



PRODUCTION OF HEALTHY BIFIDO FLAVOURED BEVERAGES FROM MILK BYPRODUCTS

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Keywords: *Bifidobacterium lactis* Bb-12, Skim milk, Sweet whey, Fruits, Healthy beverages

ABSTRACT

Healthy beverages were prepared from skim milk or sweet whey as byproducts by using 0.1% *Bifidobacterium lactis* Bb-12 and incubated at 37°C for 3 h. Fresh strawberry and guava juices were added separately at rate 20% (w/w) and 15% sucrose was added to both beverages. The resultant beverages were freshly analyzed for pH, total solids, total protein, mineral contents, amino acid contents, protein efficiency ratio (PER) and biological value (BV). Organoleptic properties were also evaluated. Microbiological examinations were conducted on the stored samples until 15 days. The results showed that guava beverages had gained higher score either for skim milk or sweet whey for colour, flavor as well as appearance compared to strawberry beverages. Strawberry beverages had higher essential amino acid contents, PER and BV than that contain guava. Same trend had been observed for mineral contents. Over storage; *Bifidobacterium lactis* Bb-12 was increased by time increasing, reaching the highest level after 10 days in sweet whey flavoured with guava.

INTRODUCTION

Whey is the by-product of cheese making, it contains lactose, whey proteins, minerals, its total solids content is typically 6.4% which is 75% is lactose. Whey applications include ice cream, bev-

erages, soups, sauces and spreads. Collectively, whey proteins have all the essential amino acids and in higher concentrations (Marshall, 2004). Many attempts have been done for utilization of whey in the formulation of some foods, but still there is a lot of scope to explore the possibilities for its utilization in beverage industries (Patel *et al* 2007). Whey beverages have been recognized as a genuine thirst quencher, light, refreshing, healthful and nutritious (Prendergast, 1985). Whey based fruits beverages are more suitable for health as compared to other drinks (Divya and Kumari, 2009). Skimmed milk is a by-product obtained during the manufacture of cream. It is a cheaper source for nutritious nonfat milk solids than whole milk, so it could be considered for development into a beverage (Shukla *et al* 2003).

Neutral antioxidants, particularly in fruits and vegetables, have been of increasing interest to both consumers and scientists, because the frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular diseases and cancer (Renaud *et al* 1998; Temple, 2000). Guava fruit contains a high level of antioxidant compounds, such as vitamin C (Nakasone & Pauli, 1998), carotenoids, (Mercadante *et al* 1999). Addition of guava adds excellent nutritive value, flavor and medicinal properties. It is useful in scurvy, digestion and cough (Divya & Kumari, 2009). Strawberries are a good source of natural antioxidants, there is a positive correlation between the antioxidant activity and total phenolic content (Wang *et al* 1996; Wang & Lin, 2000). In addition to the usual nutrients, such as vitamins

(Received October 7, 2010)

(Accepted October 20, 2010)

and minerals, strawberries are rich in anthocyanins, flavonoids, and phenolic acids (Heinonen *et al* 1998; Rice-Evans & Miller, 1996), and they have shown a remarkably high scavenging activity toward chemically generated radicals, making them effective in inhibiting oxidation of human low-density lipoproteins (Rice-Evans & Miller, 1996). Strawberries are highly valued for their sweetness, flavor and pleasant color, and are one of the most frequently consumed fruits (Golaszewski *et al* 1998).

On the other hand, bifidobacteria have anti-tumoral activity and anti-cholesterolemic as well as immune system activation effects (Trojanova & Rada, 2005). The number of bifidobacteria is further reduced during old age, accompanied by increases of *Clostridium* and other species. The most important physiological effect is the action of intestinal conditioning, which includes improvement of the intestinal flora, inhibition of intestinal putrefactive substances, and improvement of the faecal properties and defecation state (Ishibashi *et al* 1997).

However, fermented milk products represent a rich source of nutrients and may improve lactose digestion through splitting lactose into glucose and galactose by bacterial enzymes (Kolars *et al* 1984). Fermented milk products also help enhance the immune system via modulation of the cellular immune response through bioactive peptides whose activity may extend beyond the immune functions by some mechanisms still unclear (Trojanova & Rada, 2005).

Today, consumers are looking for ways to promote their own health, to enhance their "health span", not just their life span. In recent years, rising medical costs have forced people to find cheaper and effective means of protecting their health, and, thus, interest in functional food products has increased (Ozer & Kirmaci, 2010). Hence, the issue of this work was to prepare healthy beverages either from skim milk or sweet whey inoculated with *Bifidobacterium lactis* Bb-12, and flavoured with guava or strawberry juice.

