



A novel hypromellose capsule, with acid resistance properties, permits the targeted delivery of acid-sensitive products to the intestine



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ARTICLE INFO

Article history:

Received 17 July 2013

Received in revised form

7 July 2014

Accepted 31 August 2014

Available online 19 September 2014

Keywords:

Simulator of the Human Intestinal Microbial Ecosystem (SHIME[®])

Targeted delivery

Acid resistant

Dissolution

Gut

ABSTRACT

The delivery of acid sensitive actives in the gastrointestinal tract (GIT) requires specific formulation technology that can act as protection against harsh physiological conditions. Recently, vegetarian capsules based on hypromellose have become commercially available (for example, Vcaps[®], Vcaps[®] plus and DRcaps[™]). Among them, DRcaps[™] shows a dissolution profile that is resistant at low pH (1.2).

The objective of this study is to assess the efficacy the DRcaps[™] in protecting and delivering pure probiotic and enzymatic activities under gastrointestinal relevant conditions such as fasted and fed status, using the Simulator of the Human Intestinal Microbial Ecosystem (SHIME[®]) technology platform. When compared with other formulations, DRcaps[™] show – especially under fasted conditions – an increased ability to protect both probiotic microorganisms (at least an increase of 1 Log) and acid-sensitive enzymes, i.e. lipase and amylase (at least an activity increase of 1.5 times over the other formulations) during gastrointestinal digestion.

Additional trials with DRcaps[™] also show a 100% residual viability for probiotic bacteria in a 24-month shelf-life test (temperature of 25 °C and 30 °C/13.8 and 18.2 g/m³ relative humidity, respectively). Finally, customers perceive that this new type of capsule has a superior organoleptic performance, assessed by the reduction or absence of an aftertaste, when compared with compressed tablets.

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1. Introduction

Delivering acid sensitive actives to the intestine requires complex formulation expertise. The efficiency of typical acid sensitive products, such as probiotics (live microorganisms which confer a health benefit on the host, according to the definition of the World Health Organization), depends on dose level and viability (Ariful, Yun, Choi, & Cho, 2010). If the latter is not maintained along production, storage and transit in the gastrointestinal tract, their main activity (i.e. help to alleviate intestinal disorders and stimulate the immune system – Gareau, Sherman, & Walker, 2010) may be irreversibly compromised (Ariful et al., 2010). Nowadays, probiotics are available in different dosage forms such as yogurts, sachet, vials and tablets. These forms have varying shelf life and there is limited proof of their delivery to the intestine (Sanders, 2003). In terms of

production, the sensitivity of probiotics to temperature, oxygen and humidity can make their formulation technically challenging and lead to higher manufacturing costs. For example, formulating a probiotic in a tablet requires a complex compression step and the subsequent need to protect the probiotic by coating and pelleting. The formulation of probiotic in stick packs requires a dedicated and expensive technology, as well as the coating/pelleting steps (De Vos, Faas, Spasmojevic, Sikkema, 2010; Mattila-Sandholm et al., 2002; Weinbreck, Bodnár, & Marco, 2010). In terms of delivery, considering that these living bacteria can be sensitive to low pH, a proper delivery method to the intestine is required which ensures their effectiveness after oral administration. In this respect, encapsulation of probiotics is established as a one of the standard techniques for probiotic oral dosage forms to cross the acidic stomach environment (Stegemann, 2011; Viernstein, Raffalt, Polheim, 2005). Nowadays, the so-called ‘microencapsulation’ is the most widely used. By means of extrusion, emulsion or drying methods it is possible to create a protective layer around the

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probiotic strains using different materials (i.e. modified alginate, whey protein gel, cellulose derivatives, chitosan, pectin, gelatin ...) (Cook, Tzortzis, Charalampopoulos, & Khutoryanskiy, 2012; Huq, Khan, Khan, Riedl, & Lacroix, 2013). It has also been shown that the size of capsule may affect the probiotic viability (Sousa et al., 2013).

Recently, vegetarian capsules based on hypromellose have become commercially available. These capsules maintain their mechanical stability in changing temperatures and low relative humidity (Ku et al., 2010). The water content of hypromellose capsules at ambient conditions is 2.5 times lower as compared to the standard hard gelatin capsules. In this respect vegetarian capsules should assure a better stability for the probiotics, as well as decreased risk of brittling (Ku et al., 2010).

In this study we will focus on hypromellose capsules characterized by different dissolution profiles in acidic media: i) immediate quick release (Vcaps® plus); ii) intermediate release (Vcaps®); iii) and very slow release in capsules resistant to gastric fluids (DRcaps™). The objective is to assess the efficacy of the DRcaps™ in protecting pure and untreated probiotics and specific enzymatic activities from an acid environment and their ability to deliver live bacteria/active enzymes to the small intestine, when compared to other formulations. To achieve this, studies were performed using the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®) technology platform. The latter has already been used to simulate the physiological conditions occurring in the upper GIT and the data obtained with this system have been proven *in vivo* with several independent studies (Marzorati, Possemiers, & Verstraete, 2009; Possemiers, Marzorati, Verstraete, & Van de Wiele, 2010). Moreover, we compared the shelf-life of different formulations – with respect to probiotic viability – and conducted a survey to understand the customers' perception of this new type of capsules.

