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MASSEY UNIVERSITY

Stability of the probiotic *Lactobacillus paracasei* CRL 431 under different environmental conditions

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Devastotra Poddar

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Abstract

Probiotics are live microorganisms which provide health benefits to the host upon consumption. There is a wealth of information available on the health benefits associated with the consumption of probiotics. However, currently probiotic microorganisms are delivered mainly through refrigerated, short shelf-life products. When incorporated into ambient shelf-life products, the products generally fail to meet the regulatory criteria, which require probiotic bacteria to be viable in high numbers at the end of shelf-life. Storage temperature, oxygen and residual moisture content often result in loss of viability of probiotics during storage and distribution.

A preliminary study was carried out to explore the effects of matrix composition (fat, protein and carbohydrate) on the probiotic bacterial (*Lactobacillus paracasei* CRL 431) viability, during fluidized bed drying and subsequent storage. The finding suggests that whole milk powder provided a superior protection to bacteria during fluidized bed drying and subsequent storage, compared to skim milk powder or milk protein isolate. Moreover, water activity of the powders during storage played a key role in determining the probiotic viability.

The effects of drying techniques, moisture content and water activity on the storage stability of *L. paracasei* in a whole milk matrix were studied. Whole milk powder-bacteria mixtures were dried using spray drying, freeze drying or fluidized bed drying and stored at 25 ºC under controlled water activity (0.11 a_w, 0.33 a_w and 0.52 a_w) for 105 days. At 0.11 a_w, cell viability loss was minimal, while at 0.52 a_w viability was lost in all powders within 22 days. At the intermediate 0.33 a_w, there were marked differences among stored powders. Further, various analytical techniques (X-ray diffraction, FT-IR, Raman, NMR spectroscopy) were used to explore why and how structural differences in the matrix-bacteria mixtures, produced using different drying technologies, under different water activity storage conditions, influence bacterial viability. The results suggest that fluidized bed drying provided a better protection to the bacteria during storage, which was attributed to unique powder structure that reduced the absorption of water. The lower absorption of water resulted in the maintenance of a more rigid structure, which limited molecular mobility.

Lactobacillus sp. is known to accumulate large amounts of inorganic manganese which apparently provides defense against oxidative damage by scavenging free radicals. The

ability of *L. paracasei* to maintain viability during long term ambient storage may be enhanced by the ability of microorganism to accumulate manganese, which may act as free radical scavenger. To investigate this hypothesis, X-ray fluorescent microscopy (XFM) was employed to determine the changes in the elemental composition of *L. paracasei* during growth in MRS medium with or without manganese as a function of physiological growth state (early log vs. stationary phase). The results revealed that lower level of manganese accumulation occurred during the early log phase of bacterial growth compared with the stationary phase cells. The lower level of manganese accumulation was found to be related to the loss in bacterial viability during storage.

Manganese has been known to possess pro- and anti-oxidant properties, and understanding of the changes in the manganese oxidation state was considered to provide some further insights into the bacterial death mechanisms. In view of the relatively high concentration of manganese in lactobacilli, it was of interest to better understand the oxidation state, coordination number and ligands of the manganese in the bacteria. It was possible to characterize the changes of manganese within bacteria using XANES. The results confirmed that manganese present within *L. paracasei* is in Mn(II) oxidation state and no changes in the manganese ligands could be observed during storage.

In summary, the thesis provides a mechanistic insight into the ways to improve the stability of probiotics for application into ambient long shelf-life products. Future studies on tracking the genetic and proteomic aspects of the bacteria during storage might be useful for further understanding and process optimization.

Keywords: Probiotics, freeze drying, spray drying, fluidized bed drying, FT-IR, NMR.

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Abbreviations

a_w	Water activity
AO	Acridine orange
CLSM	Confocal laser scanning microscopy
DSC	Differential scanning calorimetry
EDS	Energy-dispersive X-ray spectroscopy
EELS	Electron energy loss spectroscopy
ESR	Electron spin resonance spectroscopy
EXAFS	Extended X-Ray Absorption Fine Structure
FT-IR	Fourier transform infrared spectroscopy
GC	Guanidine cytosine
MPI	Milk protein isolate
PI	Propidium iodide
SEM	Scanning electron microscopy
SMP	Skim milk powder
TEM	Transmission electron microscopy
TGA	Thermo gravimetric analysis
WMP	Whole milk powder
XANES	X-ray absorption near edge structure
XAS	X-ray absorption spectroscopy
XFM	X-ray fluorescence microscopy
XRD	X-ray diffraction