121
Annals
Agric. Sci.,
Ain Shams Univ.,
Cairo, 54(1), 121-135, 2009

PRODUCTION AND PROPERTIES OF ANTIOXIDATIVE FERMENTED PROBIOTIC BEVERAGES WITH NATURAL FRUIT JUICES

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El-Nawawy[1] M.A.; W. El-Malkey[2] and I.E. Aumara[1]

1- Food Science Dept., Faculty of Agric., Ain Shams Univ., Cairo, Egypt
2- Dept. of Medical Food, National Org. for Drug control and Research (NODCAR), Giza, Egypt.
E-mail: dr_ihabaumara@yahoo.com

Keywords: Antioxidative, Probiotic, Lemon juice, Mango, Guava, *Bifidobacterium*, Permeate, Organoleptic, Properties

ABSTRACT

This study presents an investigation on permeate beverages consisting of fresh permeate and 10% sucrose (heated to 85°C for 30 min) and single or mixed probiotic cultures (*Bifidobacterium longum, Lactobacillus acidophilus* and *Lactobacillus rhamnosus*) then fresh fruit component (lemon juice, mango and guava, pulps) were added. This study was carried out to prepare antioxidative fermented probiotic beverages with high antioxidant contents chemical, microbiological and organoleptic qualities along the storage at 4°C for 60 days. Antioxidant contents for all antioxidative probiotic fermented permeate with different fruit juice was higher as compared with permeate. Vitamin C and phenolic compounds was the highest in antioxidative probiotic fermented permeate with Lemonjuice followed by guava. On the contrary, the caroteinoids and flavonoinds were the highest in antioxidative probiotic permeate with mango pulp. In all antioxidative probiotic permeate beverages with lemon juice, mango and guava pulps bifidobacteria counts slightly increased during the first 7 days of the refrigeration period, thereafter, progressively decrease was noticed till the end of the storage period. Viability of Bifidobacteria was higher in probiotic beverage with lemon juice than in probiotic beverage with mango and guava pulps. Also, the viability was higher than 1x 10^6 cfu/ml throughout the storage period. Viability of *Lb. rhamnosus* was higher than *Lb. acidophilus* along the storage period. The highest viability was noticed for *lactobacillus rhamnosus* and *Bifidobacterium longum* for antioxidative probiotic beverages with lemon-juice followed by the beverages with mango pulp. At the end of the storage, the viability of all probiotic cultures used was higher than 1x 10^6 cfu/ml. Yeast and moulds, spore forming bacteria and psychrophilic bacterial counts were the lowest in all antioxidative probiotic fermented permeate beverage in the presence of *lactobacillus rhamnosus* in a combination with *Bifidobacterium longum* or *lactobacillus acidophilus* in a combination with *Bifidobacterium longum* with mango pulp, followed by lemon juice. The highest organoleptic properties was in antioxidative probiotic fermented permeate beverage with *lactobacillus rhamnosus* in a combination with *Bifidobacterium longum* with Lemon followed by guava when fresh and along the storage period. It could be recommended that, the resulting antioxidative probiotic fermented permeate beverage with *lactobacillus rhamnosus* in a combination with *Bifidobacterium longum* and lemon juice could be recommended as new acceptable antioxidative probiotic products containing high viable counts of probiotic and antioxidant compounds.

INTRODUCTION

Permeate is the major by-product of the cheese and casein industry, obtained from ultrafiltration technique of milk. Permeate contains about 5.8-6.0 per cent total milk solids. Permeate is a source

(Received April 29, 2009)
(Accepted May 2, 2009)

of high quality soluble proteins, lactose, vitamins and minerals that are important to the human health. Permeate resembles a problem for dairy industries in its disposal as a source for environmental pollution. However, great attentions were directed for utilizing whey or permeate in the production of many useful products.

Whey composes of lactose (5%), water (93%), proteins (0.85%), minerals (0.53%) and a minimum amount of fat (0.36%). The main whey proteins are β-lactoglobulin (58%) and α-lactalbumin (13%), while immunoglobulins, serum albumins and proteose peptones are present in lesser extent **(Pescuma *et al* 2008)**. Considerable efforts have been made over the past years to find new outlets for whey utilization and reduce environmental pollution **(Martinez *et al* 2002)**. Moreover, whey proteins provide an excellent way to fortify dairy foods increasing the nutritional quality of cheese and dairy desserts **(Kenny *et al* 2001 and Whetstine *et al* 2005)**.

Orange and citrus flavour drinks are the most frequently used products with whey, whey proteins or even whey permeate However, tropical fruits are applied also fruits as apple, pear, cherry, melon or apricot in a value of 4 to 20 % **(Green *et al* 1998)**. While acidity of blends is mostly adjusted by applying of citric acid or acids released from whey fermentation. pH values ranged from 3 to 5. Also sweetness of beverages is improved by adding fructose or enzymatically cleaved lactose or sucrose in levels from 5 to 11%.

Probiotic dairy products, have been classically defined as "foods containing live micro-organisms believed to actively enhance health by improving the balance of microflora in the gut" **(Gomes and Malcata, 1999; FAO, 2002; Gardinar *et al* 2002 Tamime *et al* 2005 and Reid 2008)**. Several authors have studied the production of probiotic fermented beverages containing living microorganisms in allover the world, partially because of its good health promoting effects; restore gut health and its dietary adjuncts **(Ouwehand and Salminen, 1998; Guarner and Malagelada, 2003; Ouwehand *et al* 2003 and Cheikhyoussef *et al* 2008)**. The use of probiotics such as bifidobacteria, *lactobacillus acidophilus,* and *Lactobacillus rhamnosus* have been quickly realize the huge market potential created by the numerous positive health benefits of these probiotic bacteria. Also, dairy products containing probiotic cultures and prebiotics are currently among the best-known examples of functional foods **(Katz, 1999 and Gueimonde *et al* 2004)**. **Klupsch (1985)** found that *Bifidobacterium* counts of >10^6 CFU/ml were present 30 days after manufacture of whey drink by using acid whey from quarg production. The acid whey is mixed with sweet whey to give a whey mixture of pH 4.8-5.5 and dried whey components and fruit are optional additional ingredients. **Adikhari *et al* (2003)** found that *Bifidobacterium* is below the recommended daily intake of 10^8 $CFUg^{-1}$ in many of bio-yoghurts.

