BACTERIAL ECOLOGY OF SLICED COOKED PORK PRODUCTS ON THE BELGIAN MARKET

W. Geeraerts[1*], V. Pothakos[1], L. De Vuyst[1] and F. Leroy[1]

[1] Research Group of Industrial Microbiology and Food Biotechnology (IMDO), Faculty of Sciences and Bioengineering

Sciences, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium

*Corresponding author email: frederic.leroy@vub.be

Abstract – Cooked pork products are valuable economical products that are relatively vulnerable to bacterial spoilage. By applying cooling, adding chemical additives (E compounds), and using modified atmosphere packaging (MAP) many of the prevailing bacterial species are inhibited, thus mostly resulting in a dominance of lactic acid bacteria. In this study, 42 cured and sliced cooked pork samples were purchased from three Belgian supermarkets to assess their bacterial heterogeneity. A total of 702 bacterial isolates was identified using (GTG)$_5$-PCR fingerprinting in combination with 16S rRNA and *pheS* gene sequencing. This indicated that 97% of the isolates were lactic acid bacteria belonging to the genera *Carnobacterium*, *Lactobacillus*, and *Leuconostoc*. The remaining isolates were identified as *Brochothrix thermosphacta*. On average, the most prevalent species were leuconostocs, namely *Leuconostoc carnosum* and *Leuconostoc gelidum* subsp. *gelidum*. Differences in ecology were found between pork samples based on the production facility and the chemical additives used. Results indicated that the bacterial diversity of cooked meat products needs to be addressed based on the level of product composition and inter-batch variations.

Key Words – cooked pork products, lactic acid bacteria, modified atmosphere packaging

I.   INTRODUCTION

One quarter of the delicatessens sold in Europe consists of cooked pork products [1]. To reduce bacterial spoilage, several preservation techniques are used to prolong their shelf-life. These techniques include storage under modified atmosphere packaging (MAP) at low temperatures and the addition of chemical additives (E compounds). A more innovative route for the preservation of meat and fish is the use of bioprotective cultures of lactic acid bacteria (LAB) [2]. Yet, this requires a better understanding of the bacterial communities involved and their metabolism within the product during storage.

Generally, the bacterial composition of the final cooked pork products depends on the technology applied and the raw materials used. By applying a cold chain and by usage of MAP packaging with N_2 and CO_2, the bacterial ecology is restricted to mostly psychrotolerant and psychrophilic bacteria that can resist anaerobic conditions. Usually, this encompasses LAB species and *Brochothrix thermosphacta* [2]. The bacterial ecology of meat can be further affected due to variations in phosphate levels, salt concentration, and the use of additives [3]. Many consumers perceive chemical additives as detrimental for quality and health, although they often lead to a better preservation and technological benefits. Because of this market demand, the meat industry is implementing specific labels and criteria [4]. Yet, food products with lower amounts of chemical additives may be more prone to bacterial spoilage (*e.g.*, production of acidity, gas, slime, and/or off-odour compounds and discolouration) [2].

In the present study, a detailed analysis of cured and sliced cooked pork products on the Belgian market was carried out at their expiration date, to determine the dominant bacterial communities.

II.   MATERIALS AND METHODS

Between October 2014 and June 2015, 42 samples of cured and sliced cooked pork products were purchased from three Belgian supermarkets, belonging to 16 different product types (different labels) and originating

from nine production facilities. The product types had different cocktails of E compounds: nine product types only contained sodium nitrite (E250) and sodium ascorbate (E301), the other seven product types contained additional E compounds. All sliced pork products were packed under MAP and stored at 4°C till expiration date. Prior to pH measurement, 10-15 g of meat was diluted with peptone physiological solution [0.85 % (m/v) NaCl and 0.1 % (m/v) peptone] and subjected to mechanical treatment. Dilution series were made and pour plating was done with (i) M17 agar, (ii) de Man-Rogosa-Sharpe (MRS) agar with a pH of 5.9, (iii) modified MRS (mMRS) agar without acetate and a pH of 8.6, and (iv) plate count agar (PCA). An agar overlay was added and plates were incubated at 22°C for 120 h. Colonies were picked up and cultivated in brain heart infusion medium. Next, cell pellets were collected by centrifugation and storage of isolates at -80°C in glycerol was done. DNA extraction was performed with the NucleoSpin®96 tissue kit (Macherey-Nagel, Düren, Germany). Prior to DNA extraction, cells were lysed with a 200-µl lysis solution containing 4.0 mg of lysozyme and 100 U of mutanolysin at 37°C for 60 min, followed by a treatment with 25 µl of a proteinase K solution (29.0 mg/ml) at 56°C for 60 min. Identification was done by placing $(GTG)_5$-PCR amplicons on an agarose gel, followed by fingerprinting and cluster analysis with Bionumerics software (v.5.10; Applied Maths, Sint-Martens-Latem, Belgium), as described previously [5]. The 16S rRNA and *pheS* genes were sequenced for representative isolates from the different clusters and evaluated with the basic local alignment search tool (BLAST) and NCBI database (http://www.ncbi.nlm.nih.gov/BLAST/).

