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Bifidus Camel Milk Powder: Sensory Evaluation and Viability of Bifidobacteria

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Abstract. Bifidus camel milk powder was prepared using *Bifidobacterium longum* 2716 or *B. bifidum* 2715, these two species were selected from among six species. The milk powder stored at 21°C and 37°C for 15 and 30 d and sensory evaluation and viability of bifidobacteria were studied. The number of bifidobacteria decreased throughout the storage period and the decrease was minimal after 7 d of storage at both temperatures. The decrease of bacterial number at 37°C was higher than that at 21°C. The total number of bifidobacteria after 30 d at 37°C was 1.5 x 10^7 cfu/ml and 2 x 10^6 cfu/ml for *B. longum* 2716 and *B. bifidum* 2715, respectively. *B. longum* 2716 showed the highest proteolytic activity in comparision with the other five species. The amount of free amino groups (FAG) released by *B. longum* 2716 was about 2.2 times higher than that released by both *B. infantis* 2205 and *B. angulatum* 2237. Sensory evalution of reconstituted bifidus camel milk powder showed that the flavor of the powder was not significantly ($P<0.05$) affected by the addition of *B. longum* 2716. Bifidus camel milk powder stored at 37°C for 15 or 30 d scored low flavor in comparison with the rest of the samples.

Introduction

Bifidobacteria are predominant bacteria in the gut of breast-fed infants. They were also isolated from various animals, including honeybees [1, pp. 1418-1434]. All human species are non-sporeforming, non-motile, anaerobic, Gram-positive bacteria. Bifidobacteria had long been recognized as bacteria with probiotic, nutritive and theraputic properties (2, p. 568).

Today there is an increasing interest in the incorporation of the intestinal species of bifidobacteria into dairy products. These bacteria have been associated with health-performing effects in the human and animal intestinal tract. These probiotic effects are related to the inhibition of pathogenic bacteria, reducing the risk of colon cancer, increasing the immune response and decreasing the concentration of cholesterol in blood

plasma [3, 4]. Bifidobacteria also act as resistance factors against diarrhea [5]. These bacteria produce acetic and lactic acids in a molecular ratio of 3:2 from 2 mol of glucose in an ideal synthetic medium [6].

There is a large interest in the ingestion of dairy products containing bifidobacteria to maintain a proper balance of normal intestinal flora and to enhance the beneficial effect of bifidobacteria. Therefore, the dairy industry is introducing bifidobacteria into different dairy products. These products are labeled as containing "bifidobacteria" as a generic name [7].

Camel milk plays an important source of nutrients in desert communities. The population of camels in Saudi Arabia is estimated to be 0.60 million [8]. Camel milk is popular in Saudi Arabia and it is consumed as fresh raw milk, pasteurized and fermented.

Growth, viability and proteolytic activity of bifidobacteria in whole camel milk had been studied by Abu-Tarboush *et al.* [9]. They concluded that camel milk could be used in the production of fermented milk because of the high amount of peptides, which might be easily attacked by bifidobacterium species.

Bifidus milk powder from cow milk was prepared as a mean of preservation for bifidobacterial stability during storage [10]. However, the purpose of this study was to study the effect of storage of probiotic camel milk powder at 21^{O}C and 37 ^{O}C on the sensory evaluation and the growth and viability of bifidobacteria.

Materials and Methods

Bacterial cultures

Bifidobacterium angulatum NCFB 2237, *B. adolescentis* NCFB 2204, *B. bifidum* NCFB 2715, *B. breve* NCFB 2257, *B. infantis* NCFB 2205 and *B. longum* NCFB 2716 were purchased in lyophilized form from the National Collection of Food Bacteria (Aberdeen, UK).

Medium

The freeze-dried cultures were activated in MRS broth (Oxoid, Basingstoke, England) supplemented with 5% (w/v) lactose [11]. The cultures were incubated at 37^{O}C for 24 h in an anaerobic chamber (Gaspak System, BBL, Cockeysville, MD).

