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The Production of Bio-yoghurt with Probiotic Bacteria, Royal Jelly and Bee Pollen Grains

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Abstract

In this study, the yoghurt and bio-yoghurt were produced using probiotic bacteria (*Lb. gasseri* ATCC 33323, *Lb. rhamnosus* DSM 20245 and *Bif. angulatum* DSM 20098 and/or *Lb. delbrueckii* subsp. *bulgaricus* and *Str. thermophilus* as yoghurt starter), royal jelly (0.6%) and bee pollen grains (0.8%). The samples were analyzed for chemical, rheological, sensory evaluation and microbiological interval during storage. Addition of the probiotic, royal jelly and bee pollen grains to the yoghurt starter cultures increased the coagulation time of the produced functional yoghurt than that of the control. The total solid, ash, fat, protein and acidity contents significantly increased while, lactose contents and pH values significantly decreased during storage period up to 21 days of all treatments. From the microbiology term there was decrease of the LAB, *Str. thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. gasseri* ATCC 33323, *Lb. rhamnosus* DSM 20245 and *Bif. angulatum* DSM 20098 during storage periods. Also, the probiotic level after three weeks of storage was greater than 6 log cfu/ml. The produced functional yoghurt had better sensory and rheological characteristics than those of control yoghurt; overall, all functional yoghurt treatments were acceptable up to the end of the storage period.

Keywords: Yoghurt; Probiotic; Royal jelly; Bee pollen grains; Chemical analysis

Abbreviations:

Lb: *Lactobacillus*; ATCC: American Type Culture Collection; DSM: Deutsche Sammlung von Mikroorganismen; Bif: *Bifidobacterium*; Subsp: Subspecies; Str: *Streptococcus*; RJ: Royal Jelly; BPG: Bee Pollen Grains; LAB: Lactic Acid Bacteria

Introduction

Functional foods generally contain one or more beneficial compounds such as probiotic, prebiotic and others. These foods present a potential to promote health by maximizing physiological functions of a person and not for the cure of illnesses [1]. The majority of probiotic products available in the marketplace contain species of *Lactobacillus* and *Bifidobacterium*, which are the main genera of Gram-positive bacteria currently characterized as probiotics [1,2]. Different species of probiotic microorganisms have been employed in the food industry, such as: *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus johnsonii*, *Lactobacillus rhamnosus*, *Lactobacillus thermophilus*, *Lactobacillus reuteri*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Bifidobacterium bifidum*, *Bifidobacterium longum*, *Bifidobacterium brevis*, *Bifidobacterium infantis* and *Bifidobacterium animalis* [3]. *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Streptococcus thermophilus* are found in a number of preparations such as traditional yoghurts, frozen yoghurts, and in desserts in some places [1,4]. Interest for probiotics has arisen in recent years especially in relation to the addition of *Bifidobacterium*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus casei*, and *Lactobacillus reuteri* to the fermented dairy products such as yoghurt [5]. Yoghurts and fermented milks are still the main vehicles for incorporation of probiotic cultures. A probiotic dairy product should contain at least 6-7 log cfu.g^{-1} of viable probiotic bacteria at the time of consumption and, should be consumed regularly in a quantity of higher than 100 g per day or in other words at least 9 log cfu per day [6]. Numerous scientific papers and review articles have been published on the health benefits associated with the consumption of fermented dairy products [7]. Therefore, every year the food industry develops new fermented milk products that are required by consumers for their health benefits [8].

Pollen is the male gametophyte of flowers. Honey bees collect pollen by adding sugars from nectar to hold the grains together and then transfer them back to the colony by packing them into hairs on the corbiculae (hind legs) of bees. Potential applications of pollen include its use in apitherapy and as a functional food in the food industry due to pollen nutritional properties [9]. Its nutritional values consists of proteins, lipids, sugar, fibers, minerals salts, amino acids, traces of micronutrients and vitamins (A, B, C, D, E). The therapeutic action has been attributed to several phenolic compounds with antioxidant activity, present in these products. All bee derived products such as honey, propolis and pollen have been applied for centuries in traditional medicine as well as in nutritional supplementation [10].

