Functionalisation of biomaterial for encapsulation of probiotics & bioactives

Riitta Partanen • Hanna-Leena Alakomi • Pirkko Forssell • Martina Lille • Raija Lantto • Johanna Buchert • Maria Saarela VTT Technical Research Centre of Finland

GENERAL IDEA

Immobilisation of cells to food grade biopolymer matrixes might improve their stability under various environmental stresses in food processing, storage and consumption. Enzymatic modification of the water-soluble carrier polymers in freeze-drying can produce particles, which are insoluble in water environment:

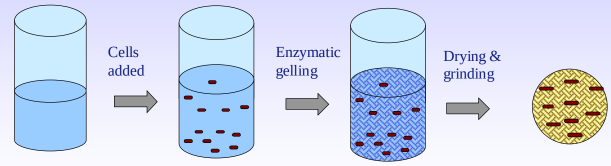
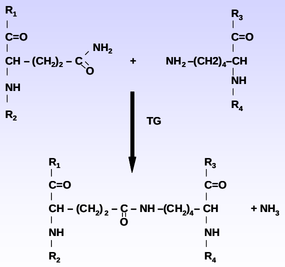


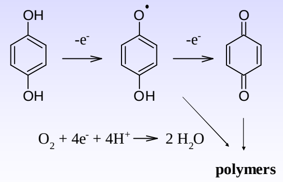
Figure 1. Entrapment of living bacteria by enzymatic gelling of biopolymers.

ENZYME TOOLS

CASE 1. Transglutaminase induces formation of a covalent bond between glutamyl and lysyl residues in protein:



CASE 2. Laccase catalyses a radical reaction, where fenolic groups are oxidised to quinones:



ENZYMATIC GELLING

At isoelectric point, aggregation of proteins leads in gelling due to lack of electrostatic repulsion under certain conditions. As compared with gelling at isoeletric point, enzymatic gelling of sodium caseinate produces a more fine-stranded, homogeneous network (Myllärinen et al. 2007, Partanen et al. 2008). Polymerisation of caseins (fig. 2) results in an elastic gel with a high tolerance to mechanical stresses (fig. 3).

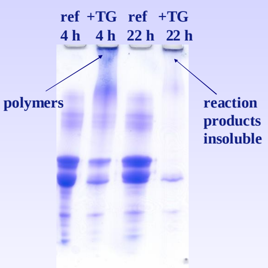


Figure 2. Cross-linking of sodium caseinate at neutral conditions by TG (Partanen et al 2008).

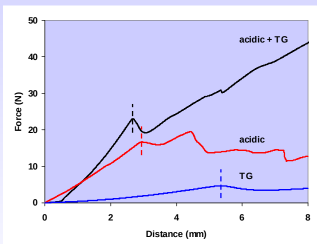


Figure 3. Rheology of enzymatically induced sodium caseinate gel as compared with acid-induced (Partanen et al. 2008).

Sugar beet pectins are substituted with feruloyl groups, which make them a substrate for laccase. Laccase-induced gelation of sugar beet pectin can be further tailored by Ca addition (Kuuva et al. 2003).

PROBIOTICS

The stability of probiotics under various environmental stresses is strain-dependent. Chain robustness together with growth history, possible stress treatments, carrier material and processing & strorage conditions determine the viability of bacteria (Alakomi et al. 2005, Saarela et al. 2005). In aqueous food products, stability is needed for weeks often in acidic environment and in dry powder form long-term stability is challenged by the presence of oxygen and moisture.

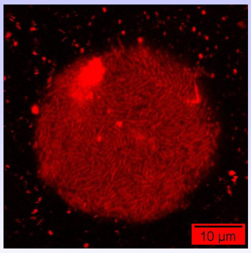


Figure 4. Collapse of spray-dried particles in aqueous environment under CLSM (bacteria in water-soluble carrier).

