Effect of probiotic bacteria on Karish Cheese production

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Abstract: *Lactobacillus rhamnosus* GR and *Bifidobacterium bifidum* DI either as free cells or entrapped cells in alginate beads were incorporated separately into Kareish cheese that stored in refrigerator (5 – 7°C) for 28 days. The count of *B. bifidum* DI in cheese made with added free cells decreased profoundly after the seventh day of storage up to the end of storage period. It decreased by about 5 logarithmic cycle within 21 days, while the entrapped cells decreased by only about 2 logarithmic cycles during the same period. Entrapment of *L. rhamnosus* GR and *B. bifidum* DI cells in alginate beads improved their survival during storage of Kareish cheese. Cheese made with adding *B. bifidum* DI contained the highest count of bifidobacteria followed by *L. rhamnosus* GR . Total bacterial count increased up to the seventh day then decreased gradually in all Kareish cheese up to the end of storage period. Mould & yeast count increased in all cheese treatments during storage period. Incorporation of *L. rhamnosus* GR and *B. bifidum* DI into Kareish cheese did not affect significantly the chemical composition except acidity, pH value and soluble nitrogen content. There were significant differences among cheese treatments in scores of organoleptic properties in all cheese treatments. Moisture content, total nitrogen content, pH value and scores of Kareish cheese were decrease as storage period progressed, while acidity and soluble nitrogen contents increased.

[Samy. F. Mahmoud, Yasser El-Halmouch and Metwally M. Montaser. **Effect of probiotic bacteria on Karish Cheese production.** *Life Sci J* 2013; 10(2): 1279-1284]. (ISSN: 1097-8135). http://www.lifesciencesite.com.

Key words: Kareish cheese, bifidobacteria, free cells, entrapped cells, Lactobacillus, alginate beads.

1. Introduction:

Kareish cheese is one of soft cheeses which are most popular in Egypt and Arabian countries owing to its high protein, low fat and reasonable price. It is an acid coagulated fresh cheese, made from skim milk with soft composition, white curd and slightly salty. Kareish cheese is considered one of the most food production rich in calcium and phosphorus. These elements are essential for bones and teeth formation. It is also rich in sodium and potassium, which play an important role in the formation of body liquids and muscles (Francois *et al.,* 2004).

Bifidobacteria are the predominant gut flora in breastfed infant (Râsic and Kurmann, 1983). These bacteria are becoming recognized worldwide because of their health and nutritional benefits such as potential beneficial roles in human intestinal tract (Kurmann and Rasic, 1991; Robinson and Samona, 1992). Antitumorigenic activity, improvement of lactose tolerance, reduction of serum cholesterol levels, reduction of ammonia and free serum phenol in patients with liver disease, synthesis of vitamins, increased immunocompetence and antagonistic effects towards enteropathogenic bacteria have all been deviled (Anand *et al.,* 1984; Kageyama *et al.,* 1984; Kebary, 1995 and Badawi and El-Sonbaty, 1997). It is estimated that over 70 products containing bifidobacteria are produced worldwide (Shah, 1997). They include fermented milk, butter milk, sour cream, frozen dessert, cheese, baby foods, pharmaceutical preparations and livestock feed supplements (Gomes *et al.,* 1995; Tamime *et al.,* 1995; Kebary, 1996; Kebary *et al.,* 1998; Badawi and Hussein 1999). Microencapsulation has been used to improve the viability of bifidobacteria in dairy products (Dinakar and Mistry,1994; Kebary *et al.,* 1998). Although various strains of lactic acid bacteria have been described as probiotic, relatively few meet the standards of the United Nations of having clinical trial documentation, and many are very sensitive to intense acidity and to the presence of bile salts in the human gastrointestinal tract. So, they die on route to the gut (Kim and Gilliland, 1984).

