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SYNBIOTIC KISHK AS FUNCTIONAL FOOD

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ABSTRACT: *Functional food products like kishk is a natural. Healthyfood stuff with respect to the environment,it has promising nutritional value which would increase the bletability and acceptapility to Egyptian consumers. The aim of this work was to prepare synbiotic kishk (SK) from buffalo's skim milk and crushed barley (2 : 1) with adding free cells and immobilized (single and double layer) alginate beads from Bif. bifidum ATCC 15696 and Bif. infantis ATCC 15697. Titratable acidity and soluble nitrogen increased gradually during storage period, while pH values dropped during storage period. Addition of bifidobacteria did not affect significantly ($p > 0.05$) the moisture, total nitrogen, carbohydrate, fat, ash and fiber contents. Free cells bifidobacteria decreased during storage period. Encapsulation of bifidobacteria improved their survival during storage of synbiotic kishk. Viability of Bif. bifidum was not significantly ($p > 0.05$) different from Bif. infantis. Adding of free and immobilized bifidobacteria inhibited the growth of moulds, yeasts and spore forming bacteria.*

Synbiotic kishk (SK) containing double layer alginate beads of bifidobacteria attained the highest organoleptic scores, while SK containing free cells gained the lowest score.

Key words: *Kishk, barley, encapsulation, bifidobacteria, survival.*

INTRODUCTION

Fermentation is an ancient way to store food and improve its safety and nutrition value. Homemade fermented foods are still nowadays commonly produced in the developing countries, especially in the rural areas, although their consumption is declining (Watson *et al.*, 1996).

Fermented dairy products has no longer considered by consumers not only in terms of taste and immediate nutritional needs, but also in terms of their ability to provide specific health benefits beyond their basic nutritional value. Currently, the fermented dairy products tailored to improve towards the balance and activity of the intestinal microflora (Saarela *et al.,* 2006).

Fermented cereal-based foods have a long tradition, although some of the processes may almost be forgotten. Cereals are, in general, a good medium for microbial fermentations. They contain a high level of carbohydrates, which can be used as a source of carbon and energy by microbes in fermentation. Besides carbohydrates, cereals also contain relatively high levels of minerals, vitamins, sterols, and other growth factors, which support growth of microbes, including the fastidious lactic acid bacteria.

Fermented milk (yoghurt)- cereal based food includes Egyptian kishk, Greek Trahanas and Turkish Tarhanas. Little has been done to develop as well as upgrade traditionally homemade fermented products especially in terms of quality, consistency, functionality, safety and shelf life (Rolle and Satin, 2000). An approach is to develop novel probiotic products from traditionally homemade fermented products.

There is growing and promising interest about using of barley in manufacture of kishk because it is improving several cardiovascular disease risk factors, lowering total and low density lipoprotein (LDL) cholesterol

and flattering the postprandial blood glucose (Behall *et al.,* 2006).

However, the survival of probiotic microorganisms in food products is a concern because a significant number of cells die or even tolerate the stress condition during processing and storage (Shah, 2000 and Gueimonde *et al.,* 2004). In order to exert their health benefits, the International Dairy Federation recommends that the minimum counts of probiotic should be around 10^6 – 10^7 cfu / ml at the end of product's shelf life (Kailasapathy, 2002). Encapsulation of probiotic bacteria is currently drawing more and more attention for being a promising method to improve the viability of probiotic organisms in functional food products (Anal and Singh, 2007 and Semyonov *et al.,* 2010).

The objectives of this work were to develop a method for preparing kishk product, to assess the possibility of incorporating barley as prepiotic and bifidobacteria as probiotic cultures in kishk and to mintor the viability of bifidobacteria and quality of synbiotic kishk during storage.

MATERIALS AND METHODS

Materials:

Fresh bulk buffalo's milk was obtained from the herd of Faculty of Agriculture, Menoufia University, Shibin El-Kom, Egypt. Whole buffalo's was separated in the pilot plant at Department of Dairy Science and Technology. Faculty of Agriculture, Menoufia University, Shibin El-Kom, Egypt.

Barley:

Barley was obtained from Department of Crops Science, Faculty of Agriculture, Shibin El-Kom, Menoufia Governorate, Egypt.

Protein 11.8, fat 1.8, carbohydrat 78.1, fiber 5.3, and ash 3.1%.

Bacterial strains and propagation:

Active *Streptococcus thermophilus* EMCC 1043, *Lactobacillus delbrueckii* subsp. *bulgaricus* EMCC 1102, were obtained from Cairo Mircen, Ain Shams University, Egypt. *Bifidobacterium bifiduim* ATCC 15696 and *Bifidobacterium infantis* ATCC 15697 were gratefully obtained from Dr. Linda J. Brady's Lab (Department of Food Science and Nutrition, University of Minnesota, USA). Bifidobacteria strains were activated individually by four successive transfers in Modified MRS broth (Difco Laboratories, Detroit, Michigan, USA) which was supplemented with 0.05% L-cystein-HCl (Sigma Chemicals Co., St. Louis, Mo, USA) according to Ventiling and Mistry (1993) followed by three successive transfers in sterile 10% reconstituted non-fat dry milk. Bifidobacteria were incubated at 37°C under anaerobic conditions using gas pak (Baltimor Biological Laboratories, Cockeysville MD, USA). *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* were activated individually by three successive transfers in sterile 10% reconstituted non-fat dry milk.