Another important bee product is royal jelly. It is a glandular secretion produced by worker bees to feed young larvae and queens. It belongs to a group of products described as "dietary supplements". In fact, the use of royal jelly is not so much linked to its high content in noble substances, but to its assumed stimulant and therapeutic value. If it was declared as a medicine, its use would become dependant on medical prescriptions and the production and marketing of royal jelly-based products would become the exclusive domain of the pharmaceutical industry. Also, various types of royal jelly exhibited antibacterial activity against food borne pathogenic bacteria [11]. FAO

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have reported that about 100-300 mg of royal jelly is the most commonly recommended daily dosage for human use [12].

The principal constituents of royal jelly are water (65%), protein (12%), sugars (15%), lipids (5%) and mineral salts. Although, they occur with notable variations, the composition of royal jelly remains relatively constant when comparing different colonies, bee races and time [13]. All amino acids essential for humans are present and a total of 29 amino acids and their derivatives have been identified, the most important being aspartic and glutamic acids [14]. A number of enzymes are also present including glucose oxidase. An insulin-like substance has been identified by Kramer et al. [15,16]. It contains thiamine, riboflavin, pantothenic acid, pyridoxine, niacin, folic acid, inositol and biotin and lipids [17].

This work aimed to supplement yoghurt with natural, nutritional, palatable and available bee products (probiotic, royal jelly and bee pollen grains) and to study the effect of adding these materials on some quality characteristics and probable changes of yoghurt during cold storage.

Materials and Methods

Strains and dairy ingredients

Fresh mixed milk (cows and buffaloes, 1:1) were obtained from the herds of Faculty of Agriculture, Moshtohor, Benha University, Toukh, Kaliobia, Egypt. Bee pollen grains (BPG) and royal jelly (RJ) was obtained during June, 2014, from the apiary of the Faculty of Agriculture, Moshtohor, Benha University, Toukh, Kaliobia, Egypt. Royal jelly was packed in opaque plastic vials, and kept frozen until use. Chemical composition of raw materials used for manufacture of the tested yoghurt is presented in Table 1.

Components		Milk	Royal Jelly	Bee pollen grains
Total solids %		14.971c	37.773a	35.910b
Ash %		0.798c	1.441b	3.001a
Fat %		3.20b	9.50a	3.90b
Protein %		3.673c	13.919b	20.894a
Total sugars %		5.00c	12.50b	8.10a
Titratable acidity %*		0.14c	0.601a	0.577b
Minerals (ppm)	Ca	1517.13a	468.77b	220.35c
	P	291.07a	279.90b	191.50c
	K	450.82a	471.02a	251.00b
	Mg	51.00a	39.90ab	11.56b
	Mn	0.052b	1.19a	0.032b
	Fe	7.92b	17.98a	6.92c
	Zn	4.00b	6.10a	3.09c

Table 1: Chemical composition of raw materials used in prepared yoghurt. *As anhydrous lactic acid, ppm: part per million, Means with the same letter are not significantly different.

Yoghurt starter cultures consisting of *Lb. delbrueckii* subsp. *bulgaricus* and *Str. thermophilus* (1:1) were obtained from Chr. Hansen's Laboratories, Copenhagen, Denmark. Probiotic bacteria including, *Bifidobacterium. angulatum* DSM 20098, *Lactobacillus gasseri* ATCC 33323 and *Lactobacillus rhamnosus* DSM 20245 were obtained from Institute of Microbiology, Federal Research Center for Nutrition and Food, Kiel, Germany.

Manufacture of probiotic yoghurt

Fresh mixed milk cows, and buffalos, (1:1) was standardized to ~3% fat, heated to 85°C for 30 min, immediately cooled to 42°C and divided into seven portions and starter culture was added as follows:

- C: 3 % yoghurt starter, (control contains *Lb. delbrueckii* subsp. *bulgaricus* and Str. *thermophilus*).
- T1: 1.5% yoghurt starters+1.5% *Lb. rhamnosus*+0.6% RJ.
- T2: 1.5% yoghurt starters+1.5% *Lb. gasseri*+0.6% RJ.
- T3:1.5% yoghurt starters+1.5% *Bif. angulatum*+0.6% RJ.
- T4: 1.5% yoghurt starters+1.5% *Lb. rRhamnosus*+0.8% BPG.
- T5: 1.5% yoghurt starters+1.5% *Lb. gasseri*+0.8% BPG.
- T6:1.5% yoghurt starters+1.5% *Bif. angulatum*+0.8% BPG.

