O3-3	**Evaluation of milk protein matrices to protect probiotic cells in simulated gastric fluids**

Burgain J[1#], Gaiani C [1*], Taube K [1], Jeandel C [1], Cailliez-Grimal C [1], Ghoul M.[1], Scher J[1]
[1] Lorraine Univ., LIBio, Lab. Ingénierie Biomolécules, Vandoeuvre les Nancy, France
* claire.gaiani@ensaia.inpl-nancy.fr # jennifer.burgain@ensaia.inpl-nancy.fr

INTRODUCTION

In recent years, consumers expect their food to be healthy and to prevent illness. It is obvious that functional foods display a rising interest with a dynamical development in the food sector. Health-based products with probiotics are getting and represent about 65% of the world functional food market. However, the probiotic market is affected by global regulatory requirements which have become stricter in recent years. For industrials, this results to an obligation to take into account cell viability and probiotic function to make a health claim.

The viability of probiotic cells is of paramount importance because to have their beneficial effects on the host's health they must stay alive as far as their site of action. Many reports indicated that there is poor survival of probiotic bacteria in products containing free probiotic cells. Providing probiotic living cells with a physical barrier to resist adverse environmental conditions is therefore an approach currently receiving considerable interest. Microencapsulation is a powerful technology which has been developed for use in the food industry and allows the protection of bacterial cells.

Probiotic bacteria have been introduced into a number of dairy products and the milk proteins have shown to be a good protector for the cells (Burgain 2011). For this reason, it is interesting to develop an encapsulation system using dairy proteins for having a biocompatible vector made of natural polymers.

MATERIALS AND METHODS

Bacterial strain and culture conditions

The two probiotic strain *Lactobacillus rhamnosus* GG and *Lactobacillus rhamnosus* GR-1 were used in this study. Harvested cells were stored in a lyophilized form and used directly for encapsulation.

Encapsulation procedure

Probiotic bacteria have been encapsulated using an emulsification method (Heidebach 2010).

The principle of the technique is based on using dairy proteins (in mixture with probiotic bacteria) which have been put in contact with rennet at low temperature. This allows keeping a liquid system where κ-casein is cleaved by the chymosin. After that, dairy proteins have been emulsified in a cold oil to form water in oil emulsion. Thermal induction of enzymatic coagulation allows proteins flocculation and provides microparticles where probiotics are dispersed in coagulated dairy proteins.

Methodology of experimental design

In this study, mixture experimental design was applied to the analysis of the microparticle resistance in simulated gastric conditions. A three-component mixture was set up, corresponding to the amount of micellar casein, whey proteins in a native or denaturated form (Table 1).

Table 1: Description of upper and lower constraints required for the construction of domain of interest

Component (w%)	Limits (%)	
	Lower	Upper
Micellar casein	80	100
Native whey protein	0	20
Denaturated whey protein	0	20

The domain is represented by a triangular region consisting of 10 experimental points (Figure 1). For the construction and analysis of the mixture design, the NemrodW software was used.

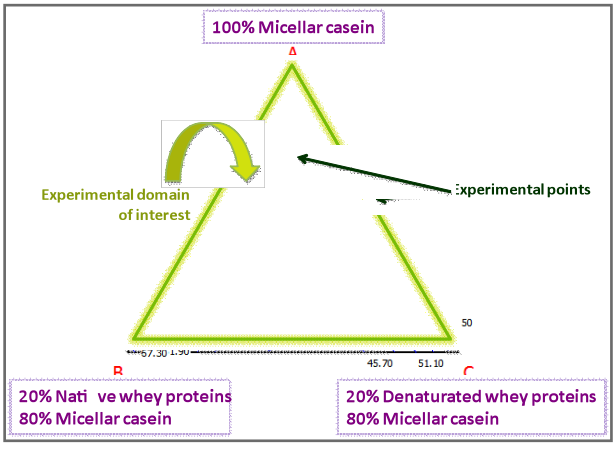

Figure 1: Graphical representation of the experimental domain and positioning of experimental points

Survival to gastric conditions

2g of microparticles were incubated in 200 mL simulated gastric fluid with pepsin at 37°C, under stirring during 120 minutes. The mixture is pumped to a particle analyser ("Qicpic") where the microparticles were analysed (Figure 2).

A laser diffraction system coupled with an image analysis processor was used for measuring the shape and the size of a representative number of microparticles.