The resulting data were processed with IBM SPSS Statistics (v.20; IBM Corporation, Armonk, NY, USA). A principal component analysis was performed using the pH values (divided along eight classes ranging from pH 5.4 to pH 6.2 with a 0.1 interval), the number of additives as E compounds (divided along three classes containing either E250 and E301 solely, E250 and E301 with additional E compounds other than E326, or E250 and E301 with E326 and possibly other E compounds), the bacterial cell counts from the four agar media [divided along eight classes ranging from 5.5 to 9.5 log (cfu g^{-1}) with a 0.5 interval], and the prevalence of the different bacterial genera.

III.   RESULTS AND DISCUSSION

Overall, the pH values of the sliced cooked pork products ranged between pH 5.41 and pH 6.22. Average bacterial cell counts ranged between < 5.0 and 9.2 log (cfu g^{-1}), whereby 64% of the samples had bacterial counts higher than 8.0 log (cfu g^{-1}) on at least one of the four agar media. With the exception of two green discoloured samples, no obvious signs of spoilage were found at expiration date, thus supporting the hypothesis that the ability to manifest spoilage is more dependent on the taxonomic distribution and metabolic activity of the bacterial consortia than on their overall numbers [6].

A total of 702 bacterial isolates was obtained from the different agar media (Table 1). The species found included *B. thermosphacta*, *Carnobacterium divergens*, *Carnobacterium funditum*, *Carnobacterium maltaromaticum, Lactobacillus curvatus/graminis*, *Lactobacillus sakei, Leuconostoc carnosum*, *Leuconostoc gelidum* subsp. *gasicomitatum,* and *Leuconostoc gelidum* subsp. *gelidum*. These meat-associated species corresponded with bacteria that have either survived the cooking process or entered the product due to post-cooking recontamination [2, 7].

Table 1 Percentile distribution (in %) of the bacterial species retrieved from sliced cooked pork samples on the Man-Rogosa-Sharpe (MRS) agar, modified MRS (mMRS) agar, M17 agar, and plate count agar (PCA)

	MRS	mMRS	M17	PCA	Total
Lactobacillus graminis/ curvatus	8	7	3	1	5
Lactobacillus sakei	11	14	7	8	10
Leuconostoc carnosum	50	48	34	64	49
Leuconostoc gelidum subsp. *gasicomitatum*	6	5	6	10	7
Leuconostoc gelidum subsp. *gelidum*	16	9	18	11	14
Carnobacterium divergens	6	10	15	2	8
Carnobacterium maltaromaticum	1	5	11	1	4
Carnobacterium funditum	1	0	0	0	0
Brochothrix thermosphacta	1	2	6	3	3

Generally, the *Leuconostoc* spp. were the most represented group on all agar media, with *Leuc. carnosum* being the most prevalent. Yet, the type of agar medium used for bacterial isolation had an obvious influence on the reconstruction of the bacterial species diversity. Carnobacteria, for instance, were less recovered from MRS agar than from M17 agar, whereas lactobacilli displayed a lower preference for the latter agar medium. These findings may be related to the presence of acetate in the M17 medium, inhibiting the growth of carnobacteria [5], whereas lactobacilli are known to prefer MRS over M17 [8].

To cluster the different samples, a principal component analysis was performed (Figure 1). The primary principal component (PC; explaining 46% of the total variance) revealed a positive correlation between higher bacterial counts (0.94-0.84), higher presence of *Leuconostoc* spp. (0.82), and lower pH values of the samples (0.63). The second PC (explaining 20% of the total variance) suggested a positive correlation between the use of more than the minimal use of chemical additives (0.87) and the presence of *Lactobacillus* spp. (0.82). A negative correlation was found with the presence of *B. thermosphacta* (-0.47) and *Carnobacterium* spp. (-0.45). A first sample cluster group (I) encompassed the samples mostly dominated by *Leuconostoc* spp.; a second group (II) was characterized by samples that were strongly dominated by *Lactobacillus* spp. and with average bacterial counts; and a final group (III) contained samples with low bacterial cell counts.

