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THE FEASIBILITY OF USING SOME PROBIOTIC BACTERIA IN MANUFACTURE OF FLAVOURED WHEY BEVERAGES

G. A. Ibrahim, Mona, A.M. Abd El Gawad and Fatma, A.M. Hassan

Food and Dairy Technology Department,
National Research Centre, Dokki, Cairo, Egypt

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ABSTRACT *Three types of probiotic starter cultures (Lactobacillus acidophilus, Bifidobacterium bifidum or ABT) were used in manufacture of whey beverages 5% fructose, 0.1% CMC, 0.22% calcium chloride, 0.05% flavour, 0.05% colors were added to sweet whey, then all the mixtures were homogenized, pasteurized and cooled to 45°C then inoculated with 2% of each starters and incubated at 37°C for 2 hours, then stored at refrigerator (5±1°) for 10 days. The resultant beverages were analyzed chemically, microbiologically and organoleptically. The results showed that total bacterial count of probiotic starter cultures (L. acidophilus and ABT) count were slightly increased during storage period. The beverage manufactured by B. bifidum contained 10^7 cfu/ml after manufacture without any obvious change during storage. This beverage had gained the highest values for color, flavour, appearance, and pH and the lowest viscosity value than other beverages. However, the pH was decreased during storage whereas viscosity took the opposite trend. Mould and yeast were not detected in all beverages when fresh and throughout storage period at refrigerator temperature.*

Key Words: *Whey beverage - Probiotic, Lactobacillus acidophilus - Bifidobacterium bifidum - ABT starter.*

INTRODUCTION

The amount of wastes of food and dairy industries is very large, causing many environmental problems. Some microorganisms can use these substances as carbon and energy sources to produce useful products (Mehanna *et al.*, 1998).

Whey is a by-product of cheese manufacture and creates a worldwide problem for waste disposal of considerable proportions. Whey contains about 6.0-6.5 per cent total solids (Kumar *et al.*, 2001). Zall (1984) reported that out of 85 million tons of global production of whey, 40% is still disposed as raw whey. In view of the low solids content of whey, there is a gross lack of interest in its utilization.

Some members of lactic acid bacteria are indigenous to the intestinal tract of men and animals and are believed to help establish and stabilize the

normal intestinal microflora of their host (Calicchia *et al.*, 1993 and Mehanna *et al.*, 1998).

During the last 20 years, the production of special fermented milk products has gained importance in many countries. In particular, there has been a rise in the production of fermented milk products produced by faecal lactic acid bacteria having nutritional, dietetic and therapeutic properties (Kisla and Ünlütürk, 1998). Benefits to health or nutrition may possibly derive from consumption of fermented milk products containing faecal lactic acid bacteria such as *Bfidobacteria*, *Lactobacillus acidophilus* and *Streptococcus salivarius* subsp. *thermophilus*, which may control of intestinal infections, anticarcinogenic activity, improved lactose digestion in persons as classified as lactose maldigestors and control of serum cholesterol levels (Kisla and Ünlütürk, 1998). The United States, Japan, South American countries and European countries produce products containing viable cells of *Bifidobacteria* and/or *Lactobacilli* on an industrial scale. Japan, a leading country in the manufacturing of these products, produces more than 70 different products containing viable cells; more than 50 of the dairy foods such as cultured milk, cultured milk beverages, fresh cheese desserts, cultured butter milk, culture added milk and powder milk plus cookies and health foods (Kim, 1988).

Several research workers tried to utilize whey in different forms of beverages (Fernandez *et al.*, 1981, Srivastava and Lohini, 1986 and Kumar *et al.*, 2001). Various kinds of whey beverages with or without fermentation are common in United States and New Zealand (Zadow, 1986). Therefore, the aim of this study was manufacture of flavoured whey beverages by using some probiotic bacteria such as *Bifidobacterium bifidum*, *Lactobacillus acidophilus* or ABT starter (*Lactobacillus acidophilus*, *Bifidiobacterium* and *Streptococcus thermophilus*) and studying the effect of these probiotic starters on some chemical, microbiological and organoleptic properties in resultant beverages.

