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# Protective effect of milk protein based microencapsulation on bacterial survival in simulated gastric juice versus the murine gastrointestinal system

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## ARTICLE INFO

### Article history:

Received 21 November 2014

Received in revised form 25

February 2015

Accepted 25 February 2015

Available online 27 March 2015

### Keywords:

Microencapsulation

Probiotics

Milk-protein matrices

Coating

Gastro-intestinal

*In vivo*

## ABSTRACT

The significant decrease of live bacteria during passage of the upper gastrointestinal system is an important drawback for probiotic functional food. Microencapsulation is frequently reported to increase bacterial survival in simulated gastric juice (SGJ) but confirmatory *in vivo* studies are lacking. The present study aimed to characterize protective effects of milk-protein-based microcapsules *in vitro* as well as in mice as model consumer. Sodium caseinate (SC) and newly developed, SGJ-resistant fat SC (FSC) capsules significantly increased survival of two *Lactobacillus* strains in SGJ. In contrast, neither SC nor FSC microcapsules increased bacterial survival in the murine gastrointestinal system 3 or 24 h after oral uptake. This lack of protection is presumably due to rapid digestion of the microcapsules in the murine stomach. The present work demonstrates that positive results from frequently applied simple *in vitro* assays cannot be extrapolated to living organisms and highlights the importance of *in vivo* analyses.

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<http://dx.doi.org/10.1016/j.jff.2015.02.046>

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## 1. Introduction

Food with health promoting aspects above its nutritional value, known as functional food, is of high interest to consumers. In this context, the supplementation of food with probiotics is a promising strategy but it is still faced with several technical problems. As per FAO/WHO definition, probiotics are living microorganisms that confer a health benefit to the host, when taken up in sufficient amounts. One major challenge for the generation of any health benefit by a probiotic functional food is, therefore, the maintenance of adequate numbers of living bacteria during shelf life. However, the product environment (high water content, low pH, osmotic stress) and the storage conditions (variable temperature, oxidative stress) of most food products are adverse conditions for bacteria, resulting in a fast reduction of viable cell counts (Burgain, Gaiani, Linder, & Scher, 2011; Heidebach, Först, & Kulozik, 2012; Krasaekoopt, Bhandari, & Deeth, 2003; Picot & Lacroix, 2004). The potential beneficial effect of the supplemented probiotics is further hampered by the fact that the remaining bacteria encounter highly stressful anti-bacterial conditions in the stomach (low pH, digestive enzymes like pepsin) and in the upper small intestine (bile acids, digestive enzymes) when the product is taken up by the consumer. In summary, the combined negative effects of food matrix, storage and gastro-intestinal passage can result in very low numbers of living bacteria that reach the respective intestinal target site.

Microencapsulation is postulated to protect bacteria from these adverse conditions in the food matrix and during the gastro-intestinal passage, resulting in an increased number of living bacteria that reach the intestinal target site in an active state. However, the choice of suitable encapsulation matrices and techniques is challenging. On the one hand, potential microencapsulation matrices need to be stable in the respective food product and in the stomach, but on the other hand, they must be digestible to ensure liberation of the probiotic load before they pass the respective intestinal target site. In the context of food, the microencapsulation matrices are additionally restricted to biopolymers that are accepted food additives. To date, mainly alginate and other biopolymers like chitosan or carrageenan are used as encapsulation matrices (Cook, Tzortzis, Charalampopoulos, & Khutoryanskiy, 2012; Heidebach et al., 2012; Nazzaro, Fratianni, Coppola, Sada, & Orlando, 2009; Yonekura, Sun, Soukoulis, & Fisk, 2014). However, due to their high technological potential the recently developed microencapsulation in milk protein matrices offers additional advantages compared to the encapsulation in polysaccharide-based matrices (Heidebach, Först, & Kulozik, 2009b; Livney, 2010). Milk proteins are normal food constituents of dairy products and they are, therefore, widely accepted by consumers. There is not even the need to declare milk proteins as food additives. From a technological point of view, milk proteins like caseins are well suited to be used as encapsulation material, as for example, sodium caseinate (SC) has a high buffer capacity, good emulsification properties and produces a high network density even at low protein concentrations. The gelation of milk protein based matrices can be performed at temperatures that are well compatible with living bacteria and the use of SC enables the generation of smaller capsules

compared to polysaccharide matrices. The latter argument is of major importance to prevent unwanted sensory effects of the microcapsules in food products (Heidebach, Först, & Kulozik, 2009a; Heidebach et al., 2009b). Furthermore, it can be assumed that the microencapsulation in digestible milk protein matrices results in earlier and more complete liberation of the bacteria in the intestine compared to polysaccharide-based capsules, some of which were already found to be highly stable (Hoad et al., 2009; Lin et al., 2008) or even excreted undigested *in vivo* (van Venrooy, 2004). First studies showed that the microencapsulation of bacteria in water-insoluble SC capsules exerts protective effects on probiotic viability under storage (Heidebach, Först, & Kulozik, 2010) as well as under low pH (Heidebach et al., 2009b) conditions. The latter one is hypothesized to be due to the maintenance of a favorable pH within the capsule (Heidebach, 2010).

Regarding the hypothesized protective effect of microencapsulation in general, there is an array of studies demonstrating increased bacterial survival in food products (Nualkaekul, Cook, Khutoryanskiy, & Charalampopoulos, 2013; Ying et al., 2013), under storage conditions (Heidebach et al., 2010; Ying et al., 2010) or in simulated gastric and/or intestinal juices (low pH, +/– digestive enzymes like pepsin, trypsin, +/– bile acids) (Burgain, Gaiani, Cailliez-Grimal, Jeandel, & Scher, 2013; Chavarri et al., 2010; Gbassi, Vandamme, Yolou, & Marchioni, 2011; Gebara et al., 2013; Gerez, Font de Valdez, Gigante, & Grosso, 2012; Maciel, Chaves, Grosso, & Gigante, 2014; Picot & Lacroix, 2004; Rodrigues et al., 2011; Shi et al., 2013). The quality of the simulated gastro-intestinal conditions is highly variable, ranging from the simple use of mere low pH buffers to complex and dynamic gastrointestinal models (Deat et al., 2009) or even to the *ex vivo* use of gastric/intestinal juices, e.g. from pigs (Doherty et al., 2012). However, whereas data concerning effects of microencapsulation within the food matrix or during product shelf life enable valuable insights into the quality of the product at the time of consumption, data derived from the use of *in vitro* systems can be assumed to be of low predictive value for the highly complex and variable digestive system in living organisms (individual feeding state, variable pH range, peristalsis, variable intestinal microbiota along the gastrointestinal tract, variable concentration of various digestive enzymes, bile acids). Albeit of pivotal importance for a sound conclusion on the protective effects of microencapsulation, there is almost no *in vivo* data available. Only few studies analyzed the protective impact of microcapsules using oral uptake of free versus encapsulated bacteria in humans or animals and the conclusions are almost exclusively based on the number of bacteria that are excreted within a certain time frame (Del Piano et al., 2010; Gardiner et al., 1999; Graff, Hussain, Chaumeil, & Charrueau, 2008; Kushal, Anand, & Chander, 2006). However, besides the fact that the number of excreted bacteria is not a conclusive readout for the initial protective effect of the microencapsulation on bacterial survival during the gastric/small intestinal passage, this kind of study does not give any insight on the location of the bacterial release. It is obvious that successful *in vivo* validation of the hypothesized target functions of microencapsulation (initial protection and liberation of the probiotics before passage of the respective intestinal target site) is a prerequisite for rational application of any microencapsulation in functional food. As it is hardly possible to

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