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Preparation, Properties and Evaluation of Folate and Riboflavin Enriched Six Functional Cereal - Fermented Milk Beverages Using Encapsulated *Lactobacillus plantarum or Streptococcus thermophilus.*

Osama M Sharaf*, Kawther El- Shafei , Gamal A Ibrahim, Hoda S El- Sayed, Jihan M Kassem, Fayza M Assem, Nabil F Tawfek, Baher A Effat, Azzat B Abd El-Khalek, and Nadia Dabiza.

Food Industries and Nutrition Division, Dairy Department (Dairy microbial.& Chem. Labs.), National Research Centre, 33 El Bohouth St. (former El Tahrir st.), Dokki, Giza,Egypt.

ABSTRACT

This study aimed to manufacture six folate and riboflavin enriched functional cereal - fermented milk beverages : barley fermented milk(BFM) , corn fermented milk(CFM) and wheat fermented milk (WFM),With the addition of strawberry and inoculated with encapsulated *Lactobacillus plantarum* or *Streptococcus thermophilus.* Samples were taken immediately after processing, three and seven days. Folate and riboflavin concentrations, microbiological, chemical and sensory properties were investigated. From a total of six fresh beverage, CFM with *Lc. plantarum* (T_2) and WFM with *S.thermophilus* (T_6)showed the highest folate levels (0.295 & 0.349 mg/L respectively). As for the results of riboflavin levels, WFM with *Lc.plantarum* (T_3)and BFM with *S.thermophilus* (T_4) showed the highest levels(2.12&1.82 mg/L respectively)as compared with others mixtures and declined thereafter during the storage period up to 7days . Viable counts of encapsulated and free cells of *Lc. plantarum* and *S .thermophilus* in all samples fresh and during storage at 7ºC for 7 days were higher than the10^8 cfu/ml. Total solids and total protein ,fat, titratable acidity , pH, soluble nitrogen, viscosity and color parameters were determined . Organoleptic properties revealed that functional wheat - fermented milk beverage with *S. thermophilus* (T_6)had the highest score in all acceptability. From the present study it can be concluded that the use of encapsulated probiotics enhanced folate and riboflavin levels in cereal - fermented milk beverages**.** Also, encapsulation of probiotics improve their gastrointestinal survival .

Key words: Fermented beverage, Cereals, Riboflavin, Folate, *Lactobacillus plantarum, Streptococcus thermophilus*, Microencapsulation.

Corresponding author

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INTRODUCTION

Functional foods are defined as foods that, in addition to their basic nutrients, contain biologically active components, in adequate amounts, that can have a positive impact on the health of the consumer [1, 2]. Functional foods have also been referred to as medicinal foods, nutritional foods, nutraceuticals, prescriptive foods, therapeutic foods, super-foods, designer foods, foodceuticals and medifoods [2]. Such foods should improve the general and physical conditions of the human organism and/or decrease the risk of occurrence of disease [3]. Vitamins are essential micronutrients that are normally found as precursors of various enzymes that are necessary for vital biochemical reactions in all living cells. Humans are incapable of synthesizing most vitamins and they consequently have to be obtained exogenously. The use of vitamin-producing microorganisms may represent a more natural and consumer-friendly alternative to fortification using chemically synthesized pseudo-vitamins ,and would allow the production of foods with elevated concentrations of vitamins that are less likely to cause undesirable side-effects. The B-group vitamin folate is involved in various essential metabolic functions such as DNA replication, repair and methylation, and synthesis of nucleotides, vitamins and certain amino acids. Riboflavin (vitamin B2) plays an essential role in cellular metabolism, being the precursor of the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which both act as hydrogen carriers in many biological redox reactions[4]. The daily recommended folate intake for an adult varies from 200 µg in Europe to 400 µg in the United States. Milk is a well-known source of folate. It contains between 20 and 50 µg of folate per liter and thus contributes significantly to the daily requirement of the average human. Some fermented milk products, especially yogurt, are reported to contain even larger amounts of folate .Up to 110 µg of folate per liter has been found in yogurt[5]. A large number of lactic acid bacteria such as *lactobacillus acidophilus, lactobacillus bulgaricus, lactobacillus plantarum, lactobacillus reuteuri, lactobacillus delbrueckii, lactobacillus rhamnosus, lactococcus lactis and lactococcus cremoris* and bifidobacterium species like *bifidobacteria infantis, bifidobacteria longum* have been reported to produce vitamins including riboflavin [6]. Although dairy products contain riboflavin, they are not considered a good source of this essential vitamin.Considering that milk contains c. 1.2 mg of riboflavin per litre, an average adult person and a pregnant woman would need to consume, respectively, 1 and 1.6 L of milk per day to meet their daily requirement.Increasing the levels of riboflavin in milk would thus be very important to prevent a riboflavinosis in populations where milk consumption is low [7]. Not only *S. thermophilus* and *L. lactis* have the ability to produce folates, but also other LAB like *Lactobacillus acidophilus* and *Lc. plantarum* have been reported to produce folate in chemically defined medium CDM [8].