Various strains of lactic bacteria are considered probiotics. Two of which are *Lactobacillus reuteri* RC-14 and *Lactobacillus rhamnosus* GR-1. They can colonize the intestine and vagina, reducing recurrences of bacterial raginosis, yeast vaginitis and urinary tract infections (Reid and Burton, 2002;

Cadieux *et al.,* 2002; Reid *et al.,* 2004 and Sharareh *et al.,* 2009). The aims of this study were to investigate the possibility of incorporating microentrapped *B. bifidum* DI and *L. rhamnosus* GR-1 in Kareish cheese, to study the effect of microentrapment on viability of *Bifidobacterium bifidum* DI and *L.s rhamnosus* GR-1, to study the behavior of *Bifidobacterium bifidum* DI and *L.rhamnosus* GR-1 during cold storage of Kareish cheese and to investigate the effect of probiotic bacteria on cheese quality.

2. Materials and Methods

Samples

Buffalo's skim milk was obtained from the Faculty of Agriculture, Cairo University, Egypt. Pure culture of *Streptococcus thermophilus* and L. *bulgaricus* was obtained from Hansen Laboratories (Denmark). *B. bifidum* DI was provided by Diversitech Inc. (Gainesville, FL), while *L. rhamnosus* GR-1 was obtained from Urex Biotech. Inc., London, Ontario, Canada.

Methods:

Preparation of microencapsulated *Bifidobacterium bifidum* DI and *L. rhamnosus* GR-1:

Microencapsulation of *Bifidobacterium bifidum* DI and *L. rhamnosus* GR-1 cells were prepared separately according to Adhikari *et al.* (2000) as the following: bacterial cells were grown in MRS broth containing L-cystein- HCl, maintained at 37°C for 24 h. The cells were harvested by centrifugation at 5000 rpm, then washed two times by sterile saline and resuspended in 10 ml of sterile normal saline. A (2% K-carageenan solution containing 0.9% NaCl). Sixty ml of K-carageenan (2%) solution was thoroughly mixed with 20 ml of cell suspension, and temporarily kept in a water bath at 47 $\pm$ °C. Ten ml of heated soybean oil containing 0.1% Tween 80 was added to the mixture of cells. The mixture was stirred for about 10 min. until emulsification and encapsulation occurred. The emulsion was removed by the addition of 150 ml of sterile KCL (0.3 M). After that, the oil phase was removed from the top of the mixture under sterilized condition. On the other hand, the capsules were harvested from the KCL solution by gentle centrifugation at 350 xg for 10 min, then capsules were washed twice with 0.3 M KCL for better stability under the same centrifugation condition and finally stored in refrigerator before use.

Cheese making:

Buffalo's skim milk was heated to 85°C. Milk was divided into 5 batches, one of them was served as control (2% normal starter), to another two batches un capsulated cells of *B. bifidum* DI and *L. rhamnosus* GR-1 were added separately at the rate $\simeq$ 1.0×10^7 cfu/ml milk plus 1.0% normal starter. The Kareish cheese was manufactured as described by Effat *et al.* (2001). The cheeses were packed in plastic bags contained salted whey and stored in refrigerator at 5 – 7°C for 4 weeks and analyzed at 0, 7, 14, 21 and 28 days.

Chemical analysis:

Fresh Kareish cheese samples analyzed for titratable acidity (TA) and soluble nitrogen (SN) as described by Ling (1963). The pH value was measured using pH meter (HANNA 8417). Moisture, fat and total protein were determined as described by AOAC (2000).

Microbiological analysis:

All samples were examined for total bacterial counts (TBC) and moulds and yeasts according to the American Public Health Association (APHA, 1992). *Bifidobacterium bifidum* DI were enumerated on modified MRS agar (Ventling and Mistry, 1993) with NNL solution (neomycin sulphate 0.2%, nalidixic acid 0.03% and Lithium chloride 6.0%) (Samona and Robinson, 1991). *Lactobacillus rhamnosus* GR-1 was enumerated using MRs pH 6.2 plus vancomycin (10 g/ml), and incubated under anaerobic conditions (Anaero Gen; Oxoid Ltd) at 37°C for 72 hr (Lankaputhra and Shah, 1996; Tharmaraj and Shah, 2003).

