ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 14 (6): 260-266, 2015
ISSN 1682-296X

RESEARCH ARTICLE OPEN ACCESS

DOI: 10.3923/biotech.2015.260.266

Effect of some Traditional Saudi Arabian Meals on the Survival of Probiotic Bacteria in Fermented Milk under *in vitro* Simulated Gastrointestinal Conditions

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ARTICLE INFO
Article History:
Received: October 14, 2015
Accepted: November 05, 2015

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ABSTRACT
This study evaluated the protective effects of incorporating the fermented milk containing probiotics *Bifidobacterium lactis* Bb-12 or *Lactobacillus acidophilus* La-5 into some traditional Saudi Arabian meals on the survival of probiotic bacteria during exposure to *in vitro* simulated gastrointestinal conditions. Six traditional Saudi Arabian meals namely cheese sandwich, liver sandwich, egg sandwich, chicken kabsah, lamb kabsah and fish kabsah were used. The chemical composition of meals were determined. Each probiotic fermented milk was mixed with each meal and exposed to *in vitro* simulated gastrointestinal conditions. All the types of Kapsa recorded the highest amount of protein and fat contents compared with other meals. There was a significant reduction ($p \leq 0.05$) in the viable count of *B. lactis* and *L. acidophilus* in fermented milk and after mixed with all tested meals during exposure to simulated gastrointestinal conditions. All the meals protected *B. lactis* and *L. acidophilus* from harsh gastrointestinal conditions compared to fermented milk. The lowest resistance of *B. lactis* was recorded in fermented milk without mixing with meals during exposure to simulated gastrointestinal conditions. The viable count of *B. lactis* was higher when the fermented milk was mixed with lamb Kapsa and cheese sandwich. The *B. lactis* exhibited higher survival rate as compared to *L. acidophilus*. The study showed the potential of some traditional Saudi Arabian meals matrices for protecting the probiotic bacteria during simulated gastrointestinal passage and may serve as transitional source for probiotic delivery.

Key words: Probiotic bacteria, traditional Arabian meals, survival, fermented milk, simulated, gastrointestinal condition

INTRODUCTION

Owing to the rapid increase concerning the knowledge of intestinal microbiota and modulation factors, interest in supplementing various types of food products with probiotic bacteria has grown significantly. A large number of Lactic Acid Bacteria (LAB) strains are well characterized and presently marketed as probiotics and the best studied strains belong to the genera *Lactobacillus* and *Bifidobacterium* (Sharma *et al*., 2014), especially *B. animalis* subsp. *lactis* and *L. acidophilus* species. These bacteria have a probiotic capacity, imparting beneficial effects on the host when administered in appropriate amounts (Araya *et al*., 2002). Probiotics are extensively utilized as food supplements due to the fact that they can enhance protection against gastrointestinal pathogens and improve the immune system. As a consequence, reduction of lactose intolerance, reduction of plasma cholesterol level and pressure, anticarcinogenic

activity and increasing nutritional value of food can result (Biavati *et al*., 2000; Parvez *et al*., 2006; Nomoto, 2005; Ogueke *et al*., 2010).

Currently the probiotics was defined as the live microorganisms which when administered in adequate amounts confer a health benefit on the host (FAO. and WHO., 2002). As such, these bacteria must be able to withstand the adverse environments encountered in the stomach and small intestine of the host in order to reach the large intestine and exert their beneficial effects (Salminen *et al*., 1993; Anal and Singh, 2007; Ding and Shah, 2009). These bacteria must tolerate acid, bile and GIT enzymes (pepsin, lipase, pancreatin) and then adhere to and colonize the intestinal epithelium at least temporarily (Vinderola *et al*., 2011; Nagpal *et al*., 2012; Saito *et al*., 2014). A low pH in the stomach, usually varying from 2.5-3.5 is the main challenge that probiotic strains need to overcome. In the small intestine, orally taken probiotics are exposed to pancreatin, bile salts and a pH level of approximately 8.0 (Ranadheera *et al*., 2012). Moreover, the functional properties of probiotics may vary due to the carrier food added to the bacteria (Saito *et al*., 2014).