All treatments were filled into leaded plastic cups (80 ml) and incubated at 42°C until the pH reached ~4.6. The yoghurt refrigerated at ~5°C and was analyzed for the chemical, rheological, microbiological analysis and sensory evaluation when fresh and after 7, 14 and 21 days, respectively.

Total solids, ash, fat and total protein contents were determined according to the methodology mentioned in AOAC, [18]. Titratable acidity was determined according to the methodology mentioned in BSI [19]. Total reducing sugars in milk and yoghurt samples were determined as described by Lawrance [20]. Minerals contents (Ca, P, K, Mg, Mn, Fe and Zn) were determined according to AOAC [19] using Perkin-elmer, and 2380 Atomic absorption spectrophotometry.

Lactic acid bacterial count (LAB), yeasts and moulds and colifrom bacteria were enumerated according to Elliker et al. [21]; IDF [22] and APHA [23], respectively. Yoghurt starter cultures (*Lb. delbrueckii* subsp. *bulgaricus* and *Str. thermophilus*) were enumerated in yoghurt samples as described by Ryan et al. [24] and also, the counts of *Bifi. angulatum, Lb. rhamnosus* and *Lb. gasser* were carried out as described by Martin et al. [25], Saxelin et al. [26] and Matijasic et al. [27] respectively.

Curd tension of yoghurt was measured as described by Kammerlehner et al. [28]. The quantity of whey which has separated from yoghurt samples after 2 h at 5°C (syneresis) of control and turmeric yoghurt was determined according to Dannenberg et al. [29].

The Sensory evaluation included flavour was given score 45 points; body and texture or consistency was score of 40 points and appearance was given score of 15 points which give a total score of 100 points [30]. The Sensory evaluation was done by 10 experienced food panelists of Food Science Department, Faculty of Agriculture, Moshtoher, Benha University.

The results were submitted to the analysis of variance (ANOVA) using the general linear model (GLM) procedure of the statistical Analysis System (SAS) [31]. The means were separated by use the least significant difference (LSD) test. Significance differences was determined at α=0.05 [32].

Results and Discussions

Coagulation time

The effect of probiotic, RJ and BPG on the coagulation time of produced yoghurt was significant ($P \leq 0.05$). From the obtained data, it was clear that the addition of probiotic, RJ and BPG to the yoghurt starter cultures increases the coagulation time of the yoghurt than that of the control (Figure 1). The obtained results are in agreement with those of Modzelewska et al. [33].

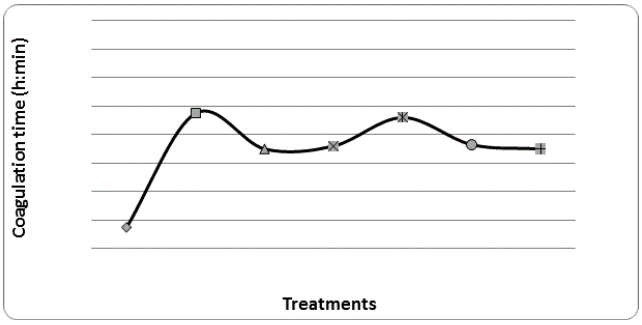

Figure 1: Effect of LAB, royal jelly and bee pollen grains on the coagulation time of produced yoghurt. C=Control (Yoghurt starter), T1=Yoghurt starter+*Bif. angulatum* DSM 20098+0.6% RJ, T2=" "+*Lb. rhamnosus* DSM 20245+0.6% RJ, T3=" "+*Lb. gasseri*, ATCC 33323+0.6% RJ, T4=" "+*Bif. angulatum* DSM 20098+0.8% BPG, T5=" "+*Lb. rhamnosus* DSM 20245+0.8% BPG, T6=" "+*Lb. gasseri* ATCC 33323+0.8% BPG.

Chemical composition of produced functional yoghurt

The main values for total solids, ash, fat, protein, total sugars and titratable acidity contents of produced functional yoghurt during storage at 5 ± 1°C for 21 days are illustrated in Table 2.