Immobilization of *Bifidobacteria*: Preparation of single layer Ca-alginate gel beads:

Bif. bifidum and *Bif. infantis* cells were grown in modified MRS broth for 18h at 37°C and harvested by centrifugation (5000 xg, for 10 min at 4°C). The cell pellets were washed twice with cold sterilized physiological saline (4°C), then with sterilized distilled water. All immobilization procedures were carried out under aseptic conditions using sterilized solutions autoclaved at 121°C for 15 min. The method of reservoir-type microcapsules of Sudekum (1976) was adopted for immobilization of bifidobacteria with some modification according to Kamaly (1998). Typically, 1 g wet cells was weighed to which 100 ml of 2% sodium alginate (BDH chemicals, Ltd, Poole, England) solution sterilized. The mixture was mixed well. Using a sering the mixture was added dropwise to an agitated (30 rpm) sterile solution of $CaCl_2$ (2% w/v). Alginate gelification occurred entrapping bifidobacteria in the form of gel

beads single layer gel beads. Beads were left in $CaCl_2$ solution for 60 min to permit hardening at room temperature. The beads were washed with sterile physiological saline to remove the excess of calcium ions and unentrapped cells Figure (1).

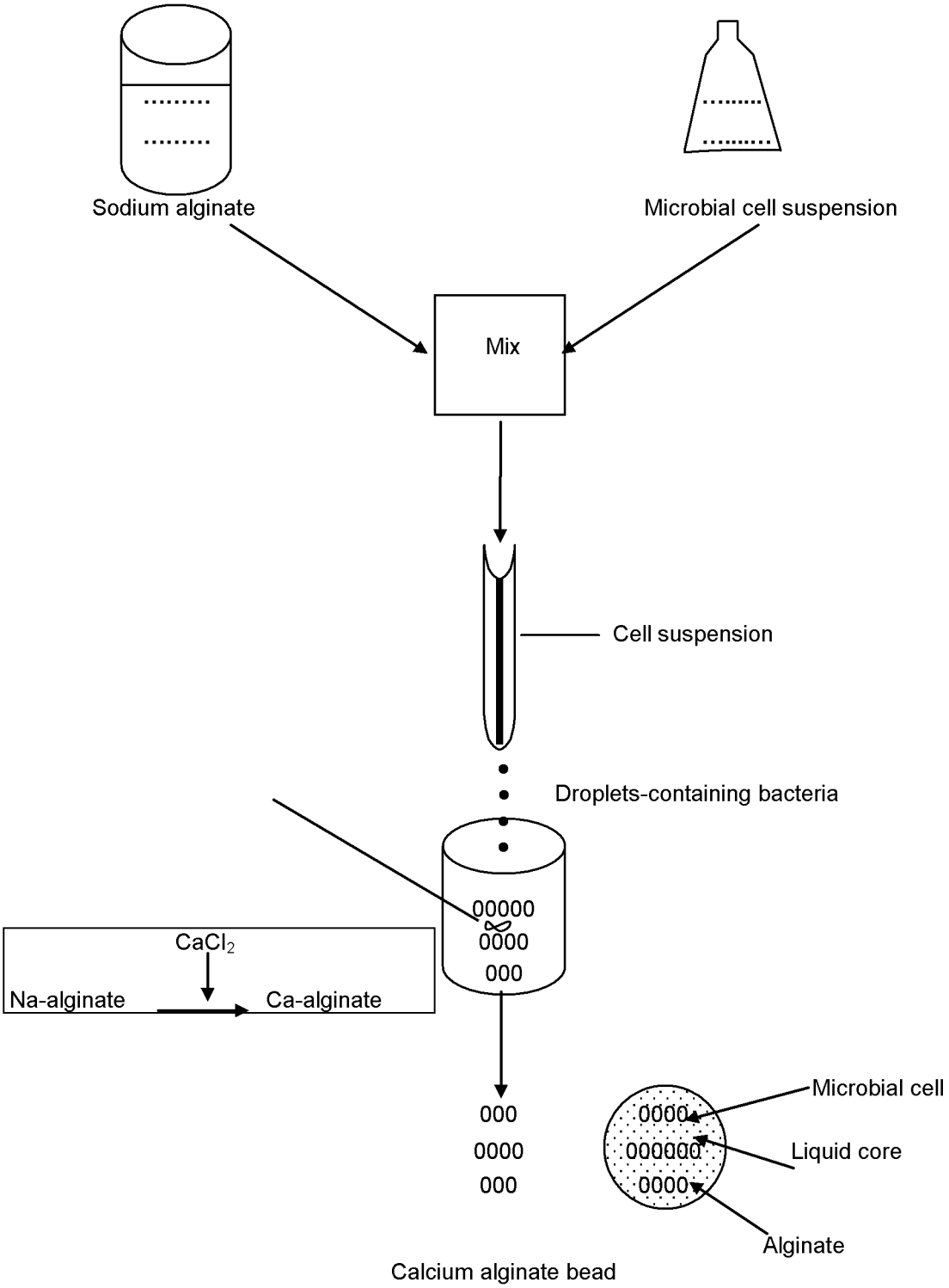

Figure (1). Flow diagram of encapsulating of bacteria by extrusion techniques.

