

## Effect of Incorporating Royal Jelly and Bee Pollen Grains on Texture and Microstructure Profile of Probiotic Yoghurt

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### Abstract

The investigation was performed to evaluate the influence of royal jelly (RJ) and/or bee pollen grains (BPG) on texture and microstructure profile of probiotic yoghurt made from mixed (1:1) standardized cow and buffalo milks (~3% fat). The probiotic bacteria used *Lb. gasserii*, *Lb. rhamnosus* and *Bif. angulatum* with the normal yoghurt starter (*Lb. delbrueckii* subsp. *bulgaricus* and *Str. thermophilus*). The yoghurts were cooled and stored for 21 days and analyzed for their textural characteristics, syneresis and microstructure. The yoghurt samples included RJ and/or BPG was appeared more stable during cold storage up on 21 days. No significant differences ( $P>0.05$ ) were recorded in springiness and cohesiveness between sample incorporated RJ and/or BPG and control sample. However, the syneresis was significantly ( $P<0.05$ ) decreased in yoghurt with RJ and/or BPG compared to control. The SEM micrograph demonstrated the samples contained RJ and/or BPG have the casein micelles relatively uniformly distributed and were similar in size.

**Keywords:** Yoghurt; Probiotic; Royal jelly; Bee pollen grains; Texture; Microstructure

### Introduction

Yoghurt is one of the most important fermented dairy products widely because it has many health benefits such as improving lactose intolerance, anticholesterolemic impacts, and reducing risk cancers [1] and other benefits related to probiotic bacteria [2]. Generally, yoghurt can be produced from a differ type of milk, such as bovine milk and/or buffalo milk [3]. While traditionally the yoghurt produced using bovine milk and *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* as starter cultures [4]. The yoghurts and fermented milks are still the main vehicles for incorporation of probiotic cultures as dietary adjuncts [5,6]. Generally, the probiotic microorganisms have been employed in the yoghurts, such as *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus johnsonii*, *Lactobacillus rhamnosus*, *Lactobacillus reuteri*, *Lactobacillus thermophiles*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Bifidobacterium brevis*, *Bifidobacterium bifidum*, *Bifidobacterium longum*, and *Bifidobacterium infantis* [7]. A probiotic dairy product should contain at least 6-7 Log CFU-g<sup>-1</sup> of viable probiotic bacteria at the time of consumption and, should be consumed regularly in a quantity of higher than 100 g per day [8].

The microstructure of yoghurt is the most important characteristics, and has a major effect on the texture and other physical properties of acid milk [9]. The common methods used to improve structure and/or texture depends on increase of total solids in the milk or addition of stabilizers [10]. The microstructure has been observed using scanning electron microscopy (SEM) and confocal scanning laser microscopy (CSLM) [9]. One study found that the polymerized whey protein has been improved firmness and syneresis of goats' milk yoghurt [11]. Another study has shown that polysaccharides derived from plant cells of fruit used as gelling agents and stabilizers in low-pH yoghurt [12]. In recent years, there has been growing interest in the utilize of natural additives and health-promoting substances into yoghurt such as royal jelly and bee pollen grains to improve the nutritive value and microstructure properties. Royal jelly (RJ) is a natural substance, produced from worker bees to feed the bee queens and young larvae also, high nutritional value, functional characteristics and biological properties [13]. Additionally, it is a good source of protein, sugars,

lipids, vitamins, enzymes, mineral, and contains about 29 amino acids, which known as beneficial effects on human health [14,15]. Generally, royal jelly is acidic medium (pH 3.1-3.9) with a high buffering capacity ranges 4 and 7 [16]. Royal jelly can be included directly in food and/or dietary supplements; furthermore, royal jelly is well known to have some medical or biological purposes [17], antibacterial [18], antifatigue [19], antioxidant activity [20], anti-allergic [21], and anti-inflammatory [22]. The RJ is affirmed with (100-300 mg) as recommended daily dosage [23].

Bee pollen grains (BPG) are a male gametophyte of flowers, and high contains of proteins, carbohydrates, fats, minerals, vitamins, and phenolic substances [24]. Widely used as an apitherapy, alternative and traditional medicine. The pollen grain used in functional foods and it is very rich in protein, which serves as material for tissue growth and tissue regeneration. The amount of protein ranges greatly, depending of the plant species. Considering chemical composition, besides protein, pollen also includes: free amino-acids, lipids, carbohydrates (sugars, starch and cellulose), minerals (Ca, Mg, P, Fe, Na, K, Al, Mn, S, Cu, etc.), vitamins (pantothenic and ascorbic acid, vitamins of B complex, etc.), various enzymes and coenzymes, etc. For the honey bees, it is the best if they have access to pollen derived from different plants, because balanced nutrition is of key significance for the development of honey bee colony [24]. This study aimed to develop a bio-yoghurt, mixing the health benefits of probiotic bacteria with the RJ and PBG. The effect of adding RJ and PBG on textural profiles, syneresis and microstructure of yoghurt during storage for 21 days at  $4 \pm 1^\circ\text{C}$  was studied.

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## Materials and Methods

### Materials

Fresh cow and buffalo milks were obtained from the Faculty herds, and bee pollen grains (BPG) and royal jelly (RJ) was obtained from the apiary of the Faculty of Agriculture, Benha University, Egypt. The RJ was packed in opaque plastic vials, and kept frozen until used. Yoghurt starter consisting of *Lactobacillus delbrueckