Survival of four commercial probiotic mixtures in full fat and reduced fat peanut butter

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ABSTRACT

A well-documented health benefit of probiotics is their ability to reduce the incidence of diarrhea in young, malnourished children in the developing countries. This study was undertaken to determine whether peanut butter, a nutritious, low-moisture food could be a carrier for probiotics by observing the survivability of selected probiotic mixtures in peanut butter under different storage conditions. Commercial probiotic mixtures (B, U, N and S) comprising of multiple strains of Lactobacillus, Bifidobacterium, Streptococcus and Lactococcus were inoculated into full fat or reduced fat peanut butter at 10⁷ CFU/g. Resulting products were stored at 4, 25 or 37 °C for 12 months. Populations of Lactobacillus, Bifidobacterium and Streptococcus/Lactococcus were determined periodically. The average viable cell counts of N and S were significantly lower than those of B and U (p < 0.05). In all probiotic products stored at different temperatures, Bifidobacterium had the greatest survivability, followed by Lactobacillus and Streptococcus/Lactococcus. The probiotics used in the study had different surviving patterns, and their survival was influenced by storage conditions. Fat content of peanut butter had no significant impacts on probiotic viability. Results suggest that peanut butter can be a vehicle to deliver probiotics for preventing diarrhea among malnourished children.

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1. Introduction

Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit to the host (FAO/WHO, 2001). A well substantiated health benefit of probiotics is the management of diarrhea (Boylston et al., 2004) which is second to pneumonia as the highest cause of mortality in children under 5 years of age in the developing countries (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 (Binns and Lee, 2010; Isolauri, 2004; Sazawal et al., 2006).

To achieve expected health benefits, the survivability of probiotic organisms is essential since only viable cells at the time of consumption have therapeutic values. Currently, the precise therapeutic level or dosage of probiotics for expected health benefits has not been established (Reid, 2008). However, it has been proposed that the viable numbers of probiotic cells at the time of consumption should be at least 10⁶–10⁷ CFU/g or mL of food product in order to reach the 10⁶–10⁹ CFU minimum daily intake level through the consumption of 100 g or mL of a food product (Cruz et al., 2009; Karimi et al., 2011; Rathore et al., 2012).

The survival and colonization of probiotics are regulated by food substrates with which probiotics are consumed. Probiotic cultures have been recently incorporated into cheese, ice-cream and butter as well as meat, cereal, fruit and vegetable based products (Cruz et al., 2009; Rivera-Espinoza and Gallardo-Navarro, 2010; Ranadheera et al., 2013). Compared to yoghurt which is the widely used vehicle for probiotic delivery, cheese was found to be a more suitable carrier for probiotics due to its denser matrix, higher fat content and pH, and lower titratable acidity (Boylston et al., 2004; Karimi et al., 2011).

Peanut butter is a shelf stable, low moisture, energy and nutrient dense product and has been used as the major ingredient of Ready to Use Therapeutic Foods (RUTFs; Diop et al., 2003; Manary, 2006; Ndekha et al., 2005) for treatment of severe childhood malnutrition which could be the consequence of chronic diarrhea (Caulfield et al., 2004). In a previous study, we observed that at an inoculation level of 10⁷ CFU/g of peanut butter, a single probiotic strain, Lactobacillus rhamnosus GG maintained a viability of 10⁶ CFU/g for 48 wk at 4 °C and for 27 wk at 25 °C (Klu et al., 2012). The aims of this study were to observe the survivability of...
four commercial probiotic mixtures, each containing 4–16 different probiotic strains, and to examine the interaction of different probiotic strains in full fat and reduced fat peanut butter at 4, 25 or 37 °C during a 12 month storage period. The ultimate goal of the research is to use probiotic peanut butter or peanut based probiotic RUTFs to control malnutrition and diarrhea concurrently.

