Short-Term Viability of Probiotics in Some Commercial Products

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Abstract

The impact of refrigeration on the growth of probiotics was studied to gain insight into the storage conditions of these commercial products. Three commercial products were purchased from a retailer: two "shelf-stable" brands (P15 and P30), and a third brand that was enteric-coated and for which refrigeration was recommended (P20). The products were tested at purchase and at 1-, 2-, 3-, and 6-month time intervals using the RCM medium under anaerobic conditions. The results revealed that all products evaluated contained viable probiotic bacteria, but only one out of the three products (P20) contained the claimed culture concentration or more. At 18 months prior to the expiration date, P20 contained 130% of its label claim, while P30 and P15 had 77% and 73% of viable probiotics only. At one year prior to the expiration date, the percentage of viable and culturable probiotics was 112%, 43%, and 20%, respectively, for P20, P30, and P15. It was also observed that a lower viability was correlated with a higher content of *Bifidobacterium* in the product. This study showed that refrigeration maintained the survivability of probiotics in stores.

Keywords

Probiotics bacteria, probiotics viability, stability, storage.

Introduction

Nowadays, it is well known that probiotics are beneficial for health.[1],[2] However, many consumers and health-care providers have a hard time identifying high-quality products in the market, and this issue remains a major concern. Probiotic products should be safe from chemical and microbial contaminants, and the probiotic content indicated on their label should meet the actual content throughout the shelf life. Elsewhere in the world, a few studies have been conducted to assess probiotic-bacteria label accuracy,[3],[4],[5] leading to the conclusion that not more than half of the tested samples met or exceeded their label claim.

The aim of the study presented herein was to analyze the content of probiotics of some commercially available probiotic products used in the Montréal region (Québec, Canada) and to evaluate the role of refrigeration in maintaining the viability of these beneficial bacteria.

Materials and methodology

Test Samples and Microorganisms

Three commercial probiotic products—labeled **P20**, **P15**, and **P30**—were obtained from a local retailer in September 2020. **P20** was a product from the company New Roots Herbal (NRH), whereas **P15** and **P30** were competitor brands. **P20** and **P30** capsules were packed in plastic opaque bottles containing a desiccant, while **P15** capsules were sealed in Aclar® blister and packed in a paper box. Samples **P15** and **P30** were kept at room temperature (19–25 °C), whereas **P20** was stored at 4 °C until used, as per manufacturer's recommendations. Information regarding investigated samples is summarized in **Table 1**. All products contain various strains of *Bifidobacterium* and *Lactobacillus* probiotics. Claims for these products are 20 billion/capsule, 15 billion/capsule, and 30 billion/capsule for **P20**, **P15**, and **P30**, respectively. They all had an expiration date of May 2022.

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

#	Brand Name	Manufacturer	Potency Claimed per Capsule	Product Composition	Bifidobacterium (%)
P20	Probiotics Intensity	New Roots Herbal	20 billion CFU	13 *Lactobacillus* strains 4 *Bifidobacterium* strains 1 *Streptococcus* strain	11
P15	Probiotic capsules	Brand 1	15 billion CFU	10 *Lactobacillus* strains 5 *Bifidobacterium* strains 1 *Lactococcus* strain	31
P30	Probiotic capsules	Brand 2	30 billion CFU	9 *Lactobacillus* strains 5 *Bifidobacterium* strains	25

Culture Media, Sample Preparation, and Incubation Conditions

The Modified Reinforced Clostridial Medium (RCM) (Oxoid, Hampshire, UK) was used for the enumeration of total viable bacteria. Test samples were prepared by decimal dilutions according to international standards and diluted appropriately to obtain between 30 and 150 colonies per plate (90 mm). Incubation was carried out for 72 ±3 h at 37 °C (±1 °C) under anaerobic conditions, which were achieved by enclosing the plated cultures with activated BD GasPak EZ anaerobe Gas-generating Pouch System with an indicator (Ref #2016683) from Becton Dickson and Co. (Sparks Glencoe, MD, USA).