Micro-encapsulation is a method of providing lactic acid bacteria (LAB) living cells with a physical barrier against adverse environmental conditions. The encapsulated cells survived better in simulated gastrointestinal conditions compared to the free cells. Sharaf et al. [9]

Used encapsulated *Propionibacterium shermanii* by sodium alginate to manufacture Tallaga cheese. Count of encapsulated *Pr. shermanii* increased during storage period and reached maximum after 15 days of storage. The maximum production of vitamin B12 was observed after 15 days of storage to reach 3.51µg/g in Tallaga cheese.

Divya and Nampoothiri [10] reported that when encapsulated *Lactococcus.lactis* CM22 was used to ferment skim milk ,144.54 µ g /L folate was obtained after 15 h fermentation at 37ºC. For encapsulated cells *L.lactis* CM28, folate yield was 130.12 µg/L after 15 h fermentation. Ice cream is also a good vehicle for probiotic delivery due to its composition and the pH of about 6 of ice cream provides ideal condition for the probiotic survival. The total folate produced by the encapsulated *L.lactis* CM22 was 173.80 µ g/L after 15 h fermentation . In the recent past, there has been significant interest in research and development of smart functional foods that maximize therapeutic health benefits to consumers. However, the food and nutraceutical industry is increasingly facing challenges in incorporating bioactive and health-promoting ingredients into food and nutraceuticals for sustained bioactivity without compromising their bioavailability and biofunctionality. Protection of bioactivity and controlled release of ingredients at the right location and at the right time is a key functionality that can be provided by encapsulation technologies.

The aim of this work was to manufacture of functional strawberry cereal - fermented milk beverages using encapsulated *Lactobacillus plantarum* or *Streptococcus thermophilus* and evaluation of folate and rboflaivin levels ,chemical,physical, microbiological and sensory properties of beverages.

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MATERIALS AND METHODS

Bactereal strains

Lactobacillus plantarum and *Streptococcus thermophilus* were isolated and identified by Dairy Science Dept.,(Dairy Microbiology Lab.),National Research Center [11]

Materials

Skim milk was obtained from Faculty of Agriculture, Cairo University, Giza, Egypt. Fine grade of strawberry fruits and sweet materials (sucrose sugar) were purchased from local market, Cairo, Egypt. Three different grains (barley, corn and wheat) were purchased from local market, Cairo, Egypt.

Preparation of cells and Encapsulation procedure

Frozen cultures *Lc. plantarum* and *S. thermopilus* were reactivated 3 times in MRS broth and M17 broth media respectively and incubated at 37 ^{0}C for 24 h. The cells were harvested by centrifugation at 4000 g for 15 min then the cells washed by sterile saline solution then used for the encapsulation. Encapsulation occurred by extrusion method using sodium alginate sterilized by autoclaving at 121^o C for 15 min. The microspheres made by using sterilized syringe through extruding a mixture of cells and sodium alginate (3%) into sterilized 0.1 M calcium chloride solution with continuous stirring at 200 rpm/min till alginate beads were formed, then the beads collected by filtration according to [12].

Preparation of cereal - based fermentation media

Corn, wheat and barley grains were used to prepare the fermentation media following the same procedure. The grains were mashed in a laboratory Falling with a sieve of size 0.5 mm. A sample (50 g) of the flour obtained was mixed with 450 ml tap water and the resulting slurry centrifuged (6000 g) for 30 min at room temperature. The starch-free supernatant fluid was collected and immediately sterilized at 121 ^{o} C for 45 min. Sedimentation of solids (possibly protein mixtures) was observed after sterilization and approx. 4–5% (w/w) of solids were present in the final fermentation media. The extraction and sterilization procedures were repeated four times[13].