2. Materials and methods

2.1. Materials

A full fat peanut butter product and a reduced fat peanut butter product were graciously provided by the American Blanching Company (Fitzgerald, GA, USA). Products were stored during the experiment in clear polyethylene terephthalate (PET) jars (4 oz) tightly covered with pressure-sensitive lined polypropylene (PP) lids (Container and Packaging Supply, Eagle, ID, USA). The full fat peanut butter contains peanuts, sugar, hydrogenated vegetable oil (rapeseed, cottonseed and soybean), salt and molasses. The ingredients for the reduced fat peanut butter include peanuts, partially defatted peanut flour, sugar, hydrogenated vegetable oil (rapeseed and cottonseed), salt, molasses, monoglycerides, tocopherol, acetate and pyridoxine HCl. Information from the manufacturer indicates that the full fat peanut butter had a protein content of 21.31% and reduced peanut butter 28.12%. The total carbohydrate content is 26.31% for full fat peanut butter and 27.51% for reduced fat peanut butter. The sugar contents for full fat and reduced fat peanut butter are 10.57% and 11.67%, respectively, and the amounts of fiber are 7.20% and 8.57% for full fat peanut butter and reduced fat peanut butter, respectively.

Four commercial probiotic mixtures, designated as B, U, N and S were used in the study. Mixtures B and U had the same probiotic strains including Lactobacillus acidophilus (CUL 60), L. acidophilus (CUL 21), Bifidobacterium bifidum (CUL 20) and Bifidobacterium lactis (CUL 34). The only known difference between the two products is that mixture B had a manufacturer’s claim of 25 billion CFU of viable cells per capsule while mixture U contained 5 billion CFU of viable cells per capsule. Mixture N had a manufacturer’s claim of 16 billion live cells per g of powder and contained 16 different bacterial strains including B. bifidum, Bifidobacterium breve, B. lactis, B. lactis Bif Relief 24-7™, Bifidobacterium longum, L. acidophilus, Lactobacillus brevis, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus gasseri, Lactobacillus paracasei, Lactobacillus plantarum, L. rhamnosus, Lactobacillus salivarius, Lactococcus lactis and Streptococcus thermophiles. Mixture S had 15 billion viable cells per capsule. According to the manufacturer, its constituents included L. acidophilus (45%), L. rhamnosus (25%), S. thermophilus (10%), L. plantarum (7%), B. bifidum (6%), L. bulgaricus (3%), B. longum (3%) and L. salivarius (1%). The exact strain ratios of product B, U and N are not available since they are considered proprietary information by the manufacturers.

2.2. The fat content of peanut butter products

The fat contents of the two peanut butter products were analyzed using the AOAC Goldfish extraction method with a Goldfish Fat and Oil Extractor (Labconco Co., Kansas City, MO, USA); triplicate assays were performed. Briefly, 4 g samples were placed into Oil Extraction Cellulose Thimbles (Fisher Scientific, Pittsburg, PA, USA) and were fixed on the instrument. A 50 mL of petroleum ether (J. T. Baker, Phillipsburg, NJ, USA) was used to extract the fat at the boiling temperature of petroleum ether (35–60 °C) for about 18 h. Fat content of the samples was derived based on the following calculation: % total fat = (weight of extracted fat/weight of dry sample × 100).

2.3. The water activity and pH of peanut butter products

The pH of peanut butter homogenate (25 g of peanut butter in 50 mL of water) was determined using a pH meter (model 8000; VWR International, PA, USA). The water activity of the peanut products was measured using the Pawkit Water Activity Meter according to the manufacturer’s instructions (Decagon Devices, WA, USA). Triplicate measurements were performed.

2.4. Hexanal analysis

Hexanal contents of peanut butter products were analyzed at the beginning as well as the end of the 12 month storage period and at each sampling point, triplicate analysis was conducted. A 3 g sample was placed into a 10 mL sample vial (Sigma–Aldrich, St. Louis, MO, USA). Exactly 50 µL of a 30 ppm 4-Heptanone standard solution (Sigma–Aldrich) was added to the sample, and the vial was closed with a PTFE/Silicone septum (Sigma–Aldrich) sealed screw cap. Vials were heated on a heating block (Barnstead Thermolyne, Dubuque, IA, USA) at 35 °C for 15 min. A 100 µm polydimethylsiloxane stable-flex solid phase micro-extraction fiber assembly was exposed to the headspace of the vial for 30 min, after which the needle in the fiber assembly was injected into a Varian 3400 Gas Chromatograph with a flame ionization detector (Varian Analytical Instruments, Walnut Creek, CA, USA). Description of hexanal occurred at 200 °C for 5 min, and Helium (20 cm/s) at 125 °C was used as a carrier. The oven temperature was initially maintained at 50 °C for 5 min and then increased to 200 °C at 10 °C increment per min. The temperatures of the injector and detector were 200 °C and 300 °C, respectively.

