

A new functional whey beverage, containing calcium and Date syrup (Dibs)

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Abstract

The study is about the use of by-products of the food industry that have functional and biological properties such as cheese whey and eggshell in the production of whey beverages. Fermented whey beverage was prepared by blending 1% eggshell, 1% egg yolk, 0.1% CMC and 0.01% vanilla with sweet whey. *Bifidobacterium animals* ssp. *Lactis*Bb-12 was used as a probiotic organism. Date syrup (Dibs) was used as a natural sweetener in different levels 10, 12.5 and 15% v/v of the beverage and analyzed for various physicochemical, microbiological properties as well as sensory properties for its overall acceptability during cold storage at $4 \pm 1^{\circ}\text{C}$ for 21 days. The obtained results indicated that pH values were decreased gradually during cold storage while the viscosity was increased. The viability of *Bifidobacterium animals* ssp. *Lactis* remained high up to 14 days then started to decrease. The study revealed that the fermented whey beverage containing 12.5% dibs showed superiority in almost all physicochemical, microbial and sensory properties as compared to the other treatments

Introduction

Specifically, beverages are by a wide margin the most dynamic functional foods category because of (i) comfort and probability to meet consumer demands for container contents, size, shape, and appearance; (ii) simplicity of distribution and better storage for refrigerated and shelf-stable products; (iii) extraordinary chance to fuse attractive supplements and bioactive mixes compounds (***Sanguansri and Augustin, 2009 and Wootton-Beard and Ryan, 2011***). The different types of commercially available products could be grouped as follows: (1) dairy-based beverages including probiotics and minerals/ ω -3 enriched drinks, (2) vegetable and fruit beverages, and (3) sports and energy drinks ***Maria et al., (2014)***.

Whey, a valuable dairy by product contains half of the milk solids and is a rich source of lactose, water soluble vitamins, minerals, and immunologically active proteins. It contains about 50-55% total milk solids, 70% of milk sugar, 20% of milk proteins and 70-90% of minerals and almost all water soluble vitamins especially vitamin B complex and vitamin C ***Durham et al., (1997), Sinha et al., (2007)***.

Probiotics are defined as 'live micro-organisms, which when consumed in adequate amounts confer a health benefit on the host' ***FAO/WHO, (2001)***. Probiotic microorganisms are generally LAB belonging to the species *Lactobacillus acidophilus*, *L. gasseri*, *L. helveticus*, *L. johnsonii*, *L. paracasei*, *L. reuteri*, *L. plantarum*, *L. rhamnosus*, and *L. fermentum*, while members of the genus *Bifidobacterium* are also used, e.g., *Bifidobacterium bifidum*, *B.*

longum, *B. animalis*, and *B. breve* (**Tamimeet et al., 2005 and Castro et al., 2015 and Linares et al., 2016**).

A huge amount of research describes the role of the *Bifidobacterium* genus in promoting human health, such as anti-hypertensive, anti-inflammatory, and anti-diabetic, anti-oxidative, immune-modulatory, anti-cholesterolemic, or microbiome modulation **Linares et al., (2017)**.

Calcium (Ca) is one of the important macronutrients necessary for the normal functioning of the human body. Calcium is not only the major component of bones and teeth, but it also participates in the regulation of hormone secretion and activation, muscle contraction, neuronal conduction via ion channels, regulation of inflammatory processes, maintaining the permeability of cell membranes, and many others **Driet et al.,(2011)**. Some studies showed beneficial effects of dietary calcium on lipid metabolism, insulin resistance, and abdominal obesity of persons with metabolic syndrome (**Jacqmainet al.,2003 and Lorenzenand Astrup, 2011**). Natural Ca sources are of interest because they contain not only Ca but also other elements (e.g., Sr and F), which may have a positive effect on bone metabolism **Olgunet al., (2015)**.

Chicken eggshell is a waste material from domestic sources such as hatcheries, poultry farms, egg product factories, homes and restaurants. Eggshell calcium is a good source of dietary calcium and excellent replacement material for important crustacean shells **Suguroet al.,(2000)**. Moreover, calcium from the crushed eggshell powder was absorbed easier than commercial CaCO₃ in the rat small intestine **Swiatkiewiczet al.,(2015)**, and readily soluble in gastric juice **Bradauskienéet al., (2016)**.