About 5 g of probiotics sample was weighed and mixed with 90 mL of buffered-peptone water using sterile blender bags with tear-off protection strip (Labplas, Sainte-Julie, Québec, Canada) for 1 min at speed 4 using a Bag Mixer (Interscience, Woburn, MA, USA). Decimal dilutions with buffered-peptone water (3M Canada, London, Ontario, Canada) were made to achieve appropriate concentrations before plating. Each replicate was plated in triplicate, and each sample was tested on two consecutive days at each testing station. After 72 h incubation, enumeration of live microorganisms was done using a Colony Counter Scan 100 (Interscience, Paris, France). The three products were tested at the time of purchase (month 0), then 1, 2, 3, and 6 months later. The viability of probiotics strains was evaluated by comparing the counts obtained at each specific time point with the product label claim.

Mathematical Analyses

Data presented in this study are transformed in percentage of viable cells using the following formula:

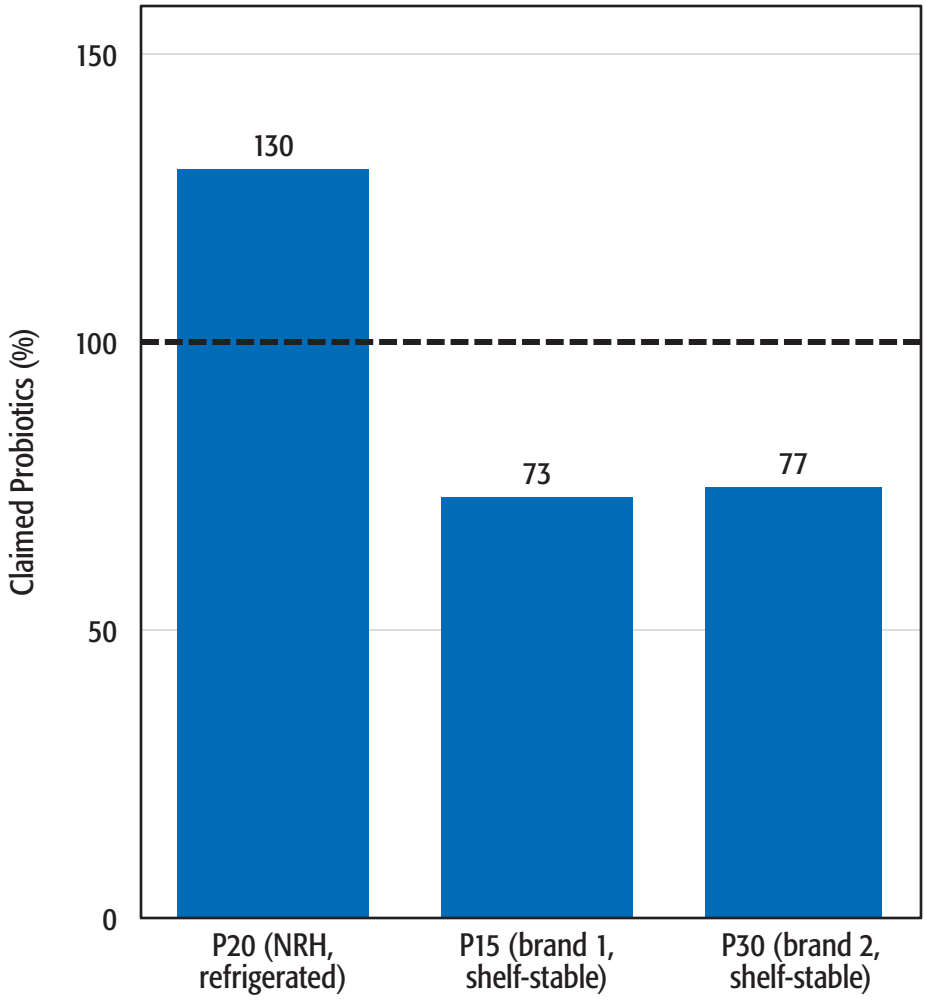
$$\text{Probiotics viability (\%)} = 100 \times \frac{\text{Probiotics determined}}{\text{Probiotics claimed}}$$

Results and Discussion

Initial Counts of Probiotics

Upon sample reception (month 0), the total probiotics in the three products were determined as 26 billion/capsule, 11 billion/capsule, and 23 billion/capsule for **P20**, **P15**, and **P30**, corresponding to 130%, 73%, and 77% of label claim, respectively. Thus, **P20** exceeded its claim, whereas **P15** and **P30** contained a significantly lower number of viable bacteria as compared to the numbers on the labels.

