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FloPro project
Biopreservation of chilled food products using their own beneficial microbiota: the Belgian white pudding case

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INTRODUCTION

Food industry players are subject to a high competition. In this way, their food products have to meet the requirements in terms of quality and safety coming from regulation, food sector, and consumers. To do that, it could be interesting to add to food products a logistical flexibility or an ecological added value (Clean Label). Meanwhile, microbial food spoilage has become a major issue in the food industry. It has been estimated to be responsible for 25 % of the world's food supply losses. In this context, the need for innovative and natural solutions to protect food products have become stronger and microbial biopreservation prove to be a promising alternative.

OBJECTIVE

The main goal of FLORPRO project is to enable industrial partners to provide on the market products with a perfect sensory and microbiological control. In order to do so, we intend to act at several levels, either in the recipe of the product, or in its packaging, but especially in its microbiological composition. Several chilled food products were selected from different sectors of the food industry. Thus, thorough studies concerning these food products and their changes during aging were realized. Through this knowledge, we were able to select part of their microbiota with no organoleptic impact in order to protect them from bacterial spoilage and extend their shelf life. We'll focus on one of these products: the Belgian white pudding.

METHODOLOGY

Food products characterisation

- Microbiology: *Pseudomonas*, yeast & mould, total aerobic mesophilic bacteria count (Total viable count), *Brochothrix thermosphac*ta, lactic acid bacteria, *Enterobacteriaceae*.
- Metagenetics: Day 0 & end of the shelf life analysis
- Physico-chemistry: pH, gaz (O_2 and CO_2), lipid & protein alteration (biogenic amine, aldehyde, etc.)

Methodologies development

- Isolation of dominant bacteria
- Characterisation of bacteria of interest
  - ✓ Bibliography
  - ✓ Bioscreen™
  - ✓ RAPD
  - ✓ Genomes
- Inoculation method of the selected bacteria
- Monitoring of the inoculated bacteria
  - ✓ qPCR
  - ✓ Metagenetics

Process validation in pilot plant

- Inoculation of bioprotective strains
  - ✓ Alone or in mix
  - ✓ Different concentrations
- Modified atmosphere packaging
  - ✓ Ratio CO_2 / 0_2 / N_2
  - ✓ Ratio weigh / volume / gaz
- Aging (1/3 at 4 °C and 2/3 at 8 °C)
- Microbiological, physico-chemical, metagenetic and sensory analysis at Day 0 and at the end of the shelf-life

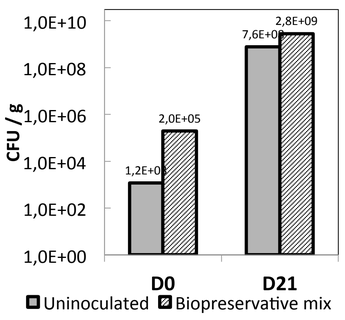
Industry trial

- Spraying on the surface of the product
- Aging (1/3 at 4 °C and 2/3 at 8 °C)
- Shelf-life analysis
  - ① **16S rDNA metagenetics → Technology: Illumina (MiSeq) ; Processing data: Mothur**
  - ② **Sensory analysis → Untrained panel (6 to 8 members) ; 2 attributes (appearance and odor) ; Scoring from 1(= dislike) to 5 (= like) ; ANOVA with post-hoc Tukey HSD**
  - ③ **Total Viable Count → PCA / 22 °C**
  - ④ **pH ; gas analysis (CO_2 and 0_2)**

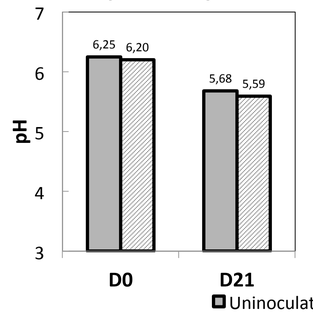
⑤ **Challenge test with bioprotective mix and *Listeria monocytogenes* (enumeration with specific media RAPID'L.mono (Biorad))**

RESULTS

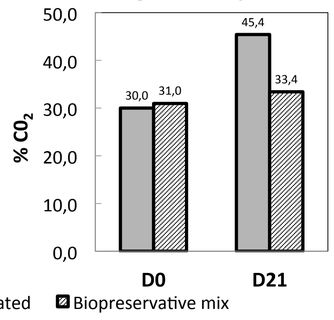
① **Total Viable Count**



② **pH analysis**



gas analysis



Tray bulging / swelling	+	-

Figure ① shows that the Total Viable Count at D_0 for the blank (uninoculated) is around 3 log CFU.g^{-1}. About he inoculated product, the biopreservative mix has been inoculated at 5 log CFU.g^{-1}. At the end of the shelf-life (D_{21}), all the samples are around 9 log CFU.g^{-1}.
Figure ② includes pH and gas analysis and shows that the pH values at D_{21} of the uninoculated and the biopreserved white pudding are very closed (5,68 vs. 5,59) and not so far from the D_0 values (6,25) so there is no over-acidification. Moreover, the % $C0_2$ of the tray containing the biopreserved product (33,4 %) is lower at D_{21} than the blank (45,4 %). Thus the swelling / bulging of the tray is stopped by the biopreservative mix.