Supplemented whey permeate , consisting of a final concentration of 60 g whey permeate 10 g yeast extract 0.2 g magnesium sulfate, and 0.05 g manganese was used with cereal extracts [14].

Preparation of fruit juice

Fresh high quality strawberry fruit was carefully washed, cut to pieces and pulped in a blender. The resultant homogenized mixture juice was used in this trail.

Preparation of fermented strawberry cereal-milk beverages

Three equal portions of supplemented whey permeate (25%)were mixed with sterilized cereals extract(25%) of barley, corn or wheat at level 1 : 1 . Then each mixture was inoculated by 1% *Lc.plantarum* beads . Another three mixtures as described previously was inoculated with 1% *S.thermophilus* beads . Each inoculated mixture was incubated at 35^{0}C/72hr. Then each fermented mixture was mixed with previous fermented milk with the free used strains at 35 ^{0}C/24 hr (25%).Twenty percent. strawberry and 5% sugar were added to each fermented beverage. The final prepared six beverages were packed into sterilized bottles and stored at refrigerator for 7 days .

Treatments

T_1: Barley fermented milk(BFM) +encapsulated *Lc. plantarum*
T_2: Corn fermented milk(CFM) +encapsulated *Lc. plantarum*
T_3: Wheat fermented milk (WFM +encapsulated *Lc.plantarum*

T_4:Barley fermented milk(BFM) +encapsulated *S. thermophilus*
T_5:Corn fermented milk(CFM) +encapsulated *S. thermophilus*
T_6:Wheat fermented milk (WFM)+encapsulated *S. thermophiles*

Beverage samples were taken at fresh , 3 and 7 days of storage and analyzed for folate & riboflavin concentrations, chemical,physical,microbiology and Organoleptic properties.

Determination of folate and riboflavin

The sample extraction procedure was carried out according to [15]A stock standard solution(100µg mL^{-1})of riboflavin and folate were prepared with water and stored at-20°C. The standard solutions required for constructing a calibration curve prepared from stock solution by serial dilution with water and were stored at 4°C before use .HPLC analysis was performed with an Agilent 1260HPLC system (Agilent Technologies ,USA) equipped with a quaternary pump auto sampler injector with20µl fixed loop injector thermostat compartment for the column and photodiode array detector . The chromatographic column was C18 Zorbax XDB (250 mm x 4.6 mm, 5 µm film thicknesses). The column was kept at room temperature at a flow rate of 0.8 ml/min with a total run time of 12 min. Separation of vitamins was carried out by gradient elution with methanol (A) and 1% TFA containing water (B). the elute composition was initially 8 % A + 92 % B, held for 2 min, and changed linearly to 92 % A + 8 % B in the next 4 min and held for 6 min. Detection wave length for detection of riboflavin and folate was set at 254 nm. The retention time of riboflavin and folate was about 7.627 min.

Microbiological analysis of beverages

Enumeration of encapsulated bacteria

The encapsulated bacteria were released by homogenizing 0.1 g of the sample in 10 ml of 0.1 sodium citrate with shaking for 10 min ,then 1 ml of the liquid was used for serial dilutions which plated on selective media . *Lc.plantarum and S.thermophilus* strains were grown in MRS agar and M17 agar respectively. Plates were incubated for 48 h at 37°C.

Enumeration of free cells

Serial dilutions of beverage samples were plated on MRS and M17 .Plates were incubated for 48 h at 37°C.

Enumeration of molds , yeasts and coliform group

Potato dextrose agar medium was used for counting yeasts and molds. Plates were incubated for 5-7 days at 25°C. Coliform group was determined using solid medium method onto plates of violet red bile agar (Difco)and incubated for 48 h at 37°C.

Chemical analysis

Cereal - fermented milk beverage samples were tested for moisture, total protein, fat and acidity as mentioned in [16].

Viscosity

Viscosity was measured in fresh samples at 7°C using a Brookfield digital viscometer (Model DV-II+VISCOMETER, Spindle-00). The speed was set from 3 to50 rpm. Three readings, 30s apart, were recorded for each sample.

Color

Color was measured using Hunter Colorimeter model D2s A-2 [17]. Tristimulus values of the color namely L, a, b & B were measured using the corresponding button on the colorimeter. Where:

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L: value represents darkness from black (0) to white (100)
a: value represents color ranging from red (+) to green (-).
b: value represents yellow (+) to blue (-).