Treatments	Total solids %	Ash %	Fat %	Protein %	Total sugars %	Titratable acidity %
Fresh						
C	15.05dc	0.78dd	3.30dd	3.85gd	4.35ea	0.73ad
T1	15.64cc	0.81cd	3.35bd	3.90fd	4.55ba	0.72gd
T2	15.65bc	0.80bd	3.35cd	3.95ed	4.45ca	0.73cd
T3	15.66bc	0.81bd	3.40ad	3.92dd	4.41da	0.71ed
T4	15.69ac	0.81ad	3.30ed	3.98bd	4.64aa	0.71fd
T5	15.70ac	0.81ad	3.30ed	4.01cd	4.60aa	0.72bd
T6	15.71ac	0.81ad	3.25fd	4.00ad	4.61aa	0.72dd
7 days						
C	15.26db	0.81dc	3.40dc	3.94gc	3.80eb	0.82ac
T1	15.75cb	0.85cc	3.45bc	4.00fc	4.11bb	0.80gc
T2	15.80bb	0.85bc	3.45cc	4.01ec	4.10cb	0.80cc
T3	15.80bb	0.85bc	3.43ac	4.05dc	4.07db	0.80ec
T4	15.89ab	0.87ac	3.30ec	4.10bc	4.20ab	0.80fc
T5	15.90ab	0.87ac	3.35ec	4.11cc	4.23ab	0.81bc
T6	15.90ab	0.87ac	3.30fc	4.15ac	4.20ab	0.81dc
14 days						
C	15.31da	0.83db	3.43db	4.00gb	3.40ec	0.99ab
T1	15.83ca	0.89cb	3.47bb	4.05fb	3.90bc	0.88gb
T2	15.90ba	0.90bb	3.45cb	4.10eb	3.85cc	0.90cb
T3	15.87ba	0.90bb	3.45ab	4.15db	3.77dc	0.90eb
T4	15.95aa	0.91ab	3.40eb	4.20bb	4.01ac	0.88fb
T5	15.98aa	0.91ab	3.35eb	4.20cb	3.98ac	0.90bb
T6	16.00aa	0.91ab	3.35fb	4.22ab	3.97ac	0.90db
21 days						
C	15.35da	0.86da	3.45da	4.05ga	3.05ed	1.20aa
T1	15.85ca	0.92ca	3.47ba	4.10fa	3.40bd	0.95ga
T2	15.90ba	0.92ba	3.45ca	4.10ea	3.30cd	1.07ca
T3	15.90ba	0.92ba	3.50aa	4.20da	3.30dd	1.0ea
T4	15.97aa	0.96aa	3.40ea	4.25ba	3.62ad	0.98fa
T5	16.00aa	0.96aa	3.40ea	4.20ca	3.58ad	1.09ba
T6	16.01aa	0.97aa	3.40fa	4.25aa	3.60ad	1.05da

Table 2: Effect of LAB, royal jelly and bee pollen grains on chemical composition of the produced yoghurt during storage at 5 ± 1°C. C=Control (Yoghurt starter), T1=Yoghurt starter +*Bif. angulatum* DSM 20098+0.6% RJ, T2=" "+*Lb. rhamnosus* DSM 20245+0.6% RJ, T3=" "+*Lb. gasseri* ATCC 33323+0.6% RJ, T4=" "+*Bif. angulatum* DSM 20098+0.8% BPG, T5=" "+*Lb. rhamnosus* DSM 20245+0.8% BPG, T6=" "+*Lb. gasseri* ATCC 33323+0.8% BPG. Means with the same letter are not significantly different.

Total solids, ash, fat and protein contents significantly ($P \leq 0.05$) increased during storage in all treatments. As, expected, the supplemented with BPG of yoghurt resulted in an increase ($P \leq 0.05$) of total solids, protein, total sugars and ash contents compared to other treatments. This is consequence of the relatively high concentration of dry matter, ash and protein in BPG. The total solids, ash, fat and protein contents slight increased during the storage and this may be due to loss of moisture content during storage. Also, the titratable acidity content significantly increased ($P \leq 0.05$) with the progress of storage periods. But, total sugars content significantly decreased during the storage in all treatments.

This decrease may be due to the microbial fermentation. These results are in accordance with that of Metry et al. [34] and Yerlikaya [35].

