

The Probiotic Viability Puzzle

Tlaleo Azael Marole, Ursula Thomashoff, Anthony Hobden, Thulani Sibanda, Elna Buys

Department of Consumer and Food Sciences, University of Pretoria, Pretoria, South Africa

Abstract

Viability is a prerequisite for any therapeutic benefits associated with the ingestion of probiotic bacteria. According to FAO and WHO guidelines, the health-promoting properties of probiotics require that there must be at least 10⁶ colony-forming units per gram of viable probiotic bacteria in the yoghurt at the time of consumption. This project focuses on the challenges in viability determination when probiotic *Lactobacillus rhamnosus* and *Bifidobacterium* species are mixed in the yoghurt together with the starter cultures and the technological challenges impacting *Bifidobacterium* species viability. A quantitative PCR (qPCR) method coupled with an improved version of propidium monoazide (PMAxxTM), and novel species-specific primers was successfully developed for the selective enumeration of the probiotics and starter cultures. The selective quantification of viable cells by PMAxxTM treatment at 100 µM, effectively inhibited and removed >99% of DNA from dead control cells. PMAxxTM-qPCR assay counts were comparable ($p > 0.05$) to the plate count for all four target organisms. Furthermore, oxidative stress pre-adaptation and high-pressure homogenization were evaluated as potential strategies to overcome the technological challenges impacting probiotic viability during yoghurt processing and storage. Oxidative stress-adapted variants of *Bifidobacterium* spp. were isolated after exposure to sublethal and lethal H₂O₂ treatments. The adapted variants were tested for their ability to withstand oxidative stress upon re-exposure (0.1 mM H₂O₂) using flow cytometry with SYTO 9 and propidium iodide (PI) fluorescent staining to measure membrane integrity as well as Cell ROX green staining to assess the oxidative state. The results suggest pre-adaptation in *Bifidobacterium* spp. can improve its resilience to oxidative stress, with a better adaptation in *B. animalis* possibly related to its genetic predisposition. The impact of high-pressure homogenization on the viability of probiotics in full-fat yoghurt under two-stage homogenization at 200, 250, and 600 bar is part of the ongoing work. These innovative approaches for viability optimization and determination would enable inline viability verification and improved probiotic quality assurance for processors.

PRESENTER BIOGRAPHY: ELNA BUYS PRESENTING ON BEHALF OF

Tlaleo Azael Marole is a Ph.D. food science student in the Department of Consumer and Food Sciences at the University of Pretoria, South Africa, under the supervision of Prof. Elna Buys and co-supervisor Dr. Thulani Sibanda. Mr Marole obtained his B.Sc. in Biotechnology at the National University of Lesotho and his M.Sc. in Food Science, Safety and Health with distinction at Heriot-Watt University, Scotland, U.K. Mr. Marole has over six years of work experience in the dairy industry and has worked for various dairy companies in South Africa where he was responsible for the management of quality and food safety systems. Mr. Marole's Ph.D. research focuses on enhancing the survival of probiotic bacteria and the production of bioactive metabolites in a multi-species synbiotic yoghurt during storage.

Ursula Thomashoff graduated with a Bachelor's degree in Food Science and a Bachelor of Science Honours degree from the University of Pretoria. She is currently completing her Master's degree at the same institution. Her expertise lies in Dairy Microbiology and Science. Ursula's groundbreaking research focuses on improving the survival of the probiotic *Bifidobacterium* spp. during yoghurt manufacturing through stress adaptation techniques. She has already presented her findings at prestigious conferences, including the 2022 ICFMH Food Micro Conference and the 2022 IDF International Conference.

Anthony Hobden is pursuing a master's degree in food science in the department of Consumer and Food Sciences at the University of Pretoria. He holds a BSc in Molecular Biology and Biotechnology from Stellenbosch University and a BSc (Hons) in Microbiology from the University of Pretoria. He has an interest in dairy technology and dairy microbiology.