Samples containing beads were suspended in 9.0 ml sterile phosphate buffer (1 m, pH 7.5) followed by gentle shaking at room temperature for 10 min to release bifidobacteria and *Lactobacillus* from beads (Sheu *et al.,* 1993).

Sensory evaluation:

The sensory evaluation of Kareish cheeses was carried out according to El-Shafei *et al.* (2008). The samples were presented to the panelists in a random order. The cheeses were evaluated organoleptically after zero, 14 and 28 days of storage period in Dairy Science Department, Food Technology Research Institute, Agriculture Research Center. Panelists evaluated cheese for appearance (20 points), body and texture (35 points), flavor (45 points) and overall acceptability (100 points).

Statistical analysis:

2×2 factorial design was used to analyze the data and Duncan's test was used to make the multiple comparisons (Steel and Torrie, 1980). Significant differences were determined at $p \leq 0.05$ level.

Table (1). Effect of *B. bifidum* DI and *L. rhamnosus* GR on moisture, fat and total protein contents of Kareish cheese during refrigerated storage.

Cheese treatments	Moisture content (%)					Fat content (%)					Total protein content (%)				
	Storage period					Storage period					Storage period				
	0.0	7	14	21	28	0.0	7	14	21	28	0.0	7	14	21	28
Control	76.57^{Aa}	75.21^{Ab}	74.12^{Ac}	73.65^{Ad}	73.00^{Ad}	0.60^{Aa}	0.60^{Aa}	0.60^{Aa}	0.50^{Aa}	0.50^{Aa}	16.49^{Aa}	16.01^{Ab}	15.50^{Ac}	15.44^{Ac}	15.38^{Ac}
B. bifidum (free cells)	76.52^{Aa}	75.23^{Ab}	74.10^{Ac}	73.59^{Acd}	72.86^{Ad}	0.60^{Aa}	0.60^{Aa}	0.60^{Aa}	0.60^{Aa}	0.60^{Aa}	16.51^{Aa}	15.99^{Ab}	15.55^{Ac}	15.45^{Ac}	15.41^{Ac}
B. bifidum (encapsulatedcells)	76.61^{Aa}	75.32^{Ab}	74.20^{Ac}	73.56^{Ac}	72.86^{Acd}	0.60^{Aa}	0.60^{Aa}	0.60^{Aa}	0.60^{Aa}	0.50^{Aa}	16.47^{Aa}	15.96^{Ab}	15.52^{Ac}	15.41^{Ac}	15.36^{Ac}
L. rhamnosus (free cells)	76.68^{Aa}	75.29^{Ab}	74.28^{Ac}	73.61^{Ac}	73.09^{Acd}	0.60^{Aa}	0.60^{Aa}	0.60^{Aa}	0.60^{Aa}	0.50^{Aa}	16.55^{Aa}	16.07^{Ab}	15.58^{Ac}	15.48^{Ac}	15.43^{Ac}
L. rhamnosus (encapsulatedcells)	76.64^{Aa}	75.26^{Ab}	74.24^{Ac}	73.54^{Acd}	73.07^{Ad}	0.60^{Aa}	0.60^{Aa}	0.60^{Aa}	0.60^{Aa}	0.60^{Aa}	16.53^{Aa}	16.06^{Ab}	15.59^{Ac}	15.49^{Ac}	15.42^{Ac}

Different Capital letters on the same column are differ significantly at $\boldsymbol{p} \leq 0.05$.

Different small letters on the same row are differ significantly at $\boldsymbol{p} \leq 0.05$.