Cantor (1999) referred to the attributes of fruits which can be exploited by fruit functions (colour, sweetness, flavour, fat replacement, functional properties, nutritional values); less apparent uses of some fruits (to add fiber, flavour and texture to foods and beverages, raisin puree/juices, fig and date products, production of novel and fresh fruit flavours); design of fruit fillings, toppings and variegates using stabilizers and developments in stabilizers; and use of oligofructose as a multifunctional ingredient providing health benefits, textural properties, body and novel crystallization profiles.

Sheehan *et al* (2007) assessed the viability of probiotic cultures, 5 *Lactobacillus* and one *Bifidobacterium* strain in orange juice (pH 3.65), pineapple juice (pH 3.40) and cranberry juice (pH 2.50). **Almeida *et al* (2008)** assumed that the blend of the co-culture (*Bifidobacterium. animalis* subsp. *lactis* and *L. rhamnosus*) and the pH level at which the fermentation was stopped are the most important aspects to be considered in order to improve the fermentation process of whey during the manufacture of probiotic lactic beverages.

The main objectives of this study were to create a new antioxidative probiotic fermented beverage from permeate with the addition of different fruit juices and probiotic cultures and study the chemical, microbiological and organoleptic properties throghout the refrigerated storage.

MATERIALS AND METHODS

Materials

Fresh milk permeate was obtained from the soft cheese factory of El-Masreen Company for dairy products, October 6th City, Giza, in which an ultrafiltration unit CARBOSEP, FRANCE was used. Mango, guava and lemon juice were obtained from the Enjoy Company for food products, Elbrageel, Giza, Egypt.

Strains and media

Bifidobacterium longum ATCC 15707, *Lactobacillus acidophilus* ATCC 4321, and *Lb. rhamnosus* DSMZ 20245 were obtained from the Egyptian

Microbial Culture Collection [EMCC] Cairo MIRCEN, Faculty of Agriculture, Ain Shams University. Active culture of *Bifidobacterium* was freshly propagated in the modified MRS medium (Difco laboratories, Detroit, MI) supplemented with 0.05% L-cystein and 0.3% lithium chloride according to the method described by **Dave and Shah (1996)**, *Lactobacillus acidophilus* count was determined using modified MRS agar supplemented with 0.2% oxagal according to **Gilliland and Walker (1990)**. While, *Lb. rhamnosus* strain was freshly propagated using MRS medium (**De Man *et al* 1960**). The flasks or plates were incubated at 37°C for 48h.

Experimental methods

Antioxidative probiotic Beverages Preparation

Fresh milk permeate was warmed to 40°C then sucrose (10%) and hydrocolloid (0.3% CMC) were added. The mixture was heat treated at 85°C for 30 min and rapidly cooled to 40°C. The resultant was inoculated with 2% of different single and mixed cultures (*Lactobacillus acidophilus* A, *Bifidobacterium longum* B, or AB or *Bif. longum* and *Lb. rhamnosus* BR). All mixtures were incubated at 37°C until pH decreased to 5.0, and as rapidly cooled to 5°C. Thereafter, 30% of heat treated Lemon juice; guava juice and mango Pulp were added. The cooled probiotic fermented beverage was filled into glass bottles (125 ml) and stored at 5°C for 90 days. Samples were taken when fresh and after 7, 15, 30, 60, and 60 days of refrigeration for chemical, microbiological and organoleptic analyses.

Chemical analyses

pH values was measured by a laboratory pH-meter (Beckman electric pH meter) with a combined electrode Model 3305. Titratable acidity expressed as lactic acid (%) was determined according to the method reported by **Ling (1963)**. Moisture content was determined using a thermostatically controlled oven at 105°C, according to the method described by **A.O.A.C. (2007)**. The total nitrogen content was determined by the semi-micro Kjeldahl method as described by **A.O.A.C. (2007)**. Ash content was determined according to the methods described by the **A.O.A.C. (2007)** using muffle furnace [Thermolyne Type 1500] at 600 °C.

Antioxidant and Minerals contents

Vitamin C, carotenoids flavonoids, phenolic compounds and antioxidant activity were determined (mg/100g) according to the method described by **A.O.A.C. (2007)**. Minerals Ca, P, Fe and Zn in all samples were determined using atomic absorption method according to the method described by atomic absorption (according to **A.O.A.C. 2007**).

Microbiological examinations

Bifidobacterial counts were anaerobically enumerated using the modified MRS agar supplemented with 0.05% L-cystein and 0.3% lithium chloride (**Dave and Shah 1996 and Roy 2001**), at 37°C for 48 h. Gas Pack BBL and Jars were used for anaerobic conditions. *Lb. acidophilus* count was determined using modified MRS agar supplemented with 0.2% oxagal according to **Gilliland and Walker (1990)**. The plates were incubated at 37°C for 48h. *Lactobacillus rhamnosus* count was determined using MRS agar according to **De Man *et al* (1960)** at 37°C for 48 h.

Yeast and mold counts were enumerated according to **Marshall (1992)** using oxitetracycline glucose yeast extract agar, at 25°C for 5 days.

Psychrophilic and aerobic sporeformers bacterial counts were determined using the pour plate technique. Aerobic sporeforming bacterial count was determined according to the method described by (**Marshall, 1992**). The plates were incubated at 32°C for 48h. Psychrophilic bacterial count was determined using plate count agar according to **Houghtby *et al* (1992)**. The plates were incubated at 10 ± 2 °C for 7days.