Preparation of *Bifidobacterium* starter

Bifidobacterium longum 2716 and *B. bifidum* 2715 were grown in MRSL for 24 h at 37^{O}C, bacteria were centrifuged at 4.000 g for 30 min at 4^{O}C (Hermlez 320 K, Germany) and the precipitated bacteria were suspended in saline solution. Each culture

was inoculated at 3% (v/v) into 100 ml of pasteurized camel milk and incubated for 8 h at 37°C in the anaerobic chamber the culture was used as *Bifidobacterium* starter.

Preparation of bifidus camel milk

Fresh, whole camel milk was obtained from Al-Faisalia farm, Riyadh, Saudi Arabia. It was pasteurized at 63°C for 30 min in a water bath. The starter was inoculated in the pasteurized camel milk at 3% (v/v) and incubated anaerobically. The bifidus milk containing (1-2 x 10^9 cfu/ml) was used throughout the following experiments.

Preparation of freeze-dried bifidus camel milk

The bifidus camel milk was poured into a stainless steel tray to a depth of approximately 1.5 cm and frozen at –30°C. The frozen milk was lyophilized using (Virtis Unitop 600 SL, Gardines, NY, USA) freeze-dryer.

Viability of bifidobacteria

The viable counts were carried out in serial dilution with 0.1% peptone-water and pour planting in triplicate using MRSL agar. The pH of the samples were determined (Corning, pH meter 240).

Stability of the bifidus camel milk powder

The freeze-dried camel milk powder was packed and sealed in polyethylene bags (200 gms). All bags were covered with aluminum foil, then were stored at 21°C and 37°C for 30 d. During this period of storage, the viable count was determined over the storage time.

Measurement of proteolysis

Proteolysis of incubated milk was determined using the o-phthaldialdehyde method (OPA) described by Church et al [12]. 10 ml of 0.75 N trichloracetic acid and 1 ml of water were added to 5 ml of sample. After 10 min of incubation at room temperature, the samples were filtered using Whatman No. 2 filter paper. The concentration of free amino groups (FAG) in the filtrate was determined. The standard curve was prepared using Leu-Gly (Sigma Chemical Co., St. Loouis, Mo, USA).

Sensory evaluation

Sensory evaluation of reconstituted bifidus (*B.longum* 2716) camel milk (11% total solids) was performed at 0, 15 and 30 days of storage at 21°C or 37°C. The milk was evaluated by a panel of 10 of university faculty and staff members, who are familiar with the taste of camel milk. Sensory attributes of flavor, texture and color were considered by panelists. A 1-5 category scale was used in this study (5= excellent, 4=good, 3=fair, 2=poor and 1=very poor) [13, p. 56]. Panelists were asked to list any defects. Samples of reconstituted bifidus camel milk were poured into pre-coded 50 ml plastic-cups.

Statistical analysis

Sensory evaluation data were compared using analysis of variance [14] and SAS programs [15].

Results and Discussion

Growth of bifidobacteria

Preliminary investigation was carried out to compare growth behaviors of six species of bifidobacteria. Initial viable counts for each culture were standardized by the use of standard curve so that they were approximately the same culture for all. The results are shown in Table 1. All species grew in camel milk, however, there was noticeable differences among *bifidobacterium* species in their growth behavior. The growth rate of *B. longum* 2716 and *B. bifidum* 2715 was higher than that of the other species. Therefore they were chosen as a starter for the preparation of bifidus camel milk. These two species were the most commonly used species in preparation of commercial dairy product [16].

Table 1. Growth of different species of bifidobacteria at 37°C for 8 h*

Species		Bacterial growth $\log_{10}$ cfu/ml
Bifidobacterium longum	2716	9.3
B. bifidum	2715	9.0
B. breve	2257	7.10
B. adolescentis	2204	7.59
B. infantis	2205	7.40
B. angulatum	2237	6.90

* Data are the average of three replicates

Although bifidobacteria are nutritionally fastidious microorganisms, they require the presence of growth factors such as amino sugars, bovine casein digest and yeast extract [17]. These bacteria could grow in camel milk, because it has more free amino acids and peptides than bovine milk [18]. These free amino acids could enhance the growth of bifidobacteria during the early stage of incubation [19].