Preparation of double layer Ca-alginate gel beads:

Double layer Ca-alginate gel beads were prepared a cording to Kamaly (1987). The method mentioned above was followed with the only exception of retaining a layer of $CaCl_2$ adhered to the surface of unwashed single layer beads on filter paper for 30 seconds. Then these single layer beads were individually dropped into a flask containing sterile stirred (30 rpm) solution of sodium alginate (2% w/v), removed and immersed into $CaCl_2$ (2% w/v) for gel strengthening and formation of the second layer (Figure 1).

Synbiotic kishk barley preparation:

Manufacturing of Kishk from barley as shown in Figure (2). The Kishk was made from buffalos' skim milk that was obtained by separating buffalo's milk in the pilot plant at Department of Dairy Science and Technology, Faculty of Agriculture, Menoufia University. Skim milk was heated to 90°C then cooled to 40°C, inoculated with 3% starter (*Streptococcus thermophilus* and *Lb. bulgaricus*, 1 : 1), incubated at 37°C for 4 h. The fermented buffalos' skim milk was divided into 6 equal portions, then free cells and encapsulated bacteria, either in single or double layer alginate beads were added at equivalent amounts to provide about 1.3 – 1.5 × 10^8 cfu/g of Kishk (fermented buffalos' skim milk and crushed barley mixed at a ratio of 2 : 1) as following:

1) Treatment I: free cells of *Bif. bifidum* ATCC 15696.
2) Treatment II: free cells of *Bif. infantis* ATCC 15697.
3) Treatment III: single layer alginate beads of *Bif. bifidum* ATCC 15696.
4) Treatment IV: single layer alginate beads of *Bif. infantis* ATCC 15697.
5) Treatment V: double layer alginate beads of *Bif. bifidum* ATCC 15696.
6) Treatment VI: double layer alginate beads of *Bif. infantis* ATCC 15697.

The Kishk samples were analyzed for chemical, microbiological and sensory evaluation, when fresh and after 30, 60, 90 days of storage period at room temperature.

Microbiological analysis:

Bifidobacterial counts were enumerated on modified MRS agar (Venting and Mistry, 1993) to each 100 ml of modified MRS of NPNL solution was added before pouring (neomycin sulfate 0.2%, paromomycin sulfate 0.2%, nalidixic acid 0.03% and Lithinum chloride 6.0%) (Samona and Robinson, 1991).

Yeasts and molds were enumerated on potato dextrose agar (acidified) medium (Difco, 1953). The count of aerobic sporeforming bacteria was carried out as described by Luck (1981).

Coliform count were enumerated according to Marshall (1992) using Violet Red Bile Agar (VRBA). The plates were incubated at 37°C for 48 h.

Chemical analysis:

The moisture content, total ash content, total nitrogen and soluble nitrogen contents, fat content, carbohydrate and fiber content were determined according to the method described by AOAC (2007).

PH was measured using laboratory pH meter [Jen way electric pH meter] with a combined glass electrode.

Titratable acidity expressed as lactic acid (%) was determined according to Ling (1963).

Determination of calorific value:

Calorific value of synbiotic kishk was calculated based on conversion factors as follows; protein 4, carbohydrate 4 and fat 9 and expressed as Kcal / 100 g.

Sensory evaluation:

Soups made from the synbiotic kishk (SK) samples were subjected to sensory evaluations. Seven people who were familiar with SK were asked to score the SK soups in terms of color, taste, odor, mouth feel and consistency using a five-point scale, with 1

being "dislike extremely" and 5 being "like extremely". SK soups were prepared by mixing 40 g SK sample with 500 ml water and simmering for 10 min with constant stirring. The cooked samples were served to the panelists at room temperature (Handan *et al.,* 2006).