Organoleptic evaluation

The organoleptic properties of the resulting antioxidative probiotic fermented beverage were evaluated by standard taste panelists of the staff-members of the Food Science Department, Faculty of Agriculture, Ain Shams University. All samples were evaluated for flavor (20 points), consistency (10 points), colour and appearance (10 points),and Overall acceptability (40 points) according to **Bodyfelt *et al* (1988)**.

Statistical analysis

The data were analyzed according to Statistical Analysis System User's Guide (**SAS, 2000**) (SAS Institute, Inc, U.S.A.). Duncan multiple ranges was used to analyze the statistical significance.

RESULTS AND DISCUSSION

Gross chemical composition and antioxidant content

Chemical analysis of all antioxidative probiotic fermented permeate with different fruit juice in **Table (1)** shows that there were no remarkable differences in moisture, total protein, carbohydrate, ash and total solids contents. Titratable acidy as lactic acid ranged from 0.75% to 0.84% **(Figs. 1, 2 and 3)**, while, pH values was less than 5.1. The chemical composition of all antioxidative probiotic fermented permeate with different fruit juices was not starter culture dependant in fresh and 60 day stored samples. Generally, the moisture content ranged from 78.2 to 82.8%, and total protein content ranged from 5.6 to 7.3%. Also, a slight decrease in moisture content was noticed after 60 days of the storage. Protein content slightly decreased in all samples after 60 days of the storage, and this decrease could be due to the proteolytic activity of the starter cultures. Furthermore, pH values gradually decreased in all samples being ranged from 4.3 to 4.0 after 60 days of the storage. There were remarkable differences in pH values when fresh and along the storage. Carbohydrates content was affected by storage in all samples, and the decrease might be due to the starter culture activity along the storage. There was a slight remarkable increase in ash as storage period progressed.

Titratable acidity was higher in BR antioxidative probiotic permeate beverage with Lemon followed by B beverage. On the contrary, antioxidative probiotic permeate beverage with Guava had the minimum acidity when fresh and along the storage. Generally, the titratable acidity gradually increased along the storage period in all treated samples.

In **Table (2)**, antioxidant contents in all antioxidative probiotic fermented permeate with different fruit juice was higher as compared with permeate. Also, the antioxidant activity increased with the use of *lactobacillus rhamnosus* in a combination with *Bifidobacterium longum* as a probiotic culture. Vitamin C and phenolic compounds was the highest in antioxidative probiotic fermented permeate with lemon followed by guava. These might be due to the high content in Guava pulp. On the contrary, the caroteinoids and flavonoinds were the highest in antioxidative probiotic fermented permeate with mango pulp.

Mineral contents results of antioxidative probiotic permeate beverages are presented in **Table (3)**. There were slight differences in mineral contents Ca, P, Fe and Zn contents of all different probiotic permeate beverages samples. There was a tendency (significant $P \le 0.05$) to increase Ca content by adding fruit juice to permeate. On the contrary, antioxidative probiotic permeate beverages with guava juice had the maximum P, Fe and Zn, values followed by the beverage with mango pulp.

Microbiological quality

The viable counts of probiotic microorganisms varied significantly ($P \le 0.05$) in the permeate beverages. Although the initial counts of the *lactobacillus* and *Bifidobacterium* **(Figs. 1, 2 and 3)** were similar when the permeate beverages was blended with lemon, mango and guava. The results suggest that the counts were higher in *lactobacillus rhamnosus* as compared with all other treatments. In mango, guava and lemon probiotic beverages, bifidobacterial count slightly increased during the first 7 days of the refrigerated storage, thereafter, gradually decreased till the end of the storage period **(Fig. 1)**.

At the beginning of the storage period, bifidobacterial counts ranged from 8.02, 8.01 and 7.85 $\log_{10}$ CFU/ml (for mango, probiotic beverage B, BR and AB, respectively), then gradual decrease was recorded (6.65, 6.17 and 6.39 $\log_{10}$ CFU/ml) at the end of the storage period of Mango probiotic beverage, respectively. Generally the viability of bifidobacteria was the highest in fermented beverages manufactured with *Bif. longum* lonely (B), followed by the combination with *lactobacillus rhamnosus* (BR). Generally, in probiotic lemon beverage, viability of bifidobacteria was higher than the viability in guava and mango probiotic beverage. Also, the viability was higher than 1×10^6 CFU/ml along the storage period. The data are confirmed with **Drgaliae *et al* (2005)** on growth of pure cultures of *L. acidophilus*, *L. casei* and *Bif. bifidum* for 24 h in reconstituted cheese whey with or without the addition of inulin.

Shah (2000) stated that probiotic viability is about 30–70% higher when stored in glass bottles than in plastic cups for the storage of fermented milks, and the main reason for that is the lower oxygen permeability of glass compared to plastic cups, that helps in maintaining microaerophilic. Also, **Lin *et al* (2006)** reported that the viable cell densities of probiotic bacteria in liquid products were higher than those in the solid products.

Table 1. Chemical Composition of antioxidative probiotic permeate beverages with different fruit juices

Beverages	Storage period (days)	Moisture %	Protein %	Carbohydrate %	Ash %	Refractive index	pH
Permeate		94.8	0.4	4.3	0.5	1.37	6.2
				Lemon Beverage			
A	Fresh	78.2	1.40	13.0	0.74	1.36	4.6
	60	77.5	1.31	12.4	0.76	1.40	4.0
B	Fresh	79.6	1.40	11.3	0.70	1.36	4.5
	60	78.9	1.33	10.8	0.73	1.40	4.2
AB	Fresh	76.5	1.50	12.6	0.76	1.36	4.6
	60	76.3	1.42	11.5	0.78	1.41	4.2
BR	Fresh	80.6	1.50	13.0	0.70	1.36	4.5
	60	80.3	1.38	11.54	0.72	1.42	4.1
				Mango Beverage			
A	Fresh	81.6	1.80	11.8	0.58	1.36	4.8
	60	79.8	1.72	11.3	0.59	1.44	4.2
B	Fresh	80.2	1.51	12.0	0.50	1.36	5.1
	60	78.5	1.38	11.1	0.51	1.42	4.6
AB	Fresh	80.1	1.42	11.4	0.52	1.36	4.9
	60	79.8	1.34	10.7	0.54	1.40	4.4
BR	Fresh	82.1	1.47	10.1	0.50	1.36	4.9
	60	80.7	1.41	9.87	0.51	1.41	4.3
				Guava Beverage			
A	Fresh	82.2	1.0	11.0	0.80	1.36	4.8
	60	80.6	1.18	10.45	0.85	1.39	4.3
B	Fresh	82.8	1.20	11.3	0.70	1.36	4.7
	60	81.3	1.34	10.8	0.76	1.38	4.4
AB	Fresh	82.2	1.40	10.6	0.84	1.36	4.7
	60	82.0	1.28	10.2	0.85	1.40	4.3
BR	Fresh	82.9	1.17	9.80	0.80	1.36	4.6
	60	81.6	1.15	9.68	0.82	1.40	4.0

