/ethylene vinyl alcohol/low density polyethylene (PET/LDPE/EVOH/LDPE), having an oxygen permeability of 2.5 $cm^3/m^2$/24 h at 23 °C and 50% relative humidity (RH). The lower film consists of a peelable barrier of polyethylene terephthalate/polyethylene/ethylene vinyl alcohol/polyethylene (PET/PE/EVOH/PE), having an oxygen permeability of 1 $cm^3/m^2$/24 h at 20 °C and 0% RH.

Samples were collected at five different stages (a-e) of the production process to determine microbial counts and, where feasible, microbial composition (Fig. 1). Raw tumbled meat was sampled to study the initial microbial load and composition (stage a). Cooked meat logs were sampled after the pasteurisation step (stage b), and prior to and after the post-pasteurisation treatment (stages c and d). The final cooked ham was sampled after slicing and packaging (stage e). At the sampling stage d, samples were taken at three different places of the ham log (top, side, and bottom) to examine heterogeneity of the final heating treatment.

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. Spoilage-induced tests consisted of provoking the outgrowth of the dominating psychrotrophic microbiota at each processing step, by storing the meat samples (obtained at stages a to e) at a constant temperature of 4 or 7 °C for a period of four weeks. At the end of this spoilage induction period, samples were taken for further analysis. At all stages investigated, ten ham logs were sampled. The whole sampling experiment was performed twice.

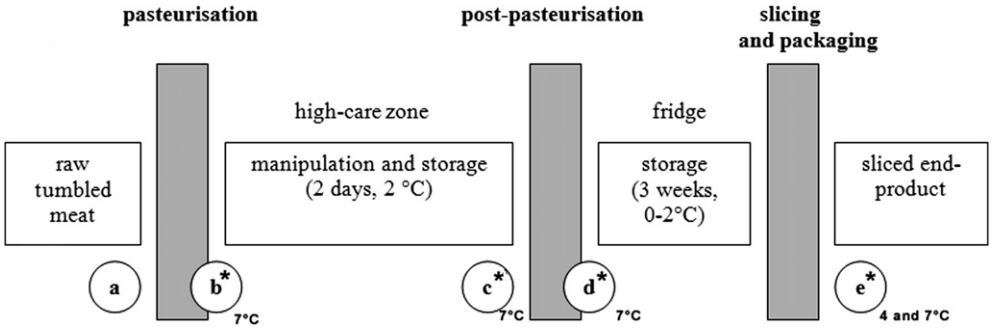


**Fig. 1.** Overview of the technological process for the production of sliced and modified-atmosphere-packaged (MAP) artisan-type cooked ham. Lower-case letters (a–e) indicate sampling points for the determination of the psychrotrophic meat-associated microbiota. Asterisks denote points where an incubation of the meat during 4 weeks at 4 or 7 °C was performed. At stage c samples were also obtained prior to incubation at 7 °C for 4 weeks as mentioned in the text.