Viability of encapsulated probiotics; effect of encapsulation materials and food matrices.

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INTRODUCTION

Probiotics are live organisms, which are associated with beneficial health effects, and may be selected for prevention and treatment of diseases (Jiménez 2008). This has stimulated interest in the development of dairy products which contain these bacteria to improve the health for the general population and children.

However, the main problem when probiotics are introduced into food matrices is their low resistance to technological and environmental conditions. Within this context, microencapsulation which is defined as a process in which the cells are retained within an encapsulating matrix or membrane, is currently drawing more attention for being a method to improve the stability of probiotic organisms in functional food products, during both processing and storage.

The aims of this study were to examine the influence of various encapsulation technologies on the viability of *Lactobacillus plantarum* (NIZO collection) during production, and to evaluate the viability of probiotics in storage tests while introduced into different types of food matrices (infant formula and yogurt).

MATERIALS AND METHODS

The spray drying process was performed with a Buchi B 290 spray dryer at constant air inlet temperature of 140 ºC and outlet temperature of 70º C. Four different microcapsules were obtained through the following combination of encapsulating agent: Inulin-Fructooligosaccharide(FOS)mixture (Orafti®Synergy 1), skimmed milk, maltodextrin (19DE), and Man, Rogosa, Sharpe agar (MRS).

The electrospinning (high voltage spinning) process was performed using a pullulan solution. The flow of the pump was set at 0.5 ml/h and the selected voltage was of 13kV.

The Encapsulation Yield (EY) for each formula was calculated (Picot 2004).

The particles were submitted to an accelerated storage test at 37ºC in the absence or presence of infant formula. Particles with the best viability and low moisture were chosen to be also introduced into two types of yogurts (yogurt 1: Dutch 'volle yoghurt'; yogurt 2: Dutch 'boeren yoghurt'). The viability of these probiotics was also evaluated during their storage for 4 weeks at RT and 4ºC, respectively. In the case of yogurts, the pH was also determined.

The morphology and particle size of the particles were observed with a microscope. The mean diameter over the volume distribution ($D_{[4.3]}$) were determined by laser diffractometry (Mastersizer 2000, Malvern).

The moisture content of spray dried powders was determined through oven drying at 102 °C until reaching constant weight.

RESULTS

Encapsulation yield

The survival rate after encapsulation process ranges from 0.12% to 24.6%. A large mortality occurred with Orafti®Synergy 1 and Maltodextrine particles as illustrated by the very low EY (<1%) (Table 1).

Table 1: Encapsulation yield

Samples	EY%
Orafti®Synergy 1	0.12±0.01
Skimmed milk	5.36±0.81
Maltodextrine 19	0.31±0.07
MRS	3.7±0.87
Pullulan	45.2±7.6

Accelerated Storage test

Survival to drying during the encapsulation process was not necessarily related to storage survival. Inulin: FOS and Skimmed milk particles showed the best viability during the accelerated storage test. Inulin: FOS and Skimmed milk may help probiotic during storage (Fritzen-Freire 2012). Prebiotics (Inulin: FOS) decrease the moisture content and water activity in the particles.

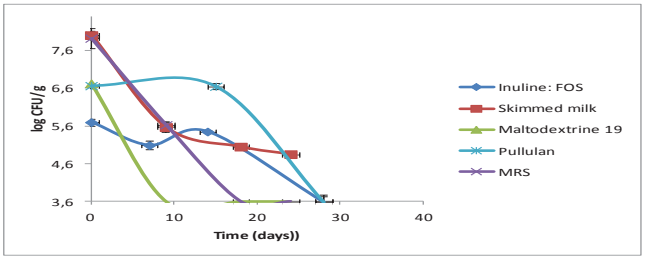

Figure 1: Accelerated storage test at 37ºC.

Properties of microcapsules

The moisture content has to be roughly less than 4% H_20/g in order to be categorized as stable (Simpson

2005).Only the powder made with Orafti®Synergy 1 complies with this requirement (data not shown).