Sensory Evaluation

Fresh and stored cereal - fermented milk beverage samples were periodically evaluated for sensory properties . Organoleptic properties were done by twelve of stuff members of National Research Centre (NRC), for appearance (10), odor (10), taste (10) and total acceptability (10).

RESULTS AND DISCUSSION

The use of vitamin-producing microorganisms may represent a more natural and consumer-friendly alternative to fortification using chemically synthesized pseudo-vitamins, and would allow the production of foods with elevated concentrations of vitamins that are less likely to cause undesirable side-effects. The application of biofortification of daily products using vitamin-producing microorganisms is an interesting alternative to the use of synthetic folic acid in fermented foods. The careful selection of folate-producing strains and the optimization of their production are essential and could lead to natural enrichment of folate in different products [5,18 ,19].

Yield of vitamins folate and riboflavin in six cereal- Fermented Milk Beverages:

Folate concentrations of the different treatments are shown in **Table(1)**. From a total of six fresh beverage, two of them ($T_2$and T_6) showed the highest folate levels (0.295 & 0.349 mg/L) as compared with others mixtures and decline thereafter was observed during the storage period.

Table 1: Folate levels in different beverages

Cereal - Fermented Milk Beverages	Fresh Folate (mg/L)	3days Folate (mg/L)	7days Folate (mg/L)
T_1:BFM +encapsulated *Lc. plantarum*	0.247	0.233	0.219
T_2:CFM+encapsulated *Lc. plantarum*	0.295	0. 288	0.263
T_3:WFM+encapsulated *Lc.plantarum*	0.258	0.247	0.231
T_4:BFM+encapsulated *S. thermophilus*	0.244	0.230	0.224
T_5:CFM+encapsulated *S. thermophilus*	0.287	0.272	0.261
T_6:WFM+encapsulated *S. thermophilus*	0.349	0.321	0.302

Milk is not considered a rich source of dietary folate. However, many dairy products are processed using microbial fermentations in which folate could be synthesized. In this regard it was previously described that certain yogurts contain a three-fold increase in folate concentrations compared to non-fermented milk [21] . Sybesma et al. [5]found that the highest folate production per biomass was found in *S. thermophilus* strain B119 (214 µg /liter/OD600 unit). In general, *Lactobacillus* strains consumed small amounts of folate and did not produce folate, with the exception of *Lactobacillus plantarum* . Laiño et al. [19] used combined *Lactobacillus delbrueckii subsp. bulgaricus* (3 strains) and *Streptococcus thermophilus* (2 strains) to elaborate 15 different yogurts. The yogurt elaborated with strains CRL871 CRL803 CRL415 and incubated at 42 ^{o}C had significantly higher folate concentrations (reaching 180 µg /L) which implies almost a 250% increase in respect to non-fermented milk, and about 125% compared to commercial yogurts. On the other hand, studies to check some LAB efficacy in fermentative fortification of skim milk and ice cream revealed an enhancement in folate and riboflavin levels. Folate fortification of ice cream and skim milk using encapsulated probiotics *Lactococcus lactis* CM22 and *L.lactis* CM28 were reported earlier as good folate producers [21,10]. The probiotic beads of both cultures were used for fermentative fortification of folate in skim milk. When encapsulated *L.lactis* CM22 was used 144.54 µ g /L folate was obtained after 15 h fermentation at 37ºC and with free cells 162.23 µ g/L total folate was present. For encapsulated cells the folate yield was 130.12 µg/L *L.lactis* CM28 and with free cells it was 96.59µg/L after 15 h fermentation. Also,the total folate produced by the encapsulated *L.lactis* CM22 in ice-cream was 173.80 µ g/L after 15 h fermentation and free cells produced 222.06 µ g/L after 8 h of fermentation at 37ºC. In the case of *L.lactis* CM28, a maximum of 172.35 µ g/L folate

was produced when encapsulated cells were allowed to ferment for 10h whereas the free cells produced 150.6µ g/L after 8 h fermentation [22]. In addition to obtaining fermented milk products using adequately selected starter cultures that can increase vitamin concentrations, it is possible to increase the folate level naturally through the addition of some fruit component [23,119]. El-Zainy et al. [24] concluded that addition of 5% strawberry to fermented milk during manufacturing process can produce acceptable probiotic milk beverages with sufficient survival rate of probiotic bacteria .