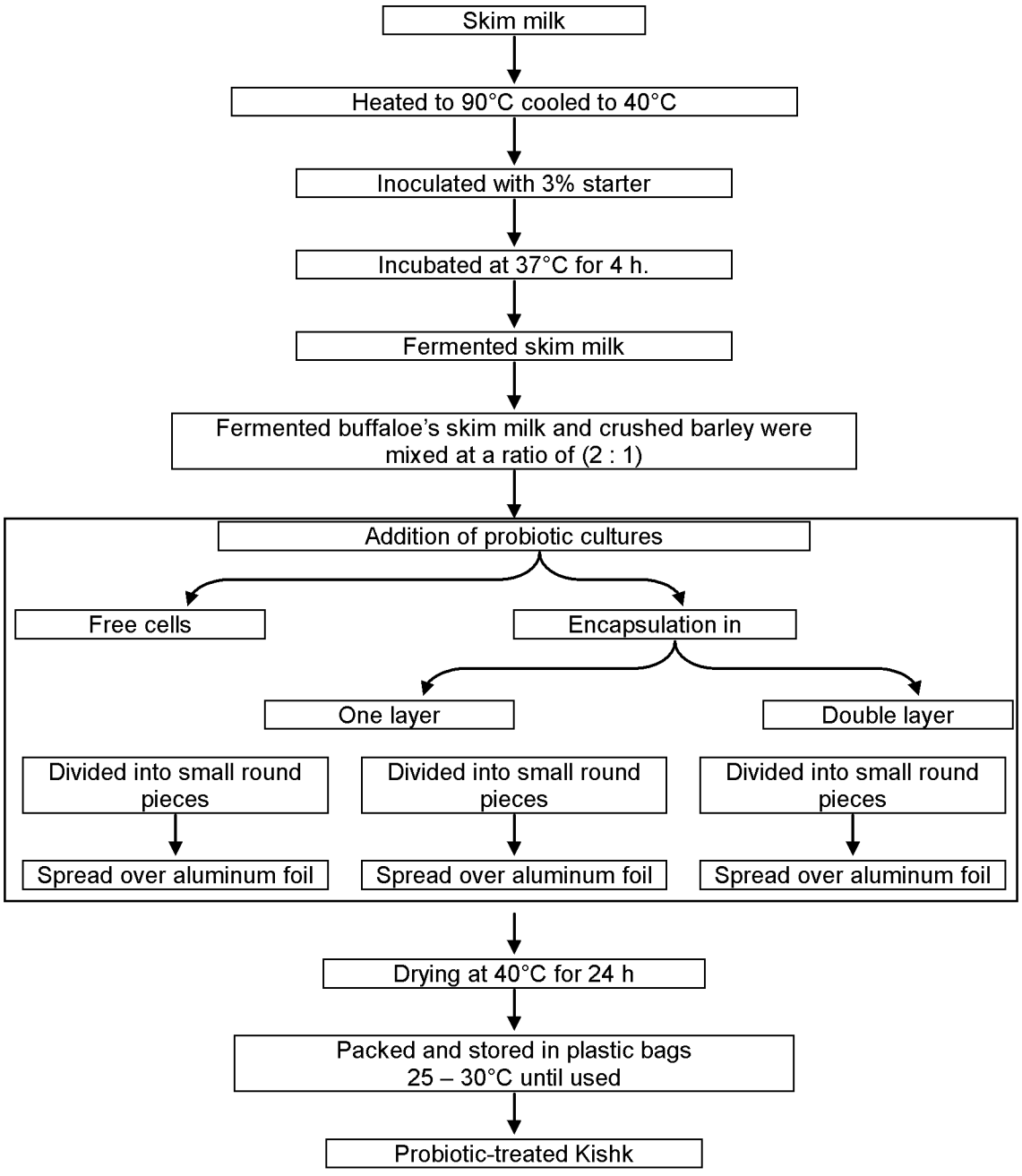

Figure (2). Manufacture of synbiotic Kishk.

Statistical analysis:

Data were analyzed using 2 × 3 factorial design. Newman-Keuls' Test was used to make the multiple comparisons (Steel and Torrie, 1980) using Costat program. Significant differences were determined at $p \leq 0.05$.

RESULTS AND DISCUSSION

Chemical composition:

The changes in moisture content of SK made from fermented milk with added free cells and immobilized single or double layer alginate beads of bifidobacteria are shown in Tables (1, 6). the moisture content of SK for different treatments did not change significantly ($p \geq 0.05$) during the storage period. This is in accordance with other researchers (Elewa and Metry, 2006 and El-Nawawy *et al.,* 2012). Furthermore, SK-containing immobilized cells (single or double layer alginate beads) of bifidobacteria cultures had a moisture content slightly higher than those of the corresponding SK-containing free cells. The improved water holding capacity of encapsulated bifidobacteria containing kishk may attributed to gelled beads that the complexing of alginate with proteins (milk-barley matrices) a slightly acid pH (Lin, 1977).

Tables (1, 6) show the effect of adding free and immobilized bifidobacteria bacteria in the fermented milk on the titratable acidity of SK during the storage period for 90 days. It is clear from these data that the acidity of SK for different treatments increased significantly ($p \leq 0.05$) throughout the storage period. These results are in accordance with those of Hussein and Kebary (1999), Elew and Aly (2006) and El-Nawawy *et al.* (2012). Furthermore, SK with single or double layer alginate beads of bifidobacteria cultures had acidity lower than those of the corresponding SK-containing free cells (Hussein and Kebary, 1999).

The acidification rate was slightly greater in SK-containing single layer alginate beads of bifidobacteria than in SK-containing double layer alginate beads of bifidobacteria. These results suggested that single layer alginate beads do not exert a diffusing limitation for substrate such as lactose or other metabolites (Larisch *et al.,* 1994). Also, the gel coating (second layer) of double layer alginate beads may offer a cell barrier to higher local detrimental acidity within the micro environment of beads (Prevost and Divies, 1992). SK made with adding *Bif. bifidum* were not significantly ($p > 0.05$) different from corresponding treatments made with adding *Bif. infantis*, which means that bacterial strains did not have significant effect on the acidity of SK treatments.

The changes in pH values of SK made from fermented milk with added free cells and immobilized single or double layer alginate beads of bifidobacteria are presented in Tables (1, 6). The pH values of SK of all treatments decreased significantly ($p \leq 0.05$) during the storage period. This possibily as a result of the formation of lactic acid. These results are in agreements with those of Hussein and Kebary (1999), Elewa and Aly (2006) and El-Nawawy *et al.* (2012). It is noteworthy that, the drop in pH value of SK could be due to the formation organic acids at the end of storage period.

Damir *et al.* (1992) identified six organic acids, namely butyric, propionic, acetic, formic, lactic and succinic were produced in fermented kishk.

Tables (1, 6) represents the changes in fat and ash contents of SK. It is clear that fat and ash contents did not change significantly ($p > 0.05$) as storage period progressed. These were no significant ($p > 0.05$) differences among SK treatments, which means neither encapsulation of bifidobacteria nor the strains of bifidobacteria had significant effect on fat and ash contents of the resulting SK treatments (Tables 1, 6). These results are in agreements with Elew and Metry (2006) and El-Nawawy *et al.* (2012).

The fiber content of SK made from skim milk with added free or immobilized single or double layer alginate beads of bifidobacteria are presented in Tables (1, 6). The previous results indicated that the fiber content did not change significantly ($p \geq 0.05$) throughout the storage period. These results are in agreement with those of Tamime (1997), Elewa and Metry (2006) and El-Nawawy *et al.* (2012).

Table (1). Chemical composition of synbiotic kishk made with free and immobilized bifidobacteria during storage period.

Synbiotic kishk properties	Storage period (days)	Free cells		Single layer beads		Double layer beads	
		Bif. bifidum	*Bif. infantis*	*Bif. bifidum*	*Bif. infantis*	*Bif. bifidum*	*Bif. infantis*
Moisture content (%)	0	8.54	8.60	8.54	8.60	8.56	8.63
	30	8.43	8.48	8.43	8.50	8.48	8.54
	60	8.34	8.40	8.36	8.44	8.41	8.48
	90	8.30	8.35	8.33	8.40	8.37	8.45
Titratable acidity (%)	0	1.01	1.05	1.01	1.06	1.02	1.05
	30	1.08	1.12	1.06	1.11	1.05	1.10
	60	1.17	1.20	1.11	1.17	1.10	1.15
	90	1.27	1.30	1.14	1.22	1.13	1.19
pH value	0	5.20	4.98	5.20	4.99	5.20	4.99
	30	5.14	4.91	5.17	4.95	5.17	4.96
	60	5.11	4.88	5.15	4.93	5.14	4.93
	90	5.08	4.85	5.13	4.91	5.12	4.92
Fat content (%)	0	2.76	2.72	2.75	2.70	2.70	2.66
	30	2.73	2.68	2.71	2.66	2.65	2.62
	60	2.70	2.65	2.68	2.64	2.63	2.60
	90	2.69	2.64	2.67	2.62	2.61	2.58
Ash content (%)	0	6.10	6.16	6.13	6.17	6.15	6.19
	30	6.14	6.20	6.17	6.20	6.18	6.22
	60	6.16	6.22	6.19	6.22	6.20	6.24
	90	6.18	6.23	6.20	6.24	6.22	6.26
Fiber content (%)	0	2.15	2.11	2.24	2.20	2.42	2.37
	30	2.19	2.14	2.27	2.24	2.45	2.41
	60	2.21	2.16	2.29	2.26	2.48	2.43
	90	2.22	2.18	2.31	2.27	2.50	2.44
Total nitrogen (%)	0	11.46	11.51	11.46	11.51	11.46	11.51
	30	11.40	11.44	11.38	11.43	11.36	11.41
	60	11.33	11.36	11.26	11.32	11.23	11.29
	90	11.20	11.21	11.11	11.14	11.07	11.10
Soluble nitrogen (%)	0	0.06	0.08	0.06	0.08	0.06	0.08
	30	0.13	0.13	0.12	0.13	0.10	0.11
	60	0.21	0.22	0.20	0.22	0.17	0.18
	90	0.35	0.39	0.33	0.36	0.27	0.29
Total carbohydrate (%)	0	69.82	69.95	69.97	70.08	70.11	70.22
	30	70.07	70.21	70.20	70.33	70.38	70.47
	60	70.27	70.40	70.41	70.52	70.59	70.67
	90	70.43	70.55	70.54	70.66	70.72	70.81
Calorific value	0	349.96	350.32	350.47	350.66	350.58	350.86
	30	350.44	350.72	350.71	351.08	350.81	351.10
	60	350.70	350.89	350.80	351.12	350.95	351.24
	90	350.73	350.80	350.63	350.78	350.65	350.92

The fiber content of different treatments were in the following order; SK-containing double layer beads > SK-containing single layer alginate beads > SK-containing free cells. The high fiber content of SK containing alginate could be explained by considering of alginate as a source of dietary fiber (Brownlee *et al.,* 2005).