Microphotographs of microparticles showed a spherical shape, with a defined limit. Some particles are adhered to each other. Spray-dried particle sizes ranged from 10.74 to 33.34 µm. In contrast, the pullulan fibres have a particle size less than 1 µm.
These particle sizes are suitable for the application in food, since it has been reported that soft, rounded particles are not perceptually gritty up to about 80 µm (Lawless 2010).

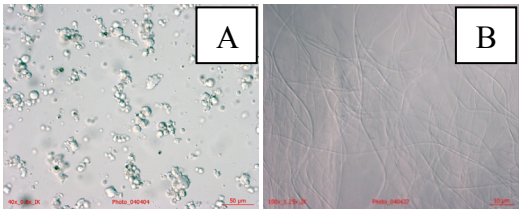

Figure 2: Microphotographs of A) Orafti® Synergy 1 particles, and B) pullulan fibres.

Food matrices storage tests

Survival during storage improved when mixed with Infant Formula. This demonstrates the importance of evaluation of survival in the food application where it will be used. Here, the difference can be explained by the difference in moisture content of the infant formula and the encapsulated particles; the infant formula can reduce the overall moisture of the sample.

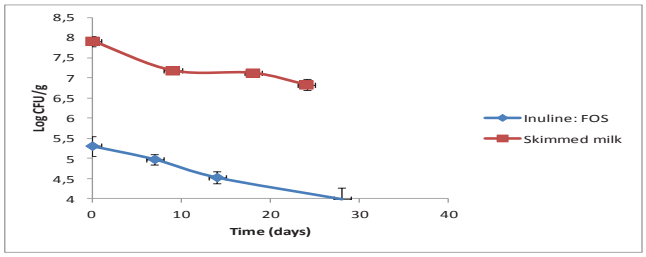

Figure 3: Accelerated storage test at 37ºC; mixed with Infant Formula.

The measurements were also performed at RT. The count of viable probiotic cells is more than 6log CFU/g (recommended levels for probiotic food throughout the whole storage time) during 4 weeks at RT in the case of skimmed milk particles (6.8±0.1 log CFU/g). The Inulin: FOS microparticles show lower level (4.0 ± 0.3 CFU/g). Fritzen- Freire (2012) use Orafti®Synergy and skimmed milk too. Our results differ from them. In our case, the EY of skimmed milk particles is higher than the EY of inulin: FOS and the storage data for this formula is better too. Here, we used a different species of bacteria (*Lactobacillus*) than Fritzen- Freire (*Bifidobacterium*). The results show that different strains can react differently to encapsulation processes and storage.
The viability of probiotic introduced into the yogurts is reduced faster than in the case of the infant formula (figure 4). It may be due to the low pH of the yogurts (≈4) and the water activity.

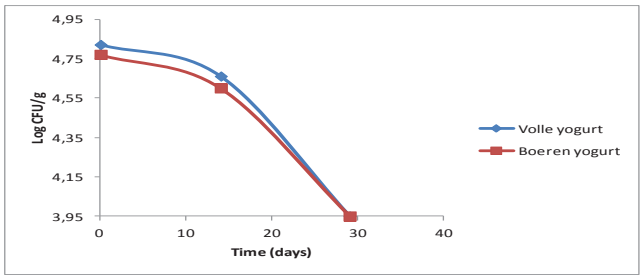

Figure 4: Storage test of Inulin: FOS introduced into the yogurts at 4ºC.

CONCLUSIONS

Comparison of the various encapsulation materials, skimmed milk is the best carrier for the microencapsulation of *L. plantarum*. Since the particle size is suitable for application in food and the viability of encapsulated probiotic stay at 6 log cfu/g during 1 month.

Furthermore, the work demonstrates that the viability of the probiotics during shelf life is influenced by the food matrix in which it is introduced, and that different species show different survival while using similar encapsulation technologies.

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