MATERIALS AND METHODS

Materials:

- Fresh sweet whey was obtained from the Food Technology Research Institute, Ministry of Agriculture during the manufacture of Ras Cheese. It contained 0.3% fat, 0.63% TP, 0.046% Ca, 6.21% TS and 4.22% lactose. The pH of sweet whey was 6.5.
- Commercial Carboxymethyl Cellulose (CMC) from BDH, U.K.
- Fructose sweetener bought from local market.
- Colours and Flavours (orange, mango and strawberry) were bought from Delta Aromatic Company.

Probiotic Starter Cultures:

- ABT culture (*Lactobacillus acidophilus, Bifidobacterium, Streptococcus thermophilus*), and *Bifidobacterium bifidum* were obtained from Chr. Hansen's Lab., Denmark.
- *Lactobacillus acidophilus* was provided by Dairy Lab., Food and Dairy Department, National Research Centre, Dokki, Cairo, Egypt.

Experimental Procedure:

Fresh sweet whey was pasteurized at 72°C for 15 sec. 5% fructose, 0.1% CMC, 0.05% fruit flavour and 0.05% fruit colour were added to the mixture. Also 0.22% calcium chloride was added to be calcium enriched beverage. The whole mixture was homogenized and then pasteurized at 72°C for 15 sec. rapidly cooled to 45°C.

The mixture was divided into three portions:

1. The first portion was inoculated with 2% *Lactobacillus acidophilus.*
2. The second portion was inoculated with 2% *Bifidobacterium bifidum.*
3. The third portion was inoculated with 2% ABT.

After incubation mango, orange or strawberry flavours were added to the mixture.

The resultant beverages were filled into sterilized bottles and incubated at 37°C for 2 hours, then stored at refrigerator (5°C±1) for 10 days. Chemical, microbiological and organoleptic properties were estimated at 0, 3, 5, 7 and 10 days of storage.

A. Chemical Analysis:

1. Total solids was determined according to IDF (International Dairy Federation, 1982).
2. pH values of beverages were measured using a digital pH meter model HANNA AT 4817.
3. Fat content was determined as described by Ling (1963).
4. Total protein was determined according to IDF (1986).
5. Lactose was measured according to Nickerson *et al.* (1976).
6. Viscosity was determined by Hoppler viscometer.

B. Bacteriological Analysis:

1. Enumeration of total bacterial count:
   Total bacterial count was enumerated with plate count agar (Difco). The plates were incubated for 48h. at 35°C.
2. Enumeration of ABT count:
   ABT were determined according to Elliker *et al.* (1956). The plates were incubated at 35°C for 48h.

3. Enumeration of *Lactobacillus acidophilus:*
   Lactobacillus acidophilus count was determined on *Lactobacillus* selective agar plus 0.2% oxgall (LBSO) (Gilliland and Walker, 1990). Plates were incubated at 37°C for 4 days.
4. Enumeration of *Bifidobacterium bifidum:*
   Bifidobacterium bifidum count was done according to Blanchette *et al.* (1996) using modified MRS agar (Oxoid) supplemented with 0.05% L. cysteine-HCL (Merck, Germany). Plates were incubated at 37°C for 48h under an anaerobic environment (BB Gas Pak, Becton Dickinson, Cockeysville MA, USA).
5. Enumeration of moulds and yeasts
   They were determined according to Harrigan and McCance (1966), using Malt Extract Agar. Plates were incubated at 30°C for 5 days.

C. Organoleptic Properties:

Organoleptic properties of beverage samples were evaluated according to Ahmed *et al.* (1992) when fresh and at 3, 5, 7 and 10 days of refrigerated storage by ten panelists of the experienced staff members of Food Technology and Dairy Science Department, National Research Center.