3. Results

In our experiment, many parameters were used to evaluate the quality of Kareish cheese. For the first three parameters; moisture, Fat and Protein content, although none of the microbial treatment was significantly differ compared to the control, remarkable statistical variations were observed in moisture and protein content according to the days of storage (Table 1). Generally a significant reduction in moisture content was recorded by encapsulated cells of *B. bifidum* DI by increasing the days of storage. About 5% of reduction was observed at 28 days of storage. In contrast, the moisture content of cheese varied insignificantly when it was treated by *B. bifidum* DI and *L. rhamnosus* GR either as free cells or encapsulated comparing with the control (Table 1).

Fat content of all Kareish cheese treatments varied insignificantly and ranged from 0.50 to 0.06% (Table 1). In case of total protein content, all the microbial treatments varied insignificantly in comparison with the control. Total protein content of Kareish cheese decreased significantly as storage period increased (Table 1). The maximum reduction was recorded by the free cells of *L. rhamnosus* GR after 28 days of storage. It was about 7% of the total protein content recorded at zero time.

Titratable acidity content (%) varied significantly within the same period of storage compared with the non-treated control. It increased directly with the period of storage (Table 2). The maximum increase was obtained by encapsulated *B. bifidum* DI after 28 days of storage.

pH value of cheese treatments varied significantly according to both microbial treatments and period of storage. All microbial treatments slightly increased the pH compared to the control, on the other hand, the pH value decreased within the treatment by increasing the storage period (Table 2).

Soluble nitrogen content of cheese varied significantly according to the microbial treatment compared with the control. it increased directly with the storage period. The maximum value was recorded by free cells of *L. rhamnosus* GR after 28 of storage. Treatment of Kareish cheese by *L. rhamnosus* GR increased soluble nitrogen content by 1.5 times over the control (at zero time)(Table 2). In general soluble nitrogen formed by the free cells of the two tested bacteria was higher than those encouraged by encapsulated forms (Table 2).

The growth of *B. bifidum* DI and *L. rhamnosus* GR during refrigerated storage of Kareish cheese increased with a peak at 7 days of storage. In contrast, it decreased dramatically with increasing the storage period until 28 days (Table 3). The highest numbers of bacteria for *B. bifidum* DI and *L. rhamnosus* GR were observed at 7 days of storage. They were 41×10^7 and 38×10^7, respectively.

The viability of *B. bifidum* DI and *L. rhamnosus* GR in cheese decreased markedly from 38×10^7 to 60×10^2 and from 40×10^7 to 51×10^2 cfu/g respectively, at the end of storage period (Table 3).

The same results of total bacterial counts were observed in Kareish cheese. Where they increased up to the seventh day of refrigerated storage, then decreased gradually up to the end of storage period (Table 4). The results show that the mould and yeast increased gradually during storage period and reached its maximum by the end of storage period for all Kareish cheese treatments. Generally, both the control and treated cheeses with free cells of *B. bifidum* DI and *L. rhamnosus* GR contained higher mould and yeast than those of encapsulated bacteria.

Table (2). Effect of *B. bifidum* DI and *L. rhamnosus* GR on titratable acidity, pH values and soluble nitrogen contents of Kareish cheese during refrigerated storage.