The growth and viability of bifidobacteria is species dependent and probably due to the difference in the proteolytic activity of the strain. The lack of proteolytic activity in some strains of bifidobacteria was the cause of inability to grow in milk [20]. The proteolytic activity of some species of bifidobacteria was studied by Ziajka and Zbikowski [21] who found that some strains were unable to utilize the hydrolyzed casein because they lack of the proteolytic system.

Bifidobacteria could not grow in bovine milk because of its deficiency of growth factors [22]. Generally, there are contradictory results regarding the growth behavior of bifidobacteria in milk. Desjardins et al, [23] found that some species of bifidobacteria could grow in milk without supplementation of growth factors, however, Klaver *et al*, [20] reported that the same strains were unable to grow in unsupplemented milk.

The change in the pH of the camel milk as affected by the growth of the six species of bifidobacteria appeared to be minimal after 8 h of incubation at 37°C (data not shown). Abu-Tarboush *et al.* [9] also found that no noticeable change in the pH of camel milk after 10 h of incubation at 37°C of four species of bifidobacteria.

Proteolytic activity

Table 2 shows the proteolytic activity of six species of bifidobacteria after 8 h of growth at 37°C. The proteolytic activity varied greatly between species. *B. longum* 2716 showed the highest activity. The amount of FAG released by this bacterium was about 2.2 times higher than that released by both *B. infantis* 2205 and *B. angulatum* 2237. In this study there was a parallel relationship between biomass production (Table 1) and proteolytic activity. This result disagree with the findings of Desjardins et al [23], who studied the proteolytic activity of four species of bifidobacteria in bovine milk, they reported that there was no correlation between the proteolytic activity and the biomass production. These researchers also found that in the first 2 or 3 h of the fermentation, the biomass was increasing faster than the number of α-amino groups released throughout the exponential and stationary phases of growth.

Table 2. Proteolytic activities of bifidobacterium species incubated at 37°C for 8 h*

Species		Free amino groups (μM)
B. longum	2716	210
B. bifidum	2715	160
B. breve	2257	130
B. adolescentis	2204	110
B. infantis	2205	95
B. angulatum	2237	95

* Data are the average of three replicates

Stability of freeze-dried bifidus camel milk

Bifidus camel milk was prepared using *B. longum* or *B. bifidum*. This milk was stored at 21°C or 37°C for 30 d. The effect of storage of bifidus milk on the growth pattern of bifidobacteria is shown in Figs. 1 and 2. The bacterial number of bifidobacteria decreased throughout the storage period and the decrease was minimal after 7 d of storage at both temperatures. However, the decrease of bifidobacteria at 37°C was higher than that at 21°C. Bifidobacteria had an optimum temperature for growth in the range of 36 to 38°C [2, p. 524], and these bacteria could not grow at a temperature below 20°C [24]. The low viable count in bifidus milk during storage at 35°C for 30 d was also reported by Nagawa *et al*, [10]. These researchers found that the loss of viable number was higher in the milk at pH 5.0 (not adjusted) compared to the milk adjusted at pH 7.0. The results obtained in Figures 1 and 2 clearly demonstrate that viable counts of both species of bifidobacteria declined during the storage period. The total number after 30 d at 37°C was 1.5 x 10^7 and 2 x 10^6 cfu/ml for *B. longum* 2716 and *B. bifidum* 2715, respectively. The loss of viability of *B. longum* 2716 was lower than that of *B. bifidum* 2715, therefore it could be concluded that *B. longum* 2716 could