Fiber content of SK treatments those made with adding *Bif. bifidum* were not significantly ($p > 0.05$) different from those of SK treatments made with adding *Bif. infantis* (Tables 1, 6).

The total nitrogen content of SK made from milk with added free and immobilized single or double layer alginate beads of bifidobacteria are shown in Tables (1, 6). the results clear that total nitrogen percent decreased significantly ($p \leq 0.05$) in SK made with free and immobilized bifidobacteria as storage period progressed. This decrease in total nitrogen was likely due to protein degradation during storage period and formation of water soluble nitrogenous compounds. Results were accordance with El-Nawawy *et al.* (2012).

SK treatments were not significantly ($p > 0.05$) different from each other, which means either bacterial strain or encapsulation of bifidobacteria did not have significant effect on total nitrogen effect on total nitrogen content of the resultant SK treatments. It seems that proteinase system of bacterial cells would be of negligible effect on protein degradation in barley kishk matrix upon immobilization. Steenson *et al.* (1987) reported that immobilize cultures of proteinase positive (prt^+) lactic streptococci were behaving as metabolically proteinase negative (prt^-) culture when grown in milk.

The effect of incorporation of free and immobilized bifidobacteria in synbiotic barley kishk on soluble nitrogen (SN) are presented in Tables (1, 6). it is obvious from these data that the levels of soluble nitrogen of all SK treatments increased significantly ($p \leq 0.05$) as storage period progressed. Generally there was a direct relationship between storage period and soluble nitrogen as previously indicated by Elewa and Metry (2006) and El-Nawawy *et al.* (2012). SK made with added *Bif. bifidum* were not significantly ($p \geq 0.05$) from those made with *Bif. infantis*, which means the ability of hydrolyzing protein is not strain dependent.

Soluble nitrogen values in free cells of bifidobacteria containing SK were substantially greater than in those of immobilized cells (single or double layer alginate beads) of bifidobacteria containing SK. Even though, the diffusion limitation encountered by the gel matrics for macromolecules such as protein has been observed by Tanaka (1984), single layer beads did not exert complete prevention of proteins in kishk matrices and protein hydrolyzing enzyme interaction, since active cells of bifidobacteria would be preferentially oriented at the peripheral area of the beads (Arnaud *et al.,* 1992).

Changes in calorific value and carbohydrate content of SK made from fermented milk with added free cells and immobilized single or double layer alginate beads of bifidobacteria are presented in Tables (1, 6). Total calories and carbohydrate content of all treatments were significant different ($p < 0.05$) as storage period progressed. However, treatments made with the addition of *Bif. bifidum* and *Bif. infantis* did not differ significantly in calorific value.

SK-containing immobilized single or double layer alginate beads were slightly higher in calorific value and carbohydrate content than that SK-containing free cells.

Sensory analysis results of soups made from synbiotic kishk barley (SK) samples are presented in Table (2). The effect of different treatments on the color, taste, odor, mouthfeel and consistency values of SK soups was statistically significant ($p < 0.05$). Color values obtained by sensory analysis of the SK soups varied between 3.40 and 3.70. Taste values obtained by sensory analysis of the SK soups varied between 4.10 and 4.71 odor values of SK soups varied between 4.22 and 4.74. Mouthfeel values obtained by sensory analysis of the SK soups varied between 3.58 – 4.27. Consistency values of the SK soups varied between 4.00 – 4.78.

Table (2). Organoleptic evaluation of synbiotic kishk made with free and immobilized bifidobacteria during storage period.

Synbiotic kishk treatments	Color	Taste	Odor	Mouthfeel	Consistency	Total score
Free cells						
Bif. bifidum	3.40	4.20	4.28	4.27	4.05	20.23
Bif. infantis	3.40	4.24	4.33	4.26	4.00	19.92
Single layer						
Bif. bifidum	3.60	4.60	4.67	3.96	4.60	21.46
Bif. infantis	3.60	4.65	4.70	3.95	4.56	21.22
Double layer						
Bif. bifidum	3.70	4.67	4.72	3.82	4.75	21.66
Bif. infantis	3.70	4.71	4.74	3.83	4.70	21.30

* For each effect the different letters in the same row means the multiple comparisons are different from each other, letter A is the highest mean followed by B, C, ... etc.

The highest total score was found in SK made with double layer alginate beads followed by SK-containing single layer beads then SK-containing free cells.