MATERIALS AND METHODS

I- Materials

Fresh skim milk (protein 3.5%, fat 0.5% and T.S. 11.2 %) and fresh sweet whey (protein 0.8% and T.S. 7.4%) were obtained from Faculty of Agric. Cairo Univ. Giza, Egypt. High quality guava and strawberry fruits were obtained from local

market. *Bifidobacterium lactis* Bb-12 was obtained from Ch., Hansen's Lab. Denmark.

Preparation of fruit juices

Fresh high quality guava and strawberry fruits were well washed, cut to pieces and then pulped in a blender. The homogenized mixtures of both types were filtered through a musline cloth, the resultant juice was filled into polyethylene bags and stored at -18°C until use.

Preparation of skim milk and whey flavoured beverages

Fifteen percent of sucrose was added to both skim milk and sweet whey. Each batch was heated to 80°C, cooled to 37°C, inoculated with 0.1% *Bifidobacterium lactis* Bb-12 and incubated anaerobically at 37°C for 3 h. Strawberry and guava juices were added separately at rate of 20% (w/w) to skim milk and sweet whey. The beverages were chemically analyzed when fresh, while microbiological examinations were carried out when fresh and during cold storage at 4°C until 15 days.

II- Methods

-Chemical analysis

Total solids (TS) and total protein (TP) contents of obtained beverages were determined according to AOAC, (2006). pH values were measured using a Laboratory pH meter (HANNA, Instrument, Portugal). Mineral contents were determined by Microwave Digestion Lab. Station, ICP technique optima 2000 DV.

Amino acids determination except methionine, cystine and tryptophan were performed according to AOAC, (2006), using an amino acid analyzer (Biochrom 30). The protein efficiency ratios (PER) of the tested samples were based on their amino acid contents according to the recommendations of Alsmeyer *et al* (1974), the following equations were used:

$$PER_1 = -0.684 + 0.456 (\text{Leucine}) - 0.047 (\text{Proline})$$

(for adults)

$$PER_2 = -0.468 + 0.454 (\text{Leucine}) - 0.105 (\text{Tyrrosine})$$

(for juveniles)

Biological value (BV) was assayed according to the following equation as recommended to Ahmed *et al* (1995):

$$BV = 49.9 + 10.53 \text{ PER}$$

-Microbiological examinations

- Total Bacterial counts (TBC) were estimated on glucose yeast extract nutrient agar medium using pouring plate technique (ISO, 2002). Plates were counted after incubation of 37°C for 24h.
- Coliform and faecal coliform counts were estimated on Violet Red Bile Lactose agar (VRBL) (ISO, 2005) using pouring plate technique. Plates were counted after 24 h at 37°C and 44.5°C for total coliform and faecal coliform counts, respectively.
- MRS agar medium supplemented with 0.09% cysteine-HCL was used to determine Bifidobacteria counts (Denis, 2001). Plates were counted after 48 h at 37 °C under anaerobic conditions.

-Sensory evaluation

Beverages were sensory evaluated using scale of 40 points for appearance, 20 points for color and 40 points for flavor.

-Statistical analysis

Statistical analysis was performed using the GLM procedure with SAS (2004) software. Duncan's multiple comparison procedure was used to compare the means. A probability to $P \leq 0.05$ was used to establish the statistical significance.

RESULTS AND DISCUSSION

Table (1) represents pH values, TS and TP contents of bifido beverages flavoured with guava or strawberry juices. Sweet whey beverages had the lowest pH values, TS and TP contents compared with skim milk beverages ($P \leq 0.05$). Fortification with guava juice caused significant increased in pH values and total solid contents, which is in accordance with those found by Divya & Kumari, (2009). No significant differences shown between guava and strawberry juices for total protein content.

For mineral contents; bifidus skim milk beverages had higher contents of Ca, P than bifidus sweet whey beverages Table (2). The average recommended dietary allowance (RDA) or adequate intake (AI) of calcium and phosphorus are about 900, 700 mg per day respectively (800 to 1000 mg, depending on the country) for adults. These RDAs are safety levels designed to provide adults with maximum protection against a negative calcium balance and, hence, against bone loss. There is no doubt that milk provides large amounts of calcium. While there is also no question of the nutritional effectiveness of the calcium provided by milk, there is still some debate as to whether this

source of calcium is biologically better than other sources (FNB, 1997; Gueguen & Pointillart, 2000). Fortification with strawberry increased Ca, K and P contents compared with these beverages fortified with guava juice. These results are in agreement with Quaranta *et al* (1986).

