Egypt. J. Food Sci. **43,** pp. 45 – 63(2015)

Production of Probiotic Nutritive Beverages Fortified with Bioactive Compounds and Antioxidants of Pumpkin and Strawberry Pulps

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IN THIS STUDY, probiotic nutritive beverages were formulated by mixing fermented milk permeate with pumpkin or strawberry pulps at a ratio of (1:1), and stored at 4±1ºC for 30 days. Milk permeate was fermented with the use of a 2% mixed starter culture (1:1:1) containing *Lactobacillus delbrueckii* subsp *bulgaricus*, an exopolysccharide (EPS) strain of *Streptococcus thermophilus*, and a probiotic organism *Bifidobacterium longum*. Changes in antioxidant activity and bioactive compounds as well as the chemical, rheological, microbiological and organoleptical characteristics of the resulting functional beverages were studied. The total solid, ash, fat, protein, fiber and acidity contents were slightly increased, while the total carbohydrate, antioxidant activity, total phenols, total flavonoids, ascorbic acid, anthocyanin and carotenoids contents and pH value were decreased during cold storage. The addition of pumpkin and strawberry pulps greatly improved but variably the Ca, P, Na, K, Mg, Fe, Cu and Zn contents in the prepared beverage. Lactic acid bacteria (LAB), *Str. thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus* or *Bif. longum* counts were not detected in prepared beverages from heated fermented permeate during cold storage, while the total viable bacteria, LAB, *Str. thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus* and *Bif. longum* counts gradually decreased in beverages produced from unheated fermented milk permeate during cold storage.

Keyword: Functional beverages, Milk permeate, Pumpkin, Strawberry, Probibtic bacteria, Exopolysccharide, Antioxidant activity, Bioactive compounds.

A fermented dairy beverage was made with milk and whey, and characterized as an important source due to the presence of protein with a high biological value that is mostly derived from whey, a major raw material (Sanmartin *et al*., 2011). The production of dairy beverages has been increasing worldwide due to their simple production technology and wide acceptance by consumers; they have been characterized as an alternative use for the whey from cow milk resulting from cheese production (Hernandez-Ledesma *et al*., 2011). In addition, lactic acid bacteria (LAB) produce different inhibitory substances that can prolong the shelf life of the fermented products. Reduction of lactose content in permeate by LAB fermentation is an effective way to avoid lactose intolerance; a serious problem for a significant sector of consumers (Geilman *et al*., 1992).

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Probiotics are alive microorganisms which when administered in adequate amounts confer a health benefit on the host (FAO/WHO, 2002) by improving microbial balance in the host's gut flora and defenses against pathogenic microorganisms. Other benefits attributed to probiotics include prevention of cancer, stimulation of the immune system, lowering of serum cholesterol levels, and improvement of vitamin synthesis (Heenan *et al*., 2004). The species which are most frequently used as probiotics belong to the genera *Lactobacillus* and *Bifidobacterium* (Isolauri, 2004).

Food produced from plants (fruits and vegetables) abounds with natural biologically active compounds, such as polyphenols, vitamin C or β-carotene, anthocyanins, flavones, have their antioxidant properties, are of great value to human health. It is thought that an adequate level of antioxidants supplied with one's diet induces immunological processes and increases defensive abilities of cells in proper way (Kalt, 2005). A well-balanced diet that will strengthen the immune system is now of great significance.

The photochemical antioxidants such as carotenoids, flavonoids and phenols ... etc., have potential health roles in the reduction of platelet aggregation, blood pressure, cardiovascular of disease and a role in modulation of cholesterol synthesis and absorption (Li, 2008). Moreover, fruits and vegetables contain protein bound polysaccharides which may increase the levels of serum insulin, reduce the blood glucose levels and improve tolerance of glucose and hence could be developed as new anti-diabetic agents (Li *et al*., 2005).

Pumpkin (*Cucurbita maxima*) is an excellent source of carotenoids, ascorbic acid, polysaccharides, mineral compounds (K, Ca, Mg and Fe), starch (1.5-20%), and pectin (4.8-12.8%). Pumpkin contains significant amounts of beta and alpha carotene and provides a very good amount of beta cryptoxanthin (Li, 2008).

Strawberries (*Fragaria xananassa*) are rich in phenols and particularly a class of phenols called anthocyanins. The anthocyanins in strawberries give the fruit its red colour and acts as a powerful antioxidant that protects the bodies' cells from free radical damage. Strawberry nutrition is an excellent source of vitamin C and B3, carbohydrates, organic acid, flavonoids, pectin and mineral compounds (Cordenunsi *et al*., 2002 and Li, 2008).

The predominant types of whey or permeate drinks are based on blends of fruit juices and unprocessed or modified whey. Today beverages are one of the most popular vehicles to deliver nutrients and phyto-nutrients for health benefits. Addition of vitamins and micronutrients to food stuffs is practiced in a number of countries' to combat deficiency diseases (Wrick, 2003).

The objective of this research study consisted of determining the impact of strawberry and pumpkin pulps on the nutritional value, bioactive compounds; antioxidant activity and sensory evaluation of fermented milk permeate beverages with probiotic bacteria.

Materials and Methods

Materials

Milk permeate was obtained from the ultrafiltration of buffalos milk using Carbo-sep, UF unit (SFEC, France) at the Animal Production Research Institute, Agriculture Research Center, Egypt. Commercial grade of granulated sugarcane was obtained from the local market, Egypt.

Pumpkin (*Cucurbita maxima* L) was obtained from the farm of Fac. of Agric. Moshtohor, and strawberry (*Fragaria xananassa*) was purchased from the local market, Egypt.

Pure cultures of *Lactobacillius delbrueckii* subsp *bulgaricus* DSM 20080, *Bifidobacterium longum* DSM 20088 and exopolysaccharides (EPS) producing strain *Streptococcus thermophilus* ASCC 1275 were kindly obtained from the Institute of Microbiology, Federal Research Center for Nutrition and Food, Kiel, Germany.