Riboflavin levels of the different treatments are shown in**Table(2)**. From a total of six fresh beverage, two of them (T_3 and T_4) showed the highest folate levels (2.12 & 1.82 mg/L) as compared with others mixtures and decline thereafter was observed during the storage period.

Table 2: Riboflavin production in different beverages

Cereal - Fermented Milk Beverages	Fresh Riboflavin (mg/L)	3days Riboflavin (mg/L)	7days Riboflavin (mg/L)
T_1:BFM +encapsulated *Lc. plantarum*	1.54	1.42	1.28
T_2:CFM+encapsulated *Lc. plantarum*	1.39	1.21	1.02
T_3:WFM+encapsulated *Lc.plantarum*	2.12	2.01	1.95
T_4:BFM+encapsulated *S. thermophilus*	1.82	1.69	1.57
T_5:CFM+encapsulated *S. thermophilus*	1.76	1.72	1.47
T_6:WFM+encapsulated *S. thermophilus*	1.71	1.62	1.44

LeBlanc et al. [4, 8] reported that riboflavin concentrations can sometimes vary in certain dairy products because of processing technologies and through the action of micro-organisms utilized during food processing. It has been shown that most yoghurt starter cultures decrease riboflavin concentrations whereas others can increase levels of this essential vitamin up to 160% of the initial concentration present in unfermented milk . Tolouie et al .[25]showed that using probiotic strains for yogurt preparation is associated with higher amounts of B2 and B3 vitamins in the yogurt. In general, probiotic yogurt is a better source of these vitamins than conventional yogurt. Riboflavin concentrations can vary in certain dairy products due to processing technologies and to the action of microorganisms during food processing. **Guru and Viswanathan** [6] observed that *Lc. acidophilus* yielded a higher riboflavin compared to *L. lactis* in milk whey medium. Further *Lc. acidophilus* yielded a maximum riboflavin content of 2930 µg/ litre on 7th day in whey and declined on 9th day of fermentation. However, *L. lactis* yielded a maximum riboflavin content of 2610µg/litre till 5th day and declined thereafter.

Bacterial viable counts in six cereals - Fermented Milk Beverages

Results obtained in Table (3) showed counts(log cfu/ml) of *Lc. plantarum* grown on MRS agar medium and *S .thermophilus* on M17 agar in six cereals - fermented Milk beverages. Data show that *Lc.plantarum* in T1 & T2 exhibited a higher maximum growth rate of count than in T_3 and T_6 for *S .thermophilus* . Differences in viable counts of encapsulated and free cells of *Lc. plantarum* and *S .thermophilus* in all samples during storage period at 7 ºC were observed.

Table 3: Counts of *Lc. plantarum* and *S .thermophilus*(log cfu/ml)in six cereal - fermented milk beverages

Cereal - Fermented Milk Beverages	Counts of free cells			Counts of beads		
	Zero	3days	7days	zero	3days	7days
T_1*Lc. plantarum*	9.9	9.8	9.1	9.8	9.8	9.5
T_2*Lc. plantarum*	9.9	9.6	9.2	9.6	9.3	9.3
T_3*Lc. plantarum*	8.9	8.4	8.1	8.9	8.9	8.8
T_4*S .thermophilus*	8.4	8.2	8.0	8.9	8.9	8.8
T_5 *S.thermophilus*	8.3	8.3	8.0	9	8.7	8.8
T_6*S .thermophilus*	8.6	8.1	8.1	9.6	9.3	9.1

Viable free cell counts of all treatments were decreased till 7 days of storage period. After 7 days, viable counts of encapsulated *Lc. plantarum* in T_1,T_2,T_3 were higher compared with free cells where reached to 9.5,9.3,8.8 log cfu/ml, and free cells reached to 9.1,9.2,8.1 log cfu/ml respectively ,also counts of encapsulated *S.thermophilus* in T_4,T_5,T_6 were 8.8,8.8,9.1 log cfu/ml and counts of free cell were 8.1,8.0,8.0 log cfu/ml .This results reflects the protective effect of microencapsulation on the viability of strains. These results are in harmony with those obtained by Sharaf et al. [9] and Champagene et al. [26] who reported that immobilized system can reach higher cell densities than classical free cell fermentations performed under the same conditions.