Tables (3, 6) show the effect of adding free and immobilized bifidobacteria in the fermented milk on the survival of bifidobacteria. It is obvious from these data that the survival of bifidobacteria of all kishk treatments increased up to the thirty day of storage then declined during the storage of SK. These results are in accordance with those reported by Dave and Shah (1997) and Micanel *et al.* (1997). SK made with free cells declined from 8.10 to 5.39 $\log_{10}$ cfu / g. Also, viability of immobilized single and double layer alginate beads declined from 8.08 to 6.48 and from 8.08 to 8.04 $\log_{10}$ cfu /g, respectively. Data indicated that immobilization of bifidobacteria had a significant effect ($p \leq 0.05$) on rate of survival. The survival of bifidobacteria of all treatments were in the following order; SK made with double layer alginate beads > SK made with single layer alginate beads > SK made with free cells. The viability of both *Bif. bifidum* and *Bif. infantis* were not significantly ($p \geq 0.05$) different from each other. These results are in accordance with Hussein and Kebary (1999).

Counts of spore forming bacteria of SK made with added free cells and immobilized single or double layer alginate beads of bifidobacteria are presented in Table (4, 6). Spore forming counts of all treatments decreased significantly ($p \leq 0.05$) as storage period progressed. At 15^{th} day of storage, spore forming counts 2.50 to 2.30 $\log_{10}$ cfu / g. There were significant differences among SK treatments which means encapsulation affected the spore forming counts. The presence of spore forming counts in SK could be due to the microflora of barley and milk base. At 90^{th} day of storage period spore forming counts declined to range between 1.59 to 1.40. The reduction of spore forming bacteria could be explained by the development of acidity and antagonistic activity of lactic acid bacteria (Lindgren, 1983). These results are in accordance with Elewa and Metry (2006) who noticed a reduction in spore forming in kishk whey and water kishk samples upon storage at temperature (22 and 37°C). Moreover, El-Gendy (1983) showed that spore forming varied from 3.3 to 5.8 $\log_{10}$ cfu / g of dry kishk which formed 57.1 to 75.0% of the total count.

Data presented in Table (5) show that SK treatments were free from yeasts and mould during the first 30 days of storage period. After that, they appeared towards the end of storage period. The obtained results cleared that moulds and yeasts were only detected at the end of storage period. Moreover,

appearance of moulds and yeasts after 30 day of storage period may be due to the post contamination. The moulds and yeasts counts of all treatments were in the following order; SK made with free cells < SK made with single layer alginate beads < SK made with double layer alginate beads. This decrease might be due to the production of antimicrobial substance by bifidobacteria. These results are in agreement with Hussein *et al.* (2006).

Coliform bacteria were determined in all synbiotic barley kishk samples using Violet Red Bile Agar (VRBA). Coliform bacteria could not be detected in all SK samples along the storage period in all samples.

On conclusion, immobilized of probiotic bacteria in alginate beads was convenient to deliver the whole living cells in appropriate number ($\simeq 10^6$ cfu / g synbiotic kishk) achieve the claimed health benefits. Also, the immobilization technique was suitable and did not alter the chemical composition and organoleptic properties of the synbiotic kishk.

Table (3). Viability (log_{10} cfu/g) of bifidobacteria in free and immobilized states incorporated in synbiotic kishk (SK) at 25 – 30°C for 90 days.

Storage period (days)	Free cells		Single layer beads		Double layer beads	
	Bif. bifidum	*Bif. infantis*	*Bif. bifidum*	*Bif. infantis*	*Bif. bifidum*	*Bif. infantis*
0	8.10	8.02	8.08	8.00	8.08	8.02
15	8.20	8.11	8.18	8.08	8.16	8.10
30	8.26	8.18	8.25	8.16	8.24	8.17
60	7.60	7.42	8.00	7.90	8.17	8.11
90	5.52	5.39	6.64	6.48	8.10	8.04

Table (4). Viability (log_{10} cfu/g) of spore forming in free and immobilized states incorporated in synbiotic kishk (SK) at 25 – 30°C for 90 days.

Storage period (days)	Free cells		Single layer beads		Double layer beads	
	Bif. bifidum	*Bif. infantis*	*Bif. bifidum*	*Bif. infantis*	*Bif. bifidum*	*Bif. infantis*
15	2.30	2.43	2.33	2.43	2.50	2.45
30	1.69	1.76	1.67	1.83	2.29	2.21
60	1.62	1.69	1.64	1.78	2.12	2.06
90	1.40	1.43	1.40	1.53	1.42	1.59

Table (5). Viability (log_{10} cfu/g) of mould and yeast counts in free and immobilized states incorporated in synbiotic kishk (SK) at 25 – 30°C for 90 days

Storage period (days)	Free cells		Single layer beads		Double layer beads	
	Bif. bifidum	*Bif. infantis*	*Bif. bifidum*	*Bif. infantis*	*Bif. bifidum*	*Bif. infantis*
0	< 1	< 1	< 1	< 1	< 1	< 1
15	< 1	< 1	< 1	< 1	< 1	< 1
30	< 1	< 1	< 1	< 1	< 1	< 1
60	< 1	< 1	< 1	< 1	1.2	1.1
90	1.5	1.2	1.5	1.3	1.6	1.4