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Vitamin D is an essential fat-soluble vitamin needed for efficient calcium absorption. The deficiency of vitamin D has been related to hypertension, diabetes, metabolic syndrome, cancer, autoimmune, and infectious diseases (**Ferguson et al., 2015; Holick and Chen, 2008**). It is synthesized when the body is exposed to sunlight or after the consumption of fortified foods and supplements. Only a few foodstuffs naturally contain it, and these are mainly of animal origin such as egg yolk, fish and liver. Egg yolk contains high biological value of proteins and other nutrients such as other vitamins, minerals, essential fatty acids and phospholipids **Anton (2013)**.

Date syrup is a product obtained from a matured product with about 67-72% solid concentration consisting of 95% reducing sugar (**Mohamed and Ahmed, 1981 and Rofehgari-Nejad et al., 2010**). Date syrup is a high energy food rich in carbohydrate, a good source of minerals; but it also contains a very complex mixture of other saccharides, amino and organic acids, polyphenols and carotenoids **Abbes et al., (2013)**. The ingredients of date syrup depend on the type of date, but generally date syrup contains fructose, glucose, moisture and a small amount of sucrose, protein, pectin, and calcium **Khosravanipouret al., (2011)**. Date syrup is used in the preparation of some traditional and industrial foods (**Mohamed and Ahmed, 1981 and Rofehgari-Nejad et al., 2010**). It is a natural sweetener that is a suitable ingredient to be used in the formulation of food products in order to improve the nutrient properties. The Date syrup is directly consumed or used as an ingredient in some food formulation such as ice cream products, drink, confectionery, bakery products, sesame paste/date syrup blends, jam and some other **Barreveld, (1993)**.

Some Studies have been done on using date syrup as a flavor in dairy products. The result of these studies have shown that low-fat cow milk prepared with palm syrup and fermented products prepared from milk being flavored with date syrup, led to good outcomes **Moneibet al., (1999)**.

The aim of the research is to produce a healthy and nutritious drink by recycling some of the nutritional secondary by-products.

Materials and methods

Materials

Milk, eggs, dibs (as sweetener) and vanilla were purchased from the local market of Cairo, Egypt . Bifidobacterium animals ssp. *lactis* Bb-12 ® was supplied by Chr . Hansen Inc . (Hoersholm,Denmark) . Carboxy methylcellulose (CMC) was obtained from Misr Food Additives-MIFAD.

All the materials were made available in the laboratory. Fresh cheese whey as obtained from the manufacturing of curd cheese was used as raw material for the beverage preparation. The eggs were cleaned by dusting, washing and allowed to dry. They were carefully deshelled and separated as egg white liquid and egg yolk liquid. The egg yolk homogenized with a metal whisk and dried at 45 °C for 5 h and allowed to cool. The egg yolk flakes was scooped, milled and sieved with a 0.09 mm size mesh according to **Kumaravelet al., (2012)**.Theeggshellswithout membranes were boiled for 20 min, then air dried, ground to a powder using a kitchen grinder and sieved to a fine powder by using a 0.09 mm size meshas described by **Fred et al.,(2006)**.Table1, shows the average composition of all ingredients used in the present study.

Table 1. Chemical composition of Sweet whey, Eggshell, Egg yolk and Date syrup (Dibs)

Content	Sweet whey	Eggshell	Egg yolk	Dibs
Total solids (%)	6.74	97.8	95.69	76.5
Protein (%)	0.96	2.3	32.2	0.95
Fat (%)	0.1	-	50	2.10
Carbohydrate (%)	4.93	-	8.1	71.88
Ash (%)	0.75	95.5	5.39	1.57

Preparation of fermented beverages

After blending the ingredients the amount of eggshell (containing 39% calcium), egg yolk (containing 6.8µg vitamin D), CMC and vanilla 1%, 0.1%, 0.01%) respectively with sweet whey, the beverage was pasteurized at 80 °C for 15min, cooled down to 40°C and inoculated with Direct Vat Set type Bb-12 culture (CHR Hansen, Denmark) containing *Bifidobacterium animalis* ssp. *lactis*. then incubated at 37 °C for 8 h and cooled to 25 °C. Dibs as a sweetener were added in different proportions such as T1(10%), T2 (12.5%) and T3(15%) to fermented whey, distributed into sterile plastic bottles (250 ml) and then stored at 4 ± 1°C (the resulting mixture were formulated under aseptic condition).