Coliform group, yeasts and molds

No growth of coliform group, yeasts and molds were detected in all beverages over the storage period at 7 ºC. This indicates that proper care was taken to avoid contamination throughout the process and the product has good quality .There was no post processing contamination.

Chemical properties:

Table 4: Chemical composition of cereal - fermented milk beverages

Samples	Total solids (TS)%	Total protein (TP) %	Fat %
T_1	11.21	1.79	1.0
T_2	11.24	172	0.5
T_3	11.43	1.76	0.7
T_4	11.48	1.89	1.0
T_5	11.34	1.75	0.5
T_6	11.10	1.84	0.7

Chemical compositions were illustrated in Table (4) for fresh cereal - fermented milk beverage samples. It had been observed that no difference between treated samples in both of total solids and total protein. Although, the samples had three different extracts (barley, corn and wheat) at same ratios, there wasn't any difference between any treated samples in most chemical compound. Skim milk was used therefore, fat percentages were small in all treated samples because it diluted with strawberry juice and three different extracts.

Table 5:Chemical composition of stored cereal - fermented milk beverage samples.

Treatments	Storage period (days)	pH	Titratable Acidity (TA)%	Soluble nitrogen (SN) %
T_1	Fresh	3.84	0.95	0.126
	3	3.66	1.05	0.140
	7	3.55	1.15	0.154
T_2	Fresh	3.70	1.00	0.130
	3	3.59	1.10	0.154
	7	3.51	1.10	0.182
T_3	Fresh	3.81	0.93	0.112
	3	3.70	1.00	0.126
	7	3.68	1.00	0.140
T_4	Fresh	4.65	0.78	0.126
	3	4.35	0.90	0.126
	7	4.23	4.35	0.140
T_5	Fresh	4.53	0.85	0.130
	3	4.45	0.87	0.140
	7	4.31	4.45	0.154
T_6	Fresh	4.38	0.88	0.098
	3	4.26	0.92	0.112
	7	4.14	4.26	0.126

Table (5) showed the chemical composition of cereal - fermented milk beverage skim milk samples during storage at fresh ,3 and 7 days at cold temperature. Slight changes were observed in pH and titratable

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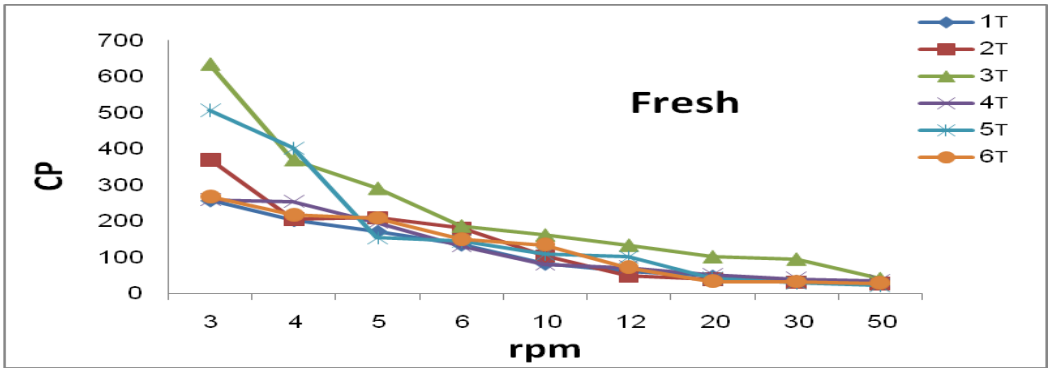
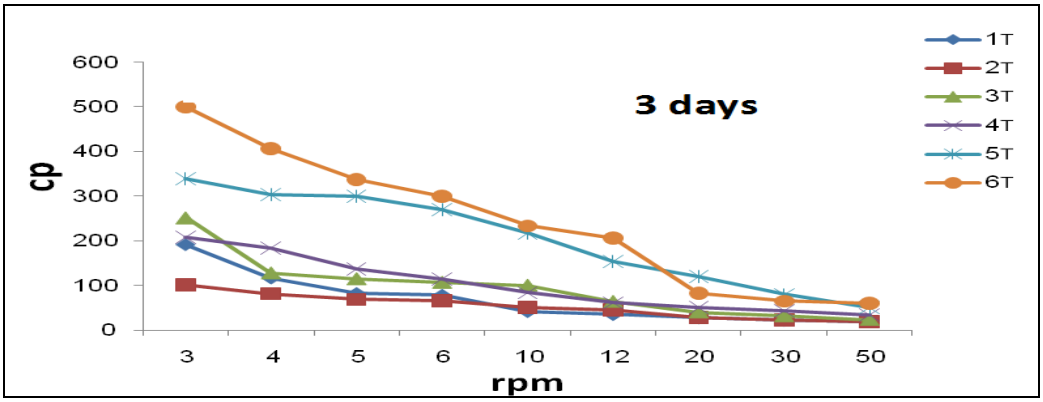
acidity in all treatments during storage period. The pH of the samples from different treatments decreased and titratable acidity increased during storage as a result of the growth and activity of skim milk strawberry beverage micro flora. Samples (T_1 ,T_2,T_3) which inculcated with *Lc.plantarum* developed higher acidity than samples (T_4 ,T_5,T_6) which inculcated with *S.thermophilus* due to *Lc.plantarum* play a major role in primary cultures whose function is to produce lactic acid from lactose [27] **.**Proteolysis in skim milk strawberry beverage was followed by determining the soluble nitrogen (SN) of samples from different treatments during storage period .It had been observed that there is increase in SN% of cereal - fermented milk beverages from different treatments during storage period. SN% increased in the samples which inculated with *Lc.plantarum* than samples which inculated with *S.thermophilus* due to the present of *Lc.plantarum* which described as proteolysis and lipolytic activity [28] while *S.thermophilus* is usually described as a poor proteolytic species due to the dependence of it's growth on the presence of amino acids released by *Lactobacillus delbreukii* ssp *bulgaricus*[28].