Microbiological analysis of produced functional yoghurt

Monitoring the viability of five strains in yoghurt over 21 days has indicated trends that are related to the different species of organism

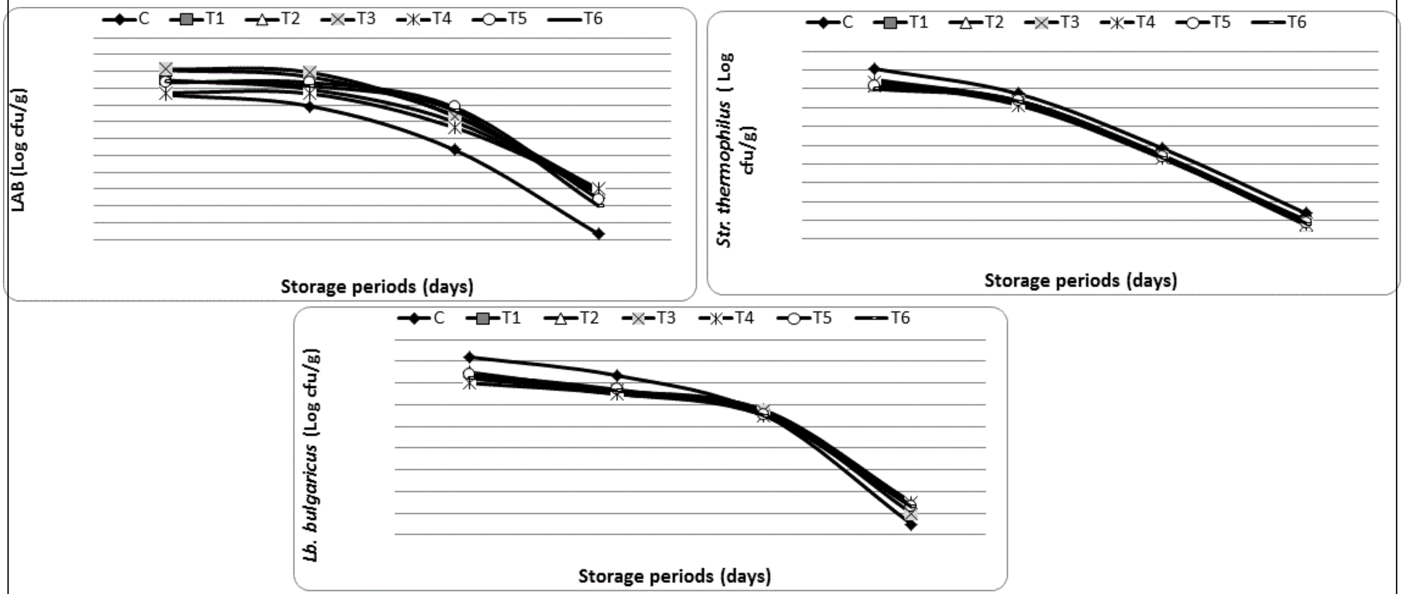

Figure 2: LAB, *Str. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* counts of produced functional yoghurt during cold storage. C=Control (Yoghurt starter), T1=Yoghurt starter+*Bif. angulatum* DSM 20098+0.6% RJ, T2=" "+*Lb. rhamnosus* DSM 20245+0.6% RJ, T3=" "+*Lb. gasseri* ATCC 33323+0.6% RJ, T4=" "+*Bif. angulatum* DSM 20098+0.8% BPG, T5=" "+*Lb. rhamnosus* DSM 20245+0.8% BPG, T6=" "+*Lb. gasseri* ATCC 33323+0.8% BPG.

tested (Figures 2 and 3). The results showed that there were significant decreases in the cell numbers of LAB; *Str. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* (Figure 2) during storage in all treatments ($P \leq 0.05$). The decrease of counts during storage of all treatments may be due to their sensitivity to the produced acidity in the product.

