Viability of Probiotics in Non–Enteric-Coated Vegetarian Capsules

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Abstract

A study was conducted on three commercial probiotic products, termed P_1, P_2 and P_3. These products were all manufactured in hypromellose capsules, but only P_3 was enteric-coated. The objective of the study was to investigate the disintegration profile and the strains survival of some probiotics products upon exposure to an acidic environment (simulated gastric fluid; SGF). Our results show that P_1 and P_2 disintegrated within 5 min of exposure, while P_3 did not disintegrate after 60 min of exposure in the SGF. In addition, after 60 min incubation in the SGF, percentages of viable cells after plating and enumeration were 3%, 8%, and 97 % for P_1, P_2, and P_3, respectively. Our results also showed that investigated products were all overbuilt at manufacturing. This overbuilding was not enough to guarantee 100% of the label claim of non–enteric-coated products. Enteric coating of vegetarian capsules is an effective way to protect probiotics from gastric acidity, and therefore to ensure that these good bacteria reach the intestine.

Keywords: Probiotics bacteria, enteric coating, simulated gastric fluid, survival, acid tolerance, vegetarian capsules.

Introduction

Several delivery systems are available for pharmaceuticals and nutraceuticals. In Canada, vitamins and minerals, amino acids, enzymes, plant extracts, and probiotics fall within the scope of the natural health products industry. Owing to their biological nature, some of these products, including enzymes and probiotics, are only amenable to hard capsules. For this purpose, contrary to gelatin capsules, vegetarian ones are widely used in the industry. In fact, vegetarians, diabetics, and patients with restricted diets, religious or ethnic groups have pushed the industry to substitute gelatin capsules with vegetarian capsules (Dagadiye et al., 2012). They are several types of vegetarian capsules such as hydroxypropylmethylcellulose (HPMC, also known as hypromellose), pullulan, starch, and polyvinylalcohol (PVA) (Kathpalia et al., 2014). Some of these capsules, known as delayed-release capsules, may supposedly withstand acidic conditions for some time (Marzorati et al., 2015). To date, vegetable capsules based on hypromellose are widely used in the food supplements industry. Some of these capsules are intended for immediate release, whereas others are supposedly meant for a delayed release. In our previous study, we reported that uncoated delayed-release capsules opened in the SGF between 45 and 60 min, and that even before opening, severe damage to probiotics could be observed as from 30 min (Kuate et al., 2017). We concluded that coating was necessary to protect probiotics in so-called “delayed-release.” In this study, we address the issue of immediate-release hypromellose capsules. The questions are: Can they be employed as delivery system for all kinds of food supplements? Is it reasonable to use these vegetarian capsules as effective delivery system for food supplements such as enzymes or probiotics? Can concomitant ingestion of food play a role in protecting these capsules and their

contents from the gastric acid environment? Do some probiotics strains have ability to survive the acidic condition of the stomach?

In this study, we will focus on three probiotic products manufactured in vegetarian capsules, of which one was enteric-coated and two non-coated. Our objective was to investigate the disintegration time in SGF and to assess the survival of probiotic strains following a one-hour stay in the simulated gastric environment.

Materials and Methodology

Test Samples and Microorganisms

Probiotic products used in this study were codified as P_1, P_2, and P_3. They were purchased from a local natural health products store and were within expiry time. Samples were kept at 4 °C until use. In all cases, probiotic strains were encapsulated in hypromellose (HPMC) capsules. Information on investigated samples are summarized in **Table 1**. Products P_1 contained four *Lactobacillus* strains and two *Bifidobacterium* strains, whereas P_2 contained eight *Lactobacillus* strains and four *Bifidobacterium* strains. Product P_3 contained thirteen *Lactobacillus* strains, four *Bifidobacterium* strains, and one *Streptococcus* strain. Enteric-coating was absent on P_1 and P_3, but present on P_3. P_1, P_2, and P_3 label claims were 10 billion per capsule, 12 billion per capsule, and 20 billion per capsule, respectively. The Modified Reinforced Clostridial Medium (RCM) (OXOID, UK) was used for the enumeration of viable bacteria at 37 °C (± 1 °C) at 58% (± 5 %) relative humidity under anaerobic conditions. Anaerobic conditions were achieved by enclosing the plated cultures with the activated BD GasPak EZ anaerobe gas-generating pouch system with indicator (Ref # 2016683) from Becton, Dickson, and Co. (Sparks Glencoe, MD, USA).