The tolerance of probiotic bacteria to these adverse conditions may be affected by the food carrier matrix and many studies in this field have been carried out in the last few years. A number of factors related to the food matrix such as fat and protein content, type of proteins, sugars, pH and some ingredients, may influence the probiotic performance by modifying their resistance to acid and bile and, consequently, their efficacy (Burns *et al*., 2014). Possemiers *et al*. (2010) reported that incorporation of probiotics in chocolate is an excellent alternative to protect them from environmental stress conditions and for the most favorable delivery. Ranadheera *et al*. (2012) demonstrated that probiotic lactobacilli, bifidobacteria and propionibacteria have high tolerance to gastrointestinal juices when incorporated in ice cream compared to plain and stirred fruit yoghurts. Casarotti *et al*. (2015) found that the milk and insulin protected *B. animalis* subsp. *lactis* BB-12 and *L. acidophilus* La-5 from *in vitro* gastrointestinal stress and *B. animalis* subsp. *lactis* BB-12 showed higher survival during the test compared to *L. acidophilus* La-5 in all tested matrices. Meira *et al*. (2015) found that the counts of *Lactobacillus acidophilus* and *Bifidobacterium lactis* were approximately 6 log CFU g^{-1} if incorporated into goat ricotta cheese under simulated gastrointestinal conditions.

Probiotics as live microorganisms provide health benefits on the host when administered in sufficient amounts (Wang *et al*., 2012). However, presence of probiotics in yogurt are beneficial for health such as improve lactose utilization (De Vrese *et al*., 2001), prevent cancer (Rafter, 2003), maintain intestinal microflora balance (Mainville *et al*., 2005) and reduce serum cholesterol level (Baroutkoub *et al*., 2010). Besides, yogurt containing *Bifidobacterium bifidum* Bb-12 improves the production of immunoglobulin A (IgA) in the intestine thus increasing the local immunity against gastrointestinal infection (Kabeerdoss *et al*., 2011). Also, It has inhibitory effects on commonly known food borne pathogens (Goderska and Czarnecki, 2007) and ability to control intestinal infections by producing inhibitory/ antimicrobial substances such as organic acids, hydrogen peroxide, deconjugated bile acids, antibiotics and bacteriocins (Schiffrin and Blum, 2001). Madureira *et al*. (2011) observed that viable numbers of probiotics should be at least 106-107 CFU g^{-1} in the final product to be accepted as the therapeutic minimum (Madureira *et al*., 2011). Many studies investigated the survival ability of probiotic cultures during refrigerated storage (Donkor *et al*., 2007; Ramchandran and Shah, 2010).

In Saudi Arabia, most people are habitual to drink fermented milk during the breakfast, lunch and dinner. The breakfast consisted of types of sandwich, while the lunch and dinner consisted of the famous meal namely the Kabsah which is the most well known traditional dish in Saudi Arabia. It contains a basmati rice with tomato and tomato paste, some kinds of spices (cinnamon, cloves, cardamom and coriander) and nuts (cashews and pine nuts) may be add. There are many types of Kabsah such as chicken Kabsah, lamb Kabsah and fish Kabsah. Shori and Baba (2015) investigated the effect of *Allium sativum* and *Cinnamomum verum* water extracts on the survival of *Bifidobacterium bifidum* after simulated gastrointestinal digestion (SGD). They found that the viable cell counts (VCC) of *B. bifidum* in fresh *A. sativum* or *C. verum* cow milk yogurt were higher (8.1×10^9 and 6.6×10^9 CFU mL^{-1}, respectively; $p<0.05$) than plain yogurt (1.9×10^9 CFU mL^{-1}).

Considering the potential influence of food matrix on probiotic strains functionality, the aim of this study was to determine the effect of the traditional Saudi Arabia meals on the survival of probiotic strains *Lactobacillus acidophilus* La-5 and *Bifidobacterium lactis* Bb-12 under simulated gastrointestinal condition.

MATERIALS AND METHODS

Meals: Different types of meals were purchased from local restaurants in Al Hofuf city. The meals components and its pH values are shown in the Table 1. Every meal was blended with equal amount of water to get homogenized meals (bones were separated from chicken, meat and fish before blended with water). The homogenized meals were kept in refrigerator at 5±1°C until used.

