

Fate of Probiotics in Peanut Butter During Simulated Gastrointestinal Passage

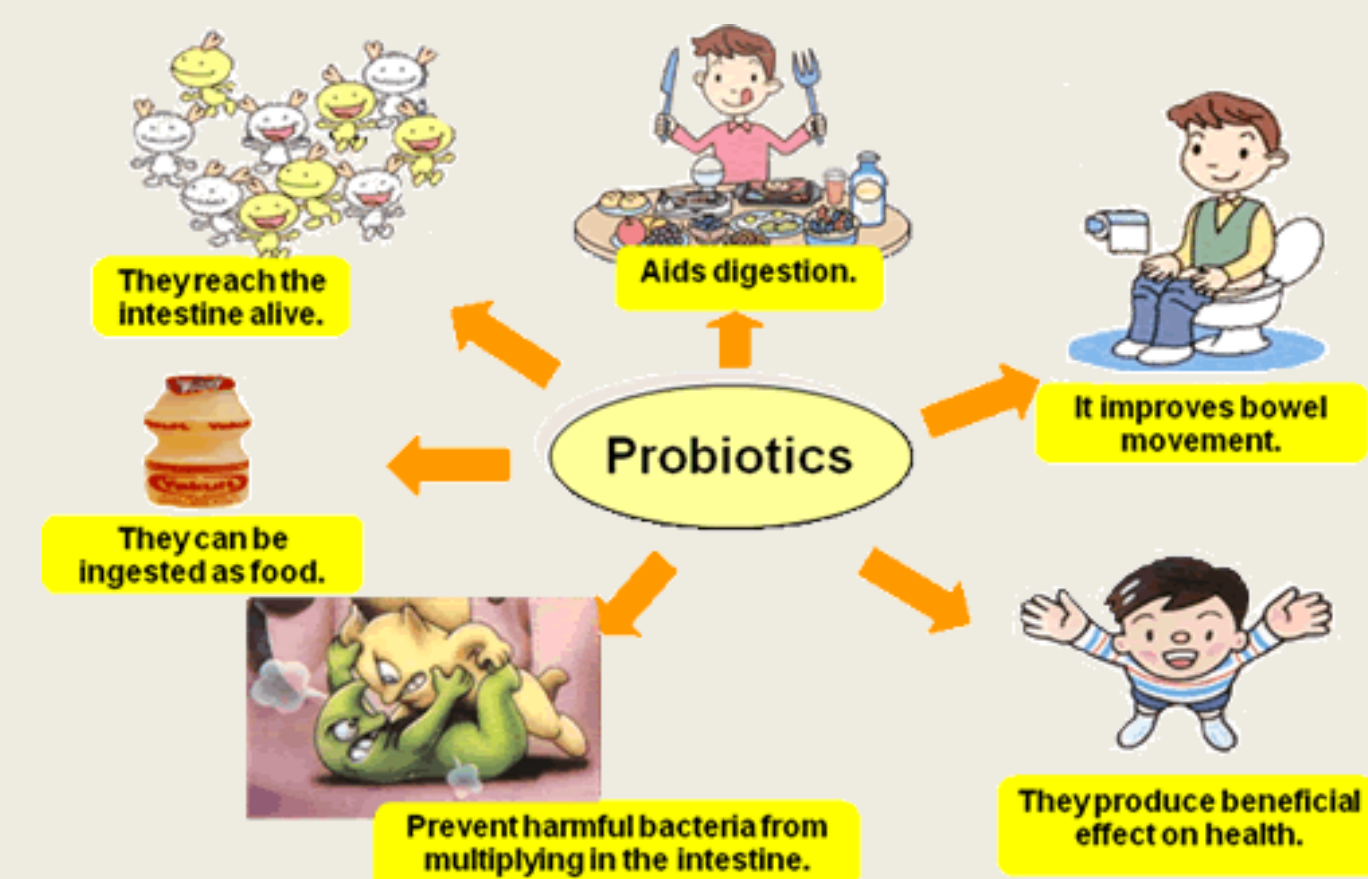
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ABSTRACT

Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host, especially by controlling diarrhea in children under 5 years of age. This study observed the effect of food matrices on the viability of probiotics during simulated gastrointestinal passage. Full fat and reduced fat peanut butter inoculated with 10⁷ CFU/g of probiotic culture U (4-strain mixture), N (16-strain mixture) or C (single-strain culture) were homogenized in 0.5% NaCl. To mimic digestion in the stomach, the pH (ca. 6) of the samples was reduced to 1.4-1.9 and pepsin and lipase were added. Samples were incubated for 2 h with agitation at 37 °C. Afterwards, digestive enzymes, bile and pancreatin were added to simulate digestion in the upper small intestine. Samples were incubated at 37 °C with agitation for 2 h after sample pH was increased to 4.3-5.2. Finally, the pH of the samples was adjusted to 6.7-7.5 to simulate conditions in the lower small intestine. Samples were incubated at 37 °C for 2 h. Aliquots were collected after every phase to enumerate bacterial populations. A similar population of probiotics that was not inoculated into peanut butter was used as controls. Decrease in probiotic cell population was more profound in samples without peanut butter (3.76 log CFU/mL) than those with peanut butter (1.54 log CFU/mL). The average probiotic counts in samples with peanut butters (4.53 log CFU/mL) were significantly ($p < 0.05$) higher than those without the protection of food matrices (2.96 log CFU/mL). Mean populations of U and N were significantly ($p < 0.05$) higher than the population of C. Fat content of peanut butter did not have significant influences on probiotic bacterial counts. Peanut butter protected probiotic bacteria under simulated gastrointestinal conditions, and thus, could serve as an effective vehicle to deliver probiotic culture/mixtures.



INTRODUCTION

Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host. A well substantiated health benefit of probiotics is the management of diarrhea (Boylston and others 2004). Diarrheal diseases is second to pneumonia as the highest cause of mortality in children under 5 years of age in the developing countries with an annual mortality of about 2.5 million (WHO, 2011). Numerous reports of clinical studies have documented the effectiveness of probiotic consumption in the prevention, control and treatment of diarrhea amongst children in this age group (Sazawal and others 2006; Binns and Lee, 2010). A probiotic bacterium must survive the acidity of the stomach, bile and enzymes present in the gastrointestinal tract and eventually colonize the colon to exert health benefits. Generally, the efficacy of probiotics is enhanced when the cultures are ingested with a food product (Alegre and others 2011). Food substrate is one of the major factors that affect and regulate the survival of probiotic cultures in the gastrointestinal tract and their subsequent colonization (Ranadheera and others 2009). In a previous research, authors observed that peanut butter is a suitable matrix in preserving the viability of *Lactobacillus rhamnosus* GG during a 1 year storage period under refrigeration and ambient conditions (Klu and others 2012).

OBJECTIVE

The objective of this study was to observe the effect of full fat peanut butter and reduced fat peanut butter on the viability of 3 different probiotic mixtures during simulated gastrointestinal passage.

MATERIALS AND METHODS

A full fat peanut butter product with a fat content of 50.10 ± 1.16% and a reduced fat peanut butter product with a fat content of 39.90 ± 0.62% were obtained from the American Blanching Company (Fitzgerald, Ga., U.S.A.). Three commercial probiotic products, designated U, N and C were used in the study. Probiotic mixture U had 2 *Lactobacillus* and 2 *Bifidobacterium* strains (Anonymous, 2011). Mixture N contained 5 strains of *Bifidobacterium*, 9 strains of *Lactobacillus*, *Lactococcus lactis* and *Streptococcus thermophilus* (Anonymous, 2011). Product C contained a single strain of *L. rhamnosus* GG. Each probiotic culture was inoculated into 1.0 kg full fat or reduced fat peanut butter (ca. 10⁷ CFU/g) at room temperature for 15 min.