RESULTS AND DISCUSSION

1. Chemical Analysis of Beverages:

1.1. Changes in pH values:

Table (1) shows the changes of pH of flavoured whey beverages by using *L. acidophilus, B. Bifidum* or ABT$_1$. It is clear that fresh orange whey beverage prepared with (ABT) had the lowest pH compared to that of the other beverages. On the other hand, beverages prepared with *B. bifidum* had the highest pH while beverages prepared with *L. acidophilus* had intermediate pH. This may be due to the effect of starter. pH value decreased gradually during storage until the end of storage. This could be due to the formation of more acids during storage as a result of microorganisms metabolism. These results are in agreement with Rao and Gandhi, 1988 and Abeid *et al.*, 2001.

1.2. Changes in viscosity:

Table (2) shows viscosity (cp) of beverages prepared with different starters and different juices. It is clear that fresh beverage manufactured by using *L. acidophilus* the a highest viscosity whereas beverage manufactured with *B. bifidum* had the lowest viscosity. On the other hand, beverages manufactured with ABT had intermediate viscosity. This may be due to the behavior of starters and to the changes of pH. Viscosity increased with the increase of storage period and reached the maximum after 10 days. This may be due to the increase of acidity during storage. Orange beverages with *L. acidophilus* had the highest viscosity when fresh compared to mango and strawberry beverages. This may be due to the high acidity of orange compared to that of mango or strawberry.

Table 1: Changes in pH values of orange, mango and strawberry whey beverages manufactured by *L. acidophilus*, *B. bifidum* or ABT during storage at refrigerator (5±1°C).

Kind of Beverage	Storage Period (Days)	Treatments		
		A	B	C
Orange	Fresh	5.68	6.08	5.24
	3	5.21	5.90	5.18
	5	5.09	5.09	5.06
	7	4.26	4.10	4.24
	10	4.09	4.06	4.18
Mango	Fresh	5.75	6.08	6.22
	3	5.29	5.10	6.21
	5	5.25	5.00	6.20
	7	5.15	4.18	5.24
	10	4.98	4.00	5.07
Strawberry	Fresh	5.82	6.07	6.27
	3	5.38	6.06	6.11
	5	5.27	6.04	6.08
	7	5.24	6.02	6.06
	10	4.27	6.01	6.00

A. Whey beverage prepared by *L. acidophilus.*
B. Whey beverage prepared by *B. bifidum.*
C. Whey beverage prepared by ABT.

Table 2: Effect of storage period (5±1°C) on viscosity (cp) of flavoured whey beverages prepared by using *L. acidophilus*, *B. bifidum* or ABT.

Kind of Beverage	Storage Period (Days)	Treatments		
		A	B	C
Orange	Fresh	3.41	2.50	3.22
	3	3.76	3.12	3.71
	5	4.24	3.85	4.03
	7	4.80	4.12	4.75
	10	5.52	4.38	5.15
Mango	Fresh	2.54	2.14	2.15
	3	3.02	2.42	2.97
	5	3.55	3.32	3.44
	7	4.10	3.57	3.79
	10	4.68	4.03	4.13
Strawberry	Fresh	2.56	2.20	2.53
	3	3.22	2.66	3.47
	5	4.00	3.42	3.68
	7	4.42	3.68	3.80
	10	4.85	4.15	4.24

A. Whey beverage prepared by *L. acidophilus.*
B. Whey beverage prepared by *B. bifidum.*
C. Whey beverage prepared by ABT.