2. Materials and methods

2.1. Capsules description

In this study four different capsules were compared for their properties in the upper GIT. These capsules are: hard gelatin capsules (HGC) and vegetarian capsules (composed of hypromellose – HPMC): the Vcaps® plus, Vcaps® and DRcaps™ (Capsugel, Colmar, France) with different dissolution behavior in a simulated gastric fluid: HGC and Vcaps® Plus dissolve rapidly at pH 1.2 while some delay in dissolution is observed for the Vcaps® at same pH; DRcaps™ are supposed to not dissolve at pH 1.2 (data not shown). All tested capsules were of size 1 (hold a content of about 400 mg). All caps were prepared by dipping stainless steel mold pins at room temperature into a hot liquid solution of the specific material (with gellan gum as gelling agent only for DR-caps™). Probiotics and enzymes were provided as dry powder directly from the producers and the capsules were manually filled before the startup of an experiment with the exception of those prepared for the stability tests which were filled on specific capsule filling machine (CFM). As described in the following paragraphs, after the first experiment, HGC capsules were replaced by Vcaps® Plus, due to the fact that they had a similar dissolution profile at pH 1.2.

2.2. Dissolution of probiotics under simulated gastrointestinal conditions

The survival of *Bifidobacterium longum* (LabMET, Gent, Belgium) encapsulated in HGC, Vcaps® or DRcaps™ was evaluated in a short-term screening assay consisting of sequential incubation of capsules under simulated fasted conditions in 100 mL Schott bottles

with rubber cap (VWR, Leuven, Belgium). The physiological conditions of the upper GIT have been sequentially simulated for the stomach (incubation at pH 1.2 for 60 min and addition of 1 g/L of pepsin) and for the small intestine (incubation at pH 6.8 for 180 min, addition of 3 g/L of Oxgall [Difco, Detroit, USA] and 0.9 g/L of pancreatin [Sigma–Aldrich, Schnellendorf, Germany]). Bottles were flushed with nitrogen to maintain anaerobiosis and incubated at 37 °C under shaking conditions (100 rpm). Each experiment was conducted in triplicate to account for biological variability and the capsules were maintained in the system with sinkers (Sotax, Kampenhout, Belgium). Samples were collected from the luminal phase as shown in Table 1.

2.3. Dissolution of probiotics and enzymes in a dynamic gastrointestinal model

In a follow up study, Vcaps®, Vcaps® Plus and DRcaps™ were tested in an adapted set-up of the SHIME® system, a computer controlled, dynamic model to study physicochemical, enzymatic and microbial parameters in the gastrointestinal tract in a controlled *in vitro* setting. It consists of five reactors, which sequentially simulate the stomach, small intestine and the three regions of the large intestine, i.e. the ascending, transverse and descending colon (Fig. 1A). The capsules were filled either with 330 mg of a mix of *Lactobacillus rhamnosus* LGG® (LGG) ($3.1 \cdot 10^{10}$ CFU/g) and *Bifidobacterium lactis* BB-12® (BB12) ($1.8 \cdot 10^{10}$ CFU/g) (Chr. Hansen, Hørsholm, Denmark) or with the following enzymes (using cellulose as an excipient to achieve a final weight of 400 mg): 157 mg amylase (6000 DU); 25 mg lipase (5000 FIP) (National Enzyme, Forsyth Missouri, USA). The adapted SHIME set-up for this study consisted of three double-jacketed vessel maintained at a temperature of 37 °C, simulating in succession the stomach (low pH, 150 rpm and addition of pepsin) and small intestine (addition of bile salts and pancreatic fluid, 150 rpm) (Fig. 1B). More specifically, simulated fasted (i.e. consumption of the product before a meal) and fed (i.e. consumption of the product during or immediately after a meal) conditions were tested. The physiology of fasted/fed status was recreated by modifying the pH profile in the gastric compartment, the concentration of bile salts in the simulated small intestine and the transition time, as described below. Under fasted conditions, the stomach was simulated with a 60-min incubation in a solution at pH 1.8, containing NaCl (6 g/L), mucin (4 g/L) and pepsin (1 g/L) (adapted from Dubois, Eerdewegh, & Gardner, 1977; Jantravid, Janssen, Reppas, & Dressman, 2008). Under fed conditions, the solution simulating the gastric compartment was composed of the standard SHIME medium (Marzorati et al., 2010), and pepsin (2 g/L). The pH decreased from a starting point of 4.2 to 1.8 within a 90-min incubation at 37 °C. The small intestine was characterized by a stable pH of 6.8, addition of pancreatin (0.9 g/L) and, alternatively, an initial bile salts

Table 1

Time points of samples collection during the preliminary dissolution study and in the following test with capsules containing probiotics or enzymes. x = sample collected; – = no sample collection. 'Fasted' refers to the physiological conditions of an empty stomach while 'fed' refers to the consumption of the capsule during a meal.