Methods

Preparation of fruit pulp

Pumpkin and strawberry were washed thoroughly with tap water. Pumpkin was hand peeled, seeds were carefully removed and the flesh was cut into "small pieces". The prepared fruits flesh was then blanched at 80°C for 10 min, according to Gupta (1998).

Fermentation of milk permeate

Milk permeate was warmed up to 40°C, sucrose was added at the ratio of 5g/100 ml, heated to 85°C for 15 min in a water bath and then rapidly cooled. The sweetened milk permeate was inoculated with 2% of mixed starter culture (1:1:1): *Lb. delbrueckii* subsp *bulgaricus*, *Bif. longum* and *Str. thermophilus*, incubated at 42°C until the pH was decreased to 5 and rapidly cooled to 4±1ºC.

Preparation of the functional beverages

Preliminary experiments were carried out to select the best formulation for the target beverages when fermented permeate (25, 50 and 75%), was mixed with pumpkin and strawberry pulps (75, 50 and 25%), respectively and sensory evaluated. Results showed that beverage formulations containing equal quantities of the fermented permeate and pumpkin and strawberry pulps yielded the highest sensory scores, which were chosen for further study.

Fermented milk permeate was divided into three portions. Functional beverages were prepared as follows:

- Firstly (4 kg) of unheated (C1) and heated (C2) fermented permeates (85°C/15min) were kept as controls.
- Secondly (2 kg) of unheated (T1) and heated (T3) fermented permeates (85°C/15min) were mixed with an equal volume of pumpkin pulp.

- Thirdly (2 kg) of unheated (T2) and heated (T4) fermented permeates (85°C/15min) were mixed with an equal volume of strawberry pulp.

The prepared beverages were filled into sterilized bottles, stored at 4±1ºC and were analyzed chemically, microbiologically and were also sensory evaluated when fresh and after 10, 20 and 30 days of cold storage. Also, the apparent viscosity of the fresh beverages was measured. The experiment was repeated three times and all analyses were carried out in duplicate.

Chemical analysis

The total solids, fat, ash, total protein, titratable acidity, pH and ascorbic acid contents were determined according to the methods described by AOAC (2000**)**. The total sugar contents were determined by Shaffer and Hartman method as described in the AOAC (2000). Crude fiber was determined by Weende method (AOAC, 2000). Carotenoids were determined according to Harvey and Catherine (1982). Minerals contents were determined according to AOAC (2000) using Perkin-elmer, 2380 Atomic absorption spectrophotometry. Antioxidant activity, total phenolic, flavonoids and anthocyanin contents were determined according to the methods described by Prieto *et al.* (1999), Shiri *et al.* (2011), Bor *et al.* (2006) and Cordenunsi *et al.* (2002), respectively. The amino acids profile of the functional beverage was performed following the protocol of Walsh and Brown (2000).

Microbiological examinations

Total lactic acid bacterial (LAB), yeasts and moulds; the total viable bacterial and colifrom bacterial counts were determined according to Elliker *et al.* (1956), IDF (1990), IDF (1991) and APHA (2001), respectively. *Lb. delbrueckii* subsp. *bulgaricus*, *Str. thermophilus* and *Bifidobacterium* sp. were enumerated according to the methods described by Ryan *et al.* (1996) and Martin & Chou (1992), respectively.

Sensory evaluation

The sensory evaluation of beverages was done by a taste panel of 10 experienced panelists from the staff-members of Food Science Department, Faculty of Agriculture, Moshtoher, Benha Univ. The samples were evaluated for taste, odour, body and texture, appearance, sweetness, acidity and colour out of 20, 20, 20, 10, 10, 10 and 10 score points (Fellers *et al*., 1986 and Bodyfelt *et al*., 1988).

Measurement of viscosity

The apparent viscosity of the prepared beverages was measured using a Brookfield viscometer Model DV11 + Pro (Brookfield unit, MA, USA) at 25ºC with a rotation speed of 60 rpm. The results are presented in milli-pascal seconds (mPa.s).

Statistical analysis

Statistical analysis for the obtained data was performed according to the methods of Clarke and Kempson (1997).

Results and Discussions

Composition of used ingredients

Chemical composition**,** antioxidant activity and bioactive compounds of raw materials used for the manufacture of the prepared functional beverages are presented in Table 1. Pumpkin pulp recorded the highest ($P \leq 0.05$) value of total solids, ash, total sugars, fiber and carotenoids contents while, milk permeate showed the lowest. In addition, milk permeate yielded the highest ($P \leq 0.05$) pH value. Fresh strawberry pulp contained generally high fat, protein, titratable acidity, antioxidant activity, total phenols, total flavonoids, ascorbic acid and anthocyanin contents ($P \leq 0.05$). Total phenols, total flavonoids, ascorbic acid and anthocyanin contents were not detected in milk permeate. The results of chemical composition for ingredients used for the preparation of produced functional beverages were in agreement with those obtained by Nawirska-Olszanska *et al*. (2011) and Abou El Samh *et al*. (2013).

TABLE 1. Chemical composition, antioxidant activity and bioactive compounds of raw materials used in prepared functional beverages.

Components	Pumpkin pulp	Strawberry pulp	Milk permeate
Total solids %	10.96^{a}	9.89^{b}	5.68^{c}
Ash %	0.47^{a}	0.45^{a}	0.26^{b}
Fat %	0.18^{b}	0.25^{a}	0.10^{c}
Protein %	0.59^{b}	0.71^{a}	0.22^{c}
Total sugars %	8.06^{a}	7.12^{b}	5.01^{c}
Fiber %	2.00^{a}	1.69^{b}	ND
Titratable acidity % as lactic acid	0.21^{b}	0.70^{a}	0.09^{c}
pH values	6.09^{b}	3.75^{c}	6.44^{a}
Antioxidant activity (mg $100g^{-1}$)	80.81^{b}	254.20^{a}	10.95^{c}
Total phenols (mg $100g^{-1}$)	42.94^{b}	200.15^{a}	ND
Total flavonoids (mg $100g^{-1}$)	3.12^{b}	16.23^{a}	ND
Ascorbic acid (mg $100g^{-1}$)	12.32^{b}	85.45^{a}	ND
Anthocyanin (mg $100g^{-1}$)	5.45^{b}	55.34^{a}	ND
Carotenoids (mg $100g^{-1}$)	10.54^{a}	1.14^{b}	ND