Cheese treatments	Titratable acidity content (%)					pH value					Soluble nitrogen content (%)				
	Storage period					Storage period					Storage period				
	0.0	7	14	21	28	0.0	7	14	21	28	0.0	7	14	21	28
Control	0.95^{Ae}	1.09^{Ad}	1.20^{Ac}	1.29^{Ab}	1.31^{Aa}	5.30^{Ba}	5.20^{Cb}	5.09^{Cc}	5.00^{Cd}	4.97^{Be}	0.41^{Ac}	0.59^{Ad}	0.81^{Ac}	0.91^{Ab}	1.04^{Aa}
B. bifidum (free cells)	0.90^{Be}	1.05^{Bd}	1.15^{Bc}	1.26^{Bb}	1.31^{Aa}	5.32^{Ba}	5.23^{Bb}	5.12^{Bc}	5.03^{Bd}	5.00^{Be}	0.42^{Ae}	0.58^{ABd}	0.78^{ABc}	0.92^{Ab}	1.02^{Aa}
B. bifidum (encapsulatedcells)	0.85^{Ce}	1.00^{Cd}	1.08^{Cc}	1.11^{Cb}	1.25^{Ba}	5.40^{Aa}	5.31^{Ab}	5.18^{Ac}	5.11^{Ad}	5.08^{Ae}	0.36^{Be}	0.52^{Bd}	0.69^{Bc}	0.80^{Bb}	0.95^{Ba}
L. rhamnosus (free cells)	0.92^{Be}	1.06^{Bd}	1.17^{Bc}	1.27^{Bb}	1.30^{Aa}	5.32^{Ba}	5.24^{Bb}	5.13^{Bc}	5.05^{Bd}	5.00^{Be}	0.42^{Ae}	0.60^{Ad}	0.80^{Ac}	0.93^{Ab}	1.07^{Aa}
L. rhamnosus (encapsulatedcells)	0.86^{Ce}	1.00^{Cd}	1.07^{Cc}	1.12^{Cb}	1.24^{Ba}	5.41^{Aa}	5.31^{Ab}	5.19^{Ac}	5.10^{Ad}	5.07^{Ae}	0.33^{Ce}	0.48^{Cd}	0.67^{BCc}	0.78^{Cb}	0.93^{Ba}

Different Capital letters on the same column are differ significantly at $p \leq 0.05$.

Different small letters on the same row are differ significantly at $p \leq 0.05$.

All treated cheeses were accepted by panelists even at the end of storage period. The scores of flavor, body & texture and appearance decreased slightly after 14 days of refrigerated storage (Table 5). Cheese of encapsulated *B. bifidum* DI and *L. rhamnosus* GR gained higher scores than those of free cells. It could be concluded that adding *B. bifidum* DI and *L. rhamnosus* GR affected insignificantly the chemical composition of Kareish cheese, while increased the acceptability of cheese. Encapsulated forms of *B. bifidum* DI and *L. rhamnosus* GR improved the cheese survival and may achieved the therapeutic effect.

Table (3). Effect of micro-entrapment on survival of *B. bifidum* DI and *L. rhamnosus* GR during refrigerated storage of Kareish cheese

Cheese treatments	Viable counts of *B. bifidum DI* and *L. rhamnosus GR* (cfu/g)				
	Storage period				
	0.0	7	14	21	28
Control	ND	ND	ND	ND	ND
B. bifidum (free cells)	21×10^6	38×10^7	52×10^4	72×10^3	60×10^2
B. bifidum (encapsulated cells)	11×10^6	22×10^7	17×10^6	83×10^5	39×10^5
L. rhamnosus (free cells)	23×10^6	41×10^7	48×10^4	61×10^3	50×10^2
L. rhamnosus (encapsulated cells)	16×10^6	23×10^7	19×10^6	77×10^5	31×10^5

ND: not detected.

Table (4). Total bacterial counts and mould and yeast of Kareish cheese treated by *B. bifidum* DI and *L. rhamnosus* GR during refrigerated storage.

Cheese treatments	Total bacterial counts $\times 10^5$					Mould and yeast $\times 10^2$				
	Storage period					Storage period				
	0.0	7	14	21	28	0.0	7	14	21	28
Control	492	541	95	89	74	13	16	25	31	35
B. bifidum (free cells)	450	511	91	80	61	14	22	32	44	51
B. bifidum (encapsulated cells)	400	462	59	50	34	13	17	25	33	38
L. rhamnosus (free cells)	452	514	85	70	61	16	27	37	52	61
L. rhamnosus (encapsulated cells)	390	454	62	48	29	123	16	27	34	39

Table (5). Sensory evaluation of Kareish cheese treated with *B. bifidum* DI and *L. rhamnosus* GR during refrigerated storage.

