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## Effect of Date Syrup (*Dips*) on Growth and Survival of Probiotic Bacteria in Milk

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### ABSTRACT

There is an increasing demand by consumers for food products with functional properties. Date Syrup (*Dips*) has high contents of total sugar and contains many functional components such as polyphenols, carotenoids and phytosterols. The objective of this study was to monitor the growth and survival of two probiotic bacteria, *Lactobacillus acidophilus* La-5 and *Bifidobacterium lactis* Bb-12 in milk with different concentrations of *dips* during fermentation. The *L. acidophilus* count increased gradually in all the samples up to 12 h of incubation. Later on, the bacterial counts decreased after 24 h of incubation in all the treatments containing *dips*. However, the count of *B. lactis* did not change significantly after 6 h. incubation for all the samples while it decreased significantly after 12 and 24 h incubation in the samples containing 10 and 15% *dips* while it increased in the control until 12 h. and decreased after 24 h incubation. Besides, an inverse relationship was observed between the *dips* concentration and the growth rate of both the probiotic bacteria. Subsequently, the acidity of milk increased in the control while it decreased with increasing the *dips* concentrations and the pH values. The results indicated that both the probiotic bacteria *B. lactis* and *L. acidophilus* can remain viable with count of  $= 10^6$  cfu mL<sup>-1</sup> in presence of *dips* concentration up to 20% during fermentation except for the treatment with 20% *dips* in the presence of *B. lactis* after the 24 h. The study results showed an excellent potential for incorporating the *dips* in other probiotic dairy products.

**Key words:** Date syrup (*dips*), milk, probiotic bacteria, fermentation, concentration, autoclave, dairy products

### INTRODUCTION

Probiotic has been defined as “a live microbial food ingredient that, when ingested in sufficient quantities, exerts health benefits” (FAO/WHO, 2001). Therefore, it can safely be concluded from this definition that at first, these microorganism must be in a vivid form and secondly, it should be taken in sufficient numbers to provide the desired health benefits. These bacteria secret many antibacterial substances against pathogenic bacteria having many health and therapeutic effects (Ouweland and Vesterlund, 2004). The bacteria such as *Helicobacter pylori* infection and *Helicobacter pylori* cause stomach ulcers and prevent antibiotic associated diarrhea (Szajewska *et al.*, 2006). Besides, the probiotic bacteria alleviate symptoms of lactose indigestion.

The consumption of fermented milk containing these bacteria reduces or avoids the possibility of occurring cancer and tumors (Abdelali *et al.*, 1995; Baricault *et al.*, 1995; Fernandes and Shahani, 1990; Abd El-Gawada *et al.*, 2004, 2005). These bacteria also play vital role in stimulating the immune system and reduce the levels of ammonia in the blood (Shah, 2007) as well as synthesize certain types of vitamins and improve microbial balance in the bowel (Capela *et al.*, 2006). Due to the health benefits of these bacteria, many different foods containing these bacteria are found all over the world. The probiotic bacteria (*L. acidophilus* and *Bifidobacterium* spp.) are a risk of death especially when the food is eaten and exposed to high acidity and bile salts. Many researchers suggested that the total count of these bacteria should not be less than  $10^6$  cfu mL<sup>-1</sup> (Kurmann *et al.*, 1992). Therefore, it is pertinent to study the food constituents and their vital effect on the presence of these bacteria. Presently, date syrup (*dips*) and dates are considered as the most important constituents of many food products. *Dips* contain high percentage of total sugars up to 81%, including 41% fructose, 39% glucose and 1% sucrose. It also contains small amounts of ash, protein and pectin up to 1.5, 2.2 and 1.8%, respectively (Al Eid, 2006). Many researchers studied the effect of different types of sweeteners such as high fructose corn syrup, honey and glucose on the growth and activity of probiotic bacteria (Curda and Plockova, 1995; Popa and Ustunol, 2011; Chick *et al.*, 2001). Moreover, sugars significantly affect the viability of bacteria whereas sucrose can cause fatal effect through the direct influence of osmotic pressure (Jay *et al.*, 2005). However, some researches reported about its protective effect on bacterial cells from death during freezing (Champagne and Rastall, 2009). In another study, Mohammadi *et al.* (2011) stated that the total effect of these sweeteners depends on the type of sugar, concentration, the vital bacteria type, storage temperature and the storage time. It is also proved that the oligosaccharide can activate these vital bacteria and are considered as prebiotic compounds such as insulin and fructooligosaccharides (Champagne and Rastall, 2009; Akin *et al.*, 2007).