These results are in accordance with those given by Metry et al. [34] and Yerlikaya [35]. Figure 3 shows the descriptive statistics for the enumeration of *Lb. rhamnosus* in yoghurt over the 21 day storage period. *Lb. rhamnosus* counts recorded 8.2 and 8.3 log cfu.g^{-1}for T2 and T5, respectively when fresh yoghurt and then significantly decreased ($P \leq 0.05$). Also *Lb. gasseri* count of fresh yoghurt had changes to be 8.23 and 8.27 log cfu.g^{-1} for T3 and T6, respectively (Figure 3) then slowly decrease was noticeable up to the end of the storage period ($P \leq 0.05$). These results agree with that obtained by Abdel-Khalek et al. [36] and Hekmat et al. [37]. *Bif. angulatum* count recorded 8.11 and 8.12 log cfu.g^{-1} for T1 and T4, respectively (Figure 3) when fresh yoghurt samples and then significantly ($P \leq 0.05$) declined until the end of storage in other treatments. The decrease of bifidobacterial counts may be due to the developed acidity during storage periods. Similar trends were obtained by Prasanna et al. [38]. Colifrom bacteria and yeasts and moulds were not detected in all yoghurt treatments either fresh or stored which are due to the high hygienic conditions during the reparation and storage of yoghurt. This was in agreement with those of Metry and Owayss [34]. Generally, the viable counts of probiotic bacteria remained above 106 cfu.g^{-1} in yoghurt treatment until the end of storage. In this respect, yoghurt must contain viable starter culture counts at the time of consumption ranging between 106-107 cfu/g to produce the health benefits of those microorganisms.

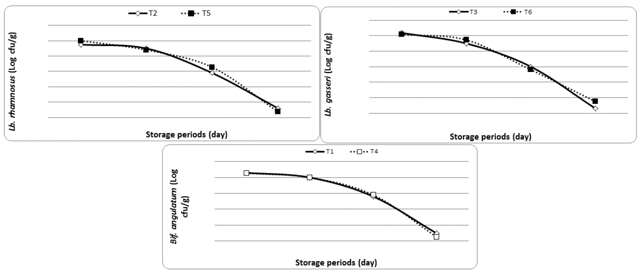

Figure 3: *Lb. rhamnosus*, *Lb. gasseri* and *Bif. angulatum* counts of produced functional yoghurt during cold storage.

Mineral content and Rheological properties of yoghurt treatments

The influence of probiotic, RJ and BPG on the mineral contents of resulting yoghurt treatments is illustrated in Table 3.

It could be noticed that incorporation of RJ and BPG to yoghurt leads to an increase in its content of minerals, considering that RJ and BPG are rich sources of minerals. The mineral content was significantly different ($P \leq 0.05$) between all functional yoghurt. Addition of RJ and BPG increased greatly but variably the Ca, P, K, Mg, Mn, Fe and Zn contents of the prepared yoghurt. Functional yoghurt with RJ was found to be a better source for Ca, P, K, Mg, Mn, Fe and Zn than that of functional yoghurt with BPG, due to the variable contents of these elements in the used ingredients.

The curd tension of functional yoghurt was measured as a penetration distance in (0.1 mm at 5 sec). The higher recorded by the

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of functional yoghurt with BPG, due to the variable contents of these elements in the used ingredients.

The curd tension of functional yoghurt was measured as a penetration distance in (0.1 mm at 5 sec). The higher recorded by the penetrometer, the less curd tension of yoghurt. Figure 4 shows the changes in the penetrometer reading (0.1 mm/5 sec) of produced yoghurt during cold storage.

Treatments	Mineral contents (ppm)						
	Ca	P	K	Mg	Mn	Fe	Zn
C	1526.10a	300.00a	455.70a	53.10a	0.065c	7.95b	4.50a
T1	1532.90a	310.20a	467.50a	57.50a	0.587b	13.55a	4.91a
T2	1532.52a	309.56a	477.32a	56.74a	0.610a	13.97a	4.85a
T3	1533.45a	312.21a	469.60a	56.45a	0.606a	13.90a	4.80a
T4	1529.65a	305.40a	459.55a	54.31a	0.071c	8.01b	4.60a
T5	1528.70a	306.32a	460.62a	55.00a	0.070c	7.99b	4.62a
T6	1530.00a	304.21a	461.77a	55.11a	0.077c	8.05b	4.65a

Table 3: Effect of LAB, royal jelly and bee pollen grains on mineral contents of produced yoghurt. C=Control (Yoghurt starter), T1=Yoghurt starter+*Bif. angulatum* DSM 20098+0.6% RJ, T2=" "+*Lb. rhamnosus* DSM 20245+0.6% RJ, T3=" "+*Lb. gasseri* ATCC 33323+0.6% RJ, T4=" "+*Bif. angulatum* DSM 20098+0.8% BPG, T5=" "+*Lb. rhamnosus* DSM 20245+0.8% BPG, T6=" "+*Lb. gasseri* ATCC 33323+0.8% BPG.