TABLE 6

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الكشك كغذاء وظيفي

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الملخص العربي :

نظراً للأهمية الصحية للشعير وأيضاً أهمية بكتريا Bifidobacteria والتي يجب أن يكون أعداد البكتريا في المنتج عالية حتى تُحقق الفوائد الصحية المرجوة. فقد هدف هذا البحث لإنتاج منتج غذائي حيوي ذو جودة عالية. وتم في هذا البحث تغليف هذه البكتريا فى صورة طبقة وطبقتين من الألجينات ودراسة تأثير هذه البكتريا المضافة سواء في صورة حرة أو في صورة مكبسلة (طبقة أو طبقتين) على عينات الكشك . وقد تم تتبع هذه الخلايا وتأثيرها على العينات على فترات مختلفة من التخزين .

ولقد أوضحت النتائج المتحصل عليها ما يلى :

1- الخلايا البكتيرية المحملة على طبقتين من ألجينات الكالسيوم أعطت أعلى معدل للحيوية عما في حالة الخلايا البكتيرية الحرة أو فى حالة الخلايا المحملة على طبقة الجينات واحدة.

2- أدى إضافة الخلايا المحملة على طبقتين من ألجينات الكالسيوم إلى الكشك للحفاظ على حيوية الخلايا البكتيرية أثناء التخزين بصورة أعلى من الخلايا الحرة وأيضاً المحملة على طبقة واحدة من ألجينات الكالسيوم .

3- تطورت الحموضة بشكلٍ أكثر وضوحاً فى الكشك المحتوى على الخلايا الحرة عنه فى حالة الخلايا المحملة فى صورة طبقة واحدة وأيضاً فى صورة طبقتين .

4- انخفض الـ pH بشكلٍ كبير فى حالة العينات المضاف إليها الخلايا الحرة يليها العينات المضاف إليها الخلايا محملة فى صورة طبقة ألجينات واحدة وأقلها العينات المضاف إليها الخلايا محملة فى صورة طبقتين .

5- لم يتأثر كل من الدهن والرماد والألياف بشكلٍ معنوي باختلاف المعاملات بين إضافة الخلايا الحرة وإضافتها فى صورة محملة .

6- أعطت المعاملات المحتوية على السلالات المحملة في صورة طبقتين من الألجينات درجات تحكيم عالية تراوحت بين 21,30 و 21,66 بينما المعاملات المحتوية على السلالات المضافة في صورة حرة أعطت درجات أقل تراوحت بين 19,92 و 20,23 .

7- لم تحتوى كل المعاملات على بكتيريا مجموعة الكوليفورم طوال فترة التخزين .

Table (6). Statistical analysis of synbiotic kishk (SK) treatments.

Synbiotic kishk properties	Effect of treatments							Effect of storage period (days)					
	Multiple comparisons•							Multiple comparisons•					
	Mean squares	Free cells		One layer		Double layer		Mean squares	0	30	60	90	
		Bif. bifidium	*Bif. infantis*	*Bif. bifidium*	*Bif. infantis*	*Bif. bifidium*	*Bif. infantis*						
Moisture (%)	0.024*	C	C	AB	AB	A	A	0.156	A	A	A	A	
Titratable acidity (%)	0.017*	A	A	B	B	C	C	0.112*	D	C	B	A	
pH values	0.059*	C	C	B	B	A	A	0.033*	A	B	C	D	
Ash (%)	0.096	A	A	A	A	A	A	0.016	A	A	A	A	
Fat (%)	0.018	A	A	A	A	A	A	0.022	A	A	A	A	
Fiber (%)	0.185*	C	C	B	B	A	A	0.017	A	A	A	A	
Total nitrogen (%)	0.016	A	A	A	A	A	A	0.403*	A	AB	BC	C	
Soluble nitrogen (%)	0.054*	A	A	AB	AB	C	C	0.159*	D	C	B	A	
Carbohydrate (%)	0.241*	B	B	AB	AB	A	A	1.194	A	A	A	A	
Calorific value	0.489*	B	B	AB	AB	A	A	0.715*	B	AB	AB	A	
Sensory evaluation	0.180*	C	D	A	B	A	B	-	-	-	-	-	
									0	15	30	60	90
Bifidobacterial counts	17.491*	C	C	B	B	A	A	8.563*	B	B	A	C	D
Spore forming counts	0.209*	A	A	B	B	C	C	2.757*	ND	A	B	C	D
Mould and yeast counts	0.185*	C	C	B	B	A	A	2.515*	c	C	C	B	A

• For each effect the different letters in the same row means the multiple comparisons are different from each other, letter A is the highest mean followed by B, C, ... etc.

* Significant at 0.05 level ($p \leq 0.05$). ND: Non detected.