ND = Not detected

$^{a-c}$ Different letters in the same row indicate significant statistical differences (Duncan's test $P \leq 0.05$)

Physicochemical composition of prepared functional beverages

Table 2 summarizes the physicochemical composition of the prepared functional beverages during storage up to 30 days at 4±1ºC. The total solids and ash contents increased slightly in all treatments during the storage periods. Generally, there were significant differences ($P \leq 0.05$) between total solids and ash contents of beverage treatments during the storage. The slight increase of total solids during storage may be attributed to the loss of some moisture content during the cold storage. These results are in accordance with those of Hashmi *et al.* (2011) and Atallah (2015).

TABLE 2. Physicochemical composition of the prepared functional beverages during storage periods at 4±1ºC.

Treatments	Total solids%	Ash %	Fat %	Protein %	Total carbohydrates %	Acidity %	pH	Fiber %
Fresh								
C1	9.09^{fA}	0.27^{cC}	0.10^{bA}	0.37^{cB}	7.59^{fA}	0.85^{bC}	4.17^{dA}	ND
C2	9.15^{eA}	0.28^{cC}	0.12^{aA}	0.39^{bB}	7.68^{eA}	0.75^{eC}	4.49^{bA}	ND
T1	10.09^{bA}	0.33^{bC}	0.10^{bA}	0.41^{bB}	7.95^{bA}	0.81^{cC}	4.29^{cA}	0.60^{dA}
T2	9.97^{dA}	0.38^{aC}	0.12^{aA}	0.50^{aB}	7.71^{dA}	0.89^{aC}	3.82^{eA}	0.85^{bA}
T3	10.31^{aA}	0.37^{aC}	0.15^{aA}	0.41^{bB}	8.21^{aA}	0.65^{fC}	4.80^{aA}	0.64^{cA}
T4	10.01^{cA}	0.40^{aC}	0.15^{aA}	0.53^{aB}	7.89^{cA}	0.76^{dC}	3.95^{fA}	1.01^{aA}
10 days								
C1	9.30^{dA}	0.29^{cB}	0.10^{bA}	0.38^{cB}	7.50^{eB}	0.90^{bB}	4.02^{dB}	ND
C2	9.27^{dA}	0.31^{cB}	0.12^{aA}	0.39^{bB}	7.61^{dB}	0.77^{eB}	4.40^{bB}	ND
T1	10.14^{bA}	0.33^{bB}	0.10^{bA}	0.41^{bB}	7.90^{bB}	0.89^{cB}	4.15^{cB}	0.60^{dA}
T2	10.01^{cA}	0.38^{aB}	0.12^{aA}	0.52^{aB}	7.60^{dB}	0.94^{aB}	3.71^{fB}	0.86^{bA}
T3	10.38^{aA}	0.37^{abB}	0.15^{aA}	0.41^{bB}	8.19^{aB}	0.72^{fB}	4.72^{aB}	0.65^{cA}
T4	10.11^{bA}	0.41^{aB}	0.15^{aA}	0.55^{aB}	7.80^{cB}	0.77^{dB}	3.90^{eB}	1.02^{aA}
20 days								
C1	9.31^{dA}	0.31^{cA}	0.10^{bA}	0.39^{cA}	7.50^{cC}	1.05^{bA}	3.88^{dC}	ND
C2	9.28^{dA}	0.31^{cA}	0.13^{aA}	0.41^{bA}	7.60^{dC}	0.83^{eA}	4.27^{bC}	ND
T1	10.14^{bA}	0.35^{bA}	0.10^{bA}	0.42^{bA}	7.78^{bC}	0.99^{cA}	3.98^{cC}	0.62^{dA}
T2	10.07^{cA}	0.40^{aA}	0.14^{aA}	0.54^{aA}	7.54^{cC}	1.08^{aA}	3.61^{fC}	0.86^{bA}
T3	10.40^{aA}	0.40^{aA}	0.16^{aA}	0.43^{bA}	8.11^{aC}	0.80^{fA}	4.60^{aC}	0.68^{cA}
T4	10.20^{bA}	0.42^{aA}	0.16^{aA}	0.57^{aA}	7.76^{bC}	0.85^{dA}	3.85^{eC}	1.03^{aA}
30 days								
C1	9.31^{dA}	0.31^{cA}	0.10^{bA}	0.39^{cA}	7.45^{cD}	1.17^{bA}	3.70^{dD}	ND
C2	9.28^{dA}	0.32^{cA}	0.13^{aA}	0.42^{bA}	7.56^{dD}	0.90^{eA}	4.15^{bD}	ND
T1	10.16^{bA}	0.36^{bA}	0.10^{bA}	0.43^{bA}	7.77^{bD}	1.08^{cA}	3.76^{cD}	0.63^{dA}
T2	10.11^{cA}	0.40^{aA}	0.15^{aA}	0.55^{aA}	7.48^{cD}	1.21^{aA}	3.56^{fD}	0.86^{bA}
T3	10.40^{aA}	0.41^{aA}	0.15^{aA}	0.43^{bA}	8.05^{aD}	0.85^{fA}	4.46^{aD}	0.68^{cA}
T4	10.20^{bA}	0.42^{aA}	0.16^{aA}	0.58^{aA}	7.73^{bD}	0.91^{dA}	3.75^{eD}	1.03^{aA}

ND= Not detected
C1 = Control 1 (unheated fermented milk permeate) C2 = Control 2 (heated fermented milk permeate)
T1 = 50% unheated fermented milk permeate + 50% pumpkin pulp
T2 = 50% unheated fermented milk permeate + 50% strawberry pulp
T3 = 50% heated fermented milk permeate + 50% pumpkin pulp
T4 = 50% heated fermented milk permeate + 50% strawberry pulp
$^{a-f \text{ or } A-D}$ Different letters in the same colum indicate significant statistical differences (Duncan's test P≤0.05)

The fat and protein contents of the produced functional beverages slightly increased (P≤0.05) during cold storage. The fat content of fresh products varied from 0.10 to 0.15% and 0.37 to 0.53% for protein, and then increased slightly up to the end of the storage period.