A review of literature showed inadequate information on the effect of date syrup (*dips*) on the growth and activity of probiotic bacteria in milk. Therefore, the aims of this study were 1) To determine the impact of addition of date syrup (*dips*) of various concentrations on the behavior of probiotic bacteria during incubation, 2) To produce enriched fermented dairy products with date syrup (*dips*).

## **MATERIALS AND METHODS**

**Materials:** Date syrup (*dips*) was obtained from the local markets. The pasteurized cow milk was obtained from a dairy company on the same manufacturing day. The milk composition as shown on the packing was 3.1% fat and 8.5% non-fat solids. The probiotic bacteria namely *Lactobacillus acidophilus* La-5 and *Bifidobacterium lactis* Bb-12 were obtained from Christian Hansen/ Denmark.

### **Methods**

**Determination of acidity and pH values:** Acidity and the pH of the fermented milk samples were determined according to the standard method (AOAC, 2000).

**Count of probiotic bacteria:** The counts of *B. lactis* and *L. acidophilus* were determined according to Adhikari *et al.* (2003) by using MRS agar medium (Oxoid, CM0361B). For bifidobacterial count, L-cysteine-HCl (Sigma chemical Co., St. Louis, Mo) was added at the rate of 0.5%, to decrease the redox potential of the medium. All plates were incubated anaerobically at 37°C for 48 h.

**Experimental design:** Cow milk was divided into five portions. The first portion was treated as control (0 syrup), while 5, 10, 15 and 20% *dips* were added to the second, third, fourth and fifth portions, respectively. Then, each portion was subdivided into two parts. The first part was inoculated with *L. acidophilus* while the other was inoculated with *B. lactis*. Both the parts were incubated at 37 and 40°C, respectively. Subsequent samples were taken after zero, 6, 12 and 24 h of incubation. The numbers of living bacteria, pH and the acidity for each treatment were determined during incubation

**Data analysis:** The data was analyzed by analysis of variance (ANOVA) and regression techniques according to SAS (2000).

## RESULTS AND DISCUSSION

### Effect of various concentrations of *dips* on the viable count of *L. acidophilus* in milk:

The changes in the logarithm numbers of viable count of *L. acidophilus* in the presence of various concentrations of *dips* during incubation periods are given in Table 1.

After direct inoculation (zero time), the count of *L. acidophilus* ranged from 6.51-6.83 log cfu mL<sup>-1</sup> in all the treatments. The bacterial count in the control sample (0-*Dips*) increased significantly after 6 (6.90-7.94 log cfu mL<sup>-1</sup>, 12 (6.82-7.94 log cfu mL<sup>-1</sup>) and 24 h (6.04-8.34 log cfu mL<sup>-1</sup>) of incubation as compared to zero time in different *dips* treatments. However, the rate of increase in the bacterial count was 1.35, 1.35 and 1.75 log cycle, respectively when compared to zero time. The viable count of *L. acidophilus* bacteria increased continuously with increasing the incubation times in all the treatments until 12 h. It was found that the log numbers of *L. acidophilus* for the treatment with 5% *dips* concentration increased significantly after 6, 12 and 24 h incubation as compared to zero time treatment. These increases were 0.62, 0.78, 0.71 log cycle for the different treatments, respectively. The increase in the count of *L. acidophilus* for the 5% *dips* was less than that found in the control sample at the same incubation time. This indicated that the addition of *dips* at 5% ratio showed a negative impact on the growth of *L. acidophilus*. Besides, there was no significant difference in the bacterial count between 5 and 15% addition of *dips* after 6 and 12 h incubation. The results showed significant differences in the bacterial counts when compared with zero time treatment. It was also observed that the numbers of bacterial counts showed decreasing trend with increasing the incubation period to 24 h. The results showed that the decrease in the bacterial count was more in 15% *dips* than

