



Vrije Universiteit Brussel

Research Group of Industrial Microbiology  
and Food Biotechnology

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# Selection for spoilage microbiota during the production and storage of artisan-type cooked ham packed under modified atmosphere

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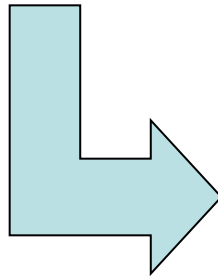
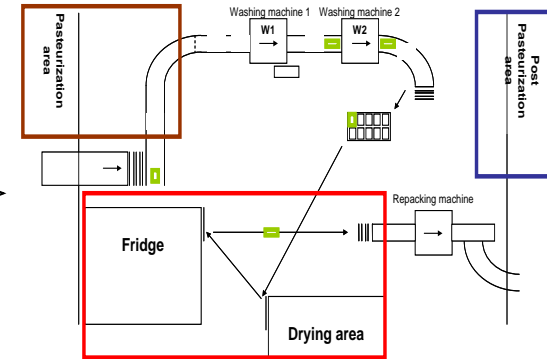
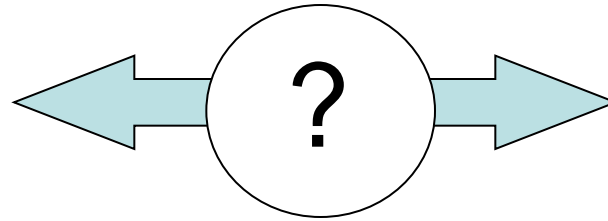
ICOMST 2009



# Outline

- ❖ Introduction and aim of the study
- ❖ Methodology
- ❖ Analysis of the final product (microbiota and metabolites)
- ❖ Analysis of the production process (microbiota)
- ❖ Conclusions

# Introduction and aim of the study



## Artisan-type cooked ham

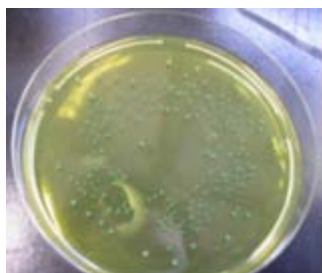
Strict quality norms

Distributed under MAP

Rather versatile product

- Monitoring of microbial population dynamics as a function of process conditions to identify specific spoilage populations
- Linking microorganisms to raw meat and/or handling operations
- Classical techniques are insufficient
- Need for complementary molecular methods

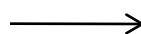
## (GTG)<sub>5</sub>-fingerprinting [culture-dependent method]



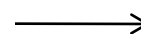
Isolates (PCA, MRS, mMRS, STAA, VRBG, KAA)



DNA extraction  
Bacterial DNA  
of isolates



PCR amplification  
of genomic DNA  
with (GTG)<sub>5</sub> primer

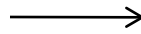


Agarose gel  
electrophoresis

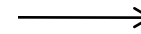
## PCR-DGGE [culture-independent method]



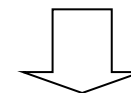
DNA extraction  
Total bacterial DNA  
of meat