Table 1: Meals and its components used in this study

Meals	Meals components	pH value
Cheese sandwich	Bread+cheese+strawberry jam+peanut butter	7.1±0.25
Liver sandwich	Bread+liver+tomato+cucumber	6.8±0.14
Egg sandwich	Bread+egg+tomato+cucumber+lettuce	5.7±0.12
Chicken kabsah	Chicken+rice	7.4±0.41
Lamb kabsah	Lamb meat+rice	6.8±0.34
Fish kabsah	Fish+rice	6.6±0.38

Chemical analysis: The moisture contents were determined according to the procedure given in AOAC (1999a). Ash was determined by combustion of the sample in a muffle furnace at 550°C for 8 h. Total nitrogen was determined by the Kjeldahl method as described by Pearson (1970) and the protein was calculated using the general factor (6.25), total fat was estimated using automated soxlet extraction based on AOAC (1999b), while total carbohydrate was calculated by difference as follows:

Carbohydrate (g/100 g) = 100-(moisture+protein+fat+ash)

pH values: The pH values of the samples were measured at 20-25°C using a pH meter (model SS-3, Beckman, Fullerton, CA, USA).

Preparation of fermented milk: Sterilized reconstituted skim milk (10%, w/w) in tow conical flasks were separately inoculated at a level of 0.07% (w/v) with a freeze-dried *Bifidobacterium lactis* Bb-12 and *Lactobacillus acidophilus* La-5 (obtained from Chr. Hansen Laboratories, Copenhagen, Denmark). The conical flasks were incubated at 37°C at pH 4.6 (6 and 4 h, respectively). The flasks were cooled in refrigerator at 5±1°C until used. Then each sample of fermented milk was mixed with each homogenized meals (1:1 w/w). The viable count of *Bifidobacterium lactis* Bb-12 and *Lactobacillus acidophilus* La-5 in fermented milk was 7.55±0.07 and 7.78±0.10 $\log_{10}$ CFU g^{-1}, respectively.

Assays of effects of simulated gastrointestinal conditions on the viability of probiotic bacteria: The tolerance of *B. lactis* Bb-12 and *L. acidophilus* La-5 in fermented milk to *in vitro* simulated gastric and enteric conditions were performed according to the method described by Buriti *et al*. (2010a, b) with some modifications as described by Da Silva *et al*. (2015). Initially, 25 g of samples were taken in conical flasks and homogenized in 225 mL of 0.5 g/100 mL NaCl solution. For the gastric phase simulation, the pH of aliquots (10 mL) was adjusted to 2.1-2.6 with 0.5 mL of HCl (0.5 mol equi/L) and 0.3 mL of pepsin solution (3 g L^{-1}, porcine stomach mucosa P6887, Sigma-Aldrich, MO, USA). Then the conical flasks were incubated at 37°C for 2 h with an agitation speed of approximately 150 rpm (Shaking Water Bath Memmert-DIN-40050\Germany).

In order to simulate enteric conditions, the pH of samples was raised to 4.9-5.4 using an alkaline solution (150 mL of 1 mol equi/L NaOH solution, 14 g of $PO_4H_2Na.2H_2O$ and distilled water up to 1 L). The Bovine bile (B3883, Sigma-Aldrich, MO, USA) and pancreatin (P3292, Sigma-Aldrich, MO, USA) were added to reach a concentration of 10 and 1 g L^{-1}, respectively. Samples were incubated again at 37°C for 2 h under agitation. After 4 h, the pH was raised to 7.5-7.7 using the same alkaline solution, bile and pancreatin concentrations were added (10 and 1 g L^{-1}, respectively) and samples were incubated again at 37°C for 2 h under agitation for achieving 6 h of assay. The enumeration of *B. lactis* and *L. acidophilus* were performed in aliquots collected after 0, 2, 4 and 6 h.

Enumeration of probiotic bacteria: Samples of 10 g were transferred aseptically in 90 mL peptone water (Oxoid, Hampshire, UK) and mixed thoroughly. Serial dilutions were done in peptone water for each sample and 1 mL of the appropriate dilutions were poured in selective media plate. The MRS agar medium (Oxoid, CM0361B) was used for enumeration of *Lactobacillus acidophilus*, while for enumeration of *Bifidobacterium lactis*, MRS agar medium containing 0.5% *L. cysteine*-HCl (Sigma chemical Co., St. Louis, Mo) was used. All plates were incubated anaerobically at 37°C for 48-72 h.

Statistical analysis: Three independent experiments were performed. All analysis and enumeration were done in duplicate. All the data were analyzed by ANOVA using the general models procedure of SAS (2010). Differences among the means were tested for significance ($p>0.05$) by Duncan's multiple range test.