Time (min)	Stomach					Small intestine							
	30'	45'	60'	90'	15'	30'	45'	60'	90'	120'	180'		
Dissolution study	x	–	x	–	–	–	–	x	–	x	x		
Fasted probiotic study	x	–	x	–	x	–	x	–	x	–	x		
Fed probiotic study		x		x	x	–	x	–	x	–	x		
Fasted enzyme study	x	–	x	–	x	x	x	x	–	x	x		
Fed enzyme study	x	–	x	x	x	x	x	x	–	x	x		

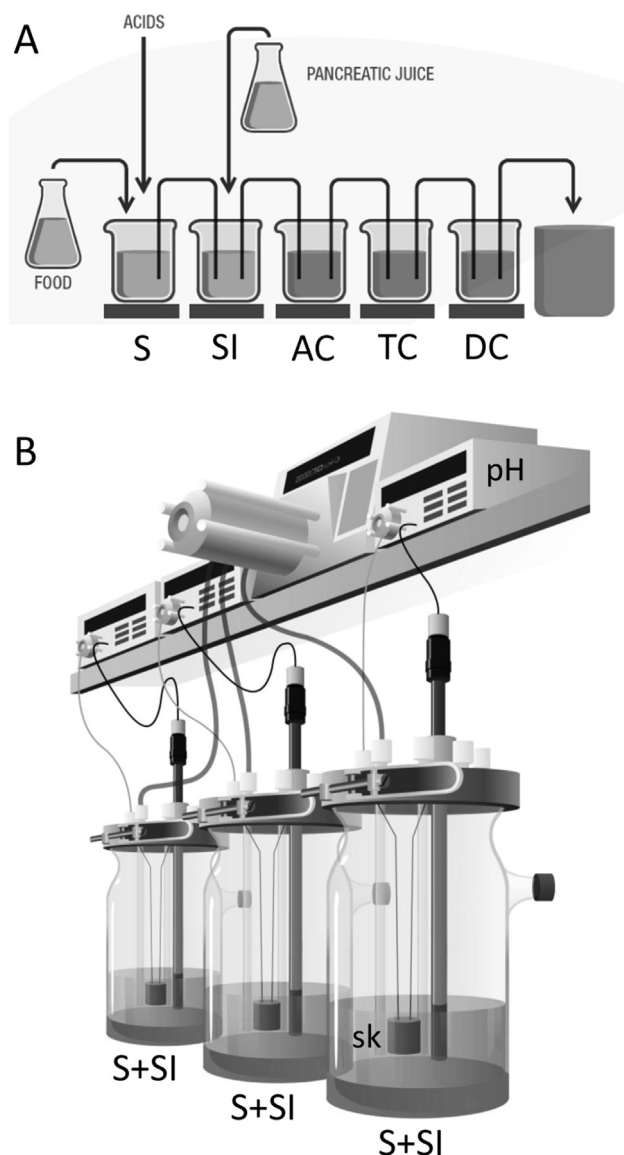


Fig. 1. A) Scheme of the SHIME® system in which 5 reactors simulate the stomach (S), small intestine (SI) and the three regions of the large intestine, i.e. the ascending (AC), transverse (TC) and descending (DC) colon. B) Design of the adapted SHIME setup used in this test to simulate the physiological conditions of the upper gastrointestinal tract. A single compartment has been used to simulate in temporal succession the conditions of stomach and small intestine (S + SI). The capsules have been maintained in the system by means of sinkers (sk) and the pH profiles adjusted by means of pH controllers (pH). All the experiments have been conducted in triplicate. Black triangle = DRcaps™; gray diamond = Vcaps®; black square = Vcaps® Plus.

concentration of 4.5 mmol/L decreasing to 2.25 mmol/L in 100 min for fasted conditions or an initial bile salts concentration of 9 mmol/L decreasing to 4.5 mmol/L in 100 min for fed conditions (total incubation time of 180 min). The concentration of bile salts was decreased by adding a solution of NaHCO_3 (3 g/L). Each experiment was conducted in triplicate to account for biological variability and capsules were maintained in the system with sinkers (Sotax). Samples were collected from the luminal phase for further analyses as reported in Table 1.

2.4. Visual inspection, plate count and enzymatic assays

Visual inspection of the integrity of the capsules was conducted at different sampling times. The capsules were scored as follows: 1)

capsule intact; 2) capsule open but almost all probiotic/enzyme remains in the capsule; 3) capsule open and all probiotic/enzyme is released; 4) capsule fully dissolved.

In the dissolution study, viable *B. longum* counts were measured using plate counts on RCA (Reinforced Clostridial agar) (Oxoid, Basingstoke, UK) incubated anaerobically at 37 °C for 48 h. In the SHIME study, the concentration of both LGG and BB12 was determined on MRS (De Man, Rogosa, Sharpe) Agar (Oxoid) while tetracycline was added to MRS to quantify only BB12. Plates were incubated anaerobically for 72 h at 37 °C. All plating activity was conducted with a Spiralenter Eddy-Jet SN706 (LED Techno, Heusden-Zolder, Belgium).

Enzymatic activities were measured with commercially available kits following the instructions of the producer. Amylase was measured by means of the 'Amylase activity assay kit' (BioVision, Milpitas, California, USA); lipase was measured with the 'Lipase activity assay kit' (BioVision).