2.5. Inoculation of peanut butter with probiotic mixtures

The jars and lids described previously were sterilized under UV light for 15 min in a Level II Biosafety Cabinet (NuAire Laboratory Equipment Supply, Plymouth, MN, USA). The peanut butter products were pre-heated in a Stabil-Therm Electric Oven (Blue M Electric Co., Blue Island, IL, USA) at 37 °C for 6 h to reduce product viscosity and aid in uniform mixing. Precisely 2.5 kg peanut butter was placed into a sterile KitchenAid® mixer, and a pre-determined amount of each probiotic mixture was inoculated into the product to achieve an inoculation level of ca. 107 CFU/g. The peanut butter and probiotic culture were mixed at room temperature for 15 min at 66 and 148 rpm for orbital and beater speeds, respectively. Nitrogen gas was incorporated into the product during the mixing to remove oxygen that might cause excessive rancidification of fats in the products during storage. Peanut butter exhibits thixotropic behavior and thus after mixing, samples of the inoculated peanut butter (10 mL) were easily dispensed into the jars using a sterile syringe (Becton, Dickinson and Co., Sparks, MA, USA) which had its end altered to aid product flow. The headspace of the jars was flushed with nitrogen for 30 s to remove oxygen, and the jars were then tightly closed with lids. Inoculated peanut butter was stored at 4, 25 or 37 °C for 12 months to mimic refrigeration, ambient and abusive condition, respectively. Un-inoculated peanut butter was used as negative controls.

2.6. Enumeration of probiotic bacteria

The initial population levels of Lactobacillus, Bifidobacterium and Streptococcus/Lactococcus species were confirmed immediately after the inoculation. To ensure that the inoculated probiotic cells were evenly distributed, multiple samples of peanut butter were taken from various locations of the mixer. Samples stored at each temperature were drawn monthly during the 12 month storage.
period. Previously unopened containers that had been brought to room temperature were used at each sampling interval. Sterile 0.1% peptone water (Becton, Dickinson and Co.) warmed to 37 °C was added to samples in the containers to achieve a 2-fold dilution based upon the weight of peanut butter in each container. The samples were mixed by vigorous manual to-and-fro shaking at an arm angle of about 45° for 1 min. After mixing, 1 mL aliquot of the sample was serially diluted in 9 mL of 0.1% sterile peptone water. A 0.1 mL of appropriate dilutions was plated, and inoculated plates were incubated and colonies enumerated. All media used in the study were purchased from Becton, Dickson and Co. Lactobacillus Selection (LBS) agar and de Man, Rogosa and Sharpe (MRS) agar were used for the enumeration of Lactobacillus species. LBS agar supplemented with tomato juice (Kroger, Cincinnati, OH, USA; 200 mL/L; LBST) which had been filtered with a cheese cloth was used to enhance the growth of L. acidophilus. Both LBS and LBST were prepared with the addition of 1.3 mL of glacial acetic acid (J.T. Baker). Lactobacillus and Bifidobacterium species were incubated under anaerobic condition for 72 h at 37 °C using the BD GasPak™ EZ in a BBL GasPak® System (Becton, Dickinson and Co.), L. lactis and S. thermophilus were incubated at 30 °C and 37 °C, respectively under anaerobic condition for 48 h. Since LBS, MRS and LBST gave comparable results, the average colony counts from the three media were used as the final Lactobacillus counts. Enumeration results for L. lactis and S. thermophilus was averaged. Counts of Lactobacillus, Bifidobacterium or Lactococcus/Streptococcus were either used separately for analysis on individual genera in probiotic mixtures or added up for analysis on total populations within each probiotic mixture.