Table 1: Changes in the viable count of *L. acidophilus* in the presence of various concentrations of *dips* in milk

<i>Dips</i> added (W/V)	Count of <i>L. acidophilus</i> (log <sub>10</sub> cfu mL <sup>-1</sup> )			
	Incubation time (h)			
	0	6	12	24
0%	6.59±0.03 <sup>b</sup> <sub>c</sub>	7.94±0.03 <sup>a</sup> <sub>b</sub>	7.94±0.05 <sup>a</sup> <sub>b</sub>	8.34±0.18 <sup>a</sup> <sub>a</sub>
5%	6.83±0.21 <sup>a</sup> <sub>b</sub>	7.45±0.03 <sup>b</sup> <sub>a</sub>	7.61±0.12 <sup>bc</sup> <sub>a</sub>	7.54±0.01 <sup>b</sup> <sub>a</sub>
10%	6.51±0.07 <sup>b</sup> <sub>d</sub>	7.42±0.02 <sup>b</sup> <sub>b</sub>	7.68±0.02 <sup>b</sup> <sub>a</sub>	6.66±0.10 <sup>c</sup> <sub>c</sub>
15%	6.67±0.17 <sup>ab</sup> <sub>b</sub>	7.45±0.03 <sup>b</sup> <sub>a</sub>	7.47±0.14 <sup>f</sup> <sub>a</sub>	6.62±0.005 <sup>e</sup> <sub>b</sub>
20%	6.55±0.04 <sup>b</sup> <sub>b</sub>	6.90±0.01 <sup>c</sup> <sub>a</sub>	6.82±0.01 <sup>d</sup> <sub>a</sub>	6.04±0.26 <sup>e</sup> <sub>c</sub>

The upper different letters in each column indicates significant difference at p>0.05, The lower different letters in each row indicates significant difference at p>0.05

10% *dips* treatments. Furthermore, the bacterial count in 15% *dips* treatment after 24 h incubation was less than that recorded at zero time. The numbers of *L. acidophilus* in the 20% *dips* treatment recorded an increase of 0.35, 0.27 log cycle after 6 and 12 h of incubation, respectively. It was observed at the end of the incubation period that the count decreased by -0.51 log cycle as compared to zero time treatment. When comparing the results of *L. acidophilus* count for all the treatments after 24 h of incubation, there was a significant increase in count in the control treatment as compared to all other treatments followed by the treatment containing 5% *dips*. Moreover, there was no significant difference between 10 and 15% *dips* concentrations. The 20% *dips* treatment recorded the lowest count of these bacteria as compared to other treatments. In general, it can be concluded that the addition of *dips* may inhibit the growth and reproduction of *L. acidophilus* with increasing the incubation time. There was noticed an inverse relationship between the increase in the concentration of *dips* and the numbers of these bacteria. This could be due to the influence of the osmotic sugar (Jay *et al.*, 2005) which caused the death of bacteria during incubation and the lack of resistance to the increased concentration of *dips*. Al Eid (2006) pointed out that *dips* contain 81% sugars which are subdivided into fructose (41%), glucose (39%) and sucrose (1%). Also, the presence of certain compounds in the *dips* have a toxic effect on the growth of these bacteria. Al-Jasass *et al.* (2010) found that the *dips* contain formic acid, acetic acid and propionic acid as 3.06, 2.38 and 0.68%, respectively. These Acids have a toxic effect on the yeast bread "*Saccharomyces cerevisia*".

Although, there was a reduction in the count of *L. acidophilus* at the end of incubation period but still the number is more than  $10^6$  cfu mL<sup>-1</sup>. This might explain the health benefits that can be obtained from the consumption of such product (Kurmann *et al.*, 1992). These results are in agreement with the results of Curda and Plockova (1995), who reported about the addition of honey with and without heat treatment to milk with different concentration of fat (0, 1, 3, 5 and 10%). They found that the growth of *L. acidophilus* was inhibited in both types of honey at = 5% concentration.