Physical properties

Viscosity

The results of cereal - fermented milk beverage samples, viscosity containing three different extracts (barley, corn and wheat) during cold storage are shown in Fig. (1). There were little differences between samples when fresh. The results indicated that samples which containing *S. thermophilus* T_4, T_5 and T_6 had higher apparent viscosity than samples containing *Lc. plantarum* strain T_1, T_2 and T_3 after 3 and 7 days during cold storage. This high viscosity of samples containing *S. thermophilus* could be attributed to the effect of exopolysaccharides synthesized by this strain as mentioned in many researches [29] and Broadbent et al.[30] who indicated that the ability to produce exopolysaccharides from *S. thermophilus* dependent and significantly affected by media and growth conditions (e.g. temperature and pH). Also, these results in accordance with Abu-Jdayil and Mohameed[31] who established that viscosity was higher on d 12 than on other days. He explained that protein rearrangement was continuing, and more protein-protein contacts were being established, leading to increasing viscosity during storage.





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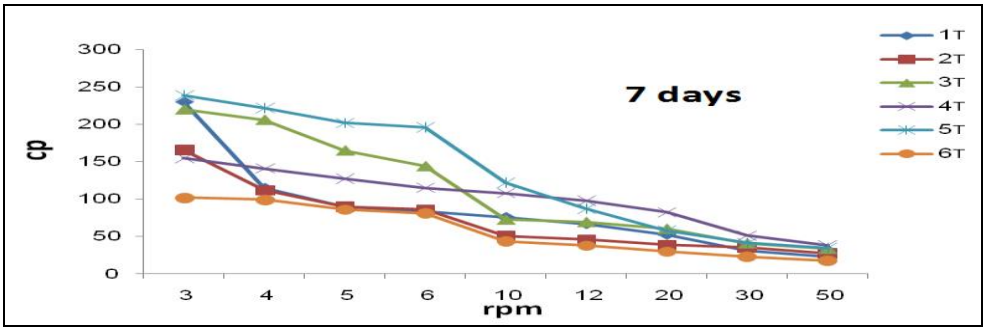

Figure 1: Viscosity of fresh and stored of strawberry cereal - fermented milk beverage samples .

Color parameters

There is a considerable interest when making beverages with fruits to achieve the right goal for consumers. So, Table (6) had indicated color parameters of strawberry cereals - fermented milk beverage samples. Obviously, there were significant differences between the parameters color of the ingredients and the samples. We could notice that skim milk had influenced the color of treated samples; it contributed to increase (L) parameter and made the samples more whiteness. Storage period slightly decreased the L parameter; it could be caused by the growth cultures of *S. thermophilus* & *Lc.plantarum*. Consequently, skim milk was a good choice for making these beverages. Addition of considerable amounts of strawberry juice had enhanced the (a) parameter which indicated the red color. There was a significant increase of (a) parameter in all treated samples and a slight decrease in the same parameter during storage period. Likewise, additional of three different extracts to the beverages made a distribute mixture with skim milk and strawberry juice had parity increased (b) parameter. Storage period hadn't affected to (b) parameter. Thus, in the present study we assumed that the performance of skim milk, strawberry and extracts had a significant influence and enhancement all the color parameters.