2. Microbiological Analysis:

Obtained data in Figs. (1), (2) and (3) show the changes in total bacterial count and *L. acidophilus* count of orange, strawberry and mango whey beverages during storage at refrigerator temperature (5°±1). It could be noticed that total bacterial count and *L. acidophilus* count were 7.45 and 7.40 log_{10}, respectively of orange beverage when fresh (0 time) and slightly increased during storage to reach 8.40 and 8.36 log_{10}, respectively, after storage period (10 days). Total bacterial count and *L. acidophilus* count of strawberry whey beverage were 7.54 and 7.60 log_{10}, respectively, when fresh (0 time) and gradually increased during the storage period to reach 8.40 and 8.36 log_{10} at the tenth day, respectively. The counts of mango beverage were 7.65 and 7.59 log_{10}, respectively, when fresh and slightly increased during the storage period to reach 8.33 and 8.30 log_{10}, respectively. The degree of survival and activity of *L. acidophilus* depend on the level of acidity in the product.

Data presented in Figs. (4), (5) and (6) indicated that the changes in total bacterial count and *B. bifidum* count of orange, strawberry and mango beverages during storage at refrigerator temperature. It could be noticed that there was no difference in total bacterial count and *B. bifidum* count during storage. The survival of *Bifidobacteria* in fermented dairy products depends on varied factors, such as the strain of bacteria used, fermentation conditions, storage, temperature and preservation methods. Reduced viability of *B. bifidum* could be due to the presence of lactic and acetic acids which inhibit the growth of *Bifidobacteria* (Gomes *et al.*, 1995). Laroia and Martin (1990) reported that at lower pH values the *Bifidobacteria* die quickly since these organisms are not acid tolerant. In general, pH higher than 5.0 could have a positive effect on the survival of *Bifidobacteria* (Daigle *et al.*, 1999). The main factors of loss of the viability of probiotic starter cultures have been attributed to the decrease in the pH of medium and accumulation of organic acids as a result of growth and fermentation.

Our results are in line with Blanchette *et al.* (1996) who reported that a fermented dairy product would have to contain 10^7 *Bifidobacteria*/g at the time of consumption for live *Bifidobacteria* to be found in the intestine after ingestion of 100g of the product and should contain between 10^7-10^8 *Bifidobacteria*/g when displayed in retail outlets.

Changes in total bacterial and ABT counts of orange, strawberry or mango whey beverage are presented in Figs. (7), (8) and (9). It is clear that total bacterial count and ABT count of orange beverage (Fig. 7) were 5.20 and 5.15 log_{10}, respectively, when fresh and gradually increased during storage to reach 6.44 and 6.30 log_{10} after ten days.

As shown in Fig. (8), total bacterial and ABT counts of strawberry whey beverage were 5.11 and 5.10 log_{10}, respectively, when fresh (0 time) and gradually increased during storage to reach 6.20 and 5.85 log_{10} at the tenth day, respectively.

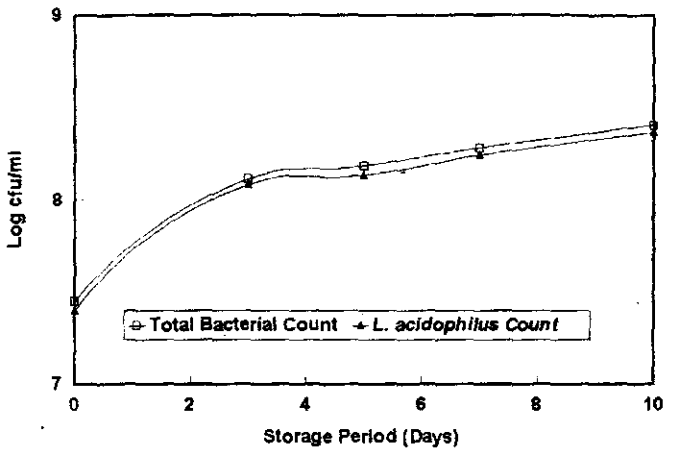

Fig.1: Changes in total bacterial count and *L. acidophilus* count of orange whey beverage during storage at refrigerator temperature.