Table 1. List of Bacteria Species Contained in Each Test Sample

#	Claimed Potency per Capsule	Product Composition	Specific Direction of Use	Enteric Coating
P_1	10 billion CFU	4 *Lactobacillus* strains 2 *Bifidobacterium* strains	After meals or on a full stomach	Absent
P_2	12 billion CFU	8 *Lactobacillus* strains 4 *Bifidobacterium* strains	None	Absent
P_3	20 billion CFU	13 *Lactobacillus* strains 4 *Bifidobacterium* strains 1 *Streptococcus* strain	None	Present

Disintegration Testing

Gastric conditions were simulated by preparing a simulated gastric fluid (SGF) according to the United States Pharmacopeia (USP). The pH of the solution was 1.2. Samples were incubated in this solution for 60 min and verified for capsule integrity at 5-minute intervals.

Simulation of the Gastric Conditions

To imitate the gastric conditions, a few capsules-equivalent to about 10 g of probiotics powder were required for each replicate. This was conducted in the SGF (pH 1.2) for enteric-coated

capsules and in a medium consisting of a mixture of the SGF and a nutritional food (Kellogg's shake) for non-enteric-coated capsules. The pH of this preparation was adjusted to 2.5 to simulate the normal gastric response to a meal or beverage. The incubation time was 60 min, reduced by the time of complete disintegration. A sinker was used to ensure that enteric-coated capsules were immerged in the SGF throughout the incubation time while the contents of the non-enteric-coated capsules were directly emptied into the prepared mixture (pH 2.5). Each set was incubated in triplicate.

Microbiological Analyses

At the end of the incubation, the mixture was immediately neutralized to 7.0 with 1N sodium hydroxide solution, whereas the capsules in the SGF were briefly washed in a buffer (pH 7.3) and immerged in a different SGF whose pH was previously adjusted to 7.0. Then, these capsules were homogenized using a disinfected blender. Then, the slurries were centrifuged at 3000 RPM for 10 min at room temperature (19 °C–23 °C). The 1 mL of supernatant was diluted into 90 mL ready-to-use buffered peptone water (3M Canada, London, ON, CAN). This solution was further diluted into 9 mL ready-to-use buffer peptone water (Biokar Diagnostics, Allonne, France) to give appropriate concentrations before plating on the agar plate. Prior to each dilution, samples were shaken to ensure the homogeneity of the contents.

As control, enumeration of viable bacteria was conducted in parallel on untreated capsules for each product. About 10 g of probiotics sample was weighed and mixed with 90 mL buffered peptone water using sterile blender bags with tear-off protection strip (Labplas, Sainte Julie, QC, CAN) for 1 min at speed 4 using a bag mixer (Interscience, Woburn, MA, USA). As above, dilutions with buffered peptone water were made to achieve appropriate concentrations before plating. Each sample was plated in triplicate. After 72 h incubation, enumeration of live microorganisms was done using a Colony Counter Scan 100 (Interscience). The survival rate was evaluated by comparing the counts obtained at each specific time point with expected counts (sample with no treatment), assuming no mortality occurred.

Mathematical Analyses

Data presented in this study are either nontransformed, or transformed in percentage of reduction (%R) (percentage of viable cells) using the following formula:

$$\% \text{ Reduction } (\%R) = (1 - M_{final} / M_{initial}) \times 100$$

Where:

M_{final} = final number of microorganisms

$M_{initial}$ = initial number of microorganisms.

Results and Discussion

Disintegration Test

P_1 and P_2 disintegrated in the simulated gastric fluid, pH 1.2. The disintegration time was 5 min for both samples. Contrariwise, none of the capsules of sample P_3 disintegrated. All six capsules remained intact at 60 min. Disintegration results are presented in **Table 2**.

Table 2. Disintegration Test Results in Simulated Gastric Fluid

#	Claimed Potency	Number of Capsules Disintegrated	
	Billion CFU per Capsule	5 min	60 min
P_1	10	6	6
P_2	12	6	6
P_3	20	0	0

Note: A product intended to act in the intestine should be able to withstand acidic conditions of the stomach.

These results show that P_3 can resist to an acidic environment, whereas P_1 and P_2 would not. In contrast to P_1 and P_2, P_3 is an enteric-coated product. This enteric coating of P_3 may account for this observation, because the polymers used in enteric coating have the property to remain unionized at low pH as that of the SGF, and therefore remain insoluble (Hussan et al., 2012). These results strongly suggest that P_3 capsules would travel through the stomach and reach the intestine undamaged, providing a reasonable physical protection to its contents. However, capsules of P_1 and P_2 disintegrated rapidly and exposed their contents to the acidic environment as early as from 5 min. Studies have demonstrated that the gastric acidity is one of the main obstacles to probiotics survival (Bezkorovainy, 2001) (**Table 3**).