The total carbohydrate of the prepared functional beverages significantly decreased during storage at 4±1ºC up to 30 days and there were significant differences (P≤0.05) between all treatments during storage period. This decrease was expectedly due to microbial activity. These results confirmed those reported by Vahedi *et al*. (2008).

Generally, data in the same table showed that the prepared functional beverages from unheated fermented permeate showed higher acidity and lower pH ($P \leq 0.05$) than beverages prepared from heated fermented permeates throughout the storage period. However, in both heated and unheated beverages, the acidity was increased and pH decreased during cold storage. This can be attributed to the presence of microorganism in all samples as apparent from the total viable counts. The presence of starter culture strains, in unheated beverages may explain the higher acidity and lower pH of these beverages compared with heated beverages. These results confirm those obtained by Hegazi *et al*. (2009).

The fiber content of fresh functional beverages of all treatments varied from 0.60 to 1.01%, and was almost unchanged during storage (Table 2). However, there were significant differences ($P \leq 0.05$) in fiber content between functional beverages and controls. The fiber in all beverages was less than 5% which may exhibit positive effects on nutrient utilization. Gronowska-Senger *et al*. (1980) found that 5% fiber in the diet had a positive effect on carotene utilization whereas 10 to 20% had a negative effect.

Antioxidant activity and bioactive compounds of functional beverages

Fermented functional beverages containing strawberry and pumpkin pulps can be considered an excellent source of antioxidant activity, total phenols, total flavonoids, ascorbic acid, anthocyanins and carotenoids which are important nutrients in the human diet.

Data in Table 3 showed that the antioxidant activity of the functional beverage containing strawberry was higher than of that containing pumpkin and control samples. However, the antioxidant activity was significantly different ($P \leq 0.05$) between all treatments during storage periods. Also, the antioxidant activity values were slightly but significantly decreased ($P \leq 0.05$) during cold storage. The results are in agreement with those given by Zhiyong *et al.* (2015).

Also, the total phenols content of functional beverages was the highest ($P \leq 0.05$) in strawberry followed by that of pumpkin. The total phenols content ranged from 21.54 to 56.32 mg $100g^{-1}$ in the fresh functional beverages. During storage up to 30 days, it decreased from 20.42 to 54.33 mg $100g^{-1}$.

In addition, the total flavonoid content in both the functional beverages showed a similar trend to that of total phenols content. The total flavonoid content was significantly higher ($P \leq 0.05$) in beverage containing strawberry pulp followed by that containing pumpkin pulp. These results may be due to the higher content of total phenols and flavonoids in strawberry than that in pumpkin pulps.

TABLE 3. Antioxidant activity and bioactive compounds of prepared functional beverages during storage periods at 4±1ºC (mg 100g^{-1}).

Treatments	Antioxidant activity	Total phenols	Total flavonoids	Ascorbic acid	Anthocyanin	Carotenoids
Fresh						
C1	11.02^{eA}	ND	ND	ND	ND	ND
C2	11.03^{eA}	ND	ND	ND	ND	ND
T1	26.98^{cA}	21.98^{cA}	1.70^{cA}	5.10^{cA}	1.75^{cA}	5.56^{aA}
T2	80.56^{aA}	56.32^{aA}	8.54^{aA}	21.05^{aA}	20.21^{aA}	0.51^{bA}
T3	24.65^{dA}	21.54^{dA}	1.65^{cA}	4.23^{dA}	1.70^{cA}	5.53^{aA}
T4	76.21^{bA}	55.89^{bA}	8.32^{bA}	19.87^{bA}	19.87^{bA}	0.50^{bA}
10 days						
C1	10.86^{eA}	ND	ND	ND	ND	ND
C2	10.85^{eA}	ND	ND	ND	ND	ND
T1	26.67^{cA}	21.65^{cA}	1.66^{cA}	4.91^{cB}	1.70^{cA}	5.54^{aA}
T2	80.51^{aA}	55.89^{aA}	8.45^{aA}	20.88^{aB}	20.01^{aA}	0.52^{bA}
T3	24.24^{dA}	21.20^{dA}	1.59^{cA}	4.00^{dB}	1.66^{cA}	5.48^{aA}
T4	76.05^{bA}	55.55^{bA}	8.10^{bA}	19.04^{bB}	19.79^{bA}	0.48^{bA}
20 days						
C1	10.81^{eA}	ND	ND	ND	ND	ND
C2	10.81^{eA}	ND	ND	ND	ND	ND
T1	26.35^{cA}	21.27^{cA}	1.54^{cA}	4.71^{cC}	1.65^{cB}	5.49^{aA}
T2	80.40^{aA}	55.25^{aA}	8.22^{aA}	20.54^{aC}	19.45^{aB}	0.47^{bA}
T3	24.13^{dA}	21.00^{dA}	1.49^{cA}	3.91^{dC}	1.51^{cB}	5.40^{aA}
T4	76.00^{bA}	55.12^{bA}	8.01^{bA}	18.89^{bC}	19.06^{bB}	0.42^{bA}
30 days						
C1	10.78^{eA}	ND	ND	ND	ND	ND
C2	10.79^{eA}	ND	ND	ND	ND	ND
T1	26.00^{cA}	20.42^{dB}	1.43^{cA}	4.21^{cD}	1.54^{cC}	5.38^{aA}
T2	80.02^{aA}	54.03^{bB}	8.12^{aA}	19.90^{aD}	18.90^{aC}	0.40^{bA}
T3	24.01^{dA}	20.52^{cB}	1.39^{cA}	3.12^{dD}	1.37^{cC}	5.35^{aA}
T4	75.97^{bA}	54.33^{aB}	7.95^{bA}	18.00^{bD}	18.50^{bC}	0.37^{bA}

[*]See footnote Table 2.
$^{a-e\ or\ A-D}$ Different letters in the same colum indicate significant statistical differences (Duncan's test P≤0.05)

The ascorbic acid contents in functional beverage containing strawberry were higher than that containing pumpkin. However, the ascorbic acid was significantly different (P≤0.05) between all treatments during storage periods. Also, ascorbic acid values were slightly but significantly decreased (P≤0.05) during cold storage.

