

# MODELLING THE DEVELOPMENT OF POTENTIAL SPOILAGE AND BIOPRESERVATIVE MICROORGANISMS ON PRECOOKED PASTA IN DIFFERENT CONDITIONS OF TEMPERATURE BASED ON CLASSICAL MICROBIOLOGY AND 16S rDNA METAGENOMIC



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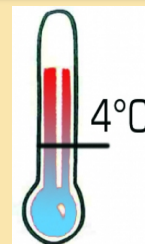
## INTRODUCTION

Food spoilage is more and more a matter of concern because of the economical loss and the non-selling of impaired products. The deterioration is mainly due to spoilage microorganisms who grow on the foodstuff and degrade it. The result can be a product with off odours, a disgusting aspect and off flavours. But this situation can easily be avoided by storing it in a temperature below 4°C. But the producer must keep in mind that the consumer's fridges are rarely at 4°C or less. This reality has to be taken in account by the professionals for the establishment of the Used-By-Date (UBD).



## OBJECTIVES

In this study we propose to observe the growth of potential spoilage or biopreservative bacteria on precooked pasta with the goal to illustrate the behaviour of these microorganisms in a temperature higher than 4°C or during a break of 4h at room temperature.



## MATERIAL

Strains used in this study were isolated in the laboratory at the UBD (14 days) from precooked pasta stored at 7°C :

- *Leuconostoc citreum* (mix of 3 isolates)
- *Lactococcus piscium*
- *Leuconostoc mesenteroides*

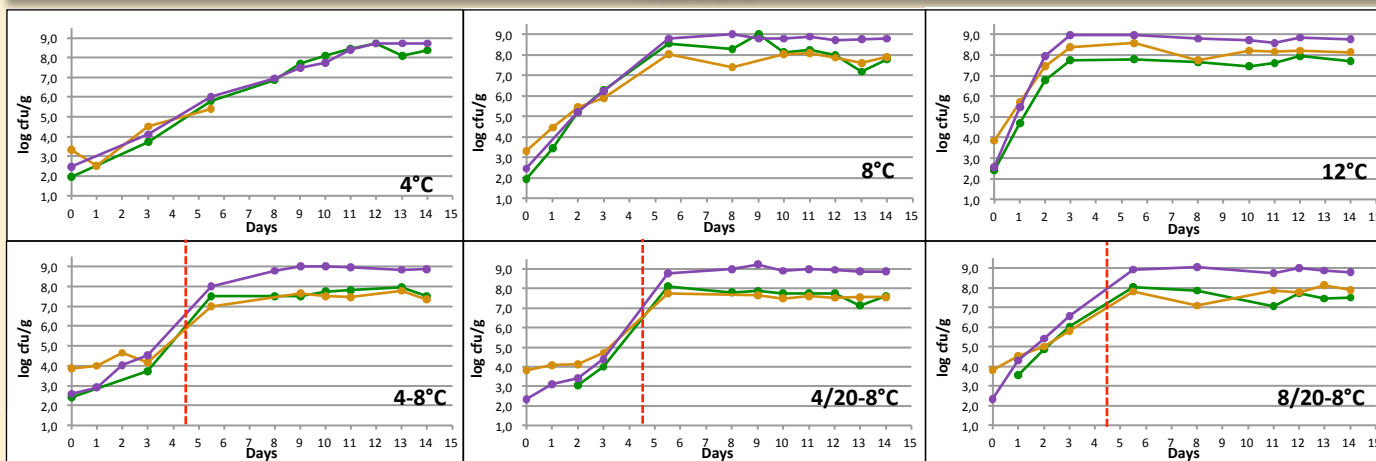


## METHODS

The pasta were precooked in a water bath at 95 ± 1°C during 3 min, cooled in sterile water (< 10°C) during 30 s and finally soaked 30 s in a solution containing all the strains with the goal of reaching a concentration of 3 ± 1 log of CFU/g on the product. After addition of 1% (v/w) of alimentary oil, they were packed under modified atmosphere (CO<sub>2</sub> 55% / N<sub>2</sub> 45%) and stored at different conditions of temperature : constant 4°C (4°C), constant 8°C (8°C), constant 12°C (12°C), 1/3 4°C – 2/3 8°C (4-8°C), 1/3 4°C – break during 4h at 20°C – 2/3 4°C (4/20-4°C) and 1/3 8°C – break during 4h at 20°C – 2/3 8°C (8/20-8°C). Each days during 14 days, 25 g of product was stomached in 225 mL of physiological water. From this mix, a total count was made on PCA (Plate Count Agar) at 22°C during 48h and an amplicon sequencing analysis based on the V1-V3 fragment of the 16S rDNA was realized on MiSeq apparatus (Illumina) (2x300b).



## RESULTS



— *Lactococcus piscium* — *Leuconostoc citreum* — *Leuconostoc mesenteroides* - - - - Breach of 4h at 20°C and/or transition to 8°C

A combination was made between the total count of the microflora on PCA at 22°C and the proportion of each strain given by targeted metagenomics, in order to obtain absolute counts for each strain. During the storage at 4°C, *L. piscium* and *L. mesenteroides* achieve the stationary phase in 12 days in contrast with the 8°C and 12°C storage where they reach it in 5 days and 3 days respectively. *L. mesenteroides* was the dominating strain at 8°C and 12°C attaining 9 log cfu/g. In opposite of 8°C incubation, *L. citreum* had a better growth than *L. piscium* at 12°C. The break at 20°C doesn't give clear changes in the growth curves, probably because it comes too late in the exponential phase of the strains.

## CONCLUSIONS

The 16S rDNA targeted metagenomics was a powerful tool for the monitoring of a microbial community at species level on a food matrix. It enables us to see the growth of each strain individually and to compare them to the one another. The behaviour of the strains at the different temperature conditions clearly shows the importance of keeping the cold chain at 4°C. *Leuconostoc mesenteroides* was identified as the dominant flora. Further studies will focus on the confirmation of the spoilage or biopreservative status of the strains by challenge-tests with chemical and sensory analysis.