Table 3. Viable Cells in Test Samples

#	Claimed	Initial Count	Overbuilding Rate	Viable After 55 min Incubation	Percentage of Viable Cells after 55 min Incubation
	(Billion CFU per Capsule)		(%)	(Billion CFU per capsule)	(%)
P_1	10	31.7	317	1.0	3.2
P_2	12	26.0	217	2.2	8.3
P_3	20	34.7	174	33.7	97.0

Initial Counts of Probiotics

Enumeration of viable cells in untreated samples showed that P_1, P_2, and P_3 contained 32, 26, and 35 billion CFU per capsule, respectively. These values are higher than the label claims that were 10, 12, and 20 billion CFU per capsule for P_1, P_2, and P_3, respectively. This represents overbuilt rates of 317 (P_1), 217 (P_2), and 174% (P_3) (**Table 3**). According to Health Canada, the regulatory body in Canada, it is the responsibility of licence holders to ensure that natural health products meet a minimum of 80% of the label claim at expiry date (Health Canada, 2015). To comply to this requirement, many companies overbuild their formulation, especially enzyme and probiotics products, as they potentially lose their potency during the shelf life.

Effect of Exposure to an Acidic Environment

The effect of exposure to an acidic environment was tested for the three products for 55 min for P_1 and P_2 and for 60 min for P_3. These times were determined based on disintegration time in SGF (**Table 2**). The acidic environment was simulated by using the simulated gastric fluid (SGF) and a 1:1 mixture of the SGF and a nutritional beverage whose pH was adjusted to 2.5. Results obtained are shown in **Table 3**. When incubated in the nutritional food mixture, viable cell counts drastically dropped from 31.7 billion CFU per capsule to 1.0 billion CFU per capsule for product P_1, and from 26.0 billion CFU per capsule to 2.2 billion CFU per capsule for P_2. These decreases represent about 3.2% and 8.3% of the initial probiotic load for products P_1 and P_2, respectively. However, it should be mentioned that in human gastrointestinal (GI) tract, stomach pH changes gradually and this process, which depends upon the type of food ingested, may last between 20 and 60 min (Tompkins et al., 2011). Therefore, these results must be viewed with some caution. The impact of the incubation directly into the SGF was worse. Only 0.04% of P_1 cells survived; so, did 0.16% of P_2 (results not shown). These results also indicate that loss did not seem to be organism-specific: *Lactobacillus* species and *Bifidobacterium* species appeared to be affected to a similar extent. On the other hand, regarding the enteric-coated sample P_3, viable cell counts after 60 min incubation in SGF were 33.7 billion CFU per capsule, corresponding to 97.0% of the initial load. **Figure 1** shows the percentage of reduction of total plate count (TPC) relatively to the initial count of probiotics in the three tested products.