PCR amplification  
of V3 region  
of 16S rRNA gene  
with universal  
primers



DGGE, gel gradient  
35%-60%



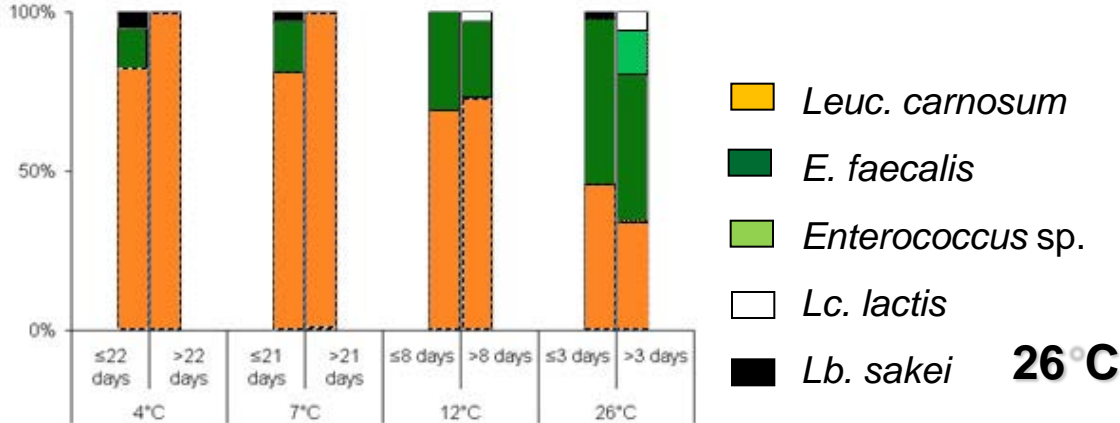
Numerical and  
image analysis

- ❖ HPLC
- ❖ SH-GC/MS
- ❖ SPME-GC/MS

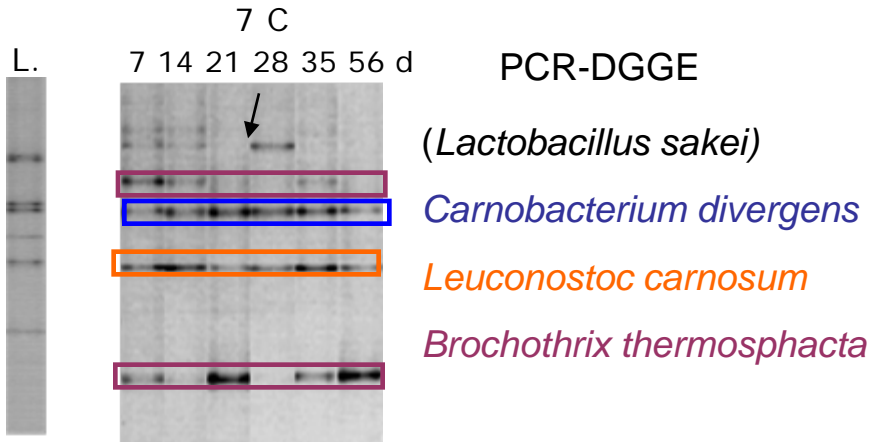
Metabolite analysis

# Final product (microbiota)

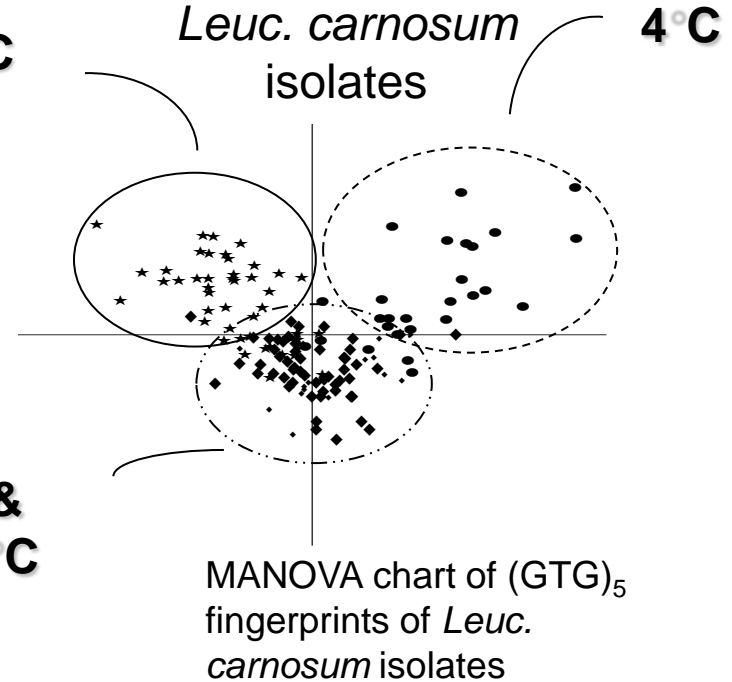
(GTG)<sub>5</sub>-fingerprinting MRS isolates



## Effect of storage temperature



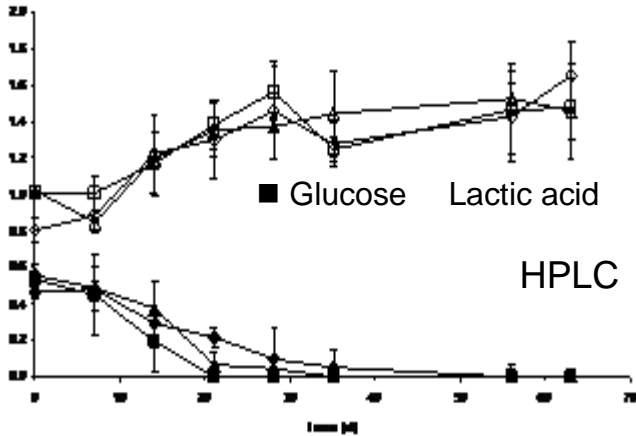
Distribution of *Leuc. carnosum* isolates