Also, strawberry functional beverage showed the highest (P≤0.05) anthocyanin contents followed by that containing pumpkin and control samples. The anthocyanins content of the fresh functional beverages ranged from 1.70 to 20.21 mg 100g^{-1} and decreased gradually during the storage period up to 30 days. The obtained results agree with that of Li (2008).

Data in the same table showed the highest carotenoid contents of beverage functional pumpkin followed by that containing strawberry and control samples. The carotenoids content of the prepared functional beverages decreased gradually (P≤0.05) during storage up to 30 days. Fruits and vegetables have always been considered an essential part of a healthy diet, with respect to its vitamin C and β-carotene contents (Li, 2008).

Minerals contents of the prepared functional beverages

Table 4 revealed that the highest ($P \leq 0.05$) Na, K, Fe, Cu and Mg contents were found in beverages containing strawberry due to the high percentage of these minerals in strawberry pulp. The Ca content was significantly different ($P \leq 0.05$) between all functional beverages, as it ranged between 35.00 to 58.00 mg $100g^{-1}$. While, the P contents ($P \leq 0.05$) of all functional beverages varied between 19.93 to 29.03 mg $100g^{-1}$. Fermented permeate was responsible for the relatively high Ca and P of the prepared beverages due to its high contents of these elements. The trace elements of Mg, Fe, Cu and Zn contents were significantly different ($P \leq 0.05$) in the prepared beverages as they ranged from 2.51 to 9.73, 0.41 to 1.02, 0.05 to 0.14 and 0.04 to 0.22 mg $100g^{-1}$, respectively, due to the variable contents of these elements in the used ingredients.

From the results of minerals it could be concluded that the prepared functional beverages can be considered a good source for some of these minerals. Trace elements are essential for growth and development, wound healing, immunity and other physiological processes (Miller, 2000). These results closely agreed with those reported by Miller (2000) and Atallah (2015).

TABLE 4. Minerals contents of produced functional beverages (mg $100g^{-1}$).

Minerals	Fresh functional beverages					
	C1	**C2**	**T1**	**T2**	**T3**	**T4**
Ca	57.00^{a}	58.00^{a}	35.00^{c}	44.00^{b}	35.41^{c}	43.80^{b}
P	28.97^{a}	29.03^{a}	19.93^{c}	24.00^{b}	20.00^{c}	24.60^{b}
Na	18.59^{a}	18.85^{a}	22.89^{b}	27.00^{a}	23.10^{b}	27.87^{a}
K	12.41^{e}	13.87^{d}	18.51^{c}	22.50^{b}	18.91^{c}	23.20^{a}
Mg	2.51^{f}	2.74^{e}	4.42^{d}	9.07^{b}	4.84^{c}	9.73^{a}
Fe	0.41^{c}	0.43^{c}	0.67^{b}	0.99^{a}	0.69^{b}	1.02^{a}
Cu	0.05^{c}	0.06^{c}	0.08^{b}	0.10^{b}	0.09^{b}	0.14^{a}
Zn	0.04^{d}	0.04^{d}	0.19^{b}	0.07^{c}	0.22^{a}	0.08^{c}

*See footnote Table 2.
[a-f] Different letters in the same row indicate significant statistical differences (Duncan's test $P \leq 0.05$)

Amino acid profiles of produced functional beverages

Table 5 shows the results for the concentrations of the essential and non- essential amino acids of fresh functional beverages. Functional beverages containing pumpkin had the highest values of essential and non-essential amino acids compared to that containing strawberry and control samples. Leucine is the major essential amino acid (109.31 mg kg^{-1}) followed by lysine and theronine with mean values 98.24 mg kg^{-1} and 65.38 mg kg^{-1}, respectively of all functional beverages. In contrary, value for non-essential amino acids of all functional beverages indicated that glutamic acid was present in the highest values (123.03 mg kg^{-1}) followed by aspartic acid (95.89 mg kg^{-1}) and proline (75.64 mg kg^{-1}). The breakdown of leucine takes place in the liver and skeletal muscles (Layman, 2003). It undergoes transamination in the muscles by transferring into glutamine or alanine that ultimately convert to glucose in the liver through gluconeogenesis; a unique pathway for the maintenance of blood glucose level (Borsheim *et al*., 2003). Hence dietary proteins rich in essential and branched chain amino acids particularly leucine provide health benefits that are not usually observed for diets containing protein from other sources (Wolfe, 2002).