2.5. Shelf life and customer satisfaction

The shelf life of different formulations (vials, hypromellose capsules and stick packs) of a mix of *Lactobacillus acidophilus* and *Bifidobacterium bifidus* (Chr. Hansen) were evaluated in terms of viability of the probiotic strains after 24 months. All the formulations were prepared according to US FDA Title 21 CFR PART 111 (Current Good Manufacturing Practice in Manufacturing, Packaging, Labeling, or Holding operations for Dietary Supplements) in a production and filling facility at max 25 °C and less than 5.7 g/m³ Relative Humidity (RH). Vials contained approx. 9×10^9 cells of the probiotic mix, 25 mg of fructo-oligosaccharides and 35 mg of hydrolyzed corn dextrin; hypromellose capsules contained approx. 6×10^9 cells of the probiotic mix, 77 mg of fructo-oligosaccharides and 68 mg of hydrolyzed corn dextrin conserved either as a blister or in a tube; and stick packs contained approx. 6×10^9 cells of the probiotic mix, 427 mg of fructo-oligosaccharides and 370 mg of hydrolyzed corn dextrin. The formulations were conserved under controlled conditions (temperature of 25 °C/13.8 g/m³ RH and 30 °C/18.2 g/m³ RH) and the probiotic viability was tested after 0, 1, 2, 3, 4.5, 6, 12, 18 and 24 months on the same media as described in Section 2.5.

The customer satisfaction survey was conducted on 175 women, aged between 20 and 60 years old (50% 20–40 years old 50% 40–60 years old), who are usually consumers of nutritional supplements. The group were divided in two arms of 88 and 87 consumers respectively. Each arm received either DRcaps™ or compressed tablets containing a mixture of a garlic extract dosed at 10 mg/g allicin (producing an after-taste and burping), black pepper extract (known for causing a burning sensation) and iron sulfate (known for producing digestive discomfort). In this home-use test, each consumer had to take a capsule/tablet, once a day, for three consecutive days. Sensory data were recorded in an online questionnaire that was completed by every participant before ingestion, each day during ingestion and after the tablet/capsule had been swallowed (Table 5).

3. Results

3.1. Dissolution of probiotics under batch gastrointestinal conditions

A dissolution study has been conducted to compare the protective effect of the different capsules. Table 2 shows the results of the visual inspection of the integrity of the capsules at different time points. With gelatin and Vcaps®, no viable *Bifidobacterium longum* was detected at any time point as a result of the rapid

Table 2

Visual scores for the preliminary dissolution test. The capsules were scored as follows: 1) Capsule intact; 2) Capsule open but almost all probiotic is still in the capsule; 3) Capsule open and all probiotic is released; 4) Capsule dissolved.

Time (min)	Stomach		Small intestine		
	30'	60'	60'	120'	180'
HGC ^a	4	4	4	4	4
Vcaps [®]	2	3	3	3	4
DRcaps TM	1	2	3	3	3

^a Vcaps[®] Plus have an identical dissolution profile (data not shown).

release of the probiotics from the capsules into the harsh simulated stomach environment (below detection limit of the technique). Visual inspection of the capsules confirmed the rapid disintegration of both types of capsules. However, whereas the gelatin capsules dissolved and disappeared completely, fragments of the Vcaps[®] remained visible throughout the entire experiment and the dissolution was the result of the fragmentation of the Vcaps[®] into smaller fragments. Conversely, the DRcapsTM remained largely intact under the conditions that were applied to simulate the stomach, leading to containment of the majority of the probiotics in the capsule. After addition of the small intestinal suspension, the capsules disintegrated leading to complete release of the probiotics (approx. 9 logs) at the beginning of the simulated small intestine. Prolonged exposure to the simulated small intestinal conditions led to a gradual decrease in the concentration of the microbial strain (i.e. 5.79 logs of total amount of viable counts after 60 min, 4.99 logs after 120 min and 4.68 logs after 180 min).

An additional dissolution test with DRcapsTM immersed in the simulated small intestine fluid showed comparable results as the test in which DRcapsTM were immersed for 2 h in simulated gastric fluid before opening in the intestinal fluid. These results confirmed that probiotics are protected by the DRcapsTM and the risk of acid solutions getting into the capsule through permeation or from between the body and the cap is limited if not absent and do not kill the probiotics. (data not shown).

3.2. SHIME study with capsules containing probiotics and enzymes

In a second experiment, the targeted release of the capsules was tested in an adapted SHIME set-up to evaluate the behavior of the different capsules under physiologically relevant conditions for the GIT. Gelatin Caps were substituted by Vcaps[®] Plus. The latter is characterized by the same dissolution profile of Gelatin capsule but is composed of the same material as Vcaps[®]. Endpoints of this experiment were the evaluation of the viability of two probiotic strains (*L. rhamnosus* GG and *Bifidobacterium lactis* BB12) and the activity of two enzymes (amylase and lipase) in the GIT, within capsules having the same matrix shell based on hydroxypropyl methylcellulose (HPMC).

Table 3 shows the results of the visual inspection of the integrity of the capsules at different time points for both the enzyme and the probiotic test. In general, under fed conditions, Vcaps[®] and Vcaps[®] Plus opened after 10 min of incubation in simulated stomach fluids, while under fasted conditions Vcaps[®] remained intact for about 25 min. DRcapsTM behaved similarly both under fasted and fed conditions: intact during stomach simulation, partially damaged after 15 min in the small intestinal simulation and completely opened after 45 min. This alternative behavior under gastrointestinal conditions led to a different protective effect on the probiotics and enzymes in the capsules, with DRcapsTM always releasing their content only in the simulated small intestine (Figs. 2 and 3).