The results showed that the curd tension of yoghurt treatments containing RJ and BPG were significantly (P ≤ 0.05) decreased, which might be due to the high content of total solids in RJ and BPG which led to an increase in the curd tension of the resulting yoghurt samples. There is an inverse relationship between the levels of total solids and syneresis (wheying off).

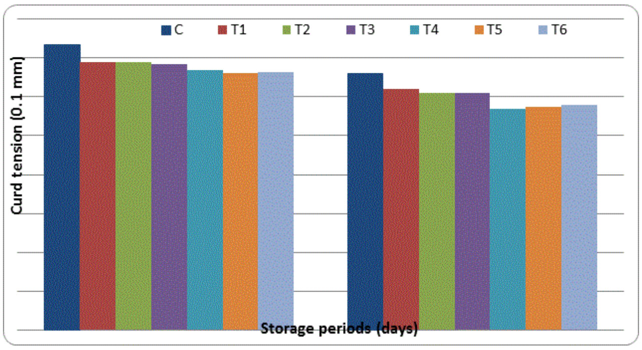

Figure 4: Curd tension of produced functional yoghurt during storage periods at 5±1°C. C=Control (Yoghurt starter), T1=Yoghurt starter+*Bif. angulatum* DSM 20098+0.6% RJ , T2=" "+*Lb. rhamnosus* DSM 20245+0.6% RJ, T3=" "+*Lb. gasseri* ATCC 33323+0.6% RJ, T4=" "+*Bif. angulatum* DSM 20098+0.8% BPG, T5=" "+*Lb. rhamnosus* DSM 20245+0.8% BPG , T6=" "+*Lb. gasseri* ATCC 33323+0.8% BPG.

Also, the addition of RJ and BPG significantly (P ≤ 0.05) decreased the syneresis value, compared to control (Figure 5). Control yoghurt had the highest value of syneresis, while T5 had the lowest value. These results are in accordance with those given by Metry et al. [34].

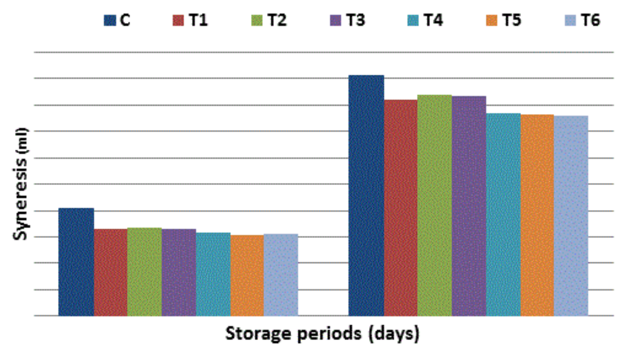

Figure 5: Syneresis of produced functional yoghurt during storage at 5±1°C. C=Control (Yoghurt starter), T1=Yoghurt starter+*Bif. angulatum* DSM 20098+0.6% RJ, T2=" "+*Lb. rhamnosus* DSM 20245+0.6% RJ, T3=" "+*Lb. gasseri* ATCC 33323+0.6% RJ, T4=" "+*Bif. angulatum* DSM 20098+0.8% BPG, T5=" "+*Lb. rhamnosus* DSM 20245+0.8% BPG, T6=" "+*Lb. gasseri* ATCC 33323+0.8% BPG.

Sensory evaluation

Data in Table 4 showed that all the functional yoghurts recorded highest scores (P ≤ 0.05) than control one when fresh and throughout the interval storage periods. Addition of probiotic, RJ and BPG significantly improved the flavour and body and texture of yoghurt compared to control. Addition of probiotic strains improved the sensory properties due to their high level of the produced flavour compounds i.e., (diacetyl, acetyl methyl carbinol, acetaldehyde, TFA, etc.) and the low level of the produced acidity. Also, additional of probiotic, RJ and BPG have the ability to decrease the sourness of yoghurt; this function can serve to increase consumer acceptability of acidic products such as yoghurt.