Table (3) illustrates that either of sweet whey or skim milk strawberry beverages had higher amino acid contents than those of guava beverages. Essential amino acids such as: Threonine, Valine, Isoleucine, Leucine, Phenylalanine, Tyrosine and Lysine, are also in higher content in either of sweet whey or skim milk strawberry beverages than in guava beverages. This trend is similar of Perez *et al* (2002); Beekwilder *et al* (2004).

On the other hand, PER and BV of bifido beverages flavoured with strawberry juice were higher than those beverages flavoured with guava juice (Table 4) for both adults and juveniles, which may be attributed to strawberry juice, due to its higher amino acids content than guava juice.

For microbiological analysis, total bacterial count (TBC) and bifidobacteria of skim milk or sweet whey beverages as affected by fortification with guava and strawberry juices are illustrated in Fig. (1) and Fig. (2), respectively. TBC of bifido beverages samples increased until 5 days and declined thereafter, the decreasing in TBC may correlated with acidity development (Matijevic *et al* 2009). Also in Fig. (2) *Bifidobacterium lactis* increased along the storage period reaching its highest level after 10 days in sweet whey beverage flavoured with guava. These results is in agreement with Masco *et al* (2005) who reported that, either Dicloxa cillin-propionic acid media or bifidobacterium media from yoghurt and dairy fruit drinks were in the ranges of $10^3 - 10^8$ CFU/ml and $10^4 - 10^6$ CFU/ml respectively. All samples are free from coliform bacteria.

Generally it seems that the incidence and the gradual increase of *Bifidobacterium lactis* during storage period played an inhibitory action (Gupta *et al* 1996) which causes the decreasing of total viable counts.

As shown in Table (5) no significant differences were found in color score between sweet whey beverages fortified with strawberry or guava juices, which were significantly higher than skim milk beverages fortified with strawberry and guava ($p \leq 0.05$). However, sweet whey beverages fortified with guava juice and skim milk fortified with strawberry juice gained higher scores for flavor and appearance ($p \leq 0.05$). These results are in agreement with these reported by Behrens *et al* (2004).

Table 1. Chemical properties of sweet whey or skim milk beverages inoculated with *Bifidobacterium lactis*, and flavoured with guava or strawberry juices

Beverage treatments*	pH	Total Solids (%)	Total Protein (%)
Sweet whey+ 20%strawberry juice	4.376 ^D	21.98 ^D	1.30 ^B
Sweet whey + 20%guava juice	4.486 ^C	23.00 ^C	1.10 ^B
Skim milk + 20%strawberry juice	4.540 ^B	25.02 ^B	3.61 ^A
Skim milk + 20%guava juice	4.630 ^A	26.04 ^A	3.72 ^A

*Average of three replicates

Table 2. Mineral contents in sweet whey or skim milk beverages inoculated with 0.1% *Bifidobacterium lactis*, and flavoured with guava or strawberry juices

Beverage treatments	Minerals	Mean (ppm)
Sweet whey+ 20% strawberry juice	Ca	500.3
	K	1524
	P	540.0
	Zn	4.799
Sweet whey + 20% guava juice	Ca	465.2
	K	1274
	P	454.9
	Zn	3.778
Skim milk + 20% strawberry juice	Ca	707.2
	K	1520
	P	660.1
	Zn	5.995
Skim milk + 20% guava juice	Ca	570.1
	K	1221
	P	522.0
	Zn	3.973

Table 3. Amino acids contents (mg/100ml) in sweet whey or skim milk beverages inoculated with 0.1% *Bifidobacterium lactis*, and flavoured with guava or strawberry juices

Amino acids	Sweet whey + 20% strawberry juice	Sweet whey+ 20% guava juice	Skim milk + 20% strawberry juice	Skim milk + 20% guava juice
Aspartic	142.08	88.67	262	204.23
Therionine *	63.79	51.98	117.03	105.09
Serine	55.03	43.28	138.50	121.82
Glutamic	267.01	205.95	592.04	526.66
Proline	68.12	59.07	248.01	227.05
Glycine	33.02	27.08	64.63	58.34
Alanine	83.34	64.62	128.93	111.08
Valine *	63.30	51.90	159.84	140.83
Iso-leucine *	53.28	43.76	127.25	112.35
Leucine *	98.70	81.17	257.59	229.50
Tyrosine *	36.88	30.12	121.55	103.89
Phenylalanine*	43.52	35.30	132.00	116.27
Histidine	23.46	19.12	71.10	62.77
Lysine *	93.10	77.24	219.39	193.85
Arginine	39.54	31.31	100.65	87.20

*: Essential amino acids

Table 4. Protein efficiency ratio (PER) and biological value (BV) of sweet whey or skim milk beverages inoculated with 0.1% *Bifidobacterium lactis*, and flavoured with guava or strawberry juices

Beverage treatments	PER ₁	PER ₂	BV ₁	BV ₂
Sweet whey + 20% strawberry juice	41.12	40.47	483	476
Sweet whey + 20% guava juice	33.55	33.22	403	340
Skim milk + 20% strawberry juice	105.12	103.72	1157	1140
Skim milk + 20% guava juice	93.29	92.82	1032	1027

1: Adults 2: Juveniles

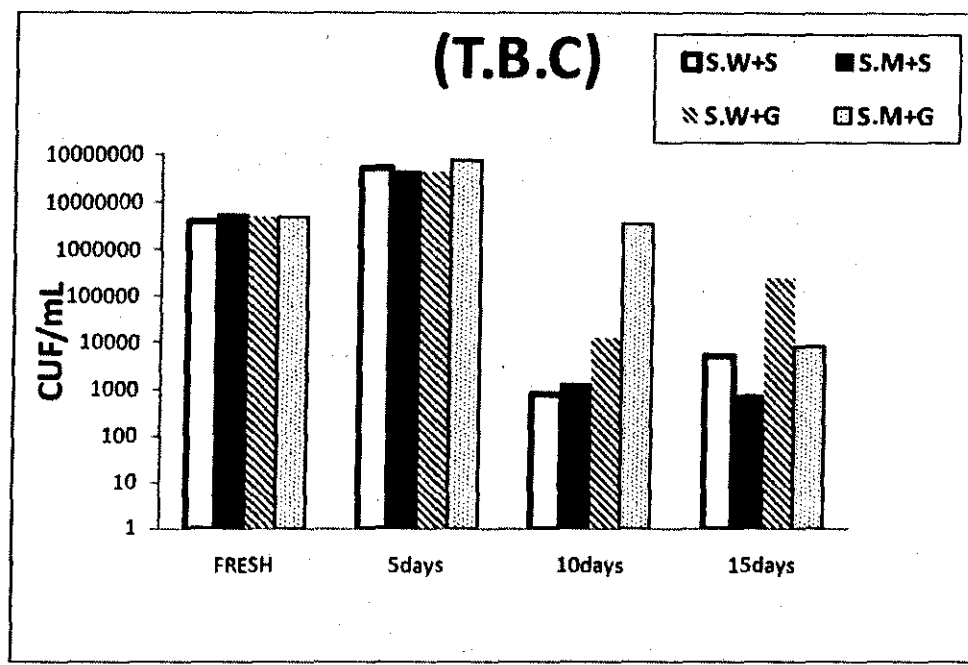


Fig. 1. Total Bacterial Count (T.B.C.) In different dairy beverages (Sweet Whey + Strawberry (S.W.+S), Skim milk + Strawberry (S.M. + S), Sweet Whey + Guava (S.W.+G) and Skim Milk + Guava (S.M. +G) during cold storage for 15 days.