Vasilopoulos *et al.* (2008) JAM 104:1341-1353

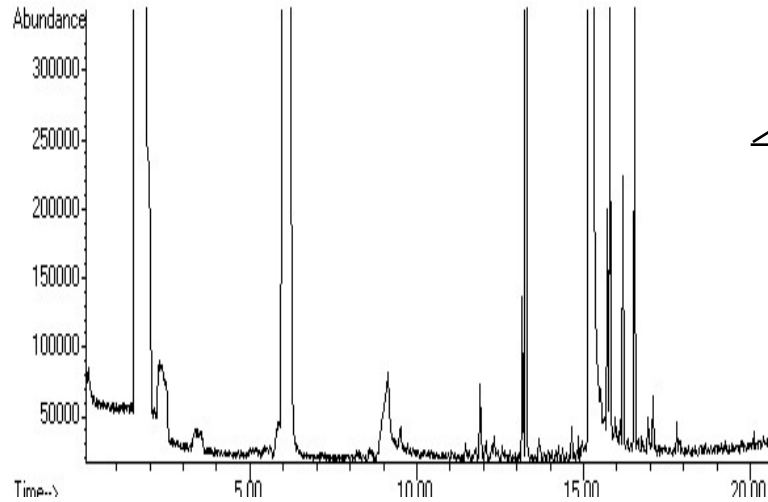
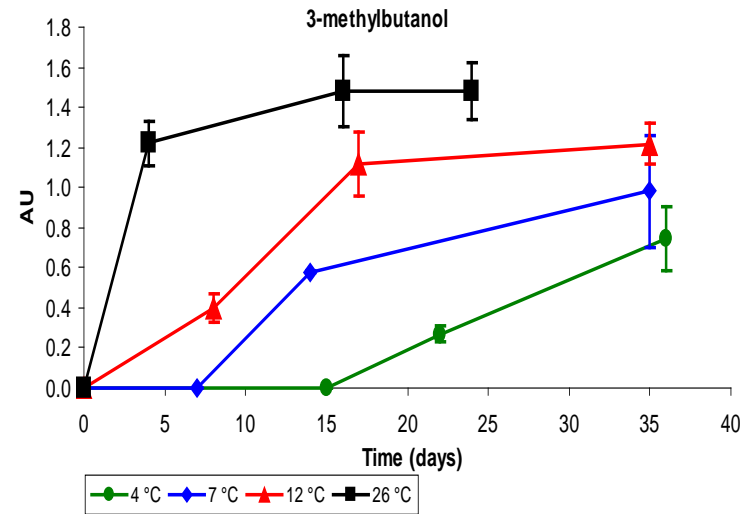
# Final product (metabolites)

Bacterial growth leads to acidification and production of volatiles in a temperature-related manner



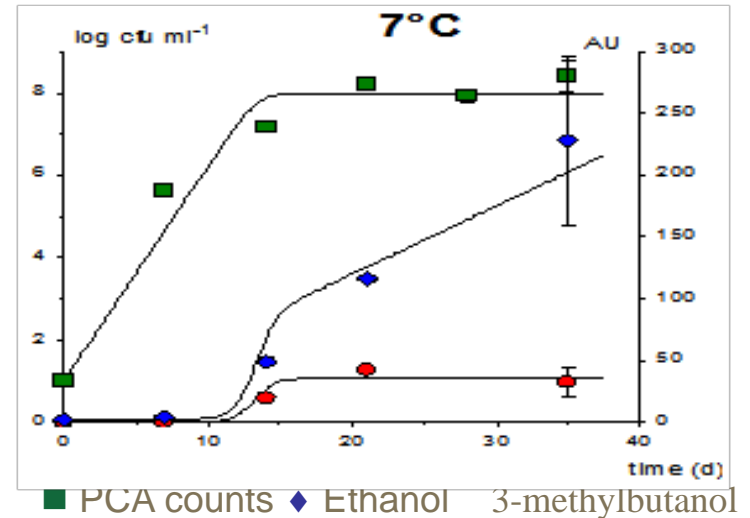
Glucose depletion led mainly to lactic acid production