Table 6: Color parameters of fresh and stored strawberry cereal - fermented milk beverage samples.

Samples	L	a	B
Ingredients			
Strawberry	22.66	37.66	27.26
Skim milk	91.17	-2.06	12.80
Wheat extract	47.88	-1.60	8.71
Barley extract	47.80	-1.56	8.70
Corn extract	47.90	-1.62	8.66
Fresh samples			
T1	75.37	6.50	13.14
T2	75.73	7.16	12.02
T3	75.17	6.54	12.71
T4	77.66	5.37	12.48
T5	76.14	5.29	12.82
T6	77.01	4.71	12.88
3 Days storage			
T1	75.97	6.31	13.12
T2	76.28	7.70	12.22
T3	75.92	6.37	12.77
T4	75.85	5.77	11.92
T5	75.26	5.59	12.76
T6	75.58	5.19	12.54
7 Days storage			
T1	76.62	6.24	13.88
T2	76.05	7.57	13.08
T3	75.92	5.80	12.88
T4	75.96	5.39	11.97
T5	76.25	4.79	13.38
T6	74.95	4.91	12.87

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Sensory Evaluation

Table 7: Organoleptic properties of fresh and stored strawberry cereal - fermented milk beverage samples .

Treatments	Storage period (days)	Appearance (10)	Odor (10)	Taste (10)	Total acceptability (10)
T_1	**Fresh**	8.47	8.14	8.0	7.26
	3	7.25	5.0	4.5	6.0
	7	6.57	4.5	4.32	5.5
T_2	**Fresh**	8.14	7.57	7.29	7.43
	3	7.5	5.0	4.75	6.25
	7	7.34	4.77	4.56	6.09
T_3	**Fresh**	8.43	7.30	7.57	7.29
	3	7.25	5.5	5.0	6.5
	7	7.09	5.20	4.87	6.33
T_4	**Fresh**	8.29	8.29	8.36	7.71
	3	7.25	6.25	7.0	7.25
	7	6.90	6.00	6.75	7.00
T_5	**Fresh**	8.25	7.86	8.0	7.14
	3	7.78	6.0	6.25	7.0
	7	7.5	5.78	6.10	6.5
T_6	**Fresh**	8.5	7.71	8.14	7.87
	3	7.0	5.5	6.25	7.32
	7	6.90	5.23	6.06	7.09

Table (7)showed that the sensory evaluation of strawberry cereal - fermented milk beverage samples when fresh , after 3 and 7days at cold storage. Data indicated that there was a slight difference in appearance between fresh samples. All stored samples had gained lower score in appearance compared to fresh. On the other hand, samples (T_1, T_2 and T_3) which inoculated with *Lc.plantarum* had scored lower in odor compared to samples (T_4, T_5 and T_6) inoculated with *S.thermophilus* when fresh as well as stored samples. Same trend was observed in taste. Samples inoculated with *S. thermophilus* had gained higher score compared with *Lc.plantarum* samples when fresh and after storage period. In the fact, wheat extract with *S.thermophilus* had the highest score in all acceptability. However, the strange flavor of samples with *Lc. plantarum* that the panelists felt during evaluated could be as a result of proteolysis activity by this strain. These data were in accordance with [33,32].

CONCLUSION

From the present study it can be concluded that the use of folate and riboflavin –producing lactic acid bacteria(*Lc.plantarum and S.thermophilus*) can be regarded as a new perspective in the specific use of probiotics. These novel bio-enriched cereals - fermented milk beverages could be able to satisfy the growing demands of consumers for more natural products and provide novel tools to prevent folate and riboflavin deficiencies without having to recur to fortification with chemicals such as folic acid. The production costs of folate and riboflavin bioenriched products would be the same as traditional ones, but the latter would have an increased economic value because they would provide consumers an additional benefit. Also,encapsulation of probiotics improve their gastrointestinal survival . Nevertheless, the conditions for folate production by the encapsulated probiotics can be further optimized to enhance the folate and riboflavin yields.

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