RESULTS AND DISCUSSION

Chemical composition of different meals: The proximate chemical composition of different meals used in these study are presented in Table 2. The moisture content of meals varied from 75.88% in egg sandwich to 66.78% in chicken Kabsah. All the types of Kabsah contained the highest amount of protein. The differences in protein contents were significant among all types of Kabsah meals and other meals at 5% level of significance. Higher concentration of protein in different types of Kabsah were from chicken, lamb and fish meat used in the preparation of this meals. The fat and carbohydrate content of meals were higher in all types of Kabsah as compared to other meals. It was noticed that the moisture contents were higher in cheese, egg and liver sandwich corresponding to a low protein contents. A reverse trend was observed for moisture contents being higher in Kabsah meals compared to low contents of protein contents. There was no significant difference in ash contents among all the tested meals. El-Jasser *et al*. (2011) reported the proximate composition (%) of commercial chicken Kabsah having a moisture of 71.6, protein as 7.32, fat as 4.48, ash as 1.5 and carbohydrate as 14.

Survival of *Bifidobacterium lactis* Bb-12 in fermented milk effected by some meals under simulated gastrointestinal condition: The survival of *B. lactis* in fermented milk affected by some meals after exposure to *in vitro* simulated

Table 2: Proximate chemical composition of different meals (fresh weight basis)

Meals	Chemical composition (%)				
	Moisture	Protein	Fat	Ash	Carbohydrate*
Cheese sandwich	79.70±0.73^{a}	5.04±0.23^{e}	3.03±0.13^{c}	1.12±0.25^{a}	11.11
Liver sandwich	77.95±0.71^{b}	7.93±0.78^{c}	4.08±0.25^{bc}	1.14±0.06^{a}	8.80
Egg sandwich	75.88±0.58^{c}	8.05±0.51^{b}	4.78±0.13^{b}	1.05±0.08^{a}	10.32
Chicken kabsah	69.78±0.61^{d}	9.82±0.45^{a}	5.71±0.35^{ab}	1.10±0.14^{a}	13.59
Lamb kabsah	66.72±1.12^{e}	9.57±0.54^{a}	5.87±0.04^{a}	1.05±0.08^{a}	16.79
Fish kabsah	67.18±0.85^{f}	9.32±0.90^{a}	5.99±1.11^{a}	1.08±0.18^{a}	16.40

Values are expressed as Mean±SD (n = 3), $^{a-f}$Means in the same column with similar letters are not significantly different ($p \leq 0.05$), *Total carbohydrate = 100-(Moisture+protein+fat+ash)

Table 3: Survival of *Bifidobacterium lactis* Bb-12 in fermented milk effected by some meals under simulated gastrointestinal condition

Meals	Viable count of *B. lactis* ($\log_{10}$ CFU g^{-1}) at difference incubation period			
	0 (h)	2* (h)	4** (h)	6** (h)
Fermented milk	7.25±0.07^{aA}	5.45±0.09^{bB}	5.01±0.19^{bB}	4.10±0.61^{cC}
Cheese sandwich	6.72±0.81^{aA}	6.12±0.44^{aB}	5.98±0.22^{aB}	5.87±0.47^{aB}
Liver sandwich	6.90±0.07^{aA}	6.54±0.02^{aA}	5.77±0.37^{aB}	5.43±0.56^{aB}
Egg sandwich	7.04±0.08^{aA}	6.35±0.14^{aB}	5.44±0.71^{abC}	5.10±0.17^{bC}
Chicken kabsah	7.20±0.01^{aA}	6.43±0.51^{aB}	5.88±0.55^{aC}	5.68±0.97^{aC}
Lamb kabsah	6.88±1.10^{aA}	6.64±0.32^{aA}	5.93±0.28^{aB}	5.86±0.68^{aC}
Fish kabsah	6.80±0.71^{aA}	6.62±0.39^{aA}	5.98±0.30^{aB}	5.79±0.23^{aB}

Values are expressed as Mean±SD (n = 3), Means in the same column or row followed by the same lowercase or capital letter, respectively are not significantly different ($p \leq 0.05$), *Simulated gastric conditions (2 h), **Simulated enteric conditions (4 and 6 h)

Table 4: Survival of *Lactobacillus acidophilus* La-5 in fermented milk effected by different meals under simulated gastrointestinal condition