Acetic acid and ethanol were found occasionally



GC/MS

More than 25 volatile compounds were detected

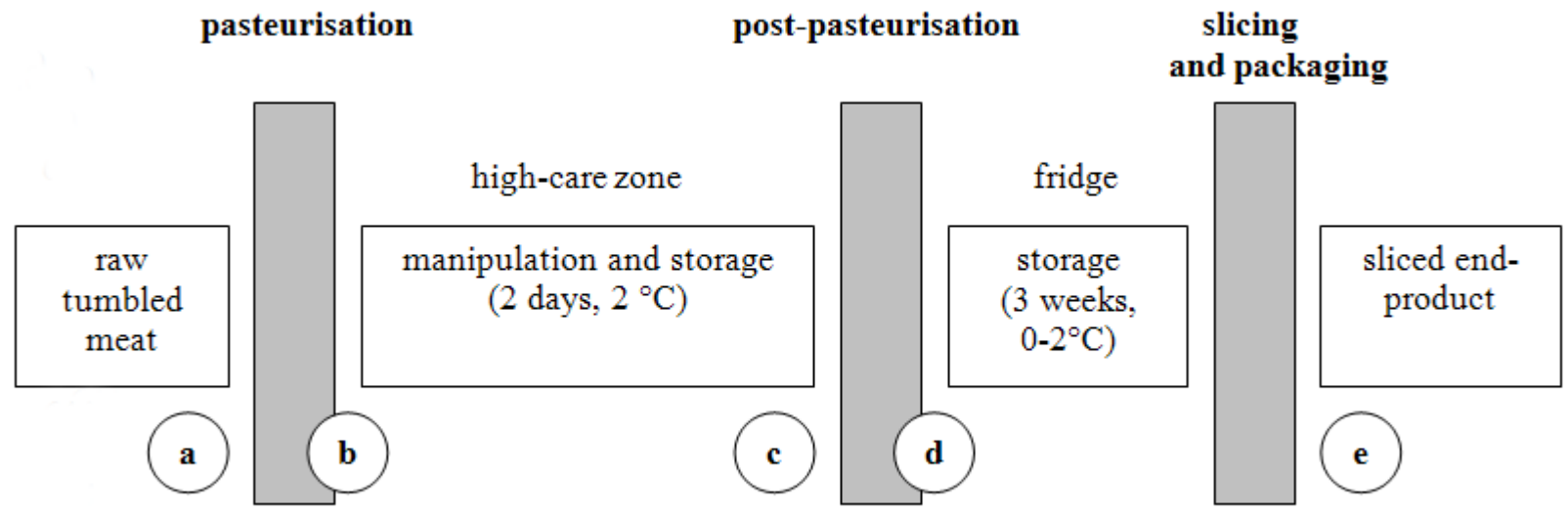


Leroy *et al.* (2009) FM 26:94-102



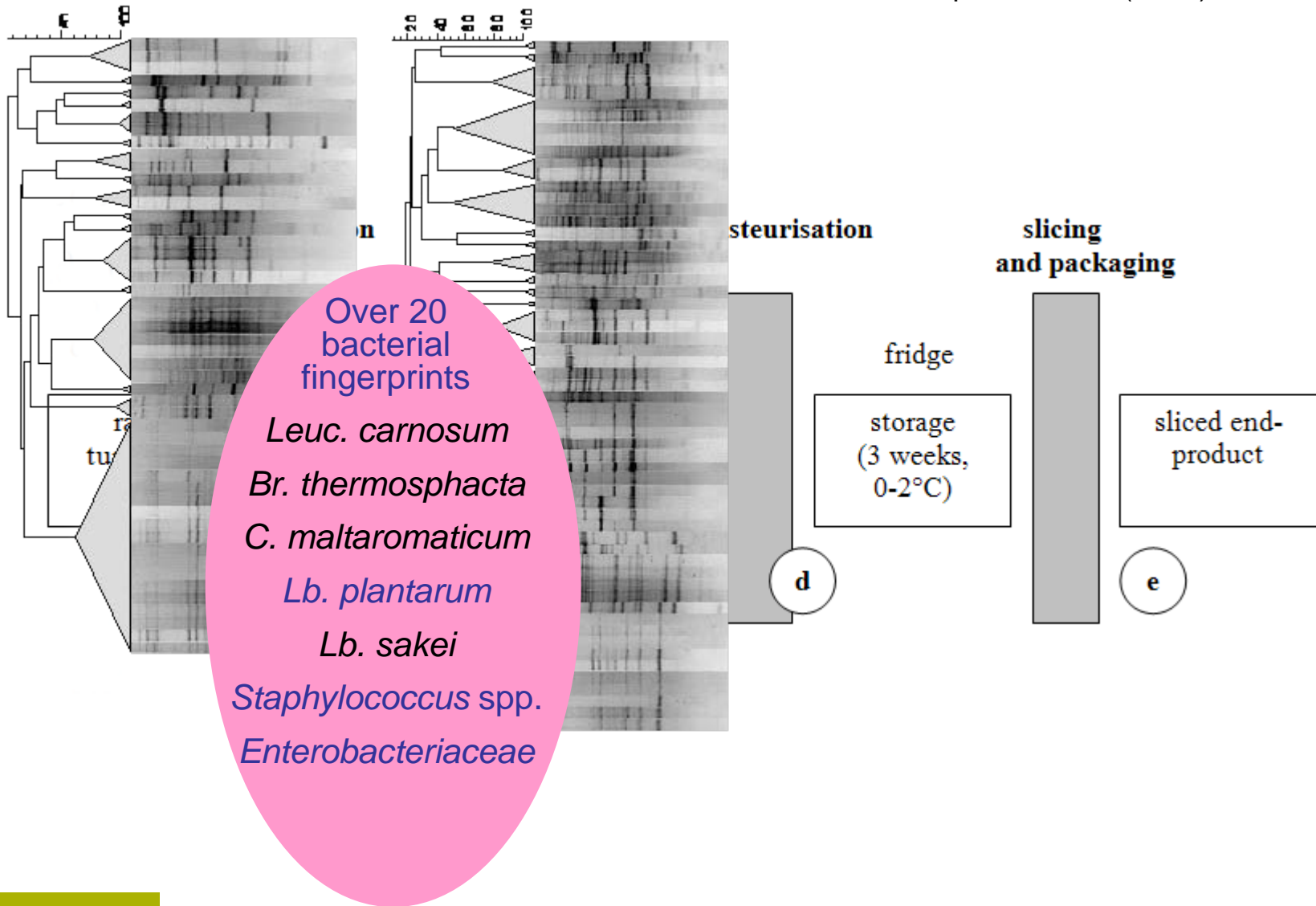
# Production process

Vasilopoulos *et al.* (2009) submitted



# Production process raw meat

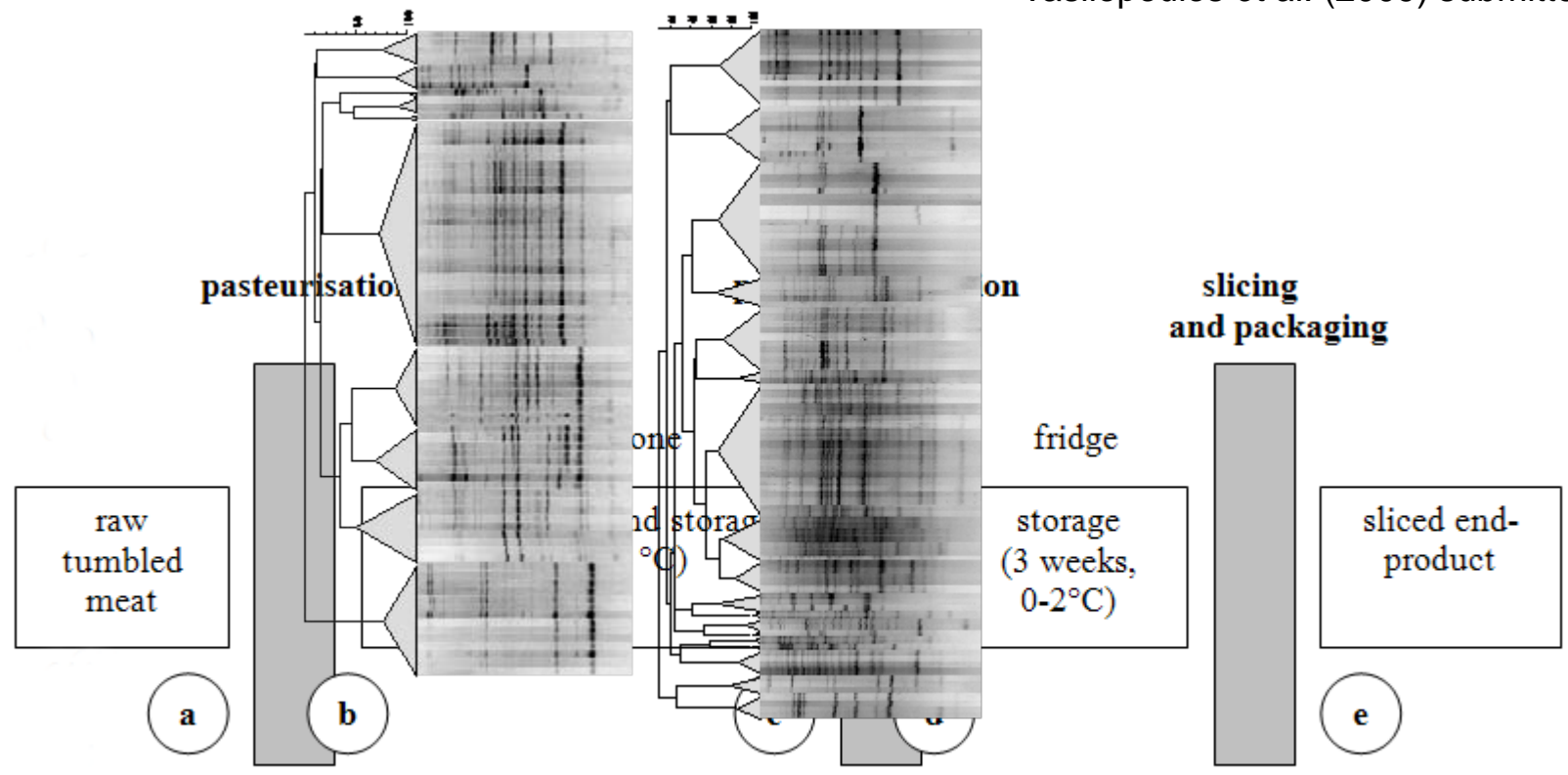
Vasilopoulos *et al.* (2009) submitted





# Production process high care zone

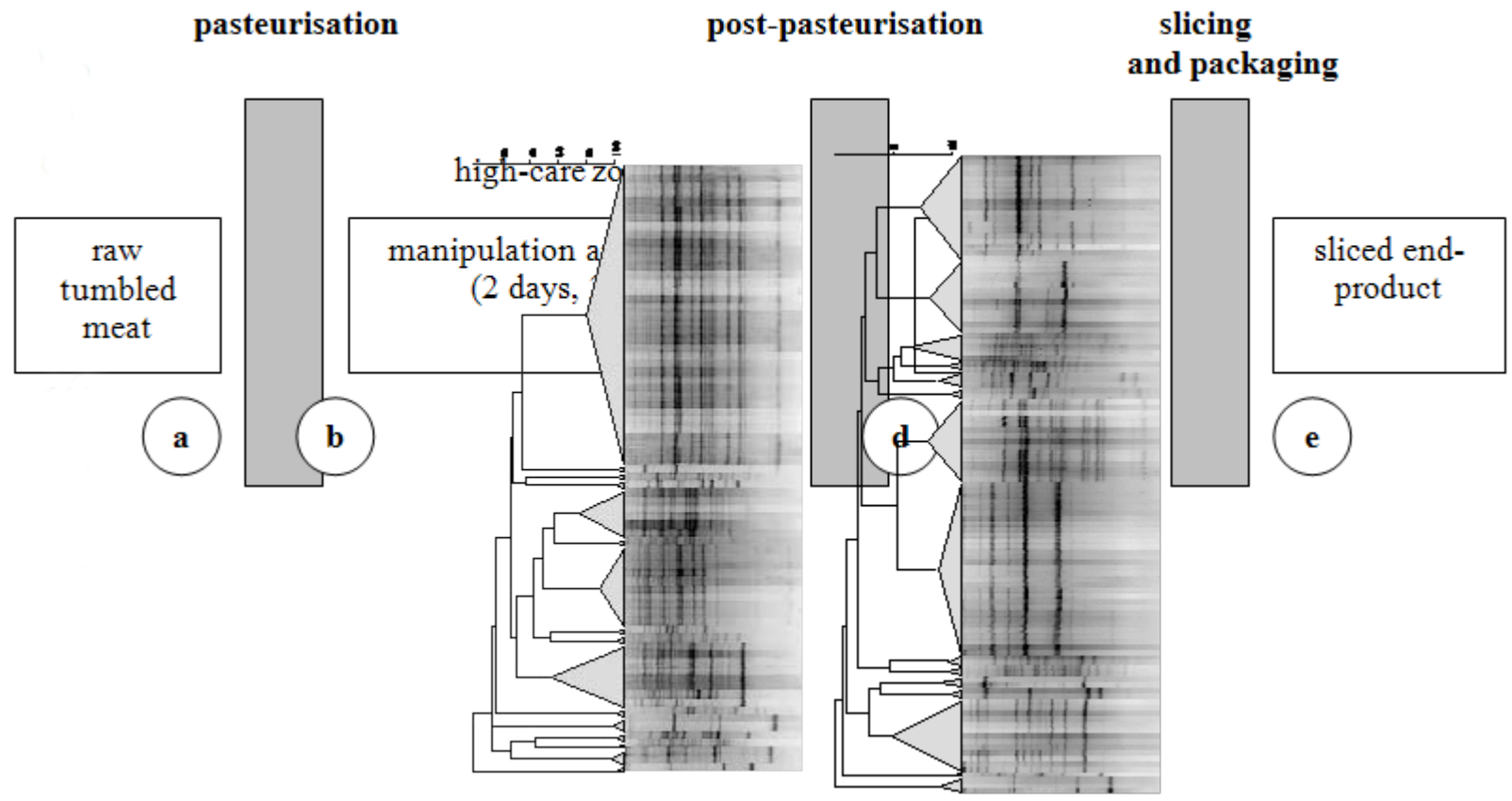
Vasilopoulos *et al.* (2009) submitted





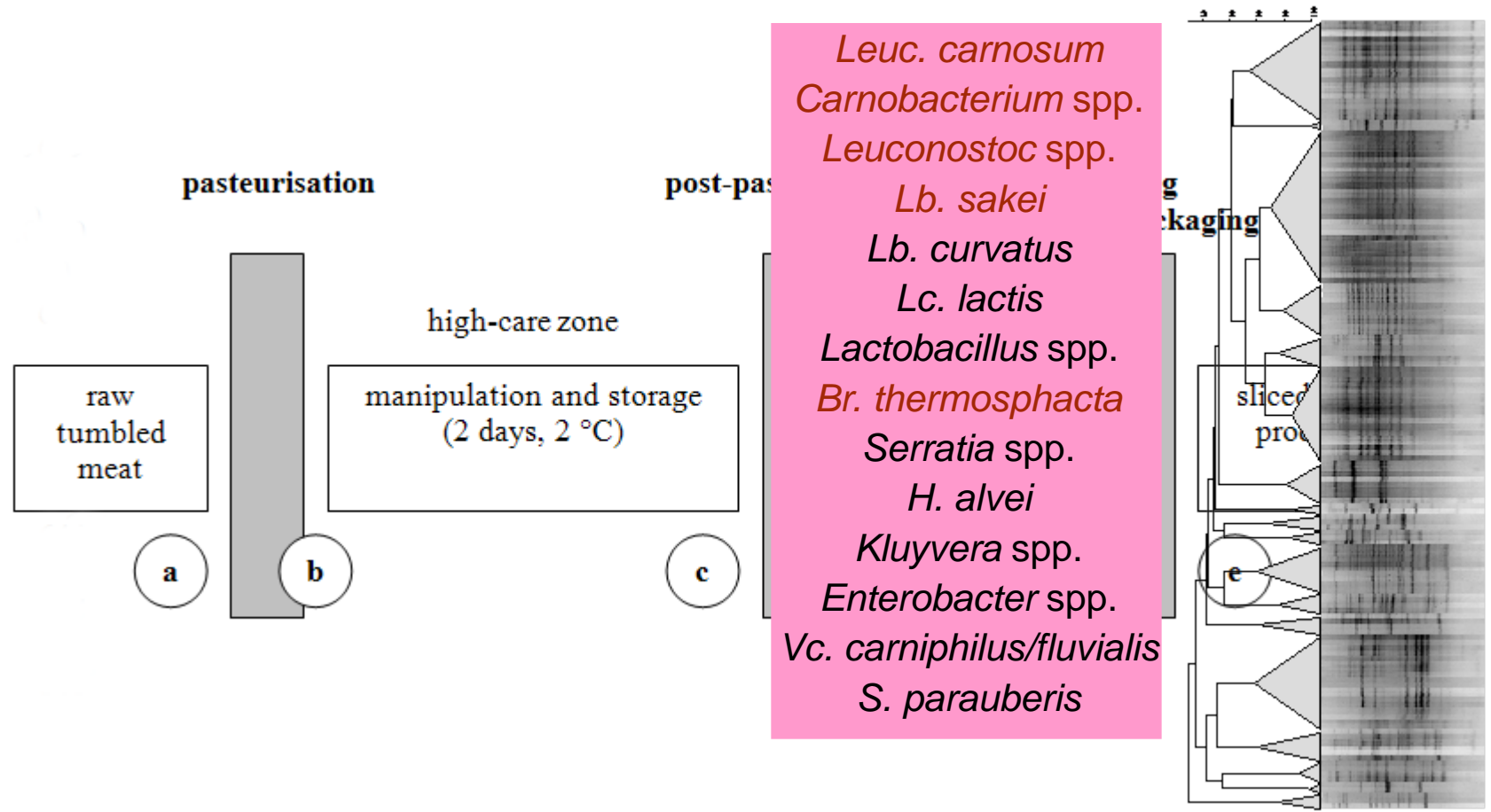
# Production process drying

Vasilopoulos *et al.* (2009) submitted

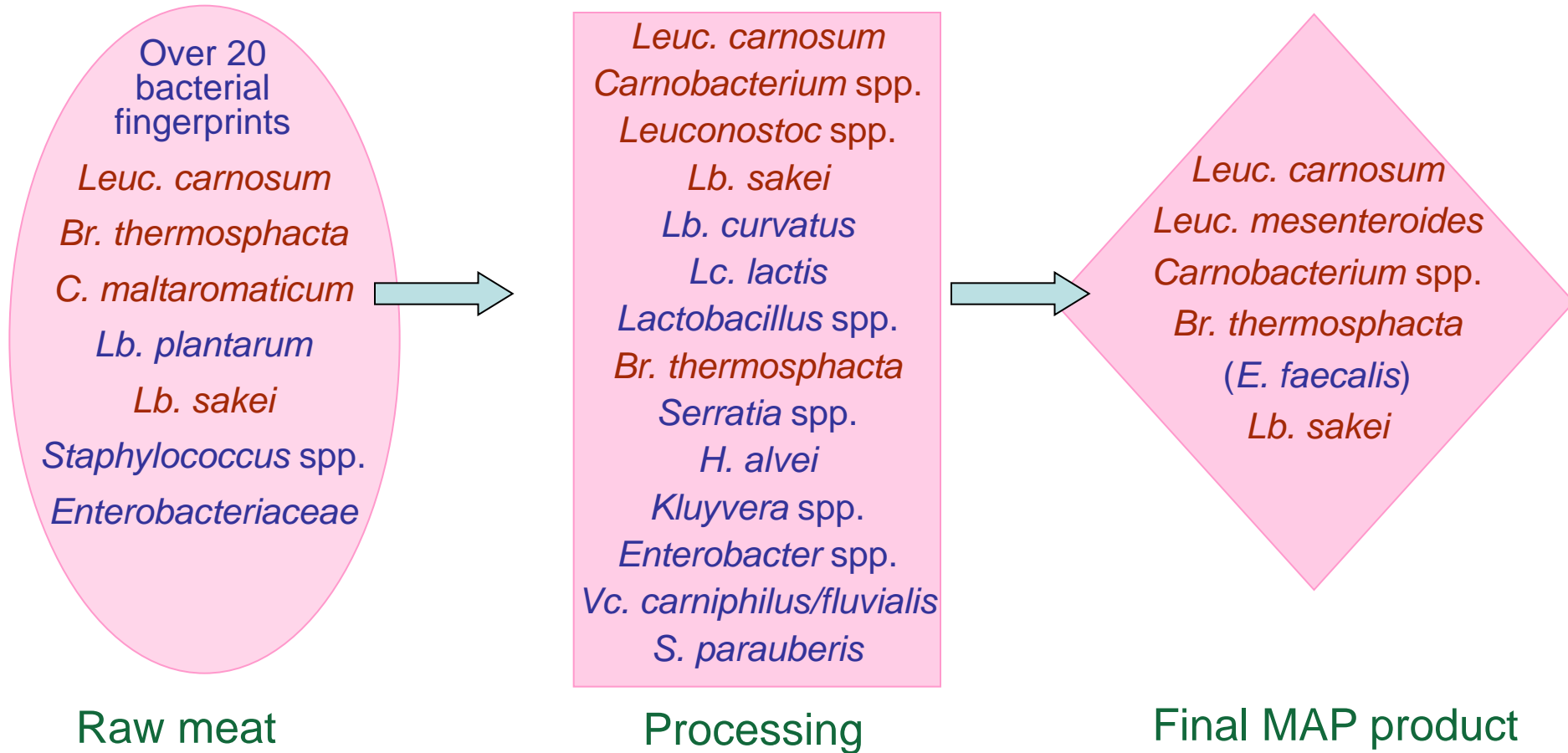


# Production process after post-pasteurisation

Vasilopoulos *et al.* (2009) submitted



# Production process overall evolution of microbiota



# Conclusions

- ❖ The processing area of artisan-type cooked ham is a harbour of microorganisms due to the interaction of the raw meat, sanitation practices, and handling by the personnel.
