

**TITLE:** DYNAMICS OF PHYSIOLOGICAL STATES OF POTENTIALLY PROBIOTIC *LIMOSILACTOBACILLUS FERMENTUM* DURING REFRIGERATION STORAGE AND EXPOSURE TO SIMULATED GASTROINTESTINAL CONDITIONS IN FRUIT JUICES

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**ABSTRACT:**

This study evaluated the dynamics of physiological states of the potentially probiotic fruit-derived *Limosilactobacillus fermentum* 139 and *L. fermentum* 263 during a 28-day refrigeration storage (4 °C) and when submitted to simulated gastrointestinal conditions in apple and orange juices. Physiological functions were monitored with multiparametric flow cytometry using propidium iodide (PI), carboxyfluorescein diacetate (cFDA) and bis-1,3-dibutylbarbutiric acid (BOX). *L. fermentum* strains had sizes of >30% of cell subpopulations with non-permeabilized membrane and enzymatic activities (viable cells, PI-cFDA+) in apple and orange juice during storage. Sizes of cell subpopulations with permeabilized membrane without enzymatic activity (dead cells, PI+cFDA-) were low (<15%) in apple and orange juices during storage. Sizes of cell subpopulations with non-permeabilized and depolarized membrane (PI-BOX+) were decreased (14%) on day 28 of storage. Behavior of permeabilized and depolarized membrane cell subpopulation (PI+BOX-) was variable among examined strains in juices during storage. Both strains maintained high PI-cFDA+ cell subpopulation sizes (>35%) after exposure to ileum condition and viable counts of  $\geq 5$  log CFU/mL. PI-BOX+ cell subpopulation sizes were low (<13%) after exposure to ileum condition. Results showed that *L. fermentum* 139 and *L. fermentum* 263 are capable of maintaining a high population of physiologically active and functional cells in apple and orange juice during a 28-day refrigeration storage and when exposed to gastrointestinal conditions.

**Keywords:** *Limosilactobacillus*, physiological functions, fruit juice, flow cytometry, survival

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