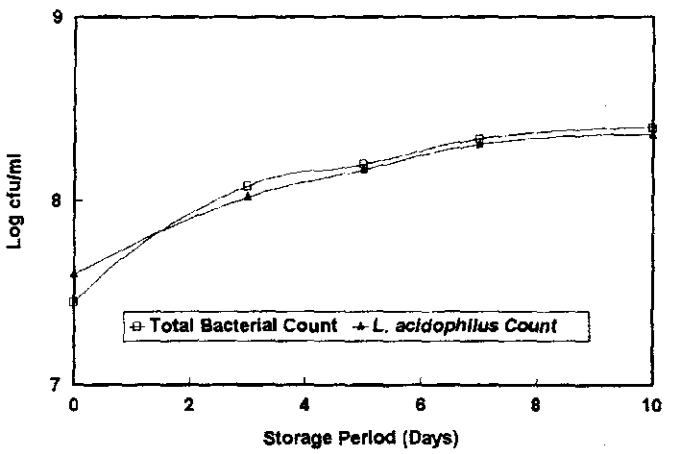

Fig. 2: Changes in total bacterial count and *L. acidophilus* count of strawberry whey beverage during storage at refrigerator temperature.

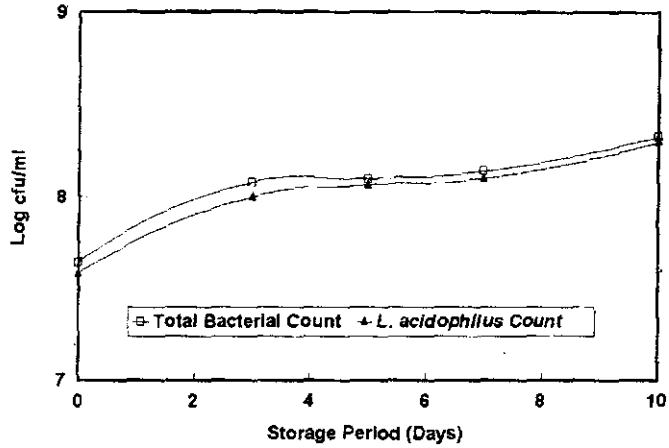

Fig. 3: Changes in total bacterial count and *L. acidophilus* count of mango whey beverage during storage at refrigerator temperature.

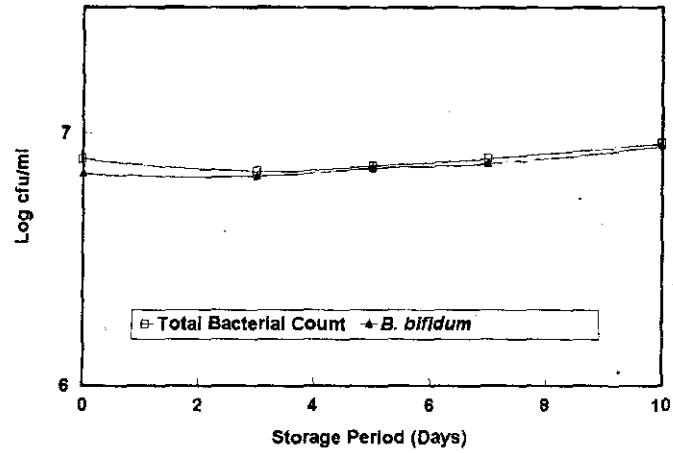

Fig. 4: Changes in total bacterial count, *Bifidobacterium bifidum* count of orange whey beverage during storage at refrigerator temperature.