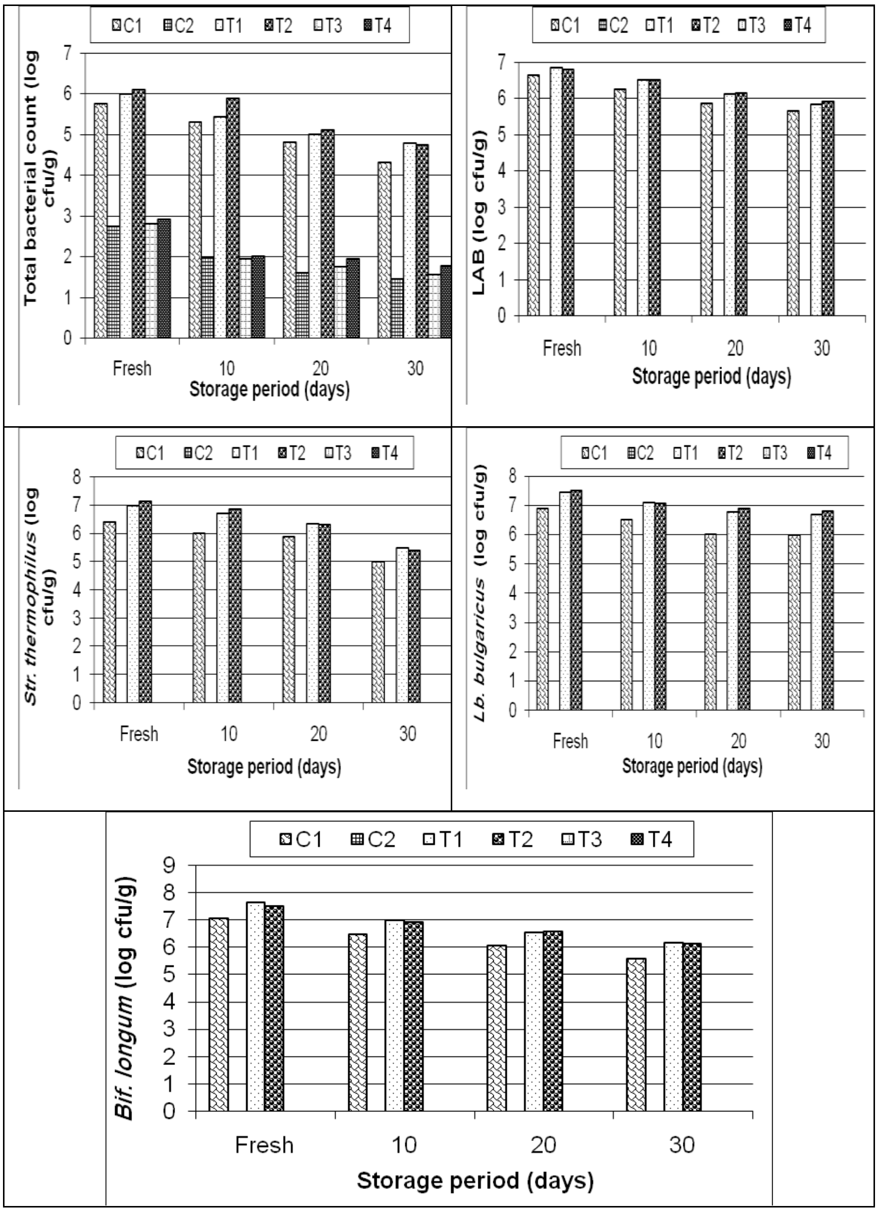
TABLE 5. Amino acids profiles of produced functional beverages (mg Kg^{-1}).

Amino acids	Fresh functional beverages		
	C1	T1	T2
Essential amino acids			
Isoleucine	55.23	65.01	56.32
Leucine	97.12	109.56	98.88
Lysine	78.56	98.24	81.84
Methionine	51.11	65.00	57.98
Phenylalanine	50.06	65.05	58.34
Theronine	58.32	67.38	64.30
Tryptophan	59.94	63.23	60.00
Valine	51.94	64.44	58.32
Arginine	54.87	63.21	57.89
Histidine	40.02	63.98	40.56
Non-essential amino acids			
Alanine	68.22	73.12	70.00
Aspartic acid	74.29	95.89	85.32
Cystein	41.26	46.23	44.39
Glutamic acid	98.36	123.03	110.23
Glycine	57.95	72.35	69.65
Proline	64.29	75.64	70.12
Serine	54.75	71.23	66.23
Tyrosine	51.97	60.77	58.77

*See footnote Table 2.

Microbiological properties

Microbiological examination of the prepared functional beverages for fresh and after 10, 20 and 30 days of storage at 4±1ºC for 30 days is shown in Fig 1. There were significant difference ($P \leq 0.05$) in the microbiological examination between functional beverages treatments and control samples during cold storage. Heat treatment reduced greatly the total viable bacterial count (TVBC) of whey permeate. The TVB and LAB counts gradually decreased up to the end of the storage period. LAB were not detected in heated fermented permeate, and heated fermented beverages throughout the storage period. The *Str. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* counts of during storage gradually decreased in all samples. The decrease of counts during storage ($P \leq 0.05$) may be due to their sensitivity to the developed acidity in the product (Atallah, 2015).



C1 = Control 1 (unheated fermented milk permeate)   C2 = Control 2 (heated fermented milk permeate)
T1 = 50% unheated fermented milk permeate + 50% pumpkin pulp
T2 = 50% unheated fermented milk permeate + 50% strawberry pulp
T3 = 50% heated fermented milk permeate + 50% pumpkin pulp
T4 = 50% heated fermented milk permeate + 50% strawberry pulp

Fig .1. Microbiological quality of produced functional beverages during cold storage at 4±1ºC.

Differences were found in *Bif. longum* counts between beverage samples from different treatments throughout the storage period. *Bif. longum* counts decreased ($P \leq 0.05$) at the end of the storage period. The decrease in bifidobacterial counts may be due to the developed acidity during storage periods. These results are in agreement with those obtained by Buriti *et al.* (2014) and Atallah (2015).

The Food and Agriculture Organization/ World Health Organization report **(**FAO/WHO 2002**)** which specifics that probiotic foods with health claims must contain per gram at least $10^6 – 10^7$ cfu at the time of consumption to exhibit their potential function.