Previous studies did not find any significant differences in the *L. acidophilus* count after 24 h of incubation between the control sample and the samples containing 5% sucrose and high fructose corn syrup or honey (Popa and Ustunol, 2011; Chick *et al.*, 2001). This difference in the results with the present study can be explained by the presence of some organic acids in *dips* which has an inhibitory effect on the growth and survival of bacteria (Al-Jasass *et al.*, 2010).

#### **Effect of various concentrations of Dips on pH of milk inoculated with *L. acidophilus*:**

The pH values of fermented milk inoculated with *L. acidophilus* bacteria in the presence of various concentrations of *dips* during different incubation period are presented in Table 2. The pH values in all the treatments decreased significantly with increasing the storage time but without any significant difference from the control during different incubation periods i.e. 6, 12 and 24 h. The pH ranged from 6.15-6.35, 5.95-6.10, 5.05-5.20 and 4.90-5.10 after 0, 6, 12 and 24 h incubation, respectively in different *dips* treatments. These results are in agreement with the finding of Chick *et al.* (2001), who did not found any significant difference in pH values between *L. acidophilus* fermented milk without sweeteners and that containing 5% sucrose and high fructose corn syrup or honey. Curda and Plockova (1995) found that the *L. acidophilus* inhibition occurred at a concentration of = 5% honey. Overall in the present study, there was no significant difference in the pH values among all the treatments with different concentrations of *dips*.

Table 2: Changes in pH values of milk inoculated with *Lactobacillus acidophilus* in the presence of various concentrations of *dips* during incubation periods

Dips added (W/V)	pH values			
	Incubation time (h)			
	0	6	12	24
0%	6.35±0.07 <sup>a*</sup>	6.10±0.28 <sup>ab</sup>	5.15±0.35 <sup>b</sup>	5.10±0.56 <sup>b</sup>
5%	6.25±0.07 <sup>a</sup>	6.05±0.21 <sup>a</sup>	5.05±0.35 <sup>b</sup>	4.75±0.21 <sup>b</sup>
10%	6.15±0.07 <sup>a</sup>	6.00±0.28 <sup>a</sup>	5.20±0.28 <sup>b</sup>	4.90±0.14 <sup>b</sup>
15%	6.10±0.14 <sup>a</sup>	5.95±0.21 <sup>a</sup>	5.20±0.28 <sup>b</sup>	4.95±0.07 <sup>b</sup>
20%	6.10±0.14 <sup>a</sup>	5.95±0.21 <sup>a</sup>	5.20±0.42 <sup>b</sup>	4.95±0.07 <sup>b</sup>

The upper different letters in each column indicates significant difference at p>0.05, The lower different letters in each row indicates significant difference at p>0.05

Table 3: Changes in acidity of milk inoculated with *Lactobacillus acidophilus* in the presence of various concentrations of *dips* during incubation periods

Dips added (W/V)	Acidity (%)			
	Incubation time (h)			
	0	6	12	24
0%	0.39±0.01 <sup>b</sup>	0.47±0.005 <sup>b</sup>	0.68±0.005 <sup>b</sup>	1.43±0.40 <sup>a</sup>
5%	0.43±0.005 <sup>c</sup>	0.51±0.005 <sup>c</sup>	0.72±0.005 <sup>b</sup>	1.53±0.15 <sup>a</sup>
10%	0.47±0.01 <sup>c</sup>	0.57±0.010 <sup>c</sup>	0.75±0.010 <sup>b</sup>	0.93±0.01 <sup>b</sup>
15%	0.55±0.01 <sup>b</sup>	0.61±0.005 <sup>b</sup>	0.77±0.005 <sup>b</sup>	0.78±0.01 <sup>b</sup>
20%	0.59±0.01 <sup>c</sup>	0.58±0.005 <sup>b</sup>	0.81±0.010 <sup>a</sup>	0.77±0.01 <sup>b</sup>