Under fed conditions, the total amount of viable LGG + BB12 at the end of the small intestine simulation was statistically higher

Table 3

Visual scores for the SHIME experiment under fasted (X in X/Y) and fed conditions (Y in X/Y). 'Fasted' refers to the physiological conditions of an empty stomach while 'fed' refers to the consumption of the capsule during a meal. The dissolution of the capsules was the same both in the test conducted with the probiotics and in that with the enzymes. 1) Capsule intact; 2) Capsule open but almost all probiotic is still in the capsule; 3) Capsule open and all probiotic is released; 4) Capsule dissolved.

Time (min)	Stomach					Small intestine							
	15'	30'	45'	60'	90'	15'	30'	45'	60'	90'	120'	180'	
Probiotic test													
Vcaps® Plus	3/3	3/-	-/4	4/-	-/4	4/4	—	4/4	—	4/4	—	4/4	
Vcaps®	3/1	3/-	-/4	4/-	-/4	4/4	—	4/4	—	4/4	—	4/4	
DRcaps™	1/1	1/-	-/1	1/-	-/1	2/2	—	4/4	—	4/4	—	4/4	
Enzyme test													
Vcaps® Plus	3/3	3/3	—	4/4	-/4	4/4	4/4	4/4	4/4	—	4/4	4/4	
Vcaps®	3/1	3/3	—	4/4	-/4	4/4	4/4	4/4	4/4	—	4/4	4/4	
DRcaps™	1/1	1/1	—	1/1	-/1	2/2	3/3	4/4	4/4	—	4/4	4/4	

when the probiotics were dosed in DRcapsTM as compared to Vcaps[®] and Vcaps[®] Plus (Fig. 2A; $P < 0.05$). The situation was the same under fasted conditions ($P < 0.05$). In the latter case, it was also possible to distinguish a higher protective effect of Vcaps[®] as compared to Vcaps[®] Plus (Fig. 2C). When considering the viability of only the strain BB12, no statistically significant differences were observed in the protective effect of the three capsules under fed conditions (Fig. 2B; $P > 0.05$). Conversely, under fasted conditions, Vcaps[®] and DRcapsTM ensured a higher BB12 viability as compared to Vcaps[®] Plus at the end of the small intestine simulation (Fig. 2D).

Fig. 3 shows the results of the residual enzymatic activities when Vcaps[®], Vcaps[®] Plus and DRcapsTM were dosed in the upper GIT. Both amylase and lipase were highly sensitive to low pH under fasted conditions (Fig. 3A and C). The enzymatic activity was preserved only with DRcapsTM. Therefore, the protection of DRcapsTM was significantly higher when compared to Vcaps[®] and Vcaps[®] Plus, as shown by statistical analysis comparing the residual enzymatic activities at the end of the simulated small intestine (squares in Fig. 3A and C; $P < 0.01$). The same trend was observed under fed conditions. The DRcapsTM provided a statistically higher protective effect of both amylase and lipase activities (Fig. 3B and D). Moreover, under these conditions it was also possible to differentiate the residual activity of amylase and lipase when the enzymes were dosed in Vcaps[®] or Vcaps[®] Plus. For both enzymes, Vcaps[®] led to a higher residual activity in the simulated small intestine as compared to Vcaps[®] Plus ($P < 0.05$).

3.3. Shelf-life and customer satisfaction

The viability of the probiotics in the different capsules was evaluated during a 24-month period stored at 25 °C and 30 °C. As shown in Table 4, after 24 months, the probiotics strains had a residual viability higher than 90% for all tested conditions with the exception of the vials. Probiotics in vegetarian capsules and packed in aluminum tubes had a residual viability of 100%.

Finally, DRcapsTM – as representative of the capsules used in the SHIME experiment – were used in a customer satisfaction trial ($N = 88$) and were compared to a compressed tablet ($N = 87$). Overall, after ingestion, DRcapsTM were preferred to the compressed tablets. Despite the size, which 57% of consumers perceived as too big, the capsules were easy to swallow (6.8 out of a scale of 9) and had a weak smell (2.3). Moreover, DRcapsTM did not leave an unpleasant taste in the mouth (82% of the consumers) and left little perception of an aftertaste (18%). In all cases, DRcapsTM and tablets produced similar discomfort during digestion (for example, difficulties in digesting the tablet or capsule, burping problems and a feeling of being bloated), which concerned around 40% of the