Gastrointestinal conditions were simulated by adopting a method described by Buriti and others (2010). Probiotic peanut butter products were suspended and homogenized in 0.5% NaCl using a stomacher blender. To mimic digestion in the stomach, the pH (ca. 6) of the samples (10 mL each) was adjusted to 1.4 -1.9 using 1 M HCl solution. Pepsin from porcine gastric mucosa and lipase from *Rhizopus arrhizus* were then added. To mimic gastric phase, samples were incubated for 2 h at 37 °C with an agitation of 150 rpm. After the incubation, the pH of the samples was adjusted to ca. 4.3 – 5.2 using an alkaline solution (150 mL of 1M NaOH, 14 g of NaH₂PO₄·2H₂O). Bile from bovine source and pancreatin from porcine pancreas was subsequently added. Samples were incubated for 2 h (enteric phase 1) under the conditions specified above. The pH of the samples was then increased to ca. 6.7 – 7.5 with the alkaline solution. Samples were incubated for another 2 h (enteric phase 2) at aforementioned conditions. All enzymes used were from Sigma-Aldrich (St. Louis, M.O., U.S.A.). At 30 min, 2 h, 4 h and 6 h intervals, aliquots was collected, serial dilutions were made when necessary, and 0.1 mL aliquots were plated on MRS/LBS, Modified Columbia Agar Base and M17 media for *Lactobacillus*, *Bifidobacterium* and *Streptococcus* respectively. Inoculated plates were incubated anaerobically for 72 h at 37 °C. For controls, probiotic cultures without the protection of peanut butter matrices were made to undergo the same experimental procedures. Additional control experiment was performed in the same manner, only using sterilized water instead of enzymes and gastric solutions. The GLM procedure was used to analyze the probiotic bacteria as influenced by individual experimental phases, presence of food matrix and the type of the matrix.

Fisher's Least Significance Design was used to compare the significant differences between the counts of the probiotic bacteria in different peanut butter products and their corresponding controls at different experimental phases. All analyses were conducted at a 95% confidence level.



RESULTS

Table 1 show that both full fat and reduced fat peanut butter products had the same protective effect on the probiotic cultures ($p > 0.05$). Overall, the average counts in the matrix-protected cultures and unprotected cultures were 4.53 and 2.96 log CFU/mL ($p < 0.05$), respectively. For both matrix-protected and unprotected probiotic bacteria, decline in probiotic cell populations were observed with time. At the end of the 6-h experiment, the mean counts in matrix-protected (5.62 log CFU/mL) and unprotected probiotic cultures (3.31 log CFU/mL) were significantly different (Table 1). On the average, the cell populations of mixture U (6.60 log CFU/ml) and N (6.45 log CFU/ml) that underwent the experimental procedures with the peanut butter products were not statistically different ($p > 0.05$). However, when the cultures were unprotected by food matrix, bacteria in N had better survivability than those in mixture U (Figures 1A & 1B and Table 1).The probiotic bacteria in C had relatively poorer survival, and the average cell population was 4.85 log CFU/mL (Figure 1C).

Table 1
Behavior of probiotic mixtures as affected by peanut butter matrix and time during a 6 h simulated gastric

Probiotic mixture	Cell population (logCFU/mL)	
	Probiotic mixture in peanut butter	Unprotected probiotic mixture
C	4.85b	4.02b
N	6.45a	5.20a
U	6.60a	4.58ab
Peanut Butter		
Full fat	5.94a	-
Reduced fat	6.00a	-
Treatment		
Control	7.41a	6.24a
Acid/Enzyme treated	4.53b	2.96b
Time (min)		
0	7.16a	7.07a
30	5.85b	4.88b
120	5.56b	4.00c
240	5.66b	3.74c
360	5.62b	3.31c