Resultant fresh yoghurt samples produced from T1 gained the highest scores (P ≤ 0.05) for overall acceptability compared to other treatments. During cold storage 5 ± 1°C, the sensory evaluation scores increased for all treatments after 7 days and then significantly (P ≤ 0.05) declined until the end of storage in other treatments.

Generally, values of flavour and body and texture were more affected (P ≤ 0.05) in fresh yoghurt samples and during storage, while the judgments did not show any significant difference in appearance and colour score by incorporation of RJ and BPG during storage compared with control samples.

Also, those findings are in agreement with Metry et al. [34] who found that the addition royal jelly up to 0.6% improved the sensory quality of resultant yoghurt without having a detrimental effect on characteristic of lactic acid bacteria.

Treatments	Flavour (45 points)	Body and texture (40 points)	Appearance (15 points)	Total (100 points)
Fresh				
C	41.0fb	36.0cc	14.5ca	91.5eb
T1	43.0ab	36.0bc	15.0aa	94.0ab
T2	42.0bb	36.0bc	15.0aa	93.0bb
T3	42.0cb	36.0bc	15.0aa	93.0cb
T4	41.5db	36.5ac	14.5ba	92.5db
T5	41.0eb	36.5ac	14.5ba	92.0db
T6	41.5db	36.5ac	14.5ba	92.5db
7 days				
C	43.0fa	38.0ca	15.0ca	96.0ea
T1	44.0aa	39.0ba	15.0aa	98.0aa
T2	44.0ba	39.0ba	15.0aa	98.0ba
T3	44.0ca	39.0ba	15.0aa	98.0ca
T4	43.5da	39.5aa	14.5ba	97.5da
T5	43.5ea	39.5aa	14.5ba	97.5da
T6	43.5da	39.5aa	14.5ba	97.5da
14 days				
C	38.0fc	37.5cb	14.0cb	89.5eb
T1	41.0ac	38.0bb	15.0ab	94.0ab
T2	41.0bc	38.0bb	15.0ab	94.0bb
T3	40.0cc	38.0bb	15.0ab	93.0cb
T4	39.5dc	39.0ab	14.5bb	93.0db
T5	39.0ec	39.0ab	14.5bb	92.5db
T6	39.0dc	39.0ab	14.5bb	92.5db
21 days				
C	25.0fd	25.0cd	10.0cc	60.0ec
T1	35.5ad	37.0bd	12.0ac	84.5ac
T2	35.5bd	37.0bd	12.0ac	84.5bc
T3	35.5cd	37.0bd	12.0ac	84.5cc
T4	34.0dd	38.0ad	12.0bc	84.0dc
T5	34.0ed	38.0ad	12.0bc	84.0dc
T6	34.0dd	38.0ad	12.0bc	84.0dc

Table 4: Sensory evaluation of produced yoghurt with LAB, royal jelly and bee pollen grains during storage periods at 5±1°C. C=Control (Yoghurt starter), T1=Yoghurt starter+*Bif. angulatum* DSM 20098+0.6% RJ, T2=" "+*Lb. rhamnosus* DSM 20245+0.6% RJ, T3=" "+*Lb. gasseri* ATCC 33323+0.6% RJ, T4=" "+*Bif. angulatum* DSM 20098+0.8% BPG, T5=" "+*Lb. rhamnosus* DSM 20245+0.8% BPG, T6=" "+*Lb. gasseri* ATCC 33323+0.8% BPG .

Conclusion

The results of this study showed that, yoghurt can be successfully made using probiotic (*Bifi. angulatum, Lb. rhamnosus* and *Lb. gasseri*), and RJ and BPG with a good sensory characteristic and nutritional quality of the resultant yoghurt during cold storage up to 21 days. The mineral content was significantly different ($P \leq 0.05$) between all functional yoghurt. Addition of RJ and BPG increased greatly but variably the Ca, P, K, Mg, Mn, Fe and Zn contents of the prepared yoghurt. Functional yoghurt with RJ was found to be a better source for Ca, P, K, Mg, Mn, Fe and Zn than that of functional yoghurt with BPG.

From the foregoing results it could be concluded that, yoghurt can be successfully made using probiotic, RJ and BPG, gave the best acceptability and nutritional quality of the resultant yoghurt during cold storage up to 21 days.

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