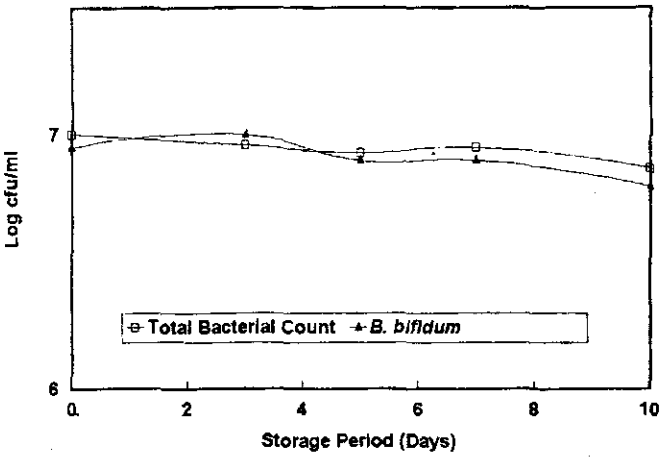

Fig. 5: Changes in total bacterial count, *Bifidobacterium bifidum* count of strawberry whey beverage during storage at refrigerator temperature.

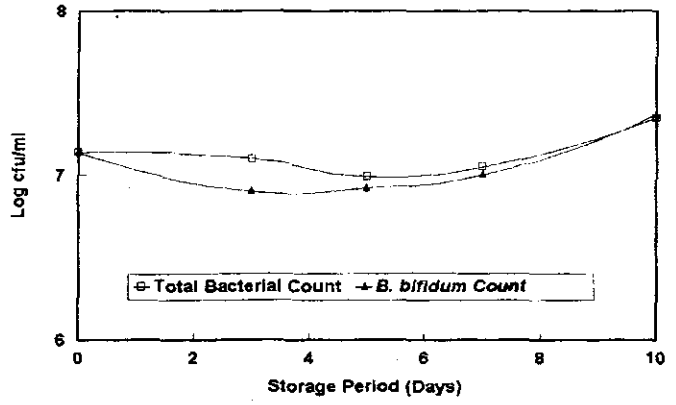

Fig. 6: Changes in total bacterial count, *Bifidobacterium bifidum* count of mango whey beverage during storage at refrigerator temperature.

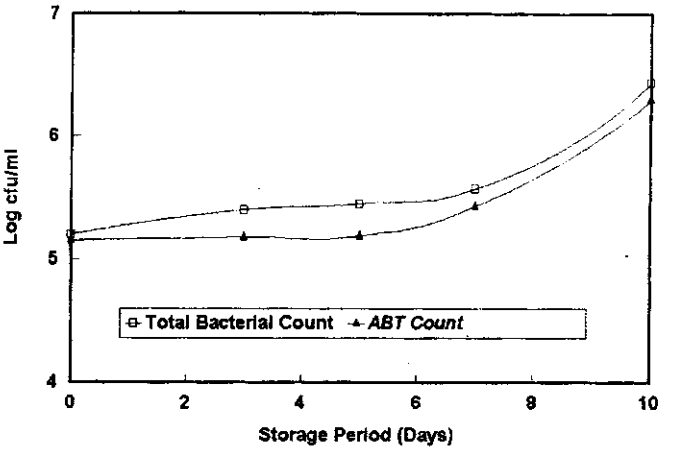

Fig. 7: Changes in total bacterial count and ABT count in orange whey beverage during storage at refrigerator temperature.

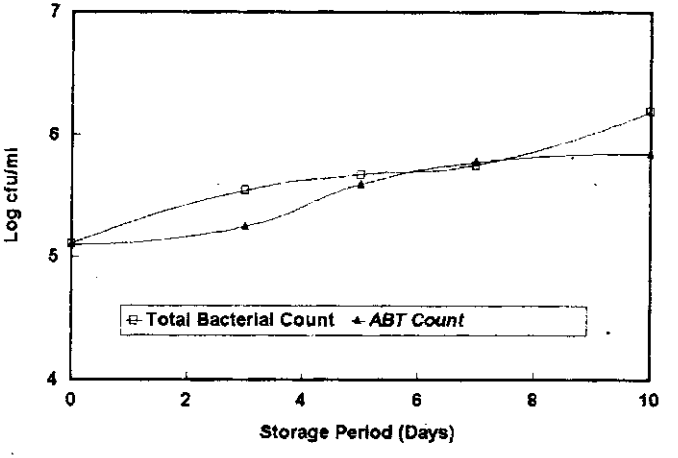

Fig. 8: Changes in total bacterial count and ABT count in strawberry whey beverage during storage at refrigerator temperature.