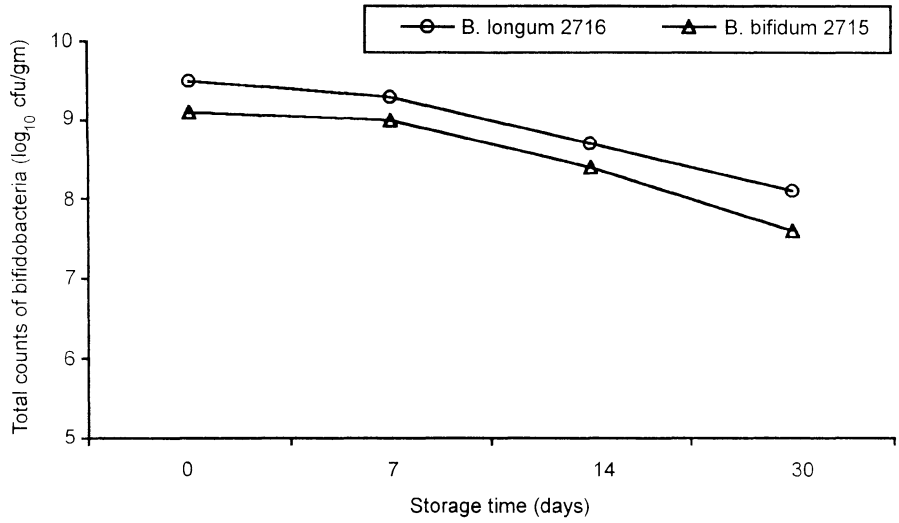

Fig 1. Total counts of bifidobacteria (*Bifidobacterium longum* -o- and *Bifidobactrium bifidum* -Δ-) in bifidus milk stored at 21°C for 30 days.

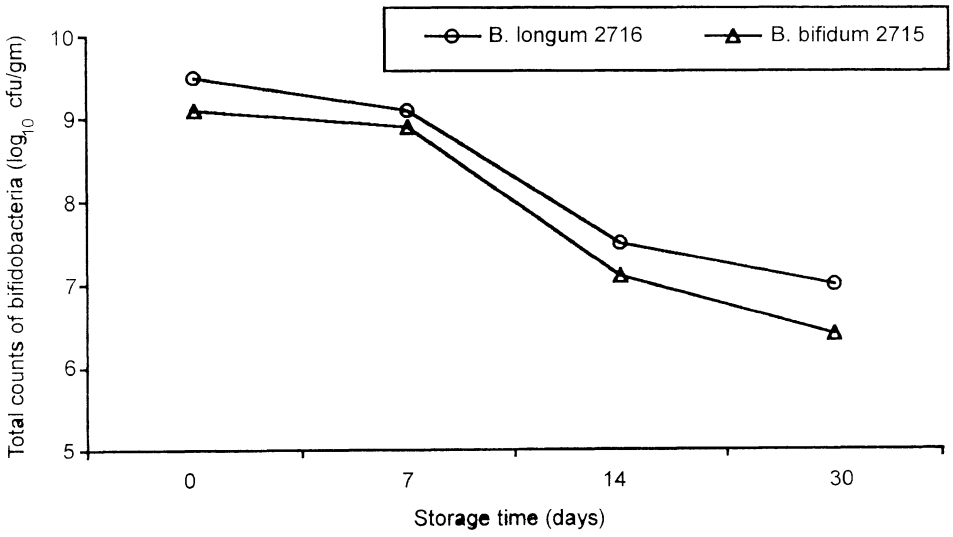

Fig 2. Total counts of bifidobacteria (*Bifidobacterium longum* -o- and *Bifidobactrium bifidum* -Δ-) in bifidus milk stored at 37°C for 30 days.

be used in the preparation of bifidus camel milk as a dietary adjuncts. It has been reported that probiotic dairy products should contain $\geq 10^6$ cfu/ml of bifidobacteria and should be consumed regularly [24].

Nagawa *et al*, [10] also recommended the use of *B. longum* E194b as a dietary adjunct. They found that the growth rate was $5x10^9$ cfu/ml after ~ 12 h post inoculation, whereas in this study *B. longum* 2716 reached 2 x 10^9 after 8 h post inoculation. The differences in growth among bifidobacteria could be related to strain differences as well as to differences in amino acids and peptides from each milk [17].