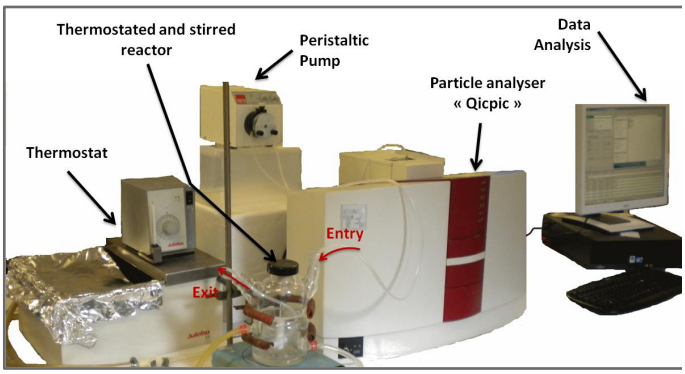

Figure 2: Installation allowing the monitoring of "in situ" microparticles digestion

RESULTS AND DISCUSSION

The use of different content in milk proteins for encapsulation shows that the obtained microparticles have different properties. First of all, the size and the shape of the particles vary as a function of composition. It results in resistance performance variables during simulated gastric conditions.

At the beginning of digestion, there is an effect of swelling of the microparticles and / or aggregation. This results in an increase in the average particle size. Subsequently, the size of the particles is reduced to a greater or lesser extent during the first 40 minutes. For the 80 minutes remaining, the size of the microparticles remains almost the same. However, loss of particle integrity during the first minutes has occurred by several physicochemical mechanisms depending on the matrix composition. Indeed, we noticed two main processes that are fragmentation or erosion.

The first case of digestion that can be observed in our study is presented on Figure 3.

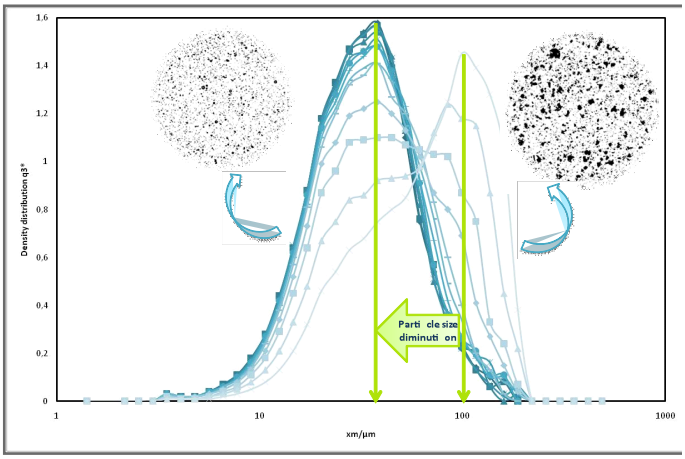

Figure 3: Monitoring of microparticle size during simulated digestion: first case

When the particles are placed in contact with the acidic environment, they tend to aggregate. After this phenomenon, the particles are fragmented and we can note a creation of a new population of particle at a lower size. Nevertheless, we notice that the decrease in size is consistent with the preservation of a structure that protects bacteria. On the video given by the apparatus, we can follow all these steps but only two images illustrate what we can observe on Figure 3.

The second case of digestion that can be observed in our study is presented on Figure 4.

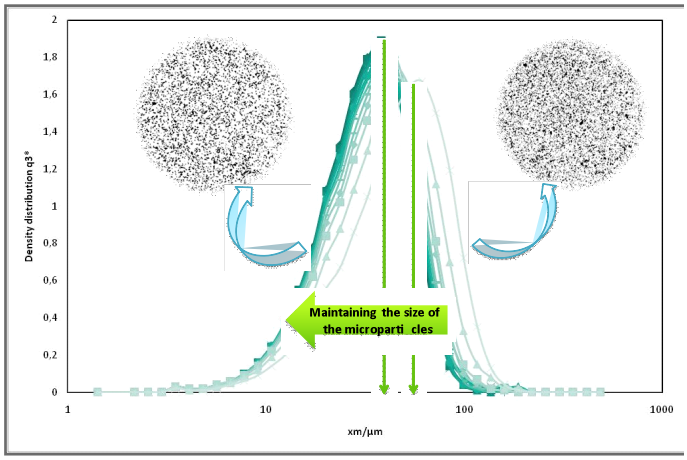

Figure 4: Monitoring of microparticle size during simulated digestion: second case

In this case, the particle size is slightly affected by the gastric conditions. With this composition, the microparticles have an impressive stability towards adverse environmental conditions. Here, digestion leads to only a light erosion of microparticles. This formulation is one of the best we got thanks to mixture design.

CONCLUSION

The use of image analysis is now promising for quantitative and qualitative evaluation of morphology and size of food materials. An original approach was developed to follow "in situ" the degradation of variable dairy matrix in simulated gastro-intestinal media. By using mixture design, we succeeded to develop a formulation only based on dairy ingredients, which best protects the bacteria when exposed to simulated gastric conditions.

REFERENCES

- Burgain J. et al. (2011) Encapsulation of probiotic living cells: From laboratory scale to industrial applications. Journal of Food engineering 104(4) 467-483.
- Heidebach T. et al. (2010) Microencapsulation of probiotic cells by means of rennet-gelation of milk proteins. Food hydrocolloids 23(7) 1670-1677.

ACKNOWLEDGEMENT

The authors thank the LPRAI Company and the team of Professor Phan Tan Luu for the gracious willing of the software NemrodW.