2.7. Statistical analysis

Two replicate experiments were conducted for bacterial enumeration. Data were analyzed using a 3-way Analysis of Variance F-test and the General Liner Model of Statistical Analysis Software (SAS Inst. Inc., Cary, NC, USA). At a confidence level of 95%, Fisher’s Least Significant Difference Design was used to compare the significance of differences among populations of probiotic mixtures (B, U, N and S) and individual groups of probiotic bacteria (lactobacilli, bifidobacteria and streptococci/lactococci) in different type of peanut butter products (full fat and reduced fat) and under various storage conditions (time and temperature). The same statistical protocol was used to determine the differences in the hexanal content of peanut butter products with respect to probiotic mixtures, type of peanut butter and storage conditions.

3. Results and discussion

3.1. Physical and chemical properties of full fat and reduced fat peanut butter products

The water activity and pH of the two peanut butter products used in the present study were comparable. The average water activity was 0.31 ± 0.03 and pH was 6.23 ± 0.12. According to the FDA, the water activity of a typical peanut butter or peanut spread should be 0.35 or less (USFDA, 2009). The average fat content of full fat peanut butter used in the present study was 50.10 ± 1.16% and that of reduced fat peanut butter was 39.90 ± 0.62%, and these values were consistent with FDA standards (USFDA, 2012).

Results of hexanal analysis showed that there was no statistically significant difference between the hexanal contents in samples stored at 4 °C and 25 °C (p > 0.05). However, a significantly higher hexanal content was detected in samples stored at 37 °C (Table 1). Although the hexanal content in full fat vs. reduced fat peanut butter stored at this temperature varied 11.12 ppm, the difference was statistically insignificant (p > 0.05; Table 1). Total hexanal content was significantly higher in samples inoculated with probiotic mixtures N and S than those inoculated with mixture B (p < 0.05; Table 1). The mean hexanal content in samples inoculated with mixture U was not significantly different from those inoculated with the other three probiotic mixtures (Table 1). The mean hexanal content of all samples tested in the present study increased from 2.15 ppm at the beginning of the experiment to 71.27 ppm after the 12 month storage period (Table 1).

Hexanal is a sensitive and reliable indicator of fatty acid oxidation that occurs during product storage, and the amount of hexanal formed in a product has a direct correlation with its storage temperature (Holse et al., 2012; Panseri et al., 2011). In peanuts, hexanal is formed mostly from the oxidation of linoleic acid (Wambura and Yang, 2010). The present study detected more hexanal in peanut butter products stored at 37 °C than at 25 and 4 °C (Table 1). Similar to what was observed in the present study, Nepote et al. (2006) reported that during storage, higher levels of lipid oxidation products were formed in dry roasted and honey roasted peanuts at 40 °C compared to −15 and 23 °C. Hexanal and other products of lipid oxidation could damage cellular protein and nucleic acid as well as cell membrane, thereby impacting the viability of probiotic cells (Dowds, 1994; Storz and Imlay, 1999). In the present study, the mean hexanal content of all samples tested in the present study increased from 2.15 ppm at the beginning of the experiment to 71.27 ppm after the 12 month storage period (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cell population (log CFU/g)</th>
<th>Hexanal (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>7.05a</td>
<td>4.71b</td>
</tr>
<tr>
<td>25</td>
<td>6.24b</td>
<td>6.43b</td>
</tr>
<tr>
<td>37</td>
<td>4.54c</td>
<td>189.83a</td>
</tr>
<tr>
<td>Peanut butter type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full fat peanut butter</td>
<td>5.87b</td>
<td>68.88a</td>
</tr>
<tr>
<td>Reduced fat peanut butter</td>
<td>6.01a</td>
<td>65.10a</td>
</tr>
<tr>
<td>Probiotic mixtures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>5.99b</td>
<td>23.55b</td>
</tr>
<tr>
<td>U</td>
<td>6.75a</td>
<td>78.90ab</td>
</tr>
<tr>
<td>N</td>
<td>5.55c</td>
<td>97.63a</td>
</tr>
<tr>
<td>S</td>
<td>5.48c</td>
<td>83.00a</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>49.67ab</td>
</tr>
<tr>
<td>Storage time (mo)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>7.33a</td>
<td>2.15b</td>
</tr>
<tr>
<td>1</td>
<td>6.94b</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>6.58c</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>6.34d</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>6.16e</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>6.10ef</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>5.97fg</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>5.82g</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>5.59h</td>
<td>ND</td>
</tr>
<tr>
<td>9</td>
<td>5.50i</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>5.22j</td>
<td>ND</td>
</tr>
<tr>
<td>11</td>
<td>5.01j</td>
<td>ND</td>
</tr>
<tr>
<td>12</td>
<td>4.68k</td>
<td>66.99a</td>
</tr>
</tbody>
</table>