Analytical methods

All the chemical analysis viz. moisture, fat, total nitrogen, ash and minerals of fermented whey beverages and content of raw materials e.g. whey, date syrup, egg yolk powder (EYP) and eggshell powder (ESP) were followed by **AOAC(2019)**. Vitamin B2 determined according to **Danish Official (1996)**. Vitamin D determined according to **Danish Official (2001)**. The pH value was measured using Lab. pH meter with a combined electrode, Hanna digital pH meter. The viscosity was measured by Brookfield DV- III viscometer using spindle SC4-21 at rpm 20. The temperature was maintained at 25° C and viscosity value was expressed in centipoises (cp).

Microbiological analysis

Bifidobacterium animals ssp. *lactis* was enumerated using MRS agar media after the incubation at 37°C for 72 h. as reported by **Dave and Shah, (1996)**.

Molds & Yeasts were determined according to Standard Methods for Examination of Dairy Products **APHA (1994)**. Coliform bacteria were enumerated according to **Harrigan and McCance, (1996)** using Violet Red Bill Agar (VRBA). The plates were incubated at 37°C for 48 hr. *Staphylococcus* spp. and *Salmonella* spp. were detected according to methods recommended by **ICMSF (1996)**.

Color analysis

Color parameters L* (lightness or brightness), a* (redness or greenness), and b* (yellowness or blueness) as determined by CIElab color scale (**Hunter, LabScan XE-Reston VA, USA**) were measured using a Spectro colorimeter **Wei et al., (2012)**

Sensory evaluation

Expert judges and consumers were selected from a staff member of the Dairy Technology Department, Food Technology Research Institute, Giza, Egypt to evaluate the texture, flavor and overall acceptability of the fermented whey beverages. They scored the sample on the basis of a nine-point hedonic scale, ranging from like extremely = 9 through like or dislike = 5 to dislike extremely = 1 as described by **Lim J., (2011)**.

Statistical analysis

The data obtained (mean of three replicates) were statistically analyzed according to the statistical analyses system user's guide **SAS (2004)**. Analysis of variance (ANOVA) and Duncan's multiple

comparison procedure were used to compare the means. A probability of $p < 0.05$ was used to establish statistical significance.

Results and Discussion

Compositional analysis

Data in Table 2 indicated that, the sweetening using dibs led to a significant difference in total solids and carbohydrates of fermented whey beverages due to addition of different levels of dibs. It confirmed that, total solids and carbohydrates increased significantly ($p < 0.05$) as the level dibs increased, due to the high carbohydrate content of dibs. The obtained results indicated the, neither protein, fat nor ash contents were influenced significantly at any level of dibs. Regarding the minerals and vitamins contents of fermented whey beverages, data stated that no significant differences ($p > 0.05$) in the minerals and vitamins contents between treatments. Furthermore, the fermented whey beverage fortified with levels of dibs was rich in minerals because data syrup is a good source of minerals **Abbes et al., (2013)**. Also it is obvious from data in **Table 2** that, these results are consistent with what the WHO/FAO has recommended for vitamin B2 daily intake.