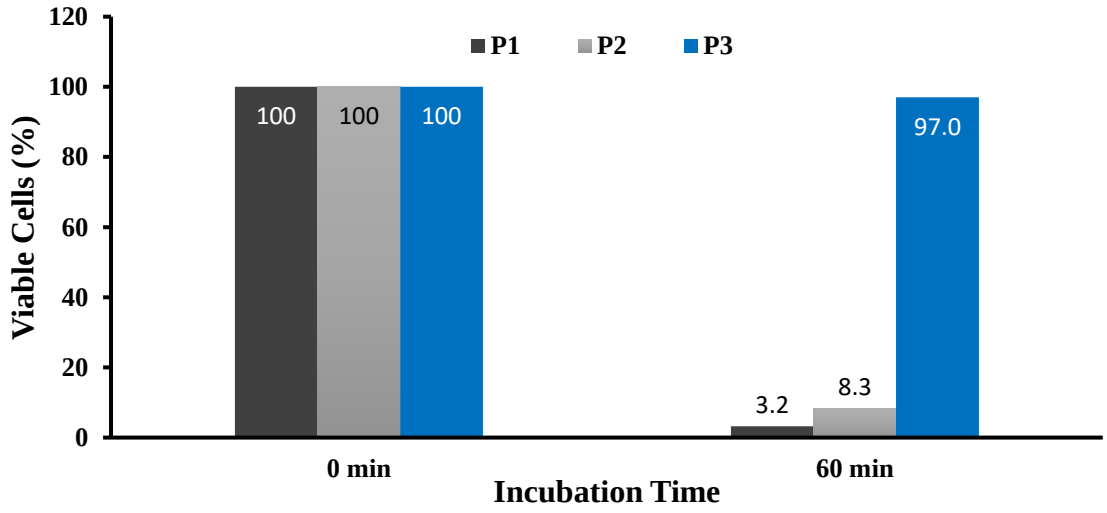

Figure 1: Cells Viability After Incubation into the Simulated Gastric Fluid

Table 3 and **Figure 1** illustrate the impact of acidic conditions on probiotics. These simulated conditions badly impaired probiotic strains in non–enteric-coated hypromellose capsules. Similar vegetarian capsules with enteric coating show a remarkable protection to acid. Not only they did not disintegrate (**Table 2**), but they also served as a protective layer for their contents because of the insolubility of the constituents of enteric-coating solutions at pH < 4.5 (Hussan et al., 2012). In fact, the pH of the gastrointestinal tract (GIT) changes throughout the small intestine: It increases gradually from a highly acidic 1.0–2.5 in the stomach (Evans et al., 1988) to about 6 in

the duodenum, then reaches 7.4 in the terminal ileum (Evans et al., 1988, Fallingborg, 1999). This increase of the pH in the intestine helps the ionization of acidic functional groups of the coating agents, which eventually become soluble, allowing the capsule to open and deliver its contents to an environment where the pH is suitable for their establishment. A similar concept is applied in the pharmaceutics oral dosage forms targeting specific areas of the intestine such as the colon (Fukui et al., 2000). According to Cole et al. (2000), HPMC capsules must be enteric-coated to achieve intestinal targeting.

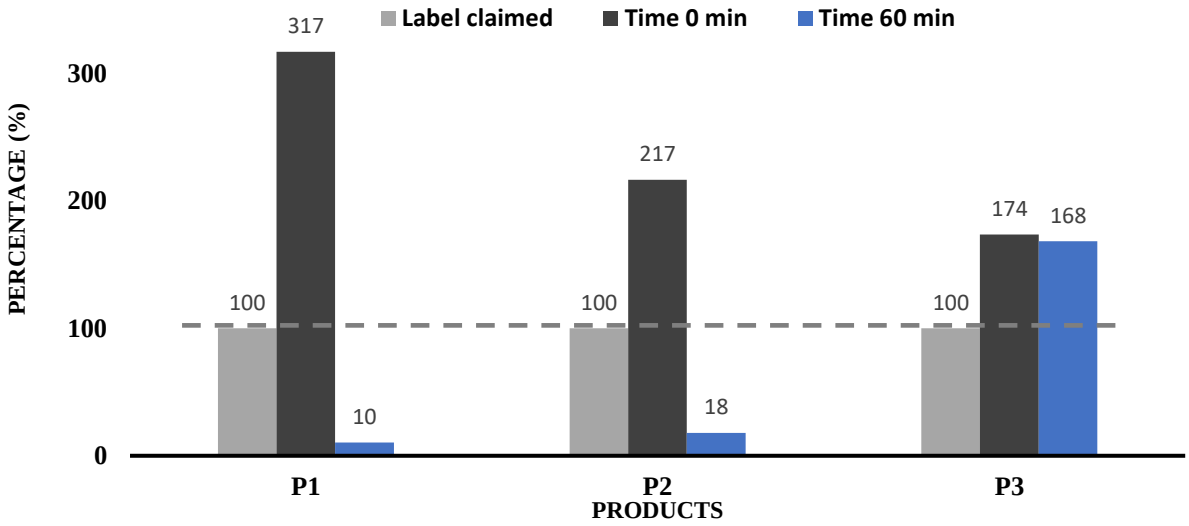

Figure 2: Percentage of Viable Cells with Respect to the Initial Counts

Figure 2 shows the percentage of initial viable cell counts and the percentage of cells after 55 min incubation relatively to the label claim. Overbuilding rates were 317%, 217%, and 174% for P_1, P_2, and P_3, giving rise to varied results. Under the conditions of the experiment, survival rates were 10% and 18% for P_1 and P_2. Thus, the overbuilding approach did not suffice to ensure the 80% minimum recommended by Health Canada for P_1 and P_2, the non–enteric-coated vegetarian capsules. Maximum dosage for probiotic products is 300% (Health Canada, 2015). Interestingly, the situation was clearly different with the product P_3 that showed a barely changed survival rate of 168%, exceeding the label claim. This is attributable to enteric coating present on P_3 capsules.

Conclusions

Non-enteric-coated hypromellose vegetarian capsules disintegrate rapidly in a simulated gastric fluid, and in so doing expose their contents to an acidic environment. In the case of probiotics, this exposure strongly affects their survival and compromises the potential benefits of these microbial organisms. This study suggests that oral delivery systems intended for intestine colonization such as probiotics should be enteric-coated to ensure maximum benefits for customers.

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