Figure 1. Percentage of Viable Probiotics Comparatively to the Label Claim



A higher-than-expected number of viable probiotics cells in **P20** suggests that this product was overbuilt at manufacturing. A similar postulate cannot necessarily be put forward with **P15** and **P30**, for which the content at the purchase was less than 80%, the minimum acceptable amount of a label claim on the shelf according to the Canadian regulatory agency for a probiotic product.[6] It is plausible that **P15** and **P30** were compliant at manufacturing, but due to the storage conditions in the store (room temperature), probiotics cells continuously died off, reaching 73% and 77%, respectively, at the time they were purchased (**Fig. 1**). Considering that the three products had 18 months remaining on their shelf life, the viable probiotics in **P15** and **P30**, both of which alleged to be "shelf stable," was unexpectedly low.

Subsequent Testing

Over the following months, the three products were tested at four different time points, namely after 1, 2, 3, and 6 months.

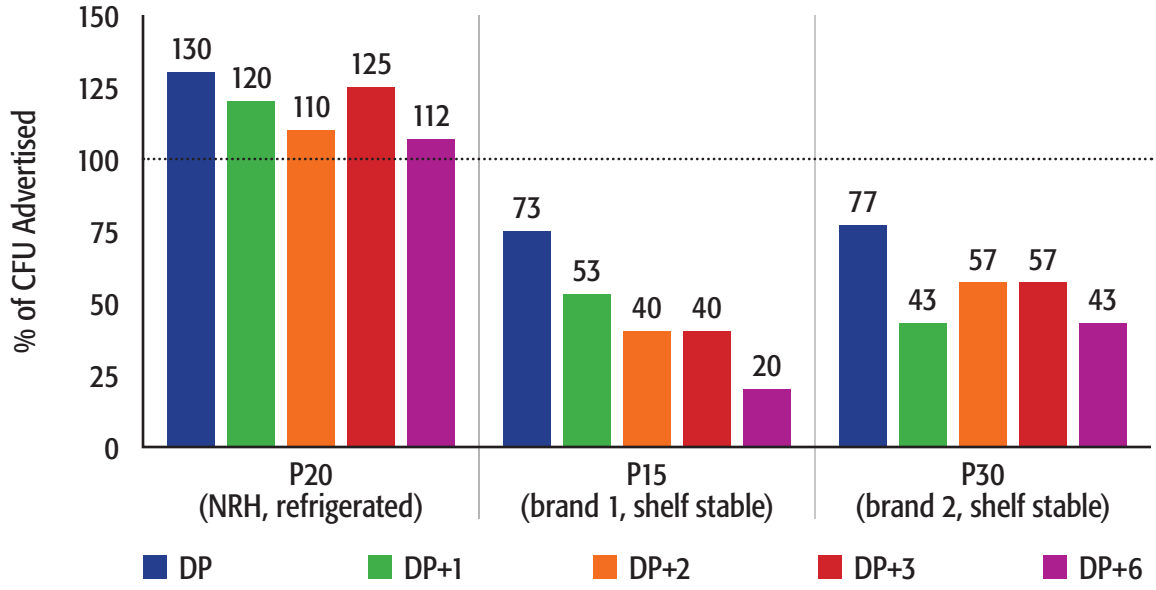
Table 1. Number of viable cells in three probiotics products over a period of six months

ID	Manufacturer	Claim	Probiotics (billion per capsule)				
			Month 0	Month 1	Month 2	Month 3	Month 6
P20	New Roots Herbal	20	26	24	22	25	22
P15	Brand 1	15	11	8	6	6	3
P30	Brand 2	30	23	13	17	17	13

During the testing period, the values obtained evolved differently for the three products. Indeed, six months later, **P20** content varied from 26 to 22 billion/capsule, representing a 15% reduction. **P30** (Brand 2) reduced from 23 to 13 billion/capsule of viable probiotics, about a 43% reduction. The most marked reduction was observed with **P15** (Brand 1), where the content dropped from 11 to 3 billion/capsule, a reduction of 73% (**Table 1**). Thus, within 6 months, **P20** reduced by 15% whereas the competitor brands reduced by 43% and 73% for **P30** and **P15**, respectively.