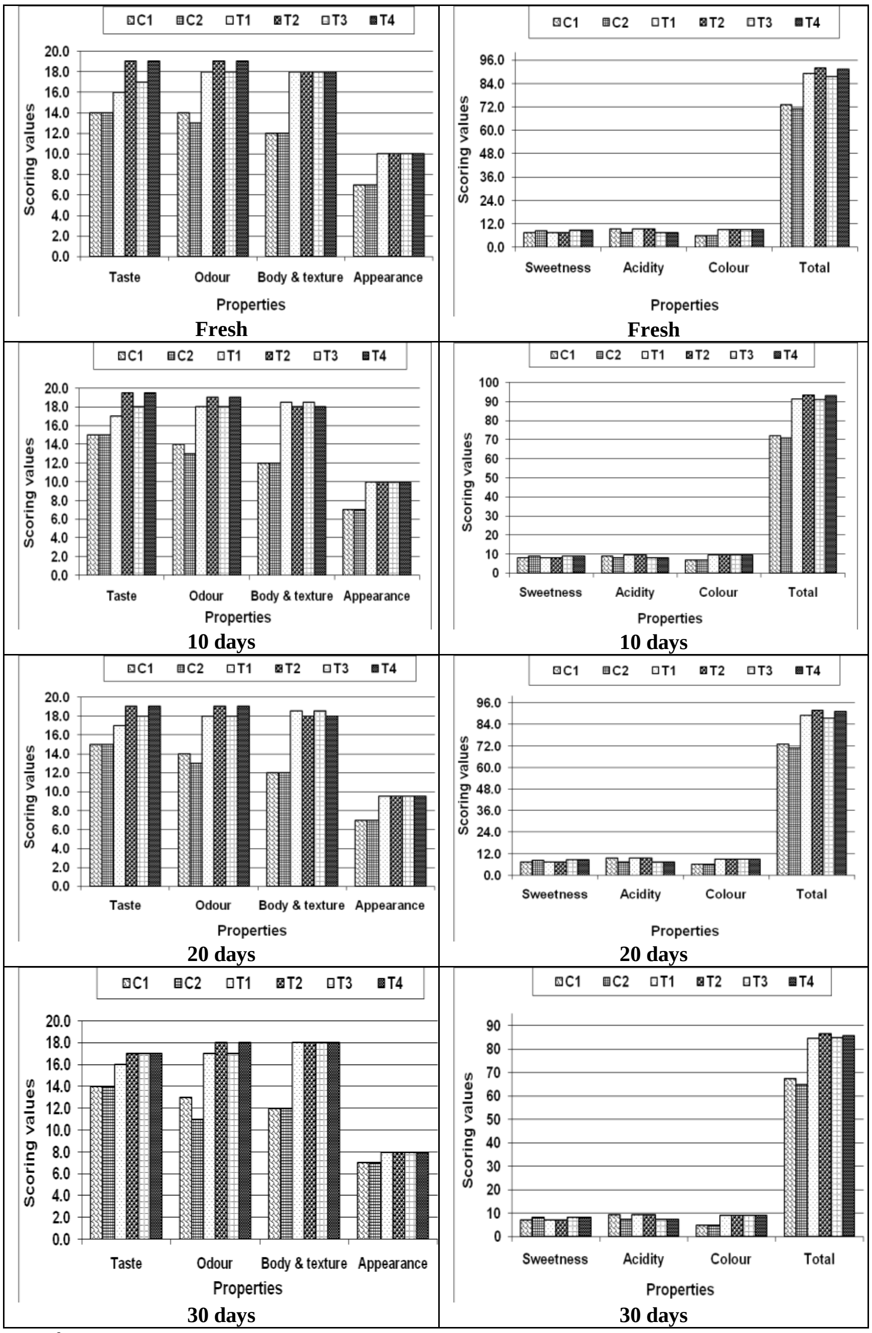
For fresh functional beverages, coliforms and yeasts & moulds are considered as indicators of plant hygiene, and sanitation quality of such products, and as a warning that the products may constitute a health risk. As a result of high hygiene conditions during processing and storage, coliform bacteria and, yeasts and moulds were not detected in all treatments when fresh and during storage. These results are in agreement with those obtained by Atallah (2015).

Sensory evaluation

Data in Fig 2 illustrated that all functional beverages containing strawberry and pumpkin have very good scores for acceptability by taste panel compared to control samples when fresh and throughout storage. The functional beverages containing probiotic strains and fruits exhibited improved sensory evaluation due to their high level of produced flavour compounds. Significant differences ($P \leq 0.05$) were found in scores for different sensory attributes between all treatments during the storage period. Functional beverage containing strawberry was ranked higher followed by beverage containing pumpkin. It is of interest that beverages based on heated or unheated fermented permeate gained close score points for the different attributes and the total score points. This can be explained on the basis of the slight changes in the composition of the products during storage. During cold storage, the organoleptic scores increased for all treatments after 10 days. No changes were observed among the treatments for all sensory characteristics up to 20 days of storage. After 30 days of cold storage, the same trend was observed for all the tested products with slight decreases in the obtained scores. Similar trends were obtained by Buriti *et al.* (2014) and Atallah (2015).

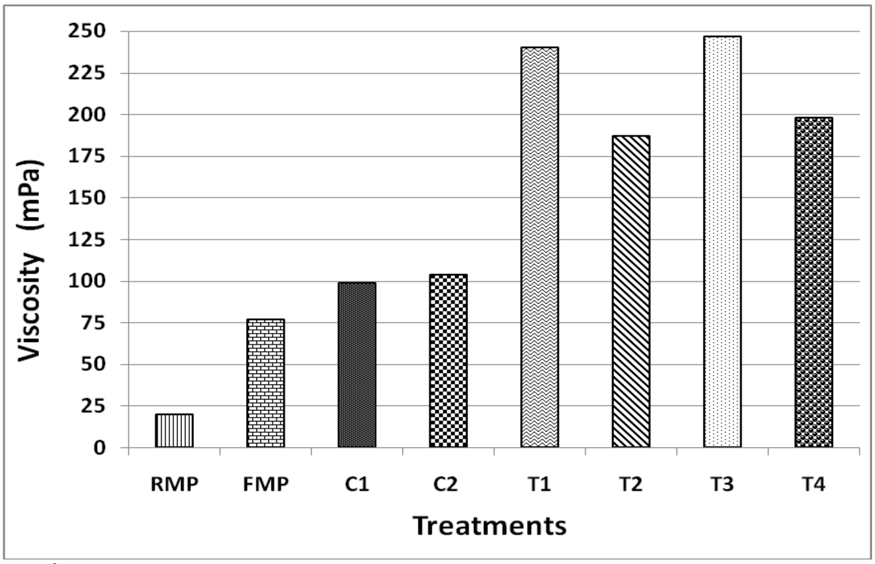
Viscosity

Data in Fig 3 indicated that control samples (C1 and C2) without additives had higher viscosity values than that milk permeate. This can be explained on the use of exopolysaccharide (EPS) producing microorganisms in the fermentation of the permeate. The EPS produced by *Str. thermophilus* strain presumably affects the texture of yoghurt and the functional beverages (Folkenberg *et al*., 2005).