- ❖ The meat does not only act as a source of spoilage-inducing bacteria but also as a carrier.
- ❖ The MAP end-products are dominated by only a few highly competitive bacterial groups, outcompeting *Enterobacteriaceae* and resulting mostly in lactic-type spoilage.
- ❖ Whereas lactic acid is the main metabolic product, other metabolites that can play a role in the organoleptic rejection of the product were also detected.
- ❖ Storage temperature of the end-product affects the composition of the microbiota and metabolite production rates.



# Acknowledgements

- ❖ Research Council of the Vrije Universiteit Brussel (OZR, BOF)
- ❖ Fund for Scientific Research – Flanders
- ❖ Institute for the Promotion of Science and Technology in Flanders
- ❖ Laboratory of Food Chemistry and Meat Technology, KaHo Sint-Lieven
- ❖ Belgian meat company



# Final product (volatiles)

## SH-GC-MS

| Compounds          | Possible origin                          |
|--------------------|--|
| <u>Acids</u>       |  |
| acetic acid        | bacterial sugar metabolism               |
| <u>Alcohols</u>    |  |
| ethanol            | bacterial sugar metabolism               |
| 1-propanol         | lipid oxidative degradation              |
| 3-methyl butanol   | bacterial amino acid metabolism          |
| 1-heptanol         | lipid oxidative degradation              |
| 3-methyl-2-octanol | lipid oxidative degradation              |
| 2-ethyl hexanol    | lipid oxidative degradation              |
| 2-nonanol          | lipid oxidative degradation              |
| <u>Aldehydes</u>   |  |
| hexanal            | lipid oxidative degradation              |
| acetaldehyde       | bacterial sugar or amino acid metabolism |
| benzaldehyde       | amino acid degradation                   |
| <u>Ketones</u>     |  |
| 2-butanon          | lipid or amino acid degradation          |
| acetoin            | bacterial sugar metabolism               |