Differences were found for the sliced cooked pork microbiota originating from the different production facilities. For eight of the nine facilities, the majority of the retrieved isolates were *Leuconostoc* species. *Lactobacillus graminis/curvatus* and *Lb. sakei* dominated the sliced cooked pork meat samples from the remaining facility (corresponding with the samples within group II). As suggested by the principal component analysis, the sliced cooked pork samples with only E250 and E301 had a relatively higher presence of *Leuc. gelidum* subsp. *gelidum* and *Carnobacterium* spp. and a lower presence of *Lactobacillus* spp. than samples with extra E compounds. *Brochothrix thermosphacta* was only found in samples that contained the technological minimum amount of additives. When analysing the effect of different E compounds added to the products, notable differences were found for the four samples containing E326. This E compound resulted in the isolation of *Lactobacillus* spp. (93%), with *Lb. curvatus*/*graminis* being the most prevalent representative, and only very minor fractions of *Carnobacterium* spp. and *Leuconostoc* spp.
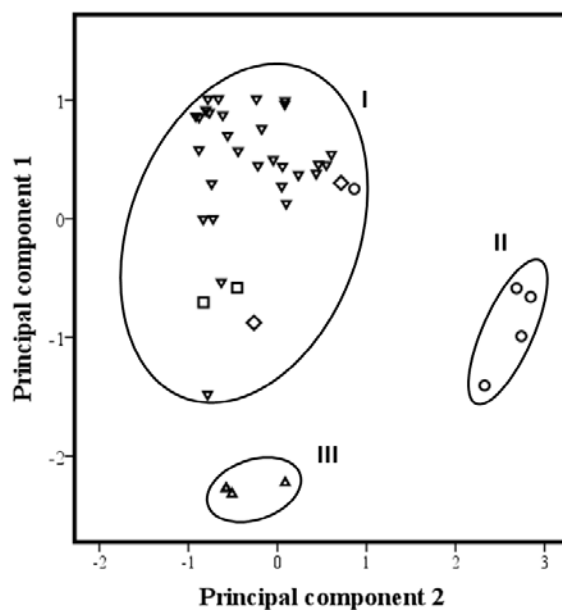
Figure 1. Principal component analysis, using pH, cell counts, the prevalence of bacterial genera, and E compounds. Samples were dominated by *Brochothrix thermosphacta* (□), *Carnobacterium* spp. (◊), *Lactobacillus* spp. (○), or *Leuconostoc* spp. (∇) or contained bacterial cell counts below 5.0 log (cfu g^{-1}) (Δ).

IV.   CONCLUSION

The diversity of the dominant bacterial species originating from sliced and cured cooked pork products acquired on the Belgian market contained the usual suspects, although in variable compositions. The results suggested the importance of intrinsic batch variations and/or specific effects of the use of additives and the production location. More dedicated studies are needed to establish the validity of these associations.

REFERENCES

1. Casiraghi, E., Alamprese, C., Pompei, C. (2007). Cooked ham classification on the basis of brine injection level and pork breeding country. Food Science and Technology 40: 164-169.
2. Vasilopoulos, C., De Vuyst, L., Leroy, F. (2015). Shelf-life reduction as an emerging problem in cooked hams underlines the need for improved preservation strategies. Critical Reviews in Food Science and Nutrition 55: 1425-1443.
3. Nieminen, T.T., Välitalo, H., Säde, E., Paloranta, A., Koskinen, K., Björkroth, J. (2012). The effect of marination on lactic acid bacteria communities in raw broiler fillet strips. Frontiers in Microbiology 3: 1-8.
4. Resconi, V.C., Keenan, D.F., Barahona, M., Guerrero, L., Kerry J.P., Hamill, R.M. (2016). Rice starch and fructo-oligosaccharides as substitutes for phosphate and dextrose in whole muscle cooked hams: sensory analysis and consumer preferences. Food Science and Technology 66: 284-292.
5. Vasilopoulos, C., Ravyts, F., De Maere, H., De Mey, E., Paelinck, H., De Vuyst, L., Leroy, F. (2008). Evaluation of the spoilage lactic acid bacteria in modified-atmosphere-packaged artisan-type cooked ham using culture-dependent and culture-independent approaches. Journal of Applied Microbiology 104: 1341-1353.

6. Leroy, F., Vasilopoulos, C., Van Hemelryck, S., Falony, G., De Vuyst, L. (2009). Volatile analysis of spoiled, artisan-type, modified-atmosphere-packaged cooked ham stored under different temperatures. Food Microbiology 26: 94-102.
7. Samelis, J., Kakouri, A., Georgiadou, K.G., Metaxopoulos, J. (1998). Evaluation of the extent and type of bacterial contamination at different stages of processing of cooked ham. Journal of Applied Microbiology 84: 649-660.
8. Yu, J., Wang, W.H., Menghe, B.L.G., Jiri, M.T., Wang, H.M., Liu, W.J., Bao, Q.H., Lu, Q., Zhang, J.C., Wang, F., Xu, H.Y., Sun, T.S., Zhang, H.P. (2011). Diversity of lactic acid bacteria associated with traditional fermented dairy products in Mongolia. Journal of Dairy Science 94: 3229-3241.