The upper different letters in each column indicates significant difference at p>0.05, The lower different letters in each row indicates significant difference at p>0.05

**Effect of Various Concentrations of *Dips* on the Acidity of Milk inoculated with *L. acidophilus*:** The acidity of *L. acidophilus* fermented milk in presence of various concentrations of *dips* during incubation period is presented in Table 3. There was a slight increase in the acidity for all the treatments during the whole incubation period. The highest acidity was found in the control and 5% *dips* concentration treatment after 24 h of incubation. This increase could be due to the increase in the *L. acidophilus* count in these treatments than the others treatments (Table 1). Furthermore, the acidity of milk increased with increasing the *dips* proportion at zero time. The change in acidity ranged between 0.39-0.59, 0.47-0.61, 0.68-0.81 and 0.77-1.53 during 0, 6, 12 and 24 h incubation, respectively in different dips treatments. This increase in acidity might be due to the presence of organic acids in *dips* such as formic acid, acetic acid and propionic acids with relative proportions of 3.06, 2.38, 0.68%, respectively (Al-Jasass *et al.*, 2010). In the present study, an inverse relationship was observed between the amount of *dips* concentrations and the acidity of milk after 24 h. incubation. Overall there was a clear relationship between the count of *L. acidophilus* and the acidity obtained as described in Table 1. The least acidity was found in the treatment containing 20% *dips* with 24 h incubation. Chick *et al.* (2001) found that the *L. acidophilus* produces lactic acid in milk containing sucrose, fructose and honey more than without their presence.

Table 4: Changes in the viable count of *Bifidobacterium lactis* in the presence of various concentrations of *dips* in milk

<i>Bifidobacterium lactis</i> (log <sub>10</sub> cfu mL <sup>-1</sup> )				
-----				
Incubation time (h)				
-----				
Dips added (W/V)	0	6	12	24
0%	7.19±0.06 <sup>a<sub>b</sub></sup>	6.99±0.04 <sup>ab<sub>c</sub></sup>	7.49±0.04 <sup>a</sup>	7.27±0.11 <sup>a<sub>b</sub></sup>
5%	7.21±0.07 <sup>a</sup>	7.17±0.04 <sup>a</sup>	7.21±0.18 <sup>b<sub>a</sub></sup>	6.62±0.14 <sup>b<sub>b</sub></sup>
10%	6.90±0.06 <sup>b<sub>b</sub></sup>	7.14±0.04 <sup>a</sup>	6.83±0.04 <sup>b<sub>b</sub></sup>	6.00±0 <sup>c</sup>
15%	7.20±0.03 <sup>a</sup>	6.88±0.18 <sup>b<sub>b</sub></sup>	6.90±0.11 <sup>b<sub>b</sub></sup>	6.02±0.02 <sup>c</sup>
20%	6.87±0.10 <sup>b<sub>a</sub></sup>	7.02±0.14 <sup>ab<sub>a</sub></sup>	6.55±0.01 <sup>b<sub>b</sub></sup>	5.45±0.10 <sup>c</sup>

The upper different letters in each column indicates significant difference at p>0.05, The lower different letters in each row indicates significant difference at p>0.05

**Effect of various Concentrations of Dips on the viable count of *Bifidobacterium lactis* in milk:** The changes in the logarithm numbers of *Bifidobacterium lactis* viable count in the presence of various concentrations of *dips* in milk during different incubation periods is given in Table 4. It was found that *B. lactis* followed different behaviors in their growth and reproduction in different treatments during different storage periods. The data indicate that the numbers of *B. lactis* was significantly high in the control treatment (without the addition of *dips*) after 12 and 24 h of incubation as compared to the rest of the treatments. The difference in the count of *B. lactis* was not significant between zero time and after 24 h of incubation. These results agree with those of Chick *et al.* (2001) who did not find significant difference between the numbers of *B. lactis* at zero and after 24 h of incubation using sucrose, fructose, honey and also without any sweetener. They further reported that the number of *B. lactis* decreased significantly (p>0.05) in all the treatments during the incubation period between 12 and 24 h. The number of bacteria ranged from 6.87-7.12, 6.88-7.17, 6.55-7.49 and 5.45-7.27 log<sub>10</sub> cfu mL<sup>-1</sup> after 0, 6, 12 and 24 h incubation in different dips treatments, respectively. This association was related to the effect of *dips* concentration. The results indicated that the number of *B. lactis* decreased with increasing concentration of *dips*. The lowest number of *B. lactis* was in the treatment with 20% *dips* concentration (5 and 45 cfu mL<sup>-1</sup>). This directly reflects the inhibitory effect of *dips* towards the growth of bacteria *B. lactis*.