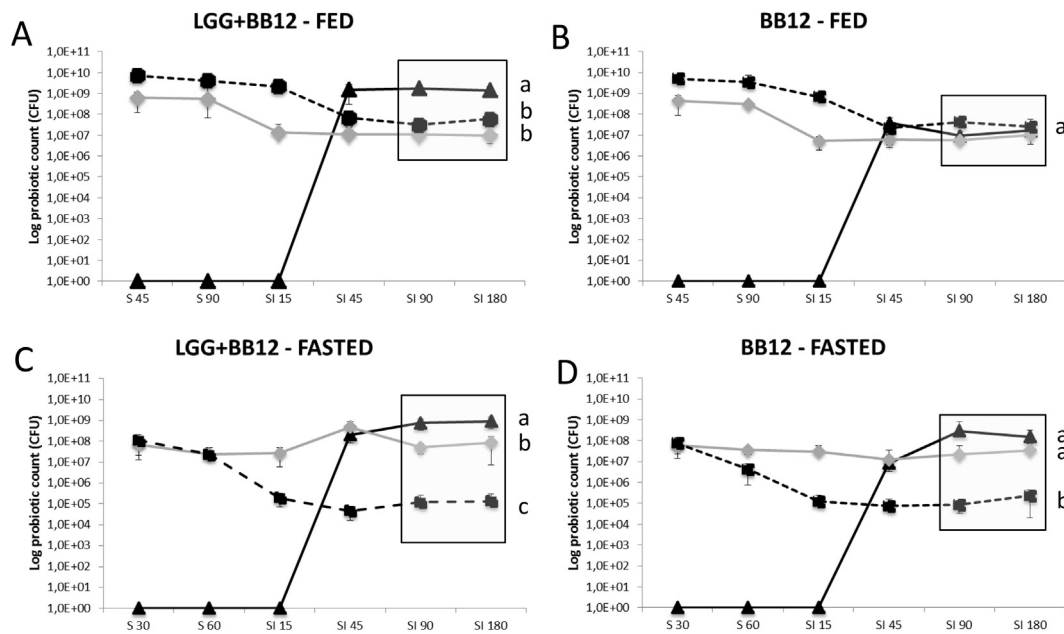


Fig. 2. Viability of *Lactobacillus rhamnosus* LGG and *Bifidobacterium lactis* BB12 at different time points in the SHIME experiments using Vcaps® Plus, Vcaps®, and DRcaps™ ($n = 3$). Physiological conditions have been simulated for the stomach (S) and small intestine (SI). Statistical differences have been assessed considering the time points within the squares by means of a Student's two-tailed t test. Samples with different letters are statistically different for $P < 0.05$. Black triangle = DRcaps™; gray diamond = Vcaps®; black square = Vcaps® Plus.

consumers (data not shown). In conclusion, DRcaps™ showed real benefits when compared to tablets of not causing an unpleasant taste in the mouth and reducing an aftertaste (Table 5).

4. Discussion

The protection of drugs, bioactives or viable microorganisms during gastrointestinal passage is gaining increasing attention from the pharma-, nutraceutical and functional food industry. The targeted delivery of a compound of interest is crucial in providing

effective therapy for chronic diseases (Yang, Chu, & Fix, 2002) or more efficient colonization of probiotic microorganisms in the lower digestive tract (Chan & Zhang, 2005). In this study, we showed that DRcaps™, when compared with conventional encapsulation materials, provide a superior ability to protect both the viability of microorganisms and the stability of enzymes under upper digestive tract conditions.

An initial indication of DRcaps™ greater protective capacity is their stability during simulated gastric digestion. Unlike the hard gelatin capsules and Vcaps®, DRcaps™ showed no disintegration in

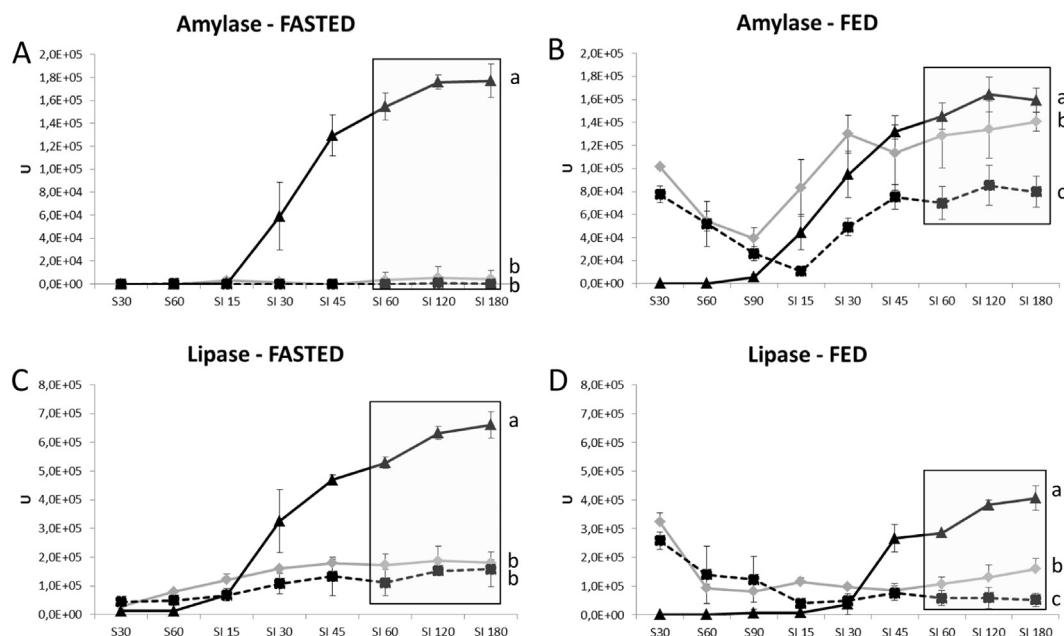


Fig. 3. Measurement of the residual enzymatic activity at different time points in the SHIME experiments using Vcaps® Plus, Vcaps®, and DRcaps™ capsules ($n = 3$). Physiological conditions have been simulated for the stomach (S) and small intestine (SI). Statistical differences have been assessed considering the time points within the squares by means of a Student's two-tailed t test. Samples with different letters are statistically different for $P < 0.05$.

Table 4

Average (Avg) and standard deviation (SD) of the shelf-life of different formulations (stick pack, vial and hypromellose capsules) conserved at 25 °C or 30 °C with respect to a probiotic mix (*L. acidophilus* and *B. bifidus*) viability during a 24-month (M) test ($n = 3$). Data are expressed as CFU/g. Tests at 30 °C were conducted only for 6 months: N.M. = not measured.