Means in the same column not followed by the same letter are significantly different (p < 0.05).

ND: Not determined.

-*: Below detectable level (<8 CFU/g).
however, a higher hexanal content of peanut butter product did not always co-relate to lower probiotic cell viability (Table 1).

### 3.2. Influence of storage conditions on the populations of different probiotic mixtures

Statistical analysis revealed that storage temperature and storage time had a significant influence on the populations of the four probiotic mixtures during storage ($p < 0.05$; Table 1). The average cell counts of the four probiotic mixtures decreased with increasing storage temperature (Table 1). By the end of the 12 month storage period, the average counts of the four probiotic mixtures in the two peanut butter products stored at the three temperatures had a 2.65 log CFU/g decrease (Table 1). Although the mean probiotic bacterial count of the four probiotic mixtures in reduced fat peanut butter was significantly higher than the count in full fat peanut butter ($p < 0.05$; Table 1), the difference between the two counts was only 0.14 log CFU/g. No background lactic acid bacteria were found in the negative controls at all storage conditions.

The graphs in Fig. 1 show the survival trends of the probiotic mixtures in peanut butter products stored at different temperatures. At 4°C, all four probiotic mixtures maintained their viabilities with the exception of mixture S whose counts decreased approximately 1 log CFU/g in both full fat and reduced fat peanut butter by the end of the 12 month storage period. Probiotic bacterial populations in samples stored at 25°C decreased approximately 1–3 log CFU/g at the end of 12 month storage period. The counts of the probiotic mixtures were relatively lower in samples stored at 37°C at the same sampling point; largely between 2.50 and 3.75 log CFU/g except for mixtures N and S. Cell counts of mixture N in full fat peanut butter fell below the detectable limit (<8 CFU/g) at the end of 11 months, and those of mixture S dropped from the initial 7 log CFU/g to approximately 1 log CFU/g in full fat peanut butter at the end of the 12 month storage period.

Previous literatures have documented a reverse relationship between storage temperature and probiotic viability. Wang et al. (2004) observed that the viabilities of *S. thermophilus* and *B. longum* decreased with increase in storage temperature in dried fermented soymilk stored at 4°C and 25°C. Higher populations of viable probiotic cells were observed in products held at 4°C (68.8%) compared to 25°C (60.8%) after a 4 month storage period. Furthermore, Champagne et al. (1996) observed significant differences in probiotic survival rate at 20°C, 25°C, and 30°C; and as storage temperature increased, mortality of probiotic cultures also increased during storage especially at 20°C. Abe et al. (2009) documented a decrease in the survivability of *Bifidobacterium* with increasing storage temperature from 5, 25, 37, 45–60°C. In a previous study in our laboratory, viability of *L. rhamnosus* GG in full fat and reduced fat peanut butter decreased as temperature increased from 4°C to 25°C and 37°C (Klu et al., 2012). It is believed that a high storage temperature results in an increase in metabolic and cellular activities which leads

### Table 2

Results of statistical analysis — average probiotic populations of B, U, N and S as affected by peanut butter type during a 12 month storage period at all three storage temperatures (4, 25 and 37°C).