Table 2:Chemical analysis of fermented whey beverages

Content	Treatments		
	T ₁	T ₂	T ₃
Total solids (%)	16.0 ^c	17.7 ^b	19.5 ^a
Protein (%)	1.5	1.49	1.48
Fat (%)	0.8	0.85	0.9
Carbohydrate (%)	11.84 ^c	13.52 ^b	15.29 ^a
Ash (%)	1.86	1.84	1.83
Ca (mg/100)	317.93	317.81	317.15
P (mg/100)	213.71	213.67	213.63
K (mg/100)	766.72	766.90	766.91
Mg (mg/100)	28.21	28.34	28.52
Na (mg/100)	58.59	58.92	58.95
Zn (mg/kg)	9.190	9.200	9.200
Mn (mg /kg)	26.054	26.054	26.057
Fe (mg/kg)	152.66	152.78	152.95
Riboflavin (mg)	1.5	1.7	1.7
Vitamin D (µg)	0.66	0.66	0.65

Means with different superscript letters (a,b,c) in the same row are significantly different (p<0.05).

Microbiological quality

Table 3 represented the changes in logarithm numbers of *Bifidobacteriumlactis* viable count of various concentrations of dibs in fermented whey beverages. The count of *Bifidobacteri* remained high until the second week and began to diminish thereafter. Viable counts were gradually decreased with the prolongation of storage periods. The difference in the count of *Bifidobacteriumlactis* was not significant ($p>0.05$) between T₂ and T₃. These results agree with the trends reviewed by **Al-Otaibiet al.,(2013)** who showed that the addition of dibs can safely be used as sweeteners for the fermented milk in the presence of the probiotic bacteria. Results also indicated that the viable counts of Bb-12 were maintained at an acceptable level to be considered as a functional food (10^6 - 10^7 CFU/g), which is the recommended minimum daily intake **Sheehan et al., (2007)**

Molds & yeasts, *Staphylococcus spp.*, *Salmonella spp.*, sporformers and coliforms were not detected in all fermented whey

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treatments, either when fresh or during the storage period. This may be due to the high hygienic conditions during production and the production of antimicrobial agents by *bifidobacteria*.

Table 3: Viability of *Bifidobacteriumlactis* (log CFU/ml) in fermented whey beverage during cold storage at $4 \pm 1^{\circ}\text{C}$

Cold storage (days)	Treatments		
	T ₁	T ₂	T ₃
Fresh	6.92 ^{b,A}	7.53 ^{a,A}	7.56 ^{a,A}
7	6.83 ^{b,A}	7.52 ^{a,A}	7.52 ^{a,A}
14	6.53 ^{b,AB}	7.40 ^{a,AB}	7.43 ^{a,AB}
21	6.10 ^{b,B}	6.90 ^{a,B}	6.92 ^{a,B}

Small letters in superscript (a,b,..) describe the significance for the factor "dibs level. While Capital letters (A,B,..) in superscript describe the significance of the storage period. Means followed by the same letter do not differ significantly ($P>0.05$).

pH values and viscosity

As appears from Table 4, the addition of dibs caused an insignificant decrease in pH value ($p>0.05$), the decrease was proportional to the addition ratio. However, the decreasing in pH values was significantly after 2 weeks ($p\leq 0.05$). This may be attributed to the high concentration of components i.e. (immunoglobulin, lysozyme, glycomacropeptide and lactoferrin) in whey.

Results given in the same table indicated that the viscosity increased slowly with addition ratio of dibs. Also, results in Table 4 show that viscosity values, in all treatments were increased in significant differences ($p<0.05$) with the prolong of storage period. That could be due to the dietary fiber content (DF) in dibs that had desirable functional properties, such as providing texture, gelling, thickening, emulsification and stabilization in DF enriched foods **Nelson(2001)**.

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Table 4: pH values and viscosity of fermented whey beverages during cold storage at $4 \pm 1^{\circ}\text{C}$

	Cold storage (days)	Treatments		
		T ₁	T ₂	T ₃
pH	Fresh	6.49 ^{a,A}	6.43 ^{ab,A}	6.40 ^{b,A}
	7	6.41 ^{a,A}	6.38 ^{ab,A}	6.33 ^{b,A}
	15	6.29 ^{a,B}	6.20 ^{ab,B}	6.17 ^{b,B}
	21	6.20 ^{a,C}	6.14 ^{ab,C}	6.00 ^{b,C}
Viscosity (cp)	Fresh	10 ^{c,D}	15 ^{b,D}	30 ^{a,D}
	7	43.33 ^{c,C}	65 ^{b,C}	85 ^{a,C}
	14	80 ^{c,B}	110 ^{b,B}	133 ^{a,B}
	21	150 ^{c,A}	210 ^{b,A}	270 ^{a,A}

Small letters in superscript (a,b,..) describe the significance for the factor "dibs level. While Capital letters (A, B, ..) in superscript describe the significance of the storage period. Means followed by the same letter do not differ significantly ($P>0.05$).