The other possible reason may be the presence of some compounds having toxic effect on the growth of bacteria as mentioned by Al-Jasass *et al.* (2010) or the osmotic effect as stated by Jay *et al.* (2005). Popa and Ustunol (2011) found that the number of *B. bifidium* rose by 0.28 log cycle in the presence of sucrose after 24 h of incubation. In the control sample (without sugar) the numbers rose by 0.36 log cycle indicating the inhibitory effect of sucrose on the growth and activity of the *B. lactis*. Though, there was a decline in the count of *B. lactis* in the end of incubation period yet the numbers exceeded to one million cells per mL of milk except that treatment containing 20% *dips*. This refers on the health effects of these bacteria (Kurmann *et al.*, 1992).

**Changes in pH values of fermented milk inoculated with *Bifidobacterium lactis*:** The pH ranged from 6.00-6.30, 6.00-6.20, 5.90-6.20 and 5.55-6.10 after 0, 6, 12 and 24 h incubation in different dips treatments, respectively (Table 5). A gradual reduction in the pH values of all the treatments was observed with different concentration of *dips* (0, 5, 10, 15 and 20%). However, this

Table 5: Changes in pH values of milk inoculated with *Bifidobacterium lactis* in the presence of various concentrations of *dips* during incubation periods

<i>Bifidobacterium lactis</i> (log <sub>10</sub> cfu mL <sup>-1</sup> )				
Incubation time (h)				
Dips added (W/V)	0	6	12	24
0%	6.30±0.0 <sup>a</sup> <sub>a</sub>	6.20±0.0 <sup>a</sup> <sub>ab</sub>	6.20±0.0 <sup>a</sup> <sub>ab</sub>	6.10±0.14 <sup>a</sup> <sub>b</sub>
5%	6.20±0.0 <sup>ab</sup> <sub>a</sub>	6.10±0.14 <sup>a</sup> <sub>a</sub>	6.10±0.0 <sup>a</sup> <sub>ba</sub>	5.55±0.19 <sup>a</sup> <sub>b</sub>
10%	6.15±0.07 <sup>bc</sup> <sub>a</sub>	6.20±0.14 <sup>a</sup> <sub>a</sub>	6.00±0.14 <sup>bc</sup> <sub>a</sub>	5.65±0.08 <sup>a</sup> <sub>b</sub>
15%	6.05±0.07 <sup>d</sup> <sub>a</sub>	6.05±0.07 <sup>a</sup> <sub>a</sub>	5.95±0.07 <sup>bc</sup> <sub>a</sub>	5.85±0.11 <sup>a</sup> <sub>b</sub>
20%	6.00±0.0 <sup>d</sup> <sub>a</sub>	6.00±0.0 <sup>a</sup> <sub>a</sub>	5.90±0.0 <sup>c</sup> <sub>b</sub>	5.85±0.07 <sup>a</sup> <sub>b</sub>

The upper different letters in each column indicates significant difference at p>0.05, The lower different letters in each row indicates significant difference at p>0.05

Table 6: Changes in acidity of milk inoculated with *Bifidobacterium lactis* in the presence of various concentrations of *dips* during incubation periods