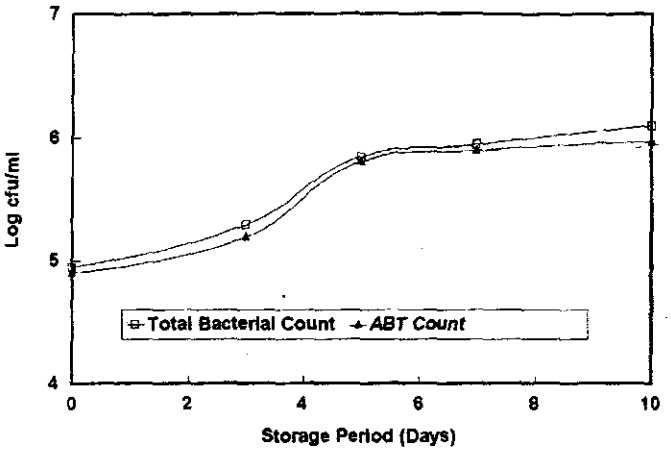

Fig. 9: Changes in total bacterial count and ABT count in mango whey beverage during storage at refrigerator temperature.

Fig. (9) shows that total bacterial and ABT counts of mango whey beverage were 4.95 and 4.90 log_{10}, respectively, when fresh (0 time) and gradually increased during storage to reach 6.1 and 5.97 log_{10} at the tenth day, respectively. Our results coincide with results obtained by Abeid *et al.* (2001) who reported that total bacterial count of mango, guava and strawberry sweet whey butter milk beverages manufactured with ABT gradually increased during storage at refrigerator temperature.

Moulds and yeasts:

Moulds and yeasts were determined in this study because these microorganisms are recognized as undesirable. Moulds and yeasts were not detected in all beverages when fresh and throughout storage period (10 days) at refrigerator temperature as the result of high hygienic condition during the preparation and storage period.

3. Organoleptic Properties:

From Table (3), it is clear that beverages prepared with mango, orange or strawberry using *Bifidobacteria* starter had gained the highest scores for colour, flavour and appearance compared to the other beverages prepared with *L. acidophilus* or ABT. Also, beverages with *Bifidobacteria* had gained the highest total score than the other beverages prepared by *L. acidophilus* or ABT. It was noticed that total scores of all types of mango, orange and strawberry beverages decreased with increasing the storage period.

Table 3: Organoleptic properties of flavoured whey beverage prepared by *L. aciodphillus, B. bifidum* or ABT during storage at refrigerator (5±1°C).

Organoleptic	Storage Period (Days)	Treatments								
		Orange			Mango			Strawberry		
		A	B	C	A	B	C	A	B	C
Colour (20)	Fresh	20	20	19	19	20	19	16	17	15
	3	20	20	19	19	20	19	16	17	15
	5	19	20	18	18	20	18	15	16	14
	7	18	19	17	18	19	17	14	15	13
	10	17	18	17	17	18	16	13	14	11
Flavour (40)	Fresh	37	39	38	38	39	37	36	37	35
	3	36	38	37	37	38	36	35	36	34
	5	35	37	36	36	38	35	34	35	33
	7	34	36	35	35	37	34	33	34	32
	10	33	35	34	34	36	33	32	33	31
Appearance (40)	Fresh	39	40	38	39	40	38	34	36	33
	3	39	40	38	39	40	38	34	36	33
	5	38	40	37	38	39	37	34	35	32
	7	38	39	36	37	39	36	33	35	31
	10	37	38	35	36	38	35	32	35	30
Total (100)	Fresh	96	99	95	96	99	94	86	90	83
	3	95	98	94	95	98	93	85	89	82
	5	92	97	91	92	97	90	83	87	79
	7	90	94	88	90	95	87	80	84	76
	10	87	91	86	87	92	84	77	82	72

A. Whey beverage prepared by *L. acidophilus.*
B. Whey beverage prepared by *B. bifidum.*
C. Whey beverage prepared by ABT.

CONCLUSION

From the foregoing results, the beverages manufactured in this study using *L. acidophilus, B. bifidum* or ABT as a starter adjunct, may be described as probiotic or "functional" foods.

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استخدام بعض بادئات المدعمات الحيوية الملائمة في تحضير مشروب الشرش

جمال أحمد إبراهيم ــ منى عبد الجواد ــ فاطمة حسن
قسم الصناعات الغذائية والألبان
المركز القومي للبحوث ــ الدقي ــ القاهرة

الملخص العربي

تـم تحضـير ٣ أنواع من مشروبات الشرش باستخدام ثلاثة أنواع مختلفة من بادئات المدعمات الحيوية وهى : ABT, *Lactobacillus acidophilus, Bifidobacterium bifidum* .

تـم إضافة ٥% فراكتوز ــ ٠,١% صمغ السليلوز ــ ٠,٢٢% كلوريد كالسيوم ــ ٠,٠٥% طعم وكذلـك ٠,٠٥% لون بعـض الفواكه وهى المانجو والبرتقال والفراولة إلى الشرش بعد الإضافة ثم التجنيس جيداً ثم البسترة والتبريد على ٤٥°م.

لقـح الشـرش بنسبة ٢% من كل بادئ من بادئات المدعمات الثلاثة والتحضين على ٣٧°م لمدة ساعتين فقط ثم الحفظ على درجة حرارة الثلاجة ٥ ± ١°م لمدة عشرة أيام.

أجـرى تحليل المشروبات كيماوياً (pH ــ اللزوجة) ــ بكتريولوجيا (العدد الكلى البكتيري ــ عدد بكـتريا البادئات المستخدمة ــ عدد الفطريات والخمائر) وحسياً بعد التصنيع مباشرة وكذلك أثناء فترة الحفظ على درجة حرارة الثلاجة ٥ ± ١°م .

وقـد أوضـحت النـتائج أن العـدد الكلـى البكتـيري لكـل من المشروب المصنع بواسطة *L. acidophilus* كذلـك ABT يزداد تدريجيا بعد التحضير وطوال مدة الحفظ على درجة حرارة الثلاجة كما لوحظ أن المشروب المحضر بواسطة*B. bifidum* يحتوى على عدد ١٠ ^٧ خلية/مل بعد التصنيع ولا يوجد زيادة معنوية في العدد خلال الحفظ على درجة حرارة الثلاجة.

كما أظهرت النتائج أن المشروب المحضر بواسطة *B. bifidum* كان له أفضل جودة حسية ، وقلة اللـزوجة وانخفاض الـ pH في الأنواع الثلاثة (البرتقال ــ المانجو ــ الفراولة) عن المحضر بواسطة البادئين الآخرين.

وقد وجد أيضاً أن هذه المشروبات المحضرة بواسطة بادئات المدعمات الحيوية كانت خالية تماماً من الفطريات والخمائر بعد التصنيع وخلال الحفظ على درجة حرارة الثلاجة.

وقـد أظهر التحليل الكيماوي للمشروبات الناتجة خلال فترة التخزين إلى انخفاض pH بينما أخذت اللزوجة اتجاهاً عكسياً حيث ارتفعت بتقدم فترة التخزين.

نسـتنتج من هذه الدراسة أن استخدام بادئات المدعمات الحيوية في تحضير مشروب من الشرش يعطيه خواص حسية جيدة بجانب الفائدة الصحية والعلاجية لهذه السلالات.