<table>
<thead>
<tr>
<th>Probiotic mixture</th>
<th>Cell population (log CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Full fat peanut butter</td>
</tr>
<tr>
<td>B</td>
<td>5.99bA</td>
</tr>
<tr>
<td>U</td>
<td>6.71aA</td>
</tr>
<tr>
<td>N</td>
<td>5.47cA</td>
</tr>
<tr>
<td>S</td>
<td>5.30cA</td>
</tr>
</tbody>
</table>

Means in the same column not followed by the same lowercase letters are significantly different ($P < 0.05$) in terms of probiotic mixture.

Means in the same row not followed by the same uppercase letters are significantly different ($P < 0.05$) in terms of type of peanut butter.

### Table 3

Results of statistical analysis — average cell populations of different probiotic species in each probiotic mixture during a 12 month storage period at all three storage temperatures (4, 25 and 37°C).

<table>
<thead>
<tr>
<th>Bacteria type</th>
<th>Cell population (log CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>5.82bb</td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>6.10bB</td>
</tr>
<tr>
<td><em>Streptococcus</em> + Lactococcus</td>
<td>4.86cA</td>
</tr>
</tbody>
</table>

Means in the same column not followed by the same lowercase letters are significantly different ($P < 0.05$) in terms of probiotic mixture.

Means in the same row not followed by the same uppercase letters are significantly different ($P < 0.05$) in terms of type of bacteria.
to the exhaustion of nutrients stored within probiotic cells and eventually cell death (Bruno and Shah, 2003).

The average counts of mixture U were higher than the counts of the other three probiotic mixtures in both peanut butter products ($p < 0.05$; Table 2). The average count of mixture B was significantly higher than the counts of mixtures N and S in full fat peanut butter. However, the average counts of mixtures N and S in full fat peanut butter were not significantly different as were the counts of mixtures B, N and S in reduced fat peanut butter ($p > 0.05$; Table 2). Food component such as fat is expected to protect probiotic cells during storage (Karimi et al., 2011; Possemiers et al., 2010). However, this phenomenon was not clearly observed in the present study (Table 2) which is consistent with the findings of Tharmaraj and Shah (2004) who reported that the addition of oil to cheese-based dips did not offer any additional protective effect for probiotics.

Previous studies have shown that *L. acidophilus* and *Bifidobacterium* species are the normal inhabitants of human gastrointestinal tracts (Shah, 2007; Champagne et al., 2005). They are

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**Fig. 2.** Ratios of probiotic species, *Lactobacillus* (Lacto) or *Bifidobacterium* (Bifi) to total probiotic population in mixture B in full fat peanut butter (A) and reduced fat peanut butter (B) and in mixture U in full fat peanut butter (C) and reduced fat peanut butter (D) during a 12 month storage period at 4, 25 or 37°C.
widely used as probiotics in food because of their health benefits (Shah, 2007; Guimeonde et al., 2004) and tolerance to acid and bile (Gomes and Malcata, 1999). However, since all four probiotic products used in this study contained Bifidobacterium and L. acidophilus, the greater survival rates of U and B must have been attributed by other factors. It is suggested that the survival rate of a particular probiotic depends on the co-existence of other probiotic bacteria in a same mixture. Tharmaraj and Shah (2004) reported that L. acidophilus survived better, in cheese based dips, in combination with Bifidobacterium animalis and L. paracasei than with B. animalis, P. shermanii and L. paracasei. B. animalis had a better survivability when in combination with L. acidophilus, L. paracasei and P. shermanii than when combined with a L. acidophilus and L. paracasei. Additionally, the authors observed a mutual antagonistic effect of B. animalis on L. rhamnosus and L. paracasei. In the present study, products N and S had 16 and 8 different probiotic bacteria, respectively. It is not clear whether the co-existence of different bacterial strains in the probiotic mixture had an effect on their viabilities during storage.

3.3. Survival of lactobacilli, bifidobacteria and streptococci/ lactococci

As shown in Table 3, the average counts of bifidobacteria were significantly higher than the counts of lactobacilli in mixtures U, B and N (p < 0.05). In mixture S however, the two populations were similar (p > 0.05). The average populations of streptococci/lactococci were significantly lower than those of bifidobacteria and lactobacilli in mixtures N and S (p < 0.05). It should be kept in mind that although the total probiotic inoculation level was kept at 10^7 CFU/g, the initial counts of Bifidobacterium, Lactobacillus and Streptococcus/Lactococcus were not exactly the same.

