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Microencapsulation of *Lactobacillus rhamnosus* by complex coacervates made from liquid whey and k-carrageenan

E. Aguirre-Mandujano¹, M. A. Herrera-Palencia², L. Hernández-Rodríguez¹, C. Lobato-Calleros¹ and A. Santos-Moreno²

¹Departamento de Preparatoria Agrícola y Posgrado en Ciencia y Tecnología Agroalimentaria, Universidad Autónoma Chapingo, Km. 38.5 Carretera México-Texcoco, 56230 Texcoco, Mexico

² Posgrado en Ciencia y Tecnología Agroalimentaria, Universidad Autónoma Chapingo, Km. 38.5 Carretera México- Texcoco, 56230 Texcoco, Mexico

Lactobacillus rhamnosus, is a microorganism having probiotic properties, it improves the microbial balance and confer beneficial effects to human intestinal health. Probiotics help prevent infections, intestinal tumors, cholesterol and stimulate immunity. However, there is a need to protect them, because the conditions of the gastrointestinal tract to which foods are subjected in probiotics, cause a significant decrease in viability. One way to protect them is microencapsulation using biopolymer wall materials. There are several studies that whey protein as microencapsulated materials are used, but there are few reports where the whey is used directly. In Mexico occur annually 10⁶ tons of whey, 47% are discharged to drain, causing major pollution. The aim of this study was to use whey for the development of a new system of protection for *L. Rhamnosus*, forming complex biopolymer whey-k-carragenin to increase cell viability when subjected to simulated gastrointestinal conditions.

Probiotic strains of *L. rhamnosus* were microencapsulated by complex coacervates (C_{P,K}) made from whey protein (P), obtained from a cheese factory and k-carrageenan (K), interacting electrostatically. The encapsulation yield and viability of the cells under simulated gastrointestinal conditions (low pH and high concentration of bile salts) were evaluated according to: (a) coacervate composition (weight ratios P/K: 2:1, 3.3:1 and 6.8:1), (b) particle size of the coacervate and (c) pH complex formation C_{P,K} (4.0 and 4.5).

The results indicate that the efficiency of protection afforded to the cells helped to maintain viability. Treatments C_{6.8:1} made to pH 4.0 showed the lowest survival rate of *L. Rhamnosus* (log CFU reduction of 2.0), and 7.1 to the free cells. The highest survival rate was showed C_{3.3:1} at pH 4.0 and 4.5 (log CFU reduction of 0.4). These treatments also showed the highest values of the viscoelastic modules (G' y G''), associated with the formation of more structured and compact networks and mechanically resistant. Therefore, this method is a good alternative for protection of probiotic cells.

Keywords: complex coacervation, probiotics, whey protein, microencapsulation

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Microorganisms of food ice cubes and their transfer to drinks

L. Settanni¹, R. Gaglio¹, N. Francesca¹, S. De Martino², C. Stucchi³ and G. Moschetti¹

¹Dipartimento Scienze Agrarie e Forestali, Università di Palermo, Viale delle Scienze 4, 90128 Palermo, Italy

²ICECUBE srl, c.da Canne Masche z.i., 90188 Termini Imerese (PA), Italy

³INGA, Istituto Nazionale Ghiaccio Alimentare, via di Capo le Case 3,00187 Roma, Italy

The present work was carried out to investigate the microbiological characteristics of the ice cubes produced at different levels: 1) home-made (HM) from domestic freezers; 2) produced by ice machines in bars and pubs (BP); 3) produced by ice industries (IN). BP samples were collected from the box stocks. HM and BP samples were transferred into sterile stomacher bags. IN samples were provided in the manufacturers' plastic bags. Samples were transported into thermal insulated boxes. Five samples per each production level, forming a total of 15 samples (HM1-HM5, BP1-BP5, IN1-IN5), were collected in duplicate in two consecutive months. Each ice sample was thawed in 1 L sterile Dhuram's bottle at room temperature and subjected to the membrane filtration analyses. Total mesophilic microorganisms (TMM), total psychrotrophic microorganisms (TPM), pseudomonads, members of the Enterobacteriaceae family, coliforms, enterococci, yeasts and moulds were investigated. When the amounts of colonies were uncountable, 1 mL of sample was directly inoculated into agar media. All results were expressed as colony forming units (CFU)/100 mL of thawed ice. TMM were in the range 100-9600, 312-6300 and 130-4000 for HM, BP and IN samples, respectively. Three HM and two IN samples were negative for the presence of TPM. The highest concentration (960) was found for IN2. Pseudomonads were detected in all HM samples but the highest levels were registered for BP1 (390) and IN2 (384). Except IN4, Enterobacteriaceae were found in all samples. All INs and 4 HM samples did not displayed coliforms. By contrast, they were hosted in all BP samples, ranging from 1 to 184. Enterococci were never found in HM samples, but detected in two INs and 3 BPs. Except IN1, moulds were always registered, while yeasts developed from the majority of HM and IN samples and two BP samples. The colonies representative for the different morphologies were randomly picked up from plates, purified to homogeneity and subjected to a phenotypic grouping. Yeasts and bacteria were subjected to the genetic identification by sequencing of D1/D2 domains of 26S rRNA gene and partial sequencing of 16S rRNA gene, respectively, while moulds were identified phenotypically. So far, the species mostly represented among bacteria, as evaluated only by the forward 16S rRNA gene sequence, were *Bacillus* spp., *Pseudomonas* spp., *Pantoea* spp., *Pantoea agglomerans*, *Enterococcus faecium*, and *Agrobacterium tumefaciens*. *Candida intermedia* and *Pichia guillermondii* were identified among yeasts and *Penicillium* spp. among moulds. The work was also aimed to monitor the microbial transfer from ice to humans through drinks. To this purpose, each microorganism was inoculated singly in sterile mineral water to produce contaminated ice cubes using disposable ice cube trays. Inoculum occurred at the highest concentrations found in the ice cubes analysed. The concentrations of the microorganisms were followed in six different types of drinks, including alcoholic (vodka and whiskey), moderate alcoholic (Martini), sparkling (tonic water), sugary (peach tea) and sugary sparkling (coke) drinks. In order to simulate the contamination of drinks by ice during consumption, six ice cubes (corresponding to 60 mL) containing each microorganism were added to 100 mL of each drink (simulating a bar administration) in sterile cups and, after 1 h, the entire volume was analysed by membrane filtration. A physiological solution was used as control. So far, the tests were performed with *Penicillium* spp. and *P. agglomerans*. *Penicillium* was not influenced by the different drinks, since, after 1 h, its level did not change. Regarding *P. agglomerans*, which is an opportunistic pathogen causing urinary tract infections, its concentration in peach tea was superimposable to that found in physiological solution, while it decreased in all other drinks. In particular, the concentration of this bacterium almost halved in vodka, coke and tonic water, diminished consistently in Martini and completely disappeared in whiskey. Experimentations are in progress to determine the behaviour of the other microorganisms in these systems. These data evidenced that the worst hygienic characteristics were found in BP samples, while the majority of ice cubes produced in specialized industries were characterized by acceptable microbiological parameters. This work indicated that the concentration of *P. agglomerans* is reduced by alcohol and CO₂, but further *in vivo* assays are necessary to better clarify their role on the other ice microorganisms.

Keywords: cross-contamination; drinks; ice cubes; microorganisms