Interestingly, despite its 15% reduction, **P20** from New Roots Herbal constantly exceeded the label claim, with 112% still available at the end of the study. In contrast, **P15**, already below the label claim at the onset of the study (73%), sharply decreased, leaving a residual probiotic viability of 20% after 6 months (**Fig. 2**). The fact that **P20** was overbuilt at manufacturing (by > 30%) could be seen as a strategy of the manufacturing company (New Roots Herbal) to ensure its product meets its label claim at expiry in order to face up to this inherent reduction. These results corroborate those reported in the literature in recent years. In a study on several *Lactobacillus* species stored at room temperature, 4 °C, and –20 °C, it was established that probiotics are affected by the temperature, greater viability being observed at a lower temperature.[7]

Figure 2. Percentage of Viable Probiotics Cells Relatively to the Label Claim



Storage temperature does not only affect *Lactobacillus* species, but also *Bifidobacterium* probiotics. Initial *Bifidobacterium* percentage in tested products varied from 11% in **P20** to 25% in **P30**. The highest concentration was present in **P15**, with 31% (**Table 1**). Remarkably, the reduction of viable probiotics was the highest in products with the highest *Bifidobacterium* content and vice versa. Although this can be investigated further by using a selective media for *Bifidobacterium*, it is likely that the higher sensitivity of *Bifidobacterium* to storage temperature may account for this observation. A study on the survivability of *Bifidobacterium lactis* and several *Lactobacillus* species found out that it was dependent on probiotic strains,

with *B. lactis* showing the less resistance at 20 °C.[8] The effect of the temperature on *Bifidobacterium* was studied by several scientists,[9],[10],[11],[12] with the conclusion that a lower temperature is beneficial for the survival of probiotics, which is in turn directly related to the therapeutic potential of these products. This therapeutic potential is exercised when viable probiotics reach the intestines.[13],[14],[15],[16] For oral products, this means passing through the gastric environment, where only enteric-coated capsules do not leak (**P20**).

Not being enteric-coated, it is anticipated that **P15** and **P30** capsules will disintegrate in the stomach and leak into the acidic gastric juice, which is notoriously detrimental to probiotics. An in vitro study on the survival of probiotic capsules in a simulated gastric environment revealed that non–enteric-coated capsules disintegrate in the stomach within 1 h and are contaminated with the gastric juice within 30 min. Accordingly, the effect of the stomach stay on the viability of probiotics determined after 30 min revealed that only 20–30% of cells remained viable.[17] After 1 hour of incubation, at least 90% of probiotics were no longer viable.[18],[19] The quality of commercial probiotics products in relation to their label accuracy was studied in several parts of the world, namely in Ontario (Canada),[20] in London (UK),[21] and very recently in Poland.[22] The results presented in the present study align with these previous works insofar as two out of the three tested products did not have the applicable number of viable bacteria declared by the manufacturer.

Although a larger sample size is recommended in further work to get a better picture of commercially available probiotics, the results of this study serve as proof of concept that in-store probiotic products, in particular those supposedly stable on the shelf, may not always meet the label claim, to the detriment of consumers.

Conclusions

The plethora of probiotics currently available on the market, combined with diverting recommendations on the storage conditions and the need for enteric coating, can be confusing for end-users. This study shows that not all probiotic products in stores meet the label claim. It also revealed that, irrespective of the storage conditions, the number of viable probiotics decreases with time but much faster when they are kept at room temperature, thereby supporting the need to refrigerate probiotic products. Refrigerated and enteric-coated probiotics, therefore, appear as the best choice for consumers looking for better-quality products.

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