ABSTRACT

Probiotics are live microorganisms that, when administered in adequate amounts, confer specific health benefits on the host. However, the harsh gastrointestinal environment and food processing conditions pose a severe challenge to their viability. To ensure the successful intestinal delivery of probiotics, encapsulation techniques are employed. The present research focused on the selection of a suitable probiotic strain, followed by matrix-based and crosslinked hydrogel modes for encapsulation. The encapsulation systems developed were characterised, and the probiotic release and survival under *in vitro* gastrointestinal conditions were evaluated. Finally, the selected encapsulation system was tested for product development.

The microbial strain, *Streptococcus thermophilus* NCDC 177, exhibited excellent probiotic potential, cell adhesion characteristics, folate production potential, and *in vitro* gastrointestinal tract survivability and was thereby selected for encapsulation studies. For the matrix-based mode of probiotic microencapsulation, ultrasonicated soy protein isolate (SPI) and sunflower oil (SO) emulsions were chosen as probiotic carriers based on the properties of emulsions [ζ-potential, Emulsifying Activity Index (EAI), Emulsifying Stability Index (ESI), Particle size, Viscosity, Conductivity, and Creaming and Foaming Properties]. After probiotic inclusion in the SPI-SO emulsions, it was subjected to electrospraying and freeze-drying to obtain microcapsules. The highest encapsulation efficiency (90.51%) and the lowest loss of probiotic viability were exhibited by the electrosprayed formulations (13% w/v SPI, 5% w/v SO, and 2% w/v Tween 80®). For the cross-linked network mode of probiotic encapsulation, calcium alginate hydrogels with κ-carrageenan and probiotic inclusion using gelation salts $CaCl_2$ and KCl were freeze-dried to obtain encapsulated probiotic beads. The optimised calcium-alginate/κ-carrageenan freeze-dried beads were composed of 3.118% w/v sodium alginate, 0.398% w/v κ-carrageenan, 3.819% w/v $CaCl_2$, and 0.503% w/v KCl, with an encapsulation efficiency of 87.81%. The probiotic microcapsules and beads obtained using different encapsulation methods were characterised using Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), Confocal Laser Scanning Microscopy (CLSM), and X-Ray Diffraction (XRD) techniques. The highest survival rate at the end of the *in vitro* gastrointestinal studies following the standardised INFOGEST protocol was exhibited by the electrosprayed SPI-SO probiotic microcapsules. The Korsmeyer-Peppas model best fitted the experimental data on probiotic release from the SPI-SO electrosprayed microcapsules. A probiotic health drink mix formula, labelled F-248, was developed by incorporating the SPI-SO microcapsules. It was composed of 40% spray-dried finger millet-milk powder flour, 20% SPI-SO microcapsules (~10.05 log $CFU \cdot g^{-1}$), 20% sugar, and 20% cocoa powder. The storage study confirmed its stability (45 days, maintaining >95% viability), *in vitro* digestion studies for the formula confirmed probiotic survival, and sensory evaluation confirmed overall acceptability of formula F-248. This research could successfully develop a suitable probiotic encapsulation technique and combine it with a drink mix with high potential as a functional food for commercial applications.

Keywords: Probiotics, Encapsulation, Microencapsulation, Soy Protein Emulsions, Calcium Alginate Hydrogels, INFOGEST *in vitro* Digestion.