Meals	Viable count of *L. acidophilus* ($\log_{10}$ CFU g^{-1}) at difference incubation period			
	0 (h)	2* (h)	4** (h)	6** (h)
Fermented milk	7.48±0.10^{aA}	5.01±0.24^{cB}	4.57±0.15^{cC}	3.12±0.21^{bD}
Cheese sandwich	7.20±0.01^{aA}	6.01±0.25^{bB}	5.99±0.24^{bB}	4.01±0.87^{aC}
Liver sandwich	6.98±0.11^{aA}	6.22±0.14^{abB}	6.10±0.64^{aB}	4.18±0.44^{aC}
Egg sandwich	7.24±0.01^{aA}	6.00±1.02^{bB}	5.89±0.57^{bB}	4.04±0.58^{aC}
Chicken kabsah	7.15±0.16^{aA}	6.87±0.58^{aAB}	6.41±0.17^{abB}	4.32±0.65^{aC}
Lamb kabsah	6.91±0.04^{aA}	6.80±0.74^{aA}	6.60±0.47^{aA}	4.66±0.71^{aB}
Fish kabsah	7.26±0.21^{aA}	6.97±0.11^{aA}	6.63±0.36^{aAB}	4.82±0.90^{aB}

Values are expressed as Mean±SD (n = 3), Means in the same column or row followed by the same lowercase or capital letter, respectively are not significantly different ($p \leq 0.05$), *Simulated gastric conditions (2 h), **Simulated enteric conditions (4 and 6 h)

gastric (for 2 h) and enteric (for 4 and 6 h) condition are illustrated in Table 3. The main objective of increasing the survival of this particular bacteria was to improve the health benefits associated with fermented milk. In general, there was a significant reduction ($p \leq 0.05$) in the viable count of *B. lactis* during exposure to simulated gastrointestinal condition. The lowest resistance of *B. lactis* was recorded in fermented milk without mixing with meals, which presented count of 5.45, 5.01 and 4.10 $\log_{10}$ CFU g^{-1} after exposure to *in vitro* stimulated gastric for 2, 4 and 6 h, respectively. Whereas the highest viable count of *B. lactis* was observed in fermented milk when mixed with all types of tested meals with significant differences ($p \leq 0.05$) when compared with the count in fermented milk alone. The viable count of *B. lactis* was higher at the end of trail when the fermented milk was mixed with lamb Kabsah and cheese sandwich. This means that the meals showed a significant effect ($p \leq 0.05$) on the *B. lactis* tolerance to gastric and enteric condition. A little protection at the end of exposure to simulated gastrointestinal condition was observed when the *B. lactis* was in the presence of egg sandwich. Similar results were reported by many researchers who found that functional properties of this probiotic bacteria may be affected by the food matrix used in delivery (Lahtinen *et al*., 2007; Ranadheera *et al*., 2012) because the buffering capacity of food would help to enhance the viability of probiotics during gastric transit (Kailasapathy and Chin, 2000; Mainville *et al*., 2005).

Survival of *Lactobacillus acidophilus* La-5 in fermented milk effected by some meals under simulated gastrointestinal condition: The survival of *L. acidophilus* in fermented milk affected by some meals after exposure to *in vitro* simulated gastric (for 2 h) and enteric (for 4 and 6 h) condition were presented in Table 4. The main purpose of increasing the survival rate was to improve the health benefits associated with this bacteria against many diseases such as cancer and others. Generally, there was a significant reduction ($p \leq 0.05$) in the viable count of *L. acidophilus* during exposure to simulated gastrointestinal condition. The good protection at the end of the incubation

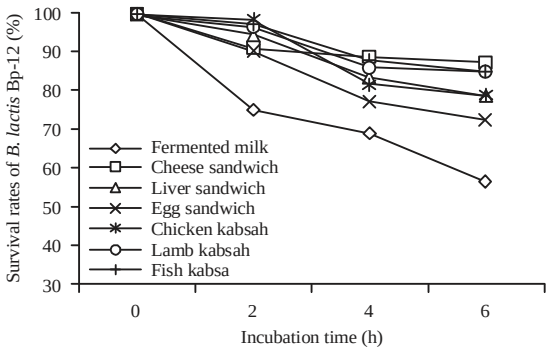

Fig. 1: Survival rats (%) of *B. lactis* under simulated gastrointestinal conditions