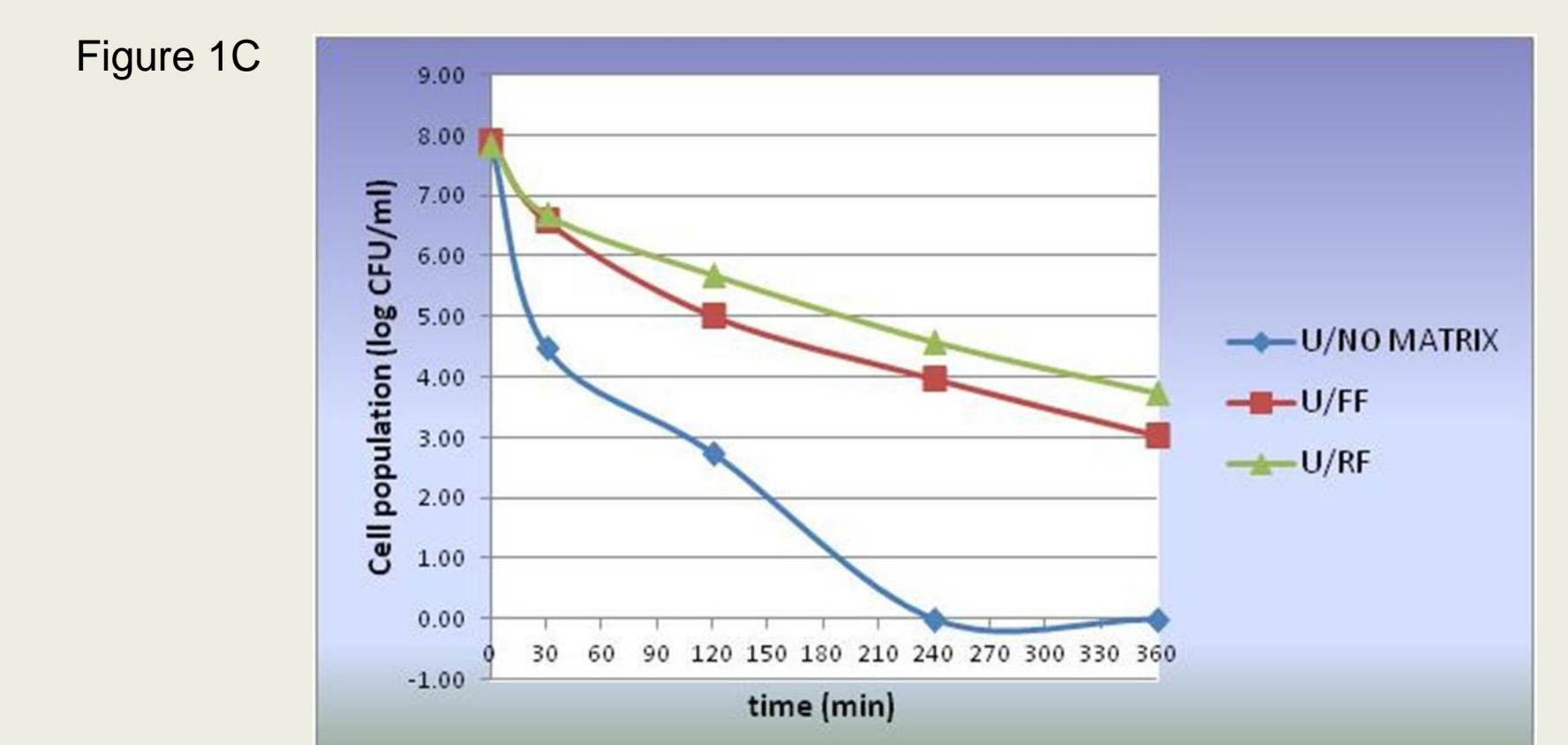
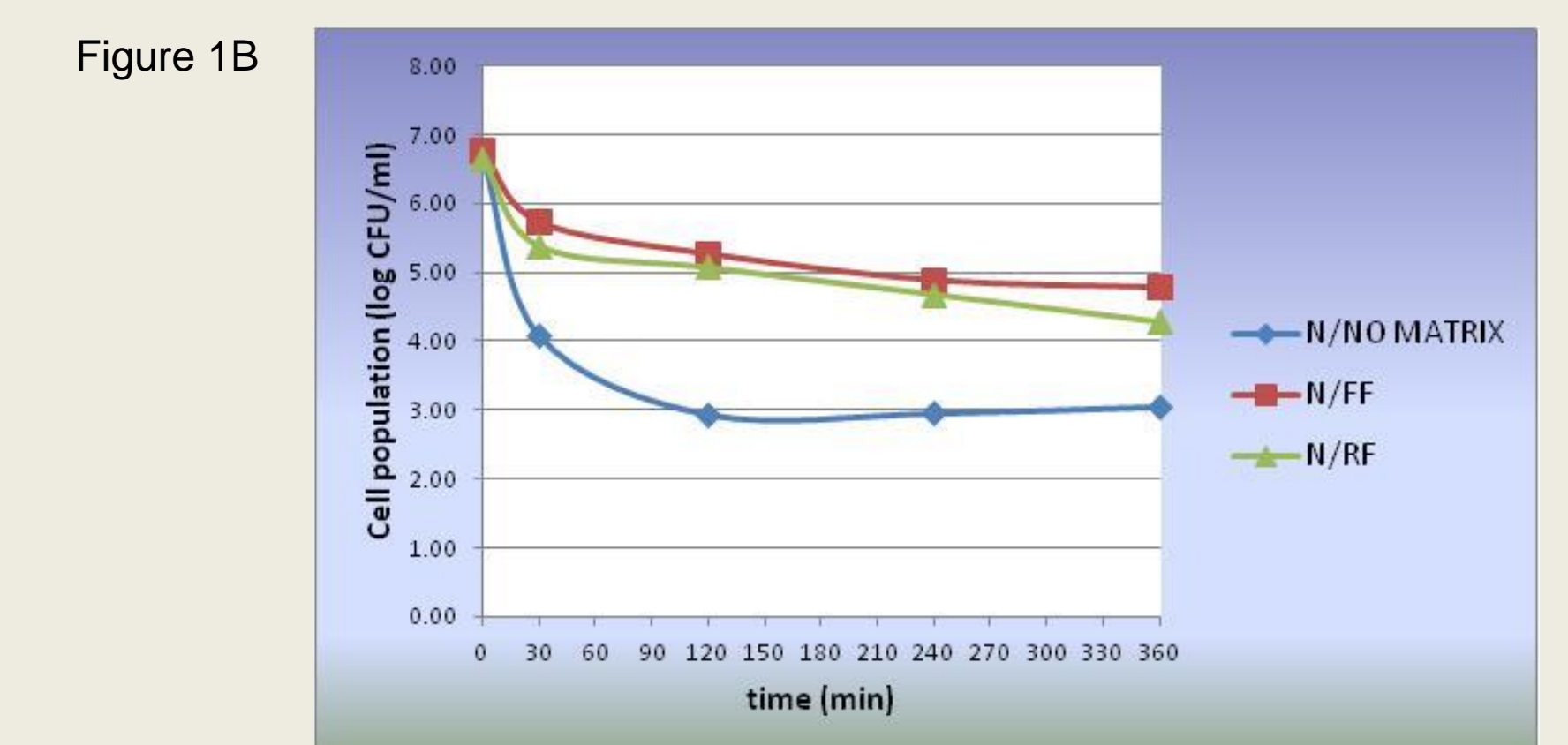
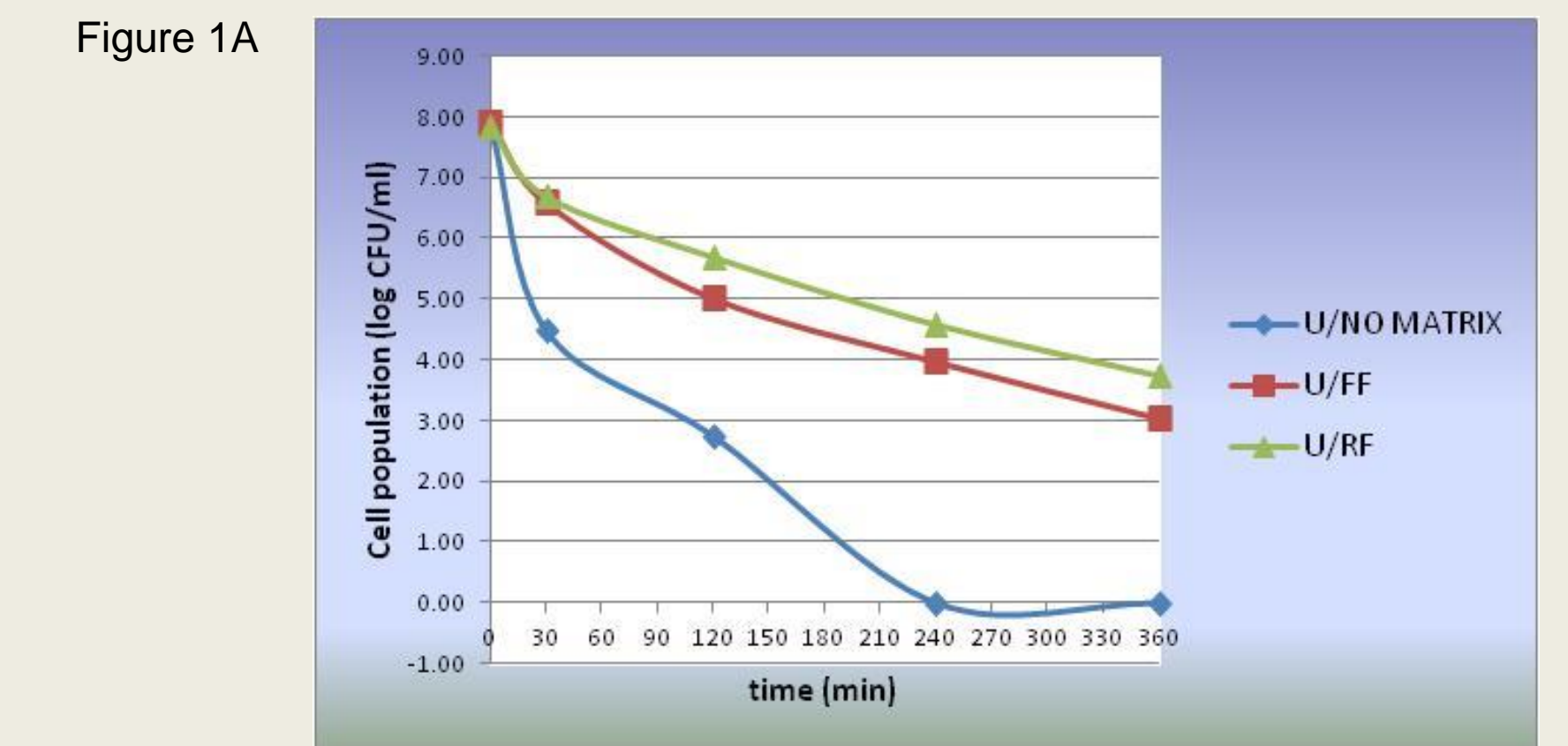


Figure 1- Survival of probiotic cell mixture U (sub-figure A), N (sub-figure B) and C (sub-figure C) that are not protected by food matrix (No MATRIX), in full fat peanut butter (FF) and in reduced fat peanut butter (RF) during a 6 h simulated gastrointestinal passage.

CONCLUSION

In this study, we observed that, peanut butter protected probiotic bacteria survival under simulated gastrointestinal conditions; with different culture mixtures exhibiting unique survival trends. This suggests that, peanut butter could serve as an effective vehicle to deliver probiotic culture to children in developing countries to help prevent and reduce the incidence of diarrheal diseases

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