Fig. 2 shows the ratios of bifidobacteria or lactobacilli to total probiotic population (sum of the counts of all present probiotic strains) in probiotic mixtures B and U in full fat and reduced fat peanut butter under different storage conditions. After 1 month storage at different conditions, Bifidobacterium species accounted for 25–32% of the total probiotic population in mixture B in full fat peanut butter (Fig. 2A) and 22–29% of the total probiotic population in reduced fat peanut butter (Fig. 2B). At the same time interval, the species accounted for 10% of the total probiotic population in mixture U in full fat peanut butter (Fig. 2C) and 45% in reduced fat peanut butter (Fig. 2D). At the 6 month sampling point the proportion of Bifidobacterium species in mixture B ranged from 50 to 74% in full fat peanut butter (Fig. 2A) and 48–75% in reduced fat peanut butter (Fig. 2B), while in mixture U, Bifidobacterium species comprised 47–74% of total probiotic bacterial populations in full fat peanut butter (Fig. 2C) and 48–78% of the probiotic population in reduced fat peanut butter (Fig. 2D). At the 12 month sampling point, Bifidobacterium proportions increased in mixture B stored at 4 and 25 °C compared with the results collected at the end of 6 month of the storage (Fig. 2A and B). In samples stored at 37 °C, however the proportions of Bifidobacterium either remained steady or slightly decreased. Bifidobacterium proportions in mixture U remained mostly steady at 25 °C between the 6 and 12 month sampling points (Fig. 2C and D). However, the proportions in reduced fat peanut butter slightly increased at 4 °C and decreased at 37 °C (Fig. 2D) while in full fat peanut butter the proportions increased slightly at both storage temperatures (Fig. 2C). After the 12 month storage period, the overall proportions of Bifidobacterium from all storage temperatures had increased from the initial 25–32% to 57–76% in mixture B. In mixture U, the increment was from the initial 10–45% to the final 51–80%.

These results suggest that Bifidobacterium strains in mixtures U and B were more persistent than Lactobacillus in peanut butter products. Similar observations have been made in earlier studies. Heenan et al. (2004) studied the survival of probiotics in a frozen vegetarian dessert (pH 7). At the end of a 25 wk storage at –20 °C, the two L. lactis strains had a higher percentage of survival (88.0 and 84.7% respectively) than did the L. acidophilus and L. paracasei strains (59.4 and 44.6% respectively) used in the study. In another study, L. acidophilus La-5 and B. lactis Bb-12 were incorporated into ice cream (pH 6.51) either separately or as a mixture and the resulting products were stored for 60 d at –25 °C (Magariños et al., 2007). When the probiotic bacteria were incorporated separately, L. acidophilus had a survival rate of 87% and B. lactis, 90%. When the two bacteria were added to the ice cream as a mixture, L. acidophilus and B. lactis had 82 and 92% survivability, respectively after the 60 d of storage. Hekmat and McMahon (1992) inoculated B. bifidum and L. acidophilus into a fermented ice cream mix (pH of 4.7). It was observed that, after a 17 wk storage period at –29 °C, L. acidophilus had reduced to 3 × 10^6 CFU/mL whiles B. bifidum to 1 × 10^7 CFU/mL from the initial number of 5 × 10^8 CFU/mL for both probiotic bacteria. As described previously, probiotic mixture N and S contained not only Lactobacillus and Bifidobacterium but also Streptococcus/ Lactococcus. Interactions among the members of each mixture in peanut butter products were complicated and no clear and consistent trend was observed.

4. Conclusions

Storage conditions played a key role in maintaining the viability of probiotic cultures, and cell survival rate decreased with increasing storage temperature and time. Overall, probiotic mixture U has the greatest survival rate followed by B, N and S. Bifido bacterium species had the highest survivability followed by Lactobacillus species and then Streptococcus/Lactococcus. Peanut butter is a suitable food matrix to deliver probiotics, and the fat content of peanut butter did not significantly influence the survivability of the probiotic mixtures included in the present study.

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