Nutritional modulation of colon cancer with a microencapsulated symbiotic

M Chávarri[1]*, I Marañón[1], F Marzo[2], E Urdaneta[2], MC Villarán[1]
[1] LEIA Technological Development Centre [2] Universidad Pública de Navarra - Vitoria, SPAIN (mariac@leia.es)

1.- Introduction

Colon cancer incidence continues increasing in the western countries and, if the precocious diagnosis favors the survival of the affected ones well, the rate of mortality still is high. For that reason, the prevention is the strategy more adapted to reduce the prevalence of this tape of cancer. This prevention is due to make on the knowledge of the nourishing components that have a chemoprotective activity. In this context, the functional foods, in special the symbiotic ones (SYM), can be of interest in the degenerative diseases among them the cancer.
Recently it has been postulated that the combination of probiotics and prebiotics, defining itself as symbiotic the new product thus obtained, is the base to elaborate new functional foods (Rastall et al., 2002). An example is the drink fermented from the "Tibetano fungus", to which antiinflammatory activity is attributed (Diniz et al., 2003). In this sense, a probiotic like *Lactobacillus gasseri* and a flavonoid like quercetin are candidates for new symbiotic products.
For probiotic bacteria to exert their therapeutic benefits, they have to reach the intestine in a viable state. This involves surviving harsh conditions in gastric acidity as well as bile salts, enzymes, toxic metabolites, bacteriophages, antibiotics and anaerobic conditions. In order to protect the cells from these detrimental factors, an approach providing probiotic cells with a physical barrier is receiving considerable interest. Micro-encapsulation is a process in which cells are retained within an encapsulating membrane to reduce cell injury or cell loss (Shah, 2002; Kailasapathy, 2002). The physical retention of cells in the encapsulating matrix facilitates the separation of cells from direct exposure to the adverse factors while at the same time allows the diffusion of nutrients in and out of the matrix and thus helps support the viability of the cells. The thinness, small diameter and semipermeable nature of the encapsulating membrane are advantageous to this purpose. Encapsulation tends to stabilize cells and can potentially enhance the viability and stability in the production, storage and handling of lactic acid cultures.
The aim of the study was to develop a method for producing chitosan-coated alginate microspheres using food grade polymers and to investigate the effect of this chitosan-coating on the survival of probiotic *lactobacillus gasseri* during exposure to the adverse environment found in the gastro-intestinal conditions.

2.- Material and methods

2.1. Materials

Sodium alginate (W201502 SIGMA), $CaCl_2$ (211221 Panreac), Chitosan (C3646 SIGMA), Acetic acid, sterile reconstituted skimmed milk (crioprotector agent), MRS Broth (69966 SIGMA), MRS Agar (69964 SIGMA), Quercetin (Q0125 SIGMA).
Simulated gastric juice (SGJ) was prepared by dissolving pepsin (Pepsin from porcine gastric mucosa, P-7000, SIGMA) in saline to a final concentration of 0.3 g/l and adjusting the pH to 2.0 with concetrated HCl. Simulated intestinal juice (SIJ) was prepared by dissolving bile (Bile extract porcine; B8631, SIGMA) in phosphate buffer and adjusting the pH to 6.0.

2.2. Preparation of probiotics for microencapsulation

Lactobacillus gasseri was purchased in lyophilized form from Spanish Type Culture Collection (CECT). The culture was rehydrated in 5 mL MRS broth and incubated at 37 ºC for 24 h to obtain a cell density of about 10^8 colony forming units per ml (cfu ml^{-1}). Harvesting of cells was done by centrifugation at 1500*g* for 5 min at 4 ºC and after discarding the supernatant of spent culture broth, the cell pellet was washed in s sterile saline solution and centrifuged again under the same conditions. The pellet was resuspended in a sterile saline solution and the concentrated cell suspension was used immediately for microentrapment.
Fresh cell suspensions were prepared for each experiment and enumerated by pour plating in MRS agar. Plates were incubated at 37 ºC for one day.

2.3. Microencapsulation of probiotics

The extrusion technique of microencapsulation was derived from Krasaekoopt (2004) using alginate as supporting matrix. Chitosan-coated alginate microspheres was prepared as described by Zhou (1998), and then the beads were suspended in crioprotector agent. The beads were then lyophilized with a freeze-drier at a condenser temperature of -40 ºC and 1 mbar vacuum for about 18 h.

2.4. Encapsulation yield

The encapsulation yield (EY), which is a combined measurement of the efficacy of entrapment and survival of viable cells during the microencapsulation procedure, was calculated as

$$EY \equiv \frac{N}{N_0} \times 100$$

Where N is the number of viable entrapped cells released from the microspheres after lyophilization, and No is the number of free cells added to the biopolymer mix during the production of the microspheres.