MASS TRANSFER CONSIDERATIONS

Dried lactic acid bacteria (LAB) cells are in dormant state and relatively stable at cold storage if residual moisture is limited between 1 and 4 % and oxygen uptake is controlled by packaging (Saarela et al. 2004). In aqueous environment (like many foods), the situation is more challenging, as concentration gradients exist in the heterogeneous system and diffusion is much less limited as compared to the dry system (Champagne et al. 2005).

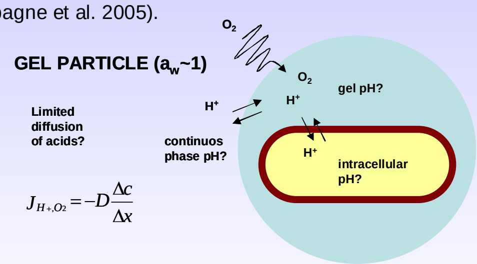


Figure 5. Cell embedded in gel matrix and the inflluence of the environment.

Water in biopolymer gels is expected to have a diffusion coefficient similar to that in bulk water (Hills et al. 1998, Mariette et al. 2002). The questions is, to what extent the transfer of small compounds like organic acids and oxygen can be controlled by tailoring the structure of the gel matrix. Would it be enough to retard the diffusion of acids to give the cells time to adjust and avoid the pH shock in some cases?

LITERATURE CITED

- Alakomi, H-L., Mättö, J., Virkajärvi, I. & Saarela, M. (2005). Application of microplate scale fluorescence assay for assessment of viability of probiotic preparations. *Journal of Microbiological Methods* Vol 62/1: 25-35.
- Champagne, C.P., Gardner, N.J., Roy, D. (2005) Challenges in the addition of probiotic cultures to foods. *Critical Reviews in Food Science and Nutrition.* 1: 61-84
- Kuuva T, Lantto R, Reinikainen T, Buchert J & Autio K (2003). Rheological properties of laccase-induced sugar beet pectin gels, *Food Hydrocolloids* 17:679-684.
- Myllärinen P, Buchert J & Autio K (2007). Effect of transglutaminase on rheological properties and microstructure of chemically acidified sodium caseinate gels. *International Dairy Journal* 17: 800-807.
- Partanen R, Autio K, Myllärinen P, Lille M, Buchert J & Forssell P (2008). Effect of transglutaminase on structure and syneresis of neutral and acidic sodium caseinate gels. *International Dairy Journal* 18:414-421.
- Mariette, F., Topgaard, D., Jönsson, B. & Soderman, O. (2002). 1H NMR diffusometry study of water in casein dispersions and gels. *Journal of Agricultural Food Chemistry* 50, 4295-4302.
- Hills, B.P., Godward, J., Manning, C.E., Biehlin, J.L. & Wright, K.M. (1998). Microstructural characterization of starch systems by NMR relaxation and q-space microscopy. *Magnetic Resonance Imaging* 16(5/6), 557-564.
- Saarela, M., Rantala, M., Nohynek, L., Virkajärvi, I. & Mättö, J. (2004) Stationary-phase acid and heat treatments for improvement of the viability of probiotic lactobacilli and bifidobacteria. Journal of Applied Microbiology. Vol. 96. No: 6, 1205 - 1214
- Saarela, M., Virkajärvi, I., Alakomi, H-L., Mattila-Sandholm, T., Vaari, A., Suomalainen, T. & Mättö, J. (2005). Influence of fermentation time, cryoprotectant and neutralisation of cell concentrate on freeze-drying survival, storage stability, and acid and bile exposure of *Bifidobacterium animalis* cells produced without milk-based ingredients. *Journal of Applied Microbiology*. 99: 1330- 1339.

VTT TECHNICAL RESEARCH CENTRE OF FINLAND
P.O. Box 1000
FI-02044 VTT
Finland

Tel. +358 40 826 5816
Fax +358 20 722 7071
riitta.partanen@vtt.fi
www.vtt.fi