Sensory properties

Mean sensory evaluation scores for bifidus milk are listed in Table 3. The data clearly demonstrate that the flavor of camel milk was not significantly ($p<0.05$) affected by the addition of *B. longum* 2716 and stored at 21°C for 15 and 30 d. Lyophilization had no effect on the other attributes of bifidus milk. However, bifidus camel milk stored at 37°C for 15 or 30 d scored low flavor in comparision with the rest of the samples. This difference probably due to the effect of high temperature (37°C) that caused high proteolytic acitivty throughout the storage period and lower the flavor acceptability.

Camel milk provided peptides that could serve as a substrate for peptidase enzyme [9]. Cheng and Nagasawa [19] concluded that peptides could be used by bifidobacteria during prolonged incubation.

Table 3. Mean* taste panel scores for bifidus camel milk

Sample	Flavor	Texture	Appearance & Color
A	4.00^{a}	3.44^{a}	4.00^{a}
B	3.89^{a}	2.89^{a}	3.44^{a}
C	3.33^{ab}	3.33^{a}	3.33^{a}
D	3.56^{ab}	3.22^{a}	3.67^{a}
E	2.89^{b}	2.78^{a}	3.33^{a}
F	2.56^{b}	3.00^{a}	3.56^{a}

* Means followed by different letters (within columns) are significantly different ($p < 0.05$)

A = Pasteurized camel milk (control)

B = Reconstituted lyophilized camel milk.

C = Reconstituted lyophilized camel milk + *B. Longum* 2716, stored at 21°C for 15 d.

D = Reconstituted lyophilized camel milk + *B. Longum* 2716, stored at 21°C for 30 d.

E = Reconstituted lyophilized camel milk + *B. Longum* 2716, stored at 37°C for 15 d.

F = Reconstituted lyophilized camel milk + *B. Longum* 2716, stored at 37°C for 30 d.

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حليب الإبل المجفف والمحتوي على البفيدوبكتريا : التقويم الحسي والحيوية للبفيدوبكتريا

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ملخص البحث. تم تصنيع حليب الإبل المجفف باستخدام كل من *B.bifidum* 2715, *Bifidobacterium longum* 2716, ولقد اختيرت هاتان السلالتان من بين ستة أنواع من البفيدوبكتريا حيث نمت بشكل جيد في حليب الإبل. تم تخزين الحليب الجاف بعد التصنيع على درجتي حرارة ٢١°م ، ٣٧°م لمدة ١٥ و ٣٠ يوماً. تم دراسة التقييم الحسي والحيوية للبفيدوبكتريا. لوحظ انخفاض العدد الكلي للبفيدوبكتريا خلال فترة التخزين ، وكان انخفاض العدد أقل ما يمكن بعد ٧ أيام من التخزين ، وكان تأثير درجة حرارة ٣٧°م أعلى منه على درجة ٢١°م في معدل انخفاض العدد الكلي للبكتريا تحت الدراسة.

لوحظ أن العد الكلي بعد التخزين على درجة ٣٧°م لمدة ٣٠ يوما هو ١٠×١,٥ ^{٧} وحدة تكوين للمستعمرة/مل، ٢ ×١٠^{٦}وحدة تكوين للمستعمرة/مل بالنسبة لـ *B. bifidum* 2715, *B. longum* 2716 على التوالي. كما أظهرت البكتريا أعلى نشاط لتحلل البروتين مقارنة بالأنواع الخمسة الأخرى. وكانت مجاميع الأمين الحرة المتكونة بواسطة هذه البكتريا حوالي ٢,٢ مرة أكبر منها في حالة كل من *B.angulalum* 2237 و *B.infantis* 2205. ولقد أوضح التقييم الحسي لحليب الإبل والمدعم بالبفيدوبكتريا أن إضافة *B.longum* 2716 لم يؤثر معنوياً على الصفات الحسية للحليب الجاف. ولقد أدى تخزين هذا الحليب على درجة ٣٧°م لمدة ١٥ و ٣٠ يوم إلى انخفاض في النكهة مقارنة بباقي العينات.