## SPME-GC-MS

| Compound                   | Possible origin                 |
|----------------------------|---------------------------------|
| <u>Alcohols</u>            |                                 |
| ethanol                    | bacterial sugar metabolism      |
| 3-methyl butanol           | bacterial amino acid metabolism |
| 1-heptanol                 | lipid oxidation derivative      |
| 6-methyl-5-heptene-2-ol    | enzymatic lipid oxidation       |
| 2-ethyl hexanol            | lipid oxidation derivative      |
| 2-nonanol                  | lipid oxidation derivative      |
| 1-octanol                  | lipid oxidation derivative      |
| 2-decanol                  | lipid oxidation derivative      |
| 1-nonanol                  | lipid oxidation derivative      |
| 3-fenoxy propanol          | cooking process derivative      |
| <u>Aldehydes</u>           |                                 |
| hexanal                    | lipid oxidative degradation     |
| nonanal                    | lipid oxidation derivative      |
| benzaldehyde               | amino acid degradation          |
| <u>Phenols</u>             |                                 |
| 2-methoxy phenol           | cooking process derivative      |
| 2-methoxy-4-methyl phenol  | cooking process derivative      |
| <u>Alkanes</u>             |                                 |
| dodecane                   | lipid oxidative degradation     |
| <u>Sulphuric compounds</u> |                                 |
| dimethyl trisulfide        | bacterial amino acid metabolism |
| <u>Furans</u>              |                                 |
| 2-methyl benzofurane       | cooking process derivative      |
| <u>Lactones</u>            |                                 |
| butyrolactone              | cooking process derivative      |
| <u>Ketones</u>             |                                 |
| acetophenone               | cooking process derivative      |

Leroy *et al.* (2009) FM 26:94-102