2.5. Survival assay of free and microencapsulated probiotics in simulated gastric and intestinal juice

Free and microencapsulated bacteria were added to SGJ and SIJ, and incubated for 5, 30, 60, 90 and 120 min at 37 ºC with shaking. Surviving bacteria after each set time interval enumerated by pour plate counts in MRS agar at 37ºC for one day.

2.6.- Statistical analysis

The mean values and the standard deviation were calculated from the data obtained with 10 trials. These data were then compared by Independent Samples T Test method.

3.- Results and Discussion

3.1. Microencapsulated

Encapsulation yields (EY) for viable cells of *L gasseri* were low for symbiotic (Quercetin+L gasseri) and for *L gasseri* without crioprotector, however, EY was significantly ($p<0.001$) higher for *L gasseri* with crioprotector (Table 1).

Microsphere type	Microsphere size (µm)	Encapsulation yield (%)
Quercetin + *L gasseri*	523,64 ± 23,58	59,1 ± 1,9
Quercetin	518,90 ± 20,98	–
L gasseri without crioprotector	352,26 ± 14,52	77,8 ± 2,3
L gasseri with crioprotector	361,18 ± 25,39	92,5 ± 3,1

Table 1 : Size and encapsulation yield of differents microspheres

3.2. Simulated Gastric and Intestinal Juice

Encapsulation in chitosan-coated alginate microspheres significantly ($p<0.001$) improved survival of *L gasseri* (Fig. 1). Cell survival after exposure to SGJ for 5 min was significantly ($p<0.001$) higher for microencapsulated *L gasseri* with crioprotector (94%) than for free L gasseri (78%).
After the initial losses, the populations of free Lactobacillus decrease for all treatment and after 2 hours incubation in SGJ free cells were not survived. By another side, after the sequential exposure to SGJ (120 min) followed by SIJ (120 min), it was observed an elevated survival percentage of microencapsulated *L gasseri*.

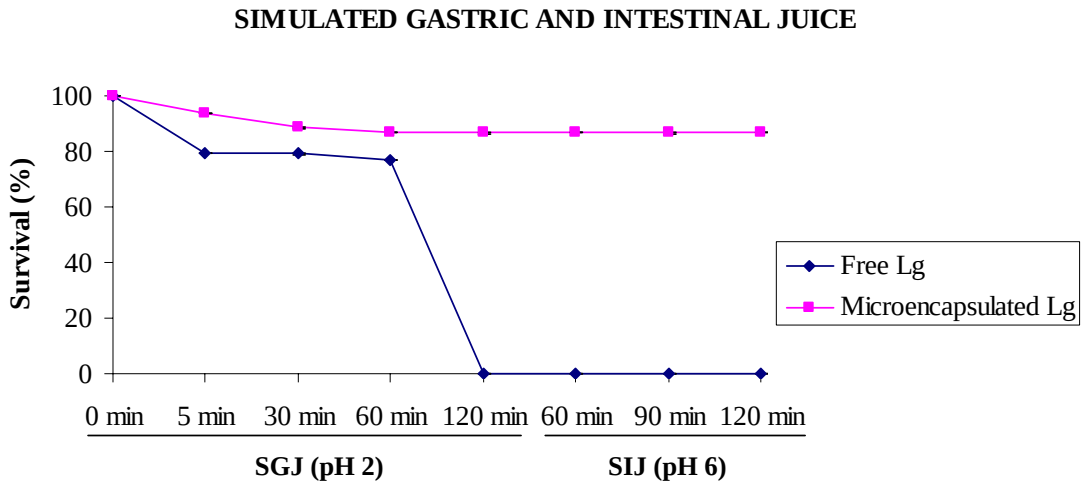

Fig.1. Survival of free and alginate encapsulated *L gasseri* during exposure to simulated gastric and intestinal juice at 37 ºC. Survival (%) represents the percentage of cells surviving relative to the initial population. Means (n=10) ± standard desviation (n-1).

3.3. Discussion

One of the technologies, initially applied in the pharmaceutical sector, the microencapsulation, appears like a viable alternative that applied in feeding could guarantee the protection of the active molecules that are added to foods (Sande, 2005).
The aim of this research was to evaluate the survival of *Lactobacillus gasseri* encapsulated in chitosan-coated alginate beads in simulated gastric and intestinal juice.
As shown in Table 1, the encapsulation yield for viable cell of symbiotic was very low. These results, together with a previous study about the survival of symbiotic during storage at 4 ºC, demonstrated that *L gasseri* microencapsulation with quercetin does not survive. Owing to this, it was produced by separated, the microencapsulation with quercetin and the microencapsulation with

L gasseri. Anyway, in order to improve encapsulation yield use crioprotective agent during freeze-drying and it was obtained to increase the survival of probiotic (Selmer-Olsen et al., 1999).

4.- Conclusions

In conclusion, the microencapsulation of *L gasseri* with alginate and a chitosan coating offers an effective means of delivering viable bacterial cells to the colon and maintaining their survival during simulated gastric and intestinal juice (Anal et al., 2007; Simonoska et al., 2008).

5.- References

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