Cheese treatments	Flavour (45)			Body and texture (35)			Appearance (20)			Total score (100)		
	Storage period (days)			Storage period (days)			Storage period (days)			Storage period (days)		
	0.0	14	28	0	14	28	0	14	28	0	14	28
Control	41^{Aa}	38^{ABab}	34^{Bbc}	31^{Aa}	30^{Aa}	27^{Bb}	19^{Aa}	18^{Aab}	17^{Ab}	90^{Aa}	86^{Bb}	79^{Cc}
B. bifidum (free cells)	41^{Aa}	36^{Bb}	33^{Bc}	31^{Aa}	30^{Aa}	25^{Bb}	19^{Aa}	19^{Aa}	17^{Ab}	90^{Aa}	85^{Bb}	77^{Dc}
B. bifidum (encapsulated cells)	41^{Aa}	41^{Aa}	36^{Ab}	31^{Aa}	31^{Aa}	30^{Aa}	19^{Aa}	19^{Aa}	18^{Aa}	90^{Aa}	89^{Aa}	86^{Ab}
L. rhamnosus (free cells)	39^{Aa}	36^{Bb}	34^{Bc}	30^{Aa}	29^{Aa}	28^{Aa}	19^{Aa}	18^{Aab}	17^{Ab}	88^{Aa}	84^{Bb}	81^{Cc}
L. rhamnosus (encapsulated cells)	41^{Aa}	38^{ABb}	36^{Ab}	31^{Aa}	30^{Aa}	30^{Aa}	19^{Aa}	18^{Aab}	17^{Ab}	90^{Aa}	86^{Bb}	84^{Bc}

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Different small letters on the same row are differ significantly at $p \leq 0.05$.

4. Discussion

Addition of *B. bifidum* DI and *L. rhamnosus* GR to Kareish cheese as a microbial treatment decreased moisture and protein content. This might be due to the development of acidity, which leads to curd contraction that helps to expel the whey from the curd (Effat *et al.,* 2001). Our results were in agreement with El-Shafei *et al.* (2008), they stated that the decrease in total protein content may due to the loss of water soluble nitrogen compounds and protein degradation during storage period. The tested microbial treatments significantly increased pH content. These results are in agreement with those reported by Farag *et al.* (1988); Kebary (1995) and Badawi and Kebary (1996 & 1998).

Microbial treatment of cheese by encapsulated *B. bifidum* DI increased the acidity content. These results were in agreement with Badawi and Kebary (1998) and Farag *et al.* (1988), where they confirmed that the increasing in acidity could be due to conversion of the residual lactose in cheese by starter bacteria. Farag *et al.* (1988); Kebary (1995) and Badawi and Kebary (1996 & 1998) reported a significant increase in pH content in Domiati cheese using *B. bifidum*.

The results indicated that the highest growth of *B. bifidum* DI and *L. rhamnosus* GR was noted at 7 days of the storage period. These results were in agreement with those of Gomes and Malcata (1998), Badawi and Hussein (1999) and Mahmoud & El-Sisi (2011).

Our work suggest that the encapsulated forms of *B. bifidum* DI and *L. rhamnosus* GR gained higher scores than those of free cells. There are many reports demonstrate that the treatment of cheese affected directly on chemical the composition of Kareish cheese, making them more acceptable.

5. Conclusion

Treatment of Kareish cheese by encapsulated forms of *B. bifidum* DI and *L. rhamnosus* GR affected directly on its chemical and physiological features like acidity, soluble nitrogen, total protein, moisture content, texture and flavor. Moisture content, total nitrogen content, pH value and scores of Kareish cheese were decrease as storage period progressed, while acidity and soluble nitrogen contents increased. These all effects of encapsulated forms made Kareish cheese more acceptable.

Acknowledgements:
This work is funded by Taif University, Taif, Kingdom of Saudi Arabia.

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