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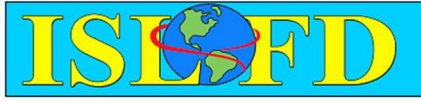
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The challenge of probiotics preservation: strategies and tools to identify stress mechanisms and sites of cell damage induced by dehydration processes

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Abstract

The market for freeze-dried lactic acid bacteria (LAB) starter cultures is constantly growing (~8 %/year) due to the increasing popularity of fermented foods and probiotics. However, many strains exhibiting interesting functionalities present low viability recovery after freeze-drying and insufficient long-shelf stability to be successfully commercialized. The stabilization steps of the manufacturing process of bacterial concentrates (freezing, freeze-drying, storage) induce several types of stresses (thermal, osmotic, mechanical, oxidative), which lead to cell injury and may cause loss of viability and functionality. Bacterial degradation can be minimized by pre-adapting bacteria during fermentation, adding protective molecules and applying favourable operating conditions during stabilization^[1], but the results are variable and highly strain-dependent. A better understanding of the preservation and degradation mechanisms appears essential for rationalizing the choice of molecules and process conditions. Moreover, since freeze-drying is a high-cost and energy-intensive process, the preservation of LAB starters needs to be revisited integrating product quality, process efficiency and environmental impact to propose sustainable stabilization processes. In this context, this presentation aims to share the strategies and tools developed in the frame of a European project to identify stress mechanisms, and sites of cell damage and improve the dehydration process conditions.

Introduction

In the frame of the European project PREMIUM (MSCA RISE, 77567), two main strategies were applied to improve bacterial functionalities and reduce environmental impacts: (i) comparing the effect of different dehydration processes (freeze-drying and spray drying) on sensitive and resistant LAB and (ii) using oligosaccharides with prebiotic properties and promising as protectants. The studied fructo-oligosaccharides and galacto-oligosaccharides obtained from the hydrolysis of plants and pulses present a diversified sugar composition in terms of molecular sizes, thus potentially protecting cells by different

mechanisms. To better understand the mechanisms of protection and identify sites of cellular damage, deep characterization of protected bacterial suspensions was applied using a multiscale approach (matrix, cell, and cell membrane) and the stabilization processes were considered as a combination of stresses.

Materials and Methods

Pilot fermentation and freeze-drying conditions were applied to identify improved stabilization conditions relevant to the industry. Physical (glass transition temperature-Tg- and water content of the matrix), biochemical (Fourier transform infrared -FTIR- features), and biophysical (phospholipid organization) properties were related to the biological activity (culturability, acidifying activity) quantified at different steps of the manufacturing process (freezing, freeze-drying, spray drying, and storage).

Results

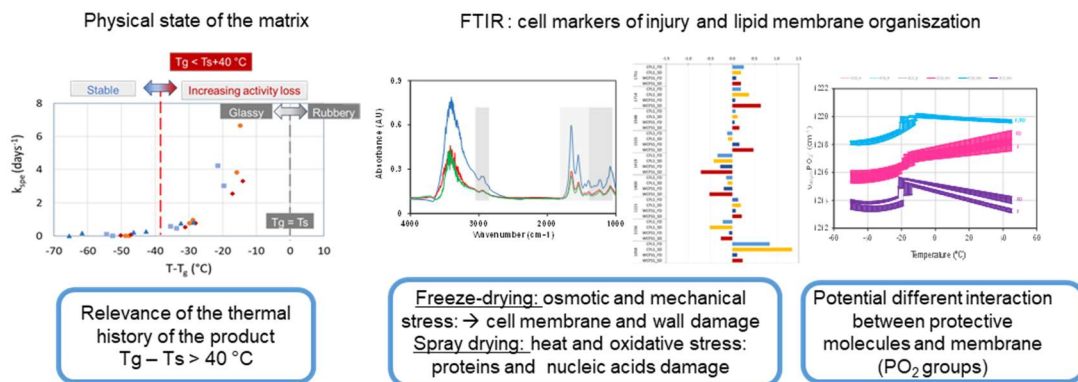


Fig. 1 Relevance of the matrix glassy state and FTIR features to identify markers of cell injury according to process stress and study interactions between protective molecules and membranes.

Conclusions

FTIR micro-spectroscopy in an aqueous environment appeared as a relevant tool to identify cellular components damaged due to exposure to process-associated-stresses. An improved formulation for protecting freeze-dried starters combined a high polymer content (high Tg) and small molecules (disaccharides). Ongoing studies involve membrane composition modulation and deeper investigation of its interaction with protective molecules during drying.

References

- [1] F. Fonseca, A. Girardeau, S. Passot. Freeze-Drying of Lactic Acid Bacteria: A Stepwise Approach for Developing a Freeze-Drying Protocol Based on Physical Properties, in: Wolkers W., Oldenhof H. (Eds) Cryopreservation and Freeze-Drying Protocols. Methods in Molecular Biology, vol 2180, New York, NY, 2021, pp.703-719.