*See footnote Fig 1.

Fig .2. Sensory evaluation of produced functional beverages during cold storage at 4±1ºC.



*See footnote Fig 1.
RMP= Raw milk permeate FMP= Fermented milk permeate without any additives

Fig. 3. Viscosity of raw milk permeate, fermented milk permeate and prepared functional beverages.

Also, the addition of fruits led to increased viscosity of the prepared beverages compared to control samples. These findings could be related to the higher total solids in functional beverages containing fruit pulp compared to control samples. Also, the addition of pumpkin significantly increased the viscosity of the beverages while strawberry significantly decreased the viscosity of the beverages. The highest viscosity values recorded for pumpkin beverages may be due to the presence of stabilizing agents (dietary fibers) in pumpkin. These have the ability to bind water, which would tend to increase the consistency of the products by increase of the water-binding capacity (Caili *et al*., 2007). A similar trend was recorded by Atallah (2015).

Conclusion

Pumpkin and strawberry pulps improved the flavour and nutritional value of probiotic beverages. A nutritious functional beverage can be successfully made by mixing fermented milk permeate with pumpkin and strawberry pulp at a ratio of 1:1 with 5% sucrose. The survival of probiotic bacteria during refrigerated conditions for at least 30 days resulted in numbers greater than 10^6 cfu g^{-1} or 6 log cfu g^{-1} in some treatments (unheated), which is essential for a product to have probiotic properties. The pulp-enriched samples were characterised by the highest content of antioxidant activity, total phenols, total flavonoids, ascorbic acid, anthocyanins and carotenoids. Functional beverages containing pumpkin had the highest essential and non- essential amino acids concentrations compared to strawberry and controls. The sensory scores of the functional beverages were high indicating acceptance. Addition of an exopolysaccharide-producing bacterial strain and other materials improved the textural and viscosity properties of the functional beverages. Generally, chemical, rheological, microbiological, organoleptical, antioxidant activity and bioactive compounds indicated that the use of pumpkin and strawberry as flavouring material in functional beverage manufacture is highly recommended. The addition of an EPS producing strain increased the viscosity of the prepared functional beverages.

Acknowledgement: The author is very grateful to Mr. Mahmoud El-Atrony for his assistance with statistical analysis of data.

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(Received
accepted ;

إنتــاج المشـروبات الغذائيــة الداعمــة للحيويــة والمدعمــة بالمركبـات الحيوية النشطة ومضادات الأكسدة في لب اليقطين و الفراولة

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في هذه الدراسة ، تم تحضير مشروبات حيوية بواسطة خلط راشح اللبن المتخمر مع لب اليقطين (القرع العسلي) و الفراولة بنسبة خلط (1:1). وتم تخمير راشح اللبن مستخدما 2٪ خليط من ثلاث مزارع بادئات بنسب خلط (1:1:1) والتى تتكون من سلالة *Lactobacillus delbrueckii* subsp *bulgaricus* DSM 20080 و سلالة *Streptococcus thermophilus* ASCC 1275 المنتجة للسكريات العديدة و سلالة البكتريا الداعمة للحيوية الـ *Bifidobacterium longum* DSM 20088 . تم خلط راشح اللبن المتخمر الحلو سواء المعامل حراريا (85ºم /15ق) و الغير معامل حراريا بصورة منفصلة مع حجم مساوى له من لب اليقطين أو الفراولة، وتم تعبئة المشروبات المحضرة فى زجاجيات معقمة ثم تم تخزينها لمدة 30 يوما على درجة حرارة 4±1مº. تم تقدير التغيرات التى حدثت في المركبات النشطة حيويا و مضادات الأكسده وكذلك الخواص الكميائية ، الميكروبيوجية ، الريولوجية والتقييم الحسى للمشروبات الوظيفية . ووجد زيادة فى محتوى المشروبات المحضرة في المواد الصلبة الكلية ومحتوى كل من الدهن ، البروتين ، الرماد ، الالياف و الحموضة بينما انخفض محتواها من الكربوهيدرات الكلية، مضادات الأكسدة، الفينول الكلى ، الفلافونات الكلية ، حمض الاسكوربيك ، الأنثوسيانين ، الكاروتينات و قيم الـ pH خلال فترة التخزين تحت تبريد . وقد وجد أن إضافة كل من لب اليقطين و الفراولة أدى إلي حدوث زيادة كبيرة ولكن بنسب مختلفة فى محتواها من الكالسيوم ، الفوسفور، الصوديوم ، البوتاسيوم ، الماغنسيوم ، الحديد ، النحاس و الزنك للمشروبات المنتجة . ولم يلاحظ وجود أى أعداد من سلالات بكتريا حمض اللاكتيك فى المشروبات المنتجة من تسخين راشح اللبن المتخمر وذلك خلال فترة التخزين بالتبريد بينما حدث انخفاض تدريجى فى العد الكلى للبكتريا و بكتريا حمض اللاكتيك و *Str. thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus* and *Bif. longum* فى المشروبات المنتجة من راشح اللبن المتخمر غير المسخن خلال فترة التخزين. فى حين ظلت أعداد البكتريا الداعمة للحيوية المستخدمة فى المشروبات المعدة من راشح اللبن غير المسخن أعلى من العدد المسموح به ($6\ \log\ cfug^{-1}$) لتحقيق التأثير المفيد والصحى حتى نهاية فترة التخزين . وقد أوضح التقييم الحسى للمشروبات الوظيفية المنتجة ارتفاع درجات القبول الحسى. وقد وجد أن إضافة سلالة البكتريا المنتجة للسكريات العديدة أدت إلى تحسين لزوجة المشروبات الوظيفية المعدة.