③ **16S rDNA metagenetic analysis**

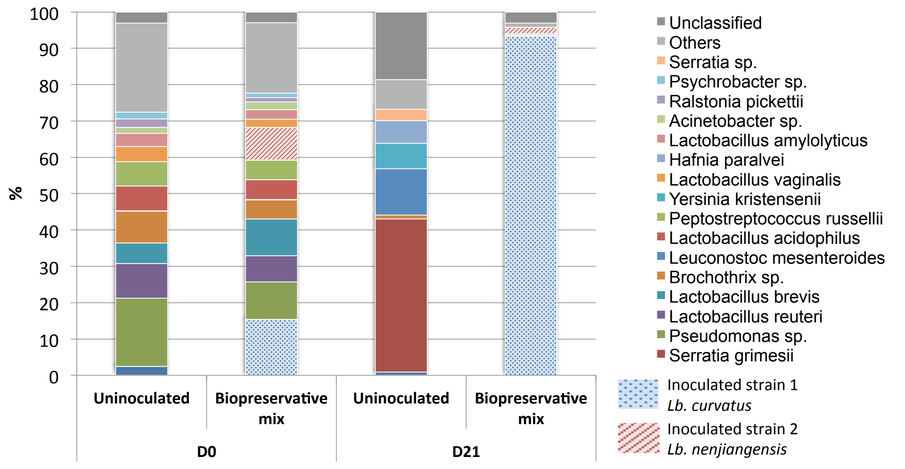


Figure ③ shows that, at D_0, white puddings are contaminated with different dominant microorganisms such as several *Lactobacillus* species, *Brochothrix* sp., and *Pseudomonas* sp.. While for the biopreservative mix, the inoculated strains are found: *Lb. curvatus* (15 %) and *Lb. nenjiangensis* (9 %). At D_{21}, the blank is dominated by *Serratia grimessi* up to around 40 % of the total microbiota but also with other species known to be involved in spoilage such as *Leuconostoc mesenteroides*, *Hafnia paralvei* and *Serratia* sp. Concerning the inoculated sample, *Lb. curvatus* is dominant (93 %). All the other species are kept below 1 % except for *Lb. nenjiangensis* (1.7 %) so the biopreservative mix allows a stabilisation of the majority species at D_{21}.

④ **Sensory analysis (global)**

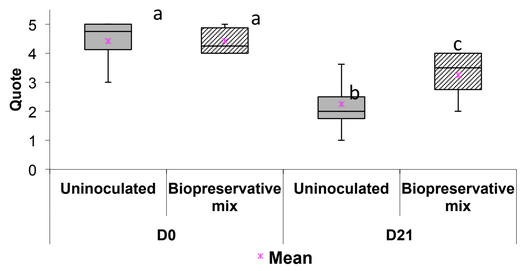
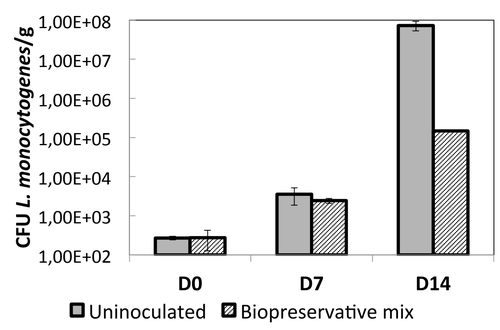


Figure ④ shows that at D_0 there is no difference in the sensory analysis (global) of the Belgian white pudding between blank and inoculated sample. At the end of the shelf life (D_{21}), biopreserved sample is significantly better quoted ($p<0.05$) than blank. Biopreservative mix seems to improve white pudding aging.
Figure ⑤ shows that when *L. monocytogenes* is inoculated between 2 and 3 log CFU.g^{-1} on the surface of a white pudding, it can grows easily to, at least, 8 log CFU.g^{-1} (D_{14}). But when *L. monocytogenes* is inoculated concurrently with the biopreservative mix (4 log CFU.g^{-1}), it grows is limited to 5 log CFU.g^{-1} showing that the mix is able to inhibit, in part (3 log), the growth of the foodborne pathogen.

⑤ **Challenge test *L. monocytogenes***



CONCLUSION

To conclude, these results show that the strategy developed in the FlorPro project, which involves the selection of bacteria naturally present in the product for a biopreservative purpose, seems to be a very promising way to enhance food products. Thus, the biopreservative mix has partially inhibited the development of potentially spoilage bacteria such as *Leuconostoc* spp. and *Serratia* spp. in the Belgian white pudding. This mix has also protected the tray from swelling and stabilised the sensory evaluation at the end of the shelf life. Moreover, a bioprotective effect has been show with a partial inhibition of *L. monocytogenes*. This strategy is being carried out on other food products with good results. This is an interesting strategy that is consistent with the current willingness of consumers of natural solutions for stabilization and protection of food products (Clean label).