Acidity (%)				
Incubation time (h)				
Dips added (W/V)	0	6	12	24
0%	0.42±0.01 <sup>c</sup> <sub>c</sub>	0.42±0.005 <sup>c</sup> <sub>c</sub>	0.45±0.01 <sup>c</sup> <sub>b</sub>	0.48±0.01 <sup>c</sup> <sub>a</sub>
5%	0.44±0.01 <sup>d</sup> <sub>c</sub>	0.47±0.005 <sup>b</sup> <sub>b</sub>	0.48±0.005 <sup>d</sup> <sub>b</sub>	0.57±0.00 <sup>d</sup> <sub>a</sub>
10%	0.52±0.01 <sup>c</sup> <sub>c</sub>	0.52±0.010 <sup>c</sup> <sub>c</sub>	0.55±0.01 <sup>b</sup> <sub>b</sub>	0.59±0.01 <sup>c</sup> <sub>a</sub>
15%	0.57±0.01 <sup>b</sup> <sub>b</sub>	0.58±0.010 <sup>b</sup> <sub>b</sub>	0.61±0.01 <sup>b</sup> <sub>a</sub>	0.61±0.005 <sup>b</sup> <sub>a</sub>
20%	0.62±0.05 <sup>c</sup> <sub>c</sub>	0.62±0.010 <sup>a</sup> <sub>c</sub>	0.67±0.01 <sup>a</sup> <sub>b</sub>	0.69±0.01 <sup>a</sup> <sub>a</sub>

The upper different letters in each column indicates significant difference at p>0.05, The lower different letters in each row indicates significant difference at p>0.05

reduction in pH was significant (p>0.05) between the *dips* (10, 15, 20%) and the control treatment during different incubation periods (0, 6, 12 and 24 h). This reduction might be due to the presence of some organic acids in the *dips* as mentioned earlier by Al-Jasass *et al.* (2010) or the acids produced by the Bifidobacteria (Scardovi and Trovatelli, 1965). The results in Table 5 also showed significant differences in the pH values (p>0.05) between zero time and 24 h incubation for all the treatments. Chick *et al.* (2001) found that the pH of fermented milk decreased with *B. bifidum* in the presence of some sweeteners, but it was significant with 5% honey as compared to the control treatment (without sweetener).

### Changes in acidity of fermented milk inoculated with *Bifidobacterium lactis* bacteria:

The change acidity of fermented milk inoculated with *Bifidobacterium lactis* ranged from 0.42-0.62, 0.42-0.62, 0.45-0.67 and 0.48-0.69 after 0, 6, 12 and 24 incubation in different dips treatments, respectively (Table 6). The acidity of the fermented milk increased with increasing incubation time for all the treatments. It can be concluded from the results in Table 6 that the highest increase in pH value was in the treatment with 5% *dips* concentration. This increase in pH reached to 29.5% as compared to all other treatments. However, this increase in pH was less in 15% and 20% *dips* concentration treatments. The values for this increase was 7 and 11.7%, respectively



for both these treatments. Moreover, the difference in acidity value was significant ( $p > 0.05$ ) among all the treatments in addition to the control at zero time incubation. The acidity values increased with increasing concentration of *dips*. The lowest value of acidity was in the control and the highest was in 20% *dips* treatments. This might be due to the reasons stated earlier by Al-Jasass *et al.* (2010), Scardovi and Trovatelli (1965) and Chick *et al.* (2001).

## CONCLUSION

The study showed that high concentrations of *dips* have inhibitory effects on the growth and survival of probiotic bacteria (*Lactobacillus acidophilus* and *Bifidobacterium lactis*). However, the viable counts of bacteria were maintained high enough at the end of incubation period (more than  $10^6$  cfu mL<sup>-1</sup>) which is highly essential to achieve the health benefits of these probiotic bacteria. This conclusion holds true for all the treatments except the treatment with 20% concentration of *dips* in the presence of *B. lactis* after the 24 h incubation. The viable number of probiotic bacteria decreased to about  $10^5$  cfu mL<sup>-1</sup>. Therefore, the *dips* can safely be used as sweeteners for the fermented milk in the presence of the probiotic bacteria.

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