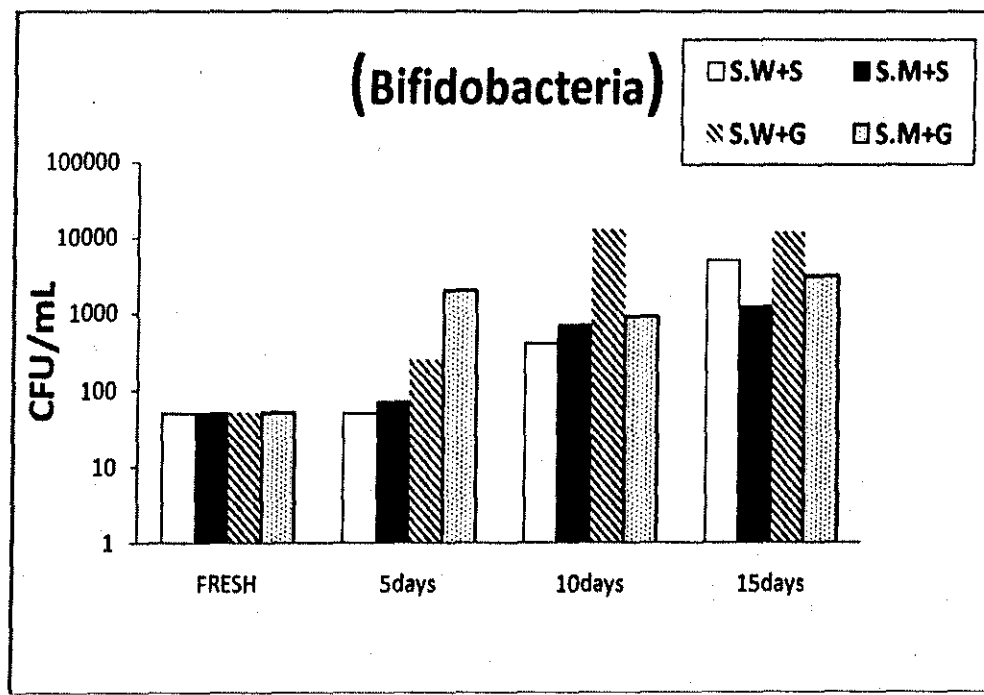


Fig. 2. Bifidobacteria count in different dairy beverages (Sweet Whey + Strawberry (S.W.+S), Skim milk + Strawberry (S.M. + S), Sweet Whey + Guava (S.W.+G) and Skim Milk + Guava (S.M. +G) during cold storage for 15 days

Table 5. Organoleptic properties of sweet whey or skim milk beverages inoculated with 0.1% *Bifidobacterium lactis*, and flavoured with guava or strawberry juices

Beverage treatments *	Color (20)	Flavor (40)	Appearance (40)
Sweet whey+ 20%strawberry juice	19.17 ^A	30.33 ^B	34.50 ^B
Sweet whey + 20%guava juice	20.00 ^A	34.63 ^A	37.66 ^A
Skim milk + 20%strawberry juice	17.50 ^B	36.17 ^A	33.50 ^B
Skim milk + 20%guava juice	17.66 ^B	36.66 ^A	37.00 ^A

*: Average of six replicates

CONCLUSION

Fortification of milk by-products, namely sweet whey and skim milk with bifidobacteria and fruit juices to produce flavoured healthy beverages, had gained different acceptability. Guava beverages had the highest scores, while strawberry beverages had higher levels of minerals such as (Ca, P, K, and Zn), amino acids, BV, PER. Inoculation with *Bifidobacterium lactis* makes these beverages healthier; and its count increased along the cold storage period reaching its highest level after 10 days with sweet whey guava beverages.

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إنتاج مشروبات البيفيدو الصحية وذات النكهة من منتجات الالبان الثانوية

[٢١]

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الموجز

ساعات ، وقد تم اضافة كلا من عصير الفراولة والجوافة منفصلين بنسبة ٢٠% (وزن/وزن)، وأحرزت نتائج التحكيم الحسى لكلا من مشروبات اللبن الفرز والشرش الحلو المدعمن بالجوافة أفضل نتائج للون والطعم والمظهر بالمقارنة بالمشروبات المدعمة بالفراولة. كما أظهرت النتائج أن المشروبات المدعمة بالفراولة بها أعلى محتوى من الأحماض الأمينية و PER و B.V. عن المحتوية على الجوافة. وقد زادت أعداد بكتيريا *Bifidobacterium lactis* Bb-12 أثناء التخزين حتى وصلت الى أعلى مستوى بعد ١٠ أيام وذلك فى مشروبات الشرش الحلو المدعمة بالجوافة. وبذلك فالتدعيم بالفراولة والجوافة مع البيفيدو بكتيريا يكسب المنتج خواص صحية وقيمة غذائية عالية.

إن الشرش واللبن الفرز من المنتجات الثانوية التي تم الاهتمام بها مؤخراً والالتفات إلى أهميتها الغذائية والصحية ، وقد كثرت محاولات انتاج منتجات لبنية مختلفة منها. وبمعرفة أهمية البيفيدو بكتيريا الصحية فقد كان الاهتمام فى هذا البحث بمحاولة اضافة هذه البكتيريا مع نوعين من هذه المنتجات الثانوية فى منتج صحى وغنى أيضا فى الاحماض الأمينية والمعادن ، عن طريق اضافة مدعمات غنية بهذه الأحماض والمعادن مثل الفراولة والجوافة. فلقد تم اعداد مشروبات ملقحة ب ٠,١% من البيفيدوبكتيريا *Bifidobacterium lactis* Bb-12 مع كلا من اللبن الفرز والشرش الحلو والتحصين لمدة ٣