Color parameters:

Whey beverages colors consider as an important factor for consumer acceptance. As seen in Table 5 the lightness (L-values) was markedly affected by the addition of dibs. The lightness degree of T₃ was higher than that of T₁ until at the end of the storage period (21 days). This tendency was recorded during the storage period. The same tendency was noticed for their redness (a-value) and yellowness (b-value) ($p<0.05$) by increasing the amount of dibs addition. Changes in color characteristics influenced by the addition of ingredients were statistically significant ($P < 0.05$), but not significant enough to have a negative effect on the product color changes.

Sensory evaluation

Sensory characteristics of fermented whey beverages with different dibs ratio during storage period are presented in Table 6. In general, sensory analysis of the treatments showed a significant difference ($P<0.05$) among the treatments for color, consistency, flavor and overall acceptability. A visual evaluation of the samples by

the panel showed that the effect of a different ration of dibs on the samples color characteristics was significant ($P < 0.05$). Also, T_2 and T_3 exhibited higher consistency scores than T_1 , the difference being significant ($P < 0.05$). As scores listed in Table 6, the flavor of whey beverages become more preferable to panelists with adding dibs in the blend up to 15% compared to 10%. The negative effect of added ingredients on flavor was noticed after 7 days. The sensory quality attributes of all treatments decreased after 14 days. These data accordance with **Sasi(2015)**. It is evident from Table (6) that, treatment T_2 gained significantly the highest average value of overall acceptability compared with other treatments.

The overall acceptability of fermented whey beverage blending with 12.5% dibs (T_2) was found to be superior. National consumer inclinations could also play an important role in sensory analysis. There are not too many whey-based drink products in the Egyptian market and there is no convention of drinking whey and whey-based beverages in the Egypt Republic. These products are more widely available and accepted by people in countries such as Germany, Austria, and Switzerland where there is a longer tradition of whey consumption.

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Table 5: Color parameters of fermented whey beverages during cold storage at $4 \pm 1^\circ\text{C}$

	Cold storage (days)	Treatments		
		T ₁	T ₂	T ₃
L	Fresh	51.45 ^{a,A}	49.82 ^{ab,A}	47.18 ^{b,A}
	7	51.33 ^{a,A}	49.51 ^{ab,A}	47.00 ^{b,A}
	14	51.20 ^{a,AB}	49.18 ^{ab,AB}	46.87 ^{b,AB}
	21	51.11 ^{a,B}	49.02 ^{ab,B}	46.69 ^{b,B}
A	Fresh	5.01 ^{b,B}	5.17 ^{ab,B}	5.35 ^{a,B}
	7	5.17 ^{b,AB}	5.23 ^{ab,AB}	5.40 ^{a,AB}
	14	5.21 ^{b,A}	5.31 ^{ab,A}	5.49 ^{a,A}
	21	5.29 ^{b,A}	5.43 ^{ab,A}	5.62 ^{a,A}
B	Fresh	18.19 ^{b,B}	18.43 ^{ab,B}	18.72 ^{a,B}
	7	18.24 ^{b,AB}	18.49 ^{ab,AB}	18.79 ^{a,AB}
	14	18.30 ^{b,A}	18.57 ^{ab,A}	18.88 ^{a,A}
	21	18.38 ^{b,A}	18.70 ^{ab,A}	18.97 ^{a,A}

Small letters in superscript (a,b,...) describe the significance for the factor "dibs level. While Capital letters (A, B, ..) in superscript describe the significance of the storage period. Means followed by the same letter do not differ significantly ($P>0.05$).