Container	Package	Storage		0 M	1 M	2 M	3 M	4.5 M	6 M	12 M	18 M	24 M
Stick pack	—	25 °C	Avg	4.20E+09	3.90E+09	3.80E+09	3.80E+09	4.50E+09	3.89E+09	1.94E+09	2.11E+09	1.95E+09
			SD	1.64E+09	1.33E+09	1.83E+09	1.27E+09	0.95E+09	1.29E+09	1.41E+09	9.72E+08	8.65E+08
		30 °C	Avg	4.20E+09	2.60E+09	2.20E+08	4.50E+08	1.00E+08	1.23E+08	N.M.	N.M.	N.M.
			SD	1.64E+09	9.47E+08	1.45E+08	9.29E+07	4.26E+07	3.44E+07			
Vial	—	25 °C	Avg	9.40E+09	4.00E+09	4.00E+09	3.28E+09	3.15E+09	2.53E+09	6.80E+04	1.00E+02	1.00E+02
			SD	2.30E+09	1.91E+09	9.17E+08	1.23E+09	1.04E+09	0.86E+09	3.39E+04	1.89E+02	1.45E+02
		30 °C	Avg	9.40E+09	3.70E+08	4.50E+08	8.70E+06	7.00E+03	9.00E+02	N.M.	N.M.	N.M.
			SD	2.30E+09	1.52E+08	0.91E+08	3.14E+06	5.66E+03	10.03E+02			
Veg. Caps	Blister	25 °C	Avg	4.60E+09	3.40E+09	1.80E+09	2.13E+09	2.93E+09	2.55E+09	1.20E+09	1.00E+09	9.00E+08
			SD	7.88E+08	1.23E+09	9.03E+08	6.59E+08	1.13E+09	6.46E+08	5.30E+08	2.31E+08	1.74E+08
		30 °C	Avg	4.60E+09	9.50E+07	8.90E+07	7.00E+07	7.50E+07	1.20E+06	N.M.	N.M.	N.M.
			SD	7.88E+08	3.60E+07	2.56E+07	2.89E+07	2.38E+07	1.35E+06			
Veg. Caps	Plastic Tube	25 °C	Avg	2.00E+09	4.10E+09	4.10E+09	4.40E+09	4.84E+09	4.26E+09	3.80E+09	3.00E+09	1.71E+09
			SD	8.65E+08	1.35E+09	8.87E+08	1.05E+09	1.44E+09	1.15E+09	1.92E+09	1.50E+09	9.10E+08
		30 °C	Avg	2.00E+09	3.50E+09	3.30E+09	2.54E+09	2.95E+09	3.75E+09	N.M.	N.M.	N.M.
			SD	8.65E+08	9.41E+08	1.25E+09	1.14E+09	7.54E+08	1.54E+09			

the acidic environment, but only opened under simulated small intestinal conditions. DRcaps™ — made of HPMC and a gelling agent — have previously been shown to be highly efficient in protecting acid-sensitive components across gastric passage (Cole et al., 2002). Moreover, the naturally low moisture content of HPMC and the lack of chemically reactive groups make it suitable for protecting water-unstable drugs (Ogura, Furuya, & Matsuura, 1998).

The higher stability of DRcaps™ upon gastric passage was also reflected by the finding of viable *Bifidobacterium longum* during simulated intestinal digestion, while HGC or Vcaps® did not demonstrate any survival for *B. longum* neither upon simulated gastric passage, nor during intestinal digestion. Yet, compared to the inoculum level, it should be noted that there is a considerable decrease in viable counts for *B. longum* after 1 h of simulated intestine digestion. We consider it unlikely that *B. longum* would have experienced acid stress given the fact that HPMC is much less permeable to water than for example, gelatin (Missaghi & Fassihi, 2006). Instead, as DRcaps™ were partly damaged (but not opened) at the end of the simulated gastric stage, it is more likely that *B. longum* was released quite soon during intestinal digestion and was thus exposed to bile salt stress. Although *B. longum* spp. are known for their resistance to bile salts due to their bile salt hydrolase activity (Tanaka, Hashiba, Kok, & Mierau, 2000), the stress may cause the strain to lose its ability to be cultured by reaching a so-called VBNC (viable but not culturable) state. This protective state is characterized by a temporary inability to be cultured on typical bacterial growth media, upon incubation under harsh environmental conditions (Zotta et al., 2012). As the bacteria are

still alive, changing the environmental conditions (partially) ends the VBNC state, again leading to the detection of viable bacteria. Yet, with respect to the protective effect, it is concluded that DRcaps™, made of HPMC, display a superior protective effect over more conventional encapsulation matrices.