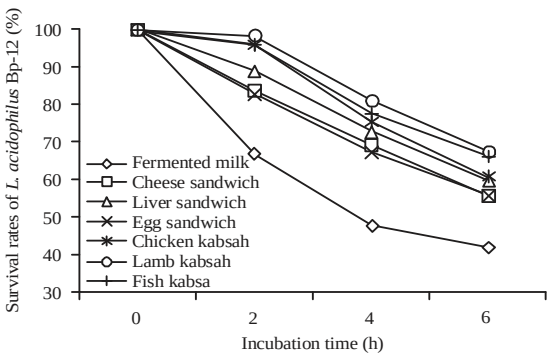

Fig. 2: Survival rats (%) of *L. acidophilus* under simulation gastrointestinal conditions

was observed when the *L. acidophilus* was incorporated into fish Kabsah meal which presented count of 4.82 $\log_{10}$ CFU g^{-1} with non significant difference among all the tested meals. The fish Kabsah showed the highest amount of fat up to 5.99% (Table 3). The fat content may provide some protection towards the probiotic survival during simulation gastric and intestine transit as it proved by the previous studies (Possemiers *et al*., 2010; Ranadheera *et al*., 2012). Whereas, Klu and Chen (2015) found that the fat content of peanut butter did not significantly influence the probiotic survivability when the full fat and reduced fat peanut butter were inoculated with commercial probiotic product. Also, protein can play an important role for the protection of probiotic from gastrointestinal stress. De Carvlho *et al*. (2009) stated that probiotic bacteria must be ingested with foods containing components with buffering capacity such as yoghurt, milk or other foods that are rich in protein. On the other hand, the pour protection of *L. acidophilus* count was found when it is incorporated into fermented milk which presented count of 3.12 $\log_{10}$ CFU g^{-1} with significant differences compared with all tested meals in the end of exposure to *in vitro* simulated gastrointestinal condition. The study results are in agreement with those of Ranadheera *et al*. (2012) who reported that, the addition of certain ingredients such as cocoa powder and stabilizers guar gum and dextrose in the ice cream enhanced the viability of probiotics by providing some protection.

The survival rate (%) of *B. lactis* in the fermented milk, cheese sandwich, liver sandwich, egg sandwich, chicken Kabsah, lamb Kabsah and fish Kabsah treatments was 56.55, 87.35, 78.70, 72.44, 78.89, 85.17 and 85.15%, respectively (Fig. 1) at the end of *in vitro* test, while the survival rate of *L. acidophilus* was 41.7, 55.69, 59.89, 55.80, 60.42, 67.44 and 66.39% in the fermented milk, cheese sandwich, liver sandwich, egg sandwich, chicken Kabsah, lamb Kabsah and fish Kabsah treatments, respectively (Fig. 2). These results proved that the *B. lactis* Bb-12 showed higher survival rate when compared to *L. acidophilus* during exposure to *in vitro* simulated gastrointestinal condition in all tested meals. These results are in agreement with other studies (Madureira *et al*., 2005; Bedani *et al*., 2013; Casarotti *et al*., 2015). The viability of *B. lactis* at the end of the *in vitro* assay decreased by 1-2 log cycle while the viability of *L. acidophilus* decreased by 2-3 log cycle. The stability of probiotic strains toward exposure to *in vitro* simulated gastrointestinal condition may due to the chemical composition and neutral pH of meals as well as the bacterial strain. Similarly, Bedani *et al*. (2013) demonstrated that the acid and bile tolerance of probiotic microorganisms is strain dependent when incorporated into different matrices. The ability to survive under acid conditions may be attributed to some strains of *Bifidobacterium* spp. This behavior is also likely to be strain dependent and is determined by the pH profile of their H^+-ATPase enzyme as well as by the composition of their cytoplasmic membrane (Mainville *et al*., 2005; Matto *et al*., 2006; Madureira *et al*., 2011).

CONCLUSION

The survival of *Bifidobacterium lactis* Bb-12 and *Lactobacillus acidophilus* La-5 in fermented milk and in a mixture with selected Saudi meals depended on the type of meals and the probiotic strains under simulated gastrointestinal conditions. The tested Saudi meals (cheese sandwich, liver sandwich, egg sandwich, chicken Kabsah, lamb Kabsah and fish Kabsah) protected the probiotic strains from the adverse conditions of the gastrointestinal conditions than the fermented milk alone. Also, both the probiotic strains showed significantly low rate of viability when exposed to *in vitro* gastric and intestinal conditions. Besides, the survival rate of *B. lactis* Bb-12 was more compared to *L. acidophilus* La-5 in all the tested meals. Therefore, further research studies are required to optimize the protective effect of these meals thus leading to a higher tolerance of probiotic strains to acid and bile and greater viability in probiotic dairy products.

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