Table 6: Sensory evaluation of fermented whey beverages during cold storage at $4 \pm 1^\circ\text{C}$

	Cold storage (days)	Treatments		
		T ₁	T ₂	T ₃
Color and Appearance	Fresh	7.13 ^{b,A}	7.82 ^{a,A}	7.81 ^{a,A}
	7	7.00 ^{b,B}	7.67 ^{a,B}	7.55 ^{a,B}
	14	6.81 ^{b,BC}	7.50 ^{a,BC}	7.50 ^{a,BC}
	21	6.55 ^{b,C}	7.44 ^{a,C}	7.36 ^{a,C}
Consistency	Fresh	7.19 ^{b,B}	7.28 ^{a,B}	7.30 ^{a,B}
	7	7.53 ^{b,A}	7.62 ^{a,A}	7.69 ^{a,A}
	14	7.21 ^{b,BC}	7.37 ^{a,BC}	7.40 ^{a,BC}
	21	7.00 ^{b,C}	7.17 ^{a,C}	7.29 ^{a,C}
Flavor	Fresh	7.0 ^{b,B}	7.5 ^{a,B}	7.3 ^{a,B}
	7	7.5 ^{b,A}	8.2 ^{a,A}	8.1 ^{a,A}
	14	6.9 ^{b,BC}	7.8 ^{a,BC}	7.7 ^{a,BC}
	21	6.4 ^{b,C}	7.23 ^{a,C}	7.21 ^{a,C}
Over all acceptability	Fresh	7.21 ^{b,C}	7.7 ^{a,B}	7.63 ^{a,B}
	7	7.45 ^{b,A}	8.10 ^{a,A}	7.94 ^{a,A}
	14	7.1 ^{b,BC}	7.6 ^{a,BC}	7.48 ^{a,BC}
	21	6.65 ^{b,C}	7.3 ^{a,C}	7.2 ^{a,C}

Small letters in superscript (a,b,...) describe the significance for the factor "dibs level. While Capital letters (A, B, ..) in superscript describe the significance of the storage period. Means followed by the same letter do not differ significantly ($P>0.05$).

Conclusion

It can be concluded that whey based drink can be prepared by using 12.5% of dibs with the highest consumer acceptability as compared to the other treatment. Whey contains about half of the milk solids and addition of eggshell, egg yolk, dibs in the preparation of fermented whey drink increased the health and nutritional value. One cup of the produced drink 250 ml will cover daily calcium requirement of an adult person.

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مشروب شرش وظيفي جديد يحتوي علي الكالسيوم ودبس البلح

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الملخص العربي

تهدف هذه الدراسة الى استخدام المنتجات الثانوية الغذائية التي لها خصائص وظيفية وبيولوجية مثل شرش الجبن وقشر البيض في إنتاج مشروبات غذائية وصحية. تم تحضير مشروب شرش اللبن المتخمر من خلال مزج ١٪ قشر بيض ، ١٪ صفار بيض ، ٠,١٪ CMC و ٠,٠١٪ فانيليا مع شرش اللبن الحلو. والتلقيح بـ *Bifidobacterium animals ssp. LactisBb* 12 كبروبيوتيك. تم استخدام دبس التمر (Dibs) كمحلي طبيعي بمستويات مختلفة ١٠ و ١٢,٥ و ١٥٪ حجم / حجم من المشروب وتم تحليله من حيث الخصائص الكيميائية، الفيزيائية والميكروبيولوجية وكذلك الخصائص الحسية أثناء التخزين البارد عند 4 ± 1 درجة مئوية لمدة ٢١ يوماً ، أوضحت النتائج التي تم الحصول عليها أن قيم الأس الهيدروجيني انخفضت تدريجياً خلال التخزين البارد في حين زادت اللزوجة. والاعدادالبكتيرية للبيفيدو ظلت مرتفعة حتى ١٤ يوماً ثم بدأت في الانخفاض. أوضحت الدراسة أن مشروب شرش الجبن المتخمر الذي يحتوي على ١٢,٥٪ من دبس التمر أظهر تفوقاً في جميع الخصائص الكيميائية، الفيزيائية، الميكروبية والخصائص الحسية مقارنة بالمعاملات الأخرى. ويعتبر مشروب مرتفع القيمة الغذائية .