The next step in our research consisted of evaluating the protective effect from DRcaps™ in a more representative gastrointestinal environment, the SHIME, a dynamic simulator of the gastrointestinal tract. This is a scientifically validated simulator of the physiological, enzymatic and microbiological conditions of the human digestive system that has been already used in several studies to evaluate the effect of pre- and probiotics in the gut (Marzorati et al., 2009, 2010, 2012; Possemiers et al., 2010; Sanchez et al., 2009). In comparison with the static and hence harsher conditions of the batch experiments or the most commonly solutions and apparatus based on the US Pharmacopeia, a SHIME-based dynamic simulation results in a more gradual implementation of gastric acid stress, transfer to small intestine and exposure to bile salts and digestive enzymes. Applying this simulation more representative for the *in vivo* conditions, we observed completely intact DRcaps™ at the end of the gastric stage, while damage to the DRcaps™ did not occur earlier than 30 min in the intestine simulation. This also resulted in the protection of a combination of two other probiotic microorganisms — *L. rhamnosus* GG and *Bifidobacterium lactis* BB12 — as well as two enzymes, lipase and amylase. Interestingly, the specific characteristics of the single strains remained of key importance to support the viability of the probiotic under different physiological conditions. For instance, under fed conditions in the simulated small intestine (i.e. double initial concentration of bile salts as compared to the fasted status), *Bifidobacterium lactis* BB12 was not significantly better protected by DRcaps, while *L. rhamnosus* GG showed a better survival rate compared to Vcaps® and Vcaps® Plus (Fig. 3). This difference may be indeed explained by the fact that, once the content of the capsule is released, *L. rhamnosus* GG has a higher resistance to bile salts as compared to *Bifidobacterium lactis* BB12 (Douillard et al., 2013; Vernazza, Gibson, & Rastall, 2006). Another important finding is that the protective effect seems to be more pronounced in the absence of a food matrix (fasted conditions). Davis, Burton, Connor, Macrae, and Wilding (2009) previously reported that the fed state of an individual, due to a longer digestion time, has an important impact on the stability of HPMC based tablets.

This is the first study in which this new type of capsules are tested as a mean to deliver acid-sensitive bio-actives in a simulated gastrointestinal tract. However, with respect to the survival of

Table 5

Results of the online questionnaire on customer satisfaction after ingestion of DRcaps™ or compressed tablets containing a mixture of a garlic, black pepper extract and iron sulfate. Customers had to take a capsule/tablet, once a day, for three consecutive days. Data in the table refers to the third and last day of treatment. * = the difference between DRcaps™ and tablets is statistically significant according to a student test ($p < 0.05$).

	DRcaps™	Tablet
Overall liking (on a scale of 9)		
Before ingestion	5.4	5.2
After ingestion	6.4*	5.1
Easy to swallow	6.8	7
Smell Intensity	2.3*	5.7
Absence of		
Perception Aftertaste	81%*	31%
Leaves unpleasant taste	18%*	68%

lactobacilli and bifidobacteria in the upper gastrointestinal tract, literature is rich in examples in which the microencapsulation technique is applied. Overall, microencapsulation tends to offer a better protection for the probiotic strains but the extent of this protection may vary a lot depending on technical parameters (Pan et al., 2013; Shamekhi, Shuhaimi, Ariff, & Manap, 2013; Tee, Nazaruddin, Tan, & Ayob, 2014; Todorov, Leblanc, & Franco, 2012; Zhang, Li, Park, & Zhao, 2013). The viability of the encapsulated bacteria is positively correlated to the dimension of the capsule layer (Anal & Singh, 2007). Conversely, the bigger is the microcapsule, the more technically difficult becomes its incorporation in the texture of food matrices, and it is not always easy to find a good balance between protection and feasibility of use (Huq et al., 2013).

A similar study has been conducted also with digestive enzymes. Exogenous enzyme supplementation is required in certain disease conditions in humans and animals (Roxas, 2008). However, limitations on their use in diet are related to the pH specificity, thermolabile nature and inhibition related to the possibility of intestinal damage. In this study, the SHIME experiments on encapsulated lipase and amylase enzymes showed a protective effect of DRcaps™ in comparison with the conventional encapsulation materials. This effect was more pronounced in the absence of a food matrix. These two enzymes have been selected because of their high sensitivity to low pH. In fact, the obtained data showed that the new DRcaps™ are highly effective in creating a separate protective environment in which the delivery of acid sensitive activities is therefore possible. As it has previously been reported, the formulation of the encapsulation material is an important factor to prevent water penetration and swelling of HPMC materials (Gao et al., 1996).

Finally, the consumer questionnaire regarding the oral uptake, sensory properties and gastrointestinal comfort was conducted with a selection of actives (i.e. garlic, black pepper and iron) aimed at creating a clear negative impact on digestive comfort. Garlic is known to have reflux effect, black pepper causes burning in the stomach while iron typically irritates the mucosa. The outcome of the study demonstrated that DRcaps™ when compared with more conventional tablets display superior organoleptic protection, as seen by a reduced or an absence of aftertaste.

Overall, we conclude that HPMC based DRcaps™ are effective in protecting both probiotic microorganisms, as well as acid-sensitive enzymes during gastrointestinal digestion. However, attention must be paid with regard to the administration strategy that is chosen to dose the DRcaps™ capsules. Depending on the gastrointestinal site where a compound of interest needs to be released, influencing factors such as nutritional background, may need to be altered to obtain the most appropriate release pattern and delivery efficiency. From our data, we conclude that primarily fasted conditions favor a better protection for both probiotics and enzymes.

Acknowledgments

Sam Possemiers and Massimo Marzorati are Postdoctoral Fellows of the Research Foundation – Flanders (FWO, Belgium). Capugel, who had no role in study design, data production and interpretation, financially supported this work. The graphical work of Tim Lacoere is kindly acknowledged.

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