A- *Lactobacillus acidophilus* **B-** *Bifidabacterium longum* **AB-** *Lactobacillus acidophilus + Bifidabacterium longum* **BR**: *Bifidabacterium longum + Lactobacillus rhamnosus*

Table 2. Antioxidant contents (mg/100g) of antioxidative probiotic permeate beverages with different fruit juices

Beverages	Vit C	Carotenoids	Flavonoids	Phenolic compounds	Antioxidant activity
Permeate	25 b	0.10 b	0.017 b	0.5 d	11.2 e
permeate Guava beverage + A	173 a	0.10 b	0.019 b	3.8 a	36.8 bc
permeate Guava beverage +B	185 a	0.10 b	0.019 b	4.0 a	44.5 a
permeate Guava beverage +AB	188 a	0.12 b	0.021 a	4.0 a	44.6 a
permeate Guava beverage +BR	185 a	0.10 b	0.018 b	4.20 a	45.8 a
permeate lemon beverage + A	28 b	0.30 a	0.22 a	1.01 c	40.1 b
permeate lemon beverage +B	30 b	0.30 a	0.22 a	0.65 c	30.7 c
permeate lemon beverage +AB	32 b	0.31 a	0.21 a	0.72 c	30.5 c
permeate lemon beverage +BR	26 b	0.30 a	0.21 a	0.95 c	28.1 d
permeate Mango beverage +A	183 a	0.10 b	0.017 b	1.40 b	41.5 ab
permeate Mango beverage +B	185 a	0.13 b	0.019 b	1.96 b	31.2 c
permeate Mango beverage +AB	185 a	0.12 b	0.018 b	1.98 b	32.4 c
permeate Mango beverage +BR	185 a	0.10 b	0.018 b	1.30 b	41.5 ab

A- *Lactobacillus acidophilus* B- *Bifidabacterium longum* AB- *Lactobacillus acidophilus* + *Bifidabacterium longum* BR: *Bifidabacterium longum* + *Lactobacillus rhamnosus*

Table 3. Mineral contents of antioxidative probiotic permeate beverages (mg/100 g)

Beverages	Ca	P	Fe	Zn
Permeate	3.1 d	0.52 d	0.14 d	0.017 b
permeate Guava beverage + A	8.5 a	10.48 a	0.34 a	0.074 a
permeate Guava beverage +B	8.9 a	10.50 a	0.30 a	0.080 a
permeate Guava beverage +AB	8.6 a	10.50 a	0.31 a	0.080 a
permeate Guava beverage +BR	9.1 a	10.50 a	0.32 a	0.081 a
permeate lemon beverage + A	6.3 c	6.42 b	0.09 c	0.013 c
permeate lemon beverage +B	5.5 c	6.42 b	0.17 c	0.013 c
permeate lemon beverage +AB	5.8 c	6.46 b	0.18 bc	0.015 c
permeate lemon beverage +BR	5.7 c	6.52 b	0.17 c	0.014 c
permeate Mango beverage +A	7.6 b	3.42 c	0.17 c	0.064 a
permeate Mango beverage +B	7.2 b	3.46 c	0.17 c	0.074 a
permeate Mango beverage +AB	7.3 b	3.48 c	0.19 b	0.070 a
permeate Mango beverage +BR	7.2 b	3.51 c	0.22 b	0.068 a

A- *Lactobacillus acidophilus* B- *Bifidabacterium longum* AB- *Lactobacillus acidophilus* + *Bifidabacterium longum* BR: *Bifidabacterium longum* + *Lactobacillus rhamnosus*

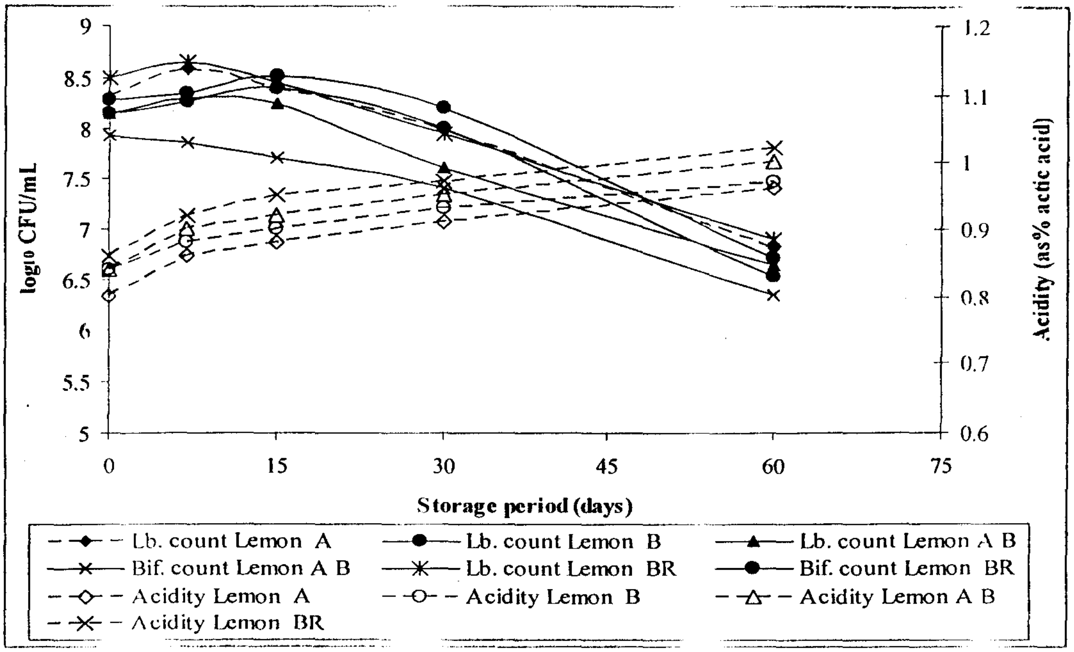

Fig. 1. Probiotic starter culture counts (log10 CFU/mL) and Titratable acidity (as % lactic acid) in different antioxidative probiotic permeate beverages with lemon along refrigerated storage at 5°C for 60 days

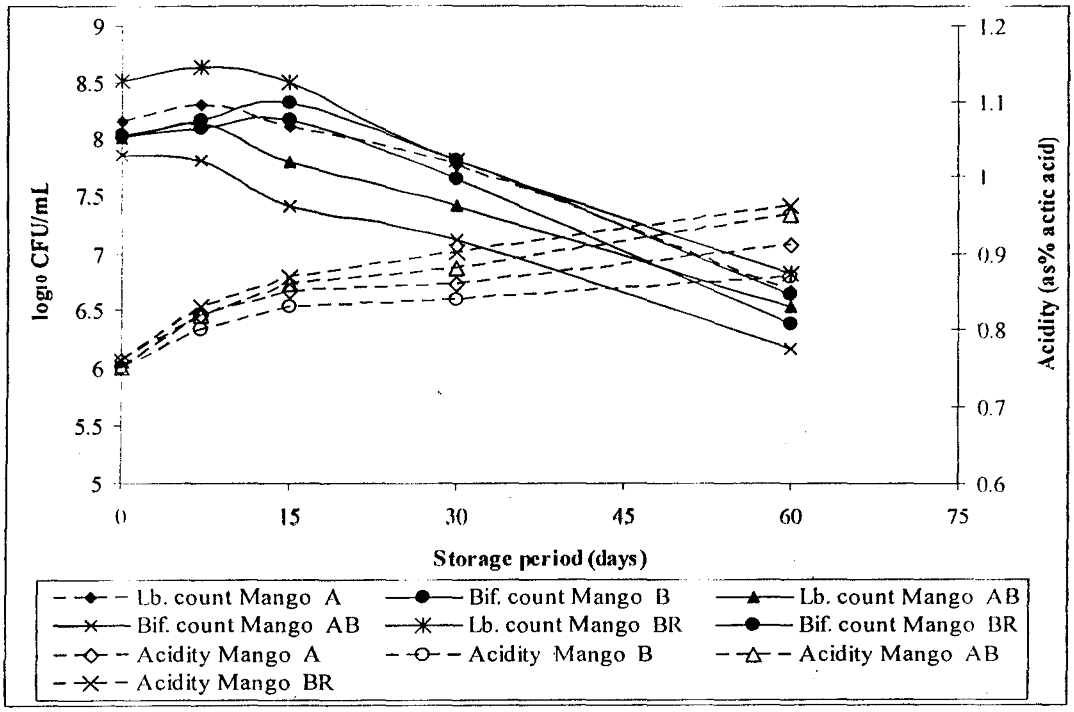

Fig. 2. Probiotic starter culture counts (log10 CFU/mL) and Titratable acidity (as % lactic acid) in different antioxidative probiotic permeate beverages with mango along refrigerated storage at 5°C for 60 days

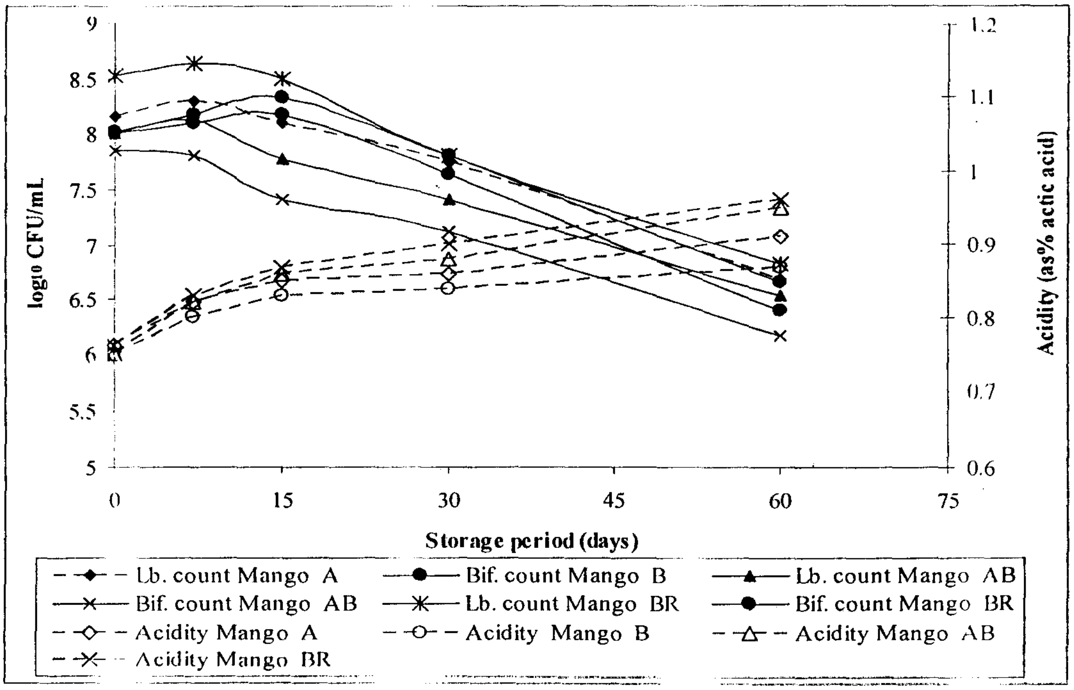

Fig. 3. Probiotic starter culture counts (log10 CFU/mL) and Titratable acidity (as % lactic acid) in different antioxidative probiotic permeate beverages with lemon along refrigerated storage at 5°C for 60 days

At the beginning of the refrigerated storage lactobacilli count ranged from 8.52 to 8.0 log_{10} CFU/ml for antioxidative fermented beverages BR (*Bif. longum* and *lactobacillus rhamnosus*) and AB (*Bif. longum* and *lactobacillus acidophilus*), respectively. Also, Lactobacilli counts slightly decreased with the increase of the storage period and gradually decreased at the end of the storage period. Lactobacilli counts reached 6.83, 6.68 and 6.53 log_{10} CFU/ml (for BR, A and AB products, respectively) at the end of the storage period. Generally, viability of *Lb. rhamnosus* was higher than *Lb. acidophilus* along the storage period. The highest viability was noticed for *lactobacillus rhamnosus* in combination with *Bifidobacterium longum* for probiotic lemon beverages, followed by the mango permeate beverage, and the viability of *Bifidobacterium longum* was the highest when fresh and along the storage period. At the end of the storage, the viability of all probiotic cultures used was higher than 1x 10^6 CFU/ml. The gradual decrease of *Bifidobacterium* and lactobacilli counts might be due to the graduall decrease in pH values (increase in titratable acidity). The data are confirmed wit **Adikhari *et al* (2003)**, who found that *Bifidobacterium* count below 10^8 $CFUg^{-1}$ in many of bio-yoghurts. The data were in agreement with those of fruit yogurts with *Lactobacillus acidophilus* and *Bifidobacterium animalis* ssp. *lactis* in stirred **(Kailasapathy *et al* 2008).**

Kar and Misra (1999) found that the viability of yoghurt starter cultures in therapeutic fermented drink were 1x 10^8 CFU/ml, and slightly increased with increasing the storage period, and still higher than 1x10^6 CFU/ml at the end of the storage. **Gokavi *et al* (2005)** found that the viability of *lactobacillus plantarum* and *lactobacillus paracasei* ssp. *casei* was higher than 1x 10^8 CFU/ml in fresh Oat beverage and slightly decreased to higher than 1x10^6 CFU/ml at the end of the storage. **Almeida *et al* (2008)** sated that the counts of *Bif. animalis* subsp. *lactis* were the highest (8.43 log_{10} CFU/ml), irrespective of the pH at which the fermentation was stopped. The counts of *L. acidophilus* and *L. delbrueckii* subsp. *bulgaricus* averaged 6.72 log10 and 7.26 log_{10} CFU/ml, respectively. In general, the highest actic acid bacteria counts were observed when the fermentation was stopped at pH 4.5, and *L. rhamnosus* had the lowest counts (5.59 log10 CFU/ml. **Sendra *et al* (2008)** found that, populations of probiotic bacteria decreased with storage time increased.

As shown in **Tables (4, 5 and 6)**, yeasts and mould counts were the lowest in all antioxidative probiotic permeate beverage with *Bifidobacterium longum* in a combination with *lactobacillus rhamnosus* or *lactobacillus acidophilus* with mango followed by lemon. Yeasts and mould counts slightly increased by increasing of the storage for all beverages, and the counts were less than 1x 10^2 CFU/ml along the first 30 days of the storage. The data suggested that the fruit juice and sugar might be the main source of the yeasts and mould.

Generally, spore forming bacteria and psychrophilic bacteria in all antioxidative probiotic fermented permeate beverages slightly increased with the increase of the storage period. Moreover, the minimum counts were in probiotic beverages with *lactobacillus rhamnosus* in a combination with *Bifidobacterium longum* or with the use of *Bifidobacterium longum* lonely when fresh and along the storage. On the contrary, the highest counts were found in probiotic beverages with *lactobacillus acidophilus* with all different fruit juices.

Organoleptic evaluation

In **Tables (7, 8, and 9)**, the highest organoleptic properties was in all antioxidative probiotic fermented permeate beverages with *lactobacillus rhamnosus* in a combination with *Bifidobacterium longum* with lemon followed by guava when fresh and along the storage period. Also, flavour and consistency scores was the highest in fresh and along the first month of the storage flowed by slightly decrease with the increase of the storage.

The lowest organoleptic properties was in antioxidative probiotic permeate beverage with *lactobacillus acidophilus* in a combination with *Bifidobacterium longum* for all lemon, guava and mango beverages. The data were in agreement with those obtained by **Garcia-Perez *et al* (2005 and 2006 and Sendra *et al* 2008)**. Generally, Fresh and refrigerated stored Lemon enriched antioxidative probiotic permeate beverage got the best results for overall acceptability followed Guava and Mango. Acceptability increased with the first 15 days of the storage period and that is probably due to the increased viability of probiotic strains in beverages.

In conclusion, the present study was undertaken to create a new antioxidative probiotic permeate beverage containing high viable suitable probiotic strains and incorporation of fruit juices. The cultures varied in their ability to survive along the refrigerated storage and in presence of sugar and fruit juices, which indicate that the selection of probiotics *Bifidobacterium longum* and *L. rhamnosus* should be cautiously undertaken.

Therefore, the resulting probiotic antioxidative probiotic fermented permeate beverage with *Bifidobacterium* in a combination with *longum lactobacillus rhamnosus* and lemon juice could be recommended as new acceptable probiotic products containing high viable counts of probiotics and antioxidant compounds with antioxidative activity.

Table 4. Microbiological properties of antioxidative fermented Guava permeate beverage during storage period (90 days at 5°C)

Treatment	Storage period (days)				
	0	7	15	30	60
	Yeast and Moulds counts (log_{10} CFU/ml)				
A	0.43	0.75	1.30	2.1	2.97
B	0.58	0.93	1.39	1.63	2.75
AB	0.39	0.67	1.04	1.53	2.61
BR	0.36	0.69	0.96	1.75	2.59
	Spore forming bacterial counts (log_{10} CFU/ml)				
A	3.22	3.4	3.64	3.58	4.45
B	2.83	3.04	3.31	3.75	4.18
AB	3.02	3.12	3.6	4.13	4.69
BR	2.75	2.94	3.28	3.7	4.12
	Psychrophilic bacterial counts (log_{10} CFU/ml)				
A	2.74	2.98	3.15	3.52	4.01
B	2.38	2.85	2.84	3.17	3.6
AB	2.43	2.82	3.10	3.36	4.0
BR	2.25	2.55	2.73	3.06	3.52

A- *Lactobacillus acidophilus* B- *Bifidabacterium longum* AB- *Lactobacillus acidophilus* + *Bifidabacterium longum* **BR**: *Bifidabacterium longum* + *Lactobacillus rhamnosus*

Table 5. Microbiological properties of antioxidative probiotic lemon permeate beverages during storage period (90 days at 5°C)

Treatment	Storage period (days)				
	0	7	15	30	60
	Yeast and Moulds counts (log_{10} CFU/ml)				
A	0.38	0.57	1.01	1.85	3.14
B	0.49	0.70	1.3	2.3	2.37
AB	0.35	0.51	1.2	2.0	3.0
BR	0.40	0.63	1.1	1.7	2.68
	Spore forming bacterial counts (log_{10} CFU/ml)				
A	2.73	2.86	3.23	3.68	4.05
B	2.44	2.8	3.02	3.41	3.85
AB	2.61	2.75	3.14	3.56	4.03
BR	2.35	2.57	2.98	3.28	3.67
	Psychrophilic bacterial counts (log_{10} CFU/ml)				
A	2.4	2.67	2.85	3.31	3.89
B	2.1	2.45	2.7	3.04	3.45
AB	2.3	2.57	2.86	3.3	3.78
BR	1.85	2.13	2.41	2.85	3.25

A: *Lactobacillus acidophilus* B: *Bifidabacterium longum*
AB: *Lactobacillus acidophilus* + *Bifidabacterium longum* BR: *Bifidabacterium longum* + *Lactobacillus rhamnosus*

Table 6. Microbiological properties of antioxidative probiotic mango permeate beverage during storage period (90 days at 5°C)

Treatment	Storage period (days)				
	0	7	15	30	60
	Yeast and Moulds counts (log CFU/ml)				
A	0.3	0.36	0.65	1.2	2.1
B	0.42	0.51	0.74	1.1	1.9
AB	0.27	0.40	0.60	1.0	1.5
BR	0.32	0.56	0.81	1.3	1.8
	Spore forming bacterial counts (log CFU/ml)				
A	2.95	3.07	3.36	3.81	4.35
B	2.71	2.96	3.11	3.66	4.04
AB	2.83	3.09	3.51	4.07	4.63
BR	2.47	2.8	3.12	3.47	3.88
	Psychrophilic bacterial counts (log CFU/ml)				
A	2.61	2.94	3.2	3.64	4.11
B	2.3	2.73	2.94	3.35	3.7
AB	2.45	2.81	3.0	3.74	4.27
BR	2.11	2.6	2.97	3.3	3.65

A: *Lactobacillus acidophilus* B: *Bifidabacterium longum*
AB: *Lactobacillus acidophilus* + *Bifidabacterium longum* BR: *Bifidabacterium longum* + *Lactobacillus rhamnosus*

Table 7. Organoleptic properties of antioxiative probiotic Guava permeate beverage along the storage period

Treatment	Storage period (days)				
	0	7	15	30	60
	A probiotic beverage				
Flavour (20)	16	16	17	16	15
Consistency (10)	8.5	9	8	8	7
Colour & Appearance (10)	8	9	8.5	8	7
Overall acceptability (40)	32.5	34	33.5	32	29
	B problotic beverage				
Flav (20)	17	17	16	15	15
Consis (10)	9	9	8.5	8	7
C & App (10)	8	9	8	7	7
Overall (10)	34	35	32.3	30	29
	AB probiotic beverage				
Flav. (20)	16	16	15	15	14
Consis (10)	8	2	8	7	7
C& App. (10)	8	8	8	7	6
Overall. (40)	32	33	31	29	27
	BR problotic beverage				
Flav. (20)	18	18	18	17	17
Consis (10)	9.5	9	9	8	8
C& App. (10)	9	10	9	8.5	8
Overall. (40)	36.5	37	36	33.5	33

A: *Lactobacillus acidophilus* B: *Bifidabacterium longum*
AB: *Lactobacillus acidophilus + Bifidabacterium longum* BR: *Bifidabacterium longum + Lactobacillus rhamnosus*

Table 8. Organoleptic properties of antioxidative probiotic lemon permeate beverage along the storage period

Treatment	Storage period (days)				
	0	7	15	30	60
Beverage with A	A problotic beverage				
Flavour (20)	16	18	18	17	15
Consistency (10)	8.5	9	9	8.5	8
Colour & Appearance (10)	8	9	8	8	7
Overall acceptability (40)	32.5	36	35	33.5	30
Beverage with B	B probiotic beverage				
Flav (20)	17	19	19	18	17
Consis (10)	9	9	9	8.5	8
C & App (10)	8.5	8.5	9	9	8
Overall (10)	34.5	36.5	37	35.5	33
Beverage with AB	AB problotic beverage				
Flav. (20)	18	18	17	16	14
Consis (10)	9	9	8	8	8
C& App. (10)	9	8.5	8	8	8
Overall. (40)	36	35.3	33	32	30
Beverage with BR	BR probiotic beverage				
Flav. (20)	20	20	20	18	16
Consis (10)	10	10	10	9	9
C& App. (10)	9	10	9	8.5	8
Overall. (40)	39	40	39	35.5	33

A: *Lactobacillus acidophilus* B: *Bifidabacterium longum*
AB: *Lactobacillus acidophilus + Bifidabacterium longum* BR: *Bifidabacterium longum + Lactobacillus rhamnosus*

Table 9. Organoleptic properties of antioxidative fermented mango permeate beverage along the storage period

Treatment	Storage period (days)				
	0	7	15	30	60
	A probiotic beverage				
Flavour (20)	14	15	17	18	16
Consistency (10)	8	8	8	8	8
Colour & Appearance (10)	7.5	7	8	7.5	7.5
Overall acceptability (40)	29.5	30	33	33.5	31.5
Beverage with B	B probiotic beverage				
Flav (20)	15	17	18	16	15
Consis (10)	8	9	9	8.5	8
C & App (10)	8	8.5	8	8	7.5
Overall (10)	31	34.5	35	32.5	30.5
Beverage with AB	AB probiotic beverage				
Flav. (20)	17	18	18	16	15
Consis (10)	8	8	7.5	7	7
C& App. (10)	9	9	8	8	7
Overall. (40)	34	35	33.5	31	29
Beverage with BR	BR probiotic beverage				
Flav. (20)	18	19	19	17.5	17
Consis (10)	8	9	9	8.5	8
C& App. (10)	9	10	9	8.5	7
Overall. (40)	35	38	37	34.5	32

A: *Lactobacillus acidophilus* B: *Bifidabacterium longum*
AB: *Lactobacillus acidophilus* + *Bifidabacterium longum* BR: *Bifidabacterium longum* + *Lactobacillus rhamnosus*

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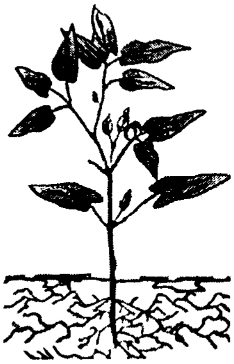
١٣٥
حوليـات العلـوم الزراعية
جامعة عين شمس ، القاهرة
مجلد(٥٤)، عدد (١)، ١٢١-١٣٥، ٢٠٠٩



إنتاج وصفات مشروب متخمر من راشح اللبن ذو صفات وقائية بعصائر الفواكه المحتوى على مضادات الأكسده

[١٠]

محمد عبد الرازق النواوي[١] - وجيه المالكى[٢] - ايهاب السيد محمد عمارة[١]

١- قسم علوم الأغذية - كلية الزراعة - جامعة عين شمس - القاهرة - مصر

٢- الهيئـة القوميـة للرقابـة والبحـوث الدوائيـة - القاهـرة - مصر

الموجـز

تم فى هذا البحث تسخين راشح اللبن الطازج (البرمييت) على ٤٠°م ثم بسترة المخلوط على ٨٥°م لمـدة ٣٠ دقيقة وتبريده سريعـاً حتى ٤٠°م. وقد تم تقسيم المخلوط إلي عدة أجزاء وتلقيح كل جزء بسلالات من بعض البادئات ذات الخواص الوقائية (مدعمات حيويه) probiotic bacteria وبعد إنتهاء التخمر (pH ٤.٨) أضيف إليه نـوع من عصائر الفاكهة (مانجو- جوافة- ليمون) بنسبة ٣٠% والمضاف إليه سكر السكروز بنسبة (١٠%) لإنتـاج مشـروب لبنى متخمـر مرتفـع فى مضادات الأكسدة وذو فوائد صحية وقد تم تخزين هذه العصائر فى زجاجات جافـة معقمة محكمة الغلق مع متابعة التغييرات التى تحدث فى خواصها الميكروبيولوجية والحسية خلال فترة التخزين على ٤°م لمدة ٦٠ يوم على فترات ٧، ١٥، ٣٠، ٦٠ يوم.

وقد إتضح أن أفضل مشروب من الناحية الحسية هو المشـروب المضـاف إليـه عصير الليمون خاصة المتخمر ببكتريـا *Bifidobacterium longum* مـع *Lactobacillus rhamnosus*.

كما كان هناك تقارب بين المشروبات المختلفة فى كل مـن نسب الرطوبـة والبروتين والكربوهيدرات ومعامـل الإنكسار مع وجـود بعض الإختلافات فى نسب المواد الصلبة الكلية والرماد ونسبة الحموضة مقدرة كحامض اللاكتيك.

وقد وجـد أن راشح اللبن المضاف إليها كل من عصير الجوافـة وعصير الليمـون هـو الأعلى فى محتـواه من فيتامين ج بينمـا راشح اللبن بعصير المانجـو هـو الأعلى فى محتواه من الكاروتينويدات والراشح المضاف إليه عصير ليمون هى الأعلى فى محتـواه من المركبات الفينولية المشروبات المتخمرة المضـاف إليها عصائـر فواكـه مختلفة متقاربة فى درجـة نشاط مضادات الأكسدة وإن كان اعلاها هو المضاف إليهـا عصيـر جوافـة والميكـروب *Lactobacillus acidophilus* يليهـا المضـاف إليها عصير ليمون وخليط البكتريا *Bifidabacterium longum* مع *Lactobacillus rhamnosus*.

وقد إحتوت جميع المشروبات االلبنيـه المتخمـره على بكتريا البادىء بأعداد معنويه اكثر مـن مليـون وحده مكونه للمستعمرات / جم خلال فترة التخـزين (٦٠ يوم) وهذايعنى إرتفاع قيمته الحيويـه كمـا أن اعداد البكتريا الأخرى والفطريات لم تبدء فى الزياده الحقيقيه حتى ٦٠ يوم فى حالة الجوافه والليمون وبعد ٣٠ يوم فى حالة المانجو وهذا يدل على أن قوة حفظ المنتج مرتفعه. وبالتالى فإن هذا المشروب هو منـتج آمن ذو فوائد صحية ويمكن التوصية بإنتاجه تجاريا .

تحكيـم: أ.د يوسف مرسى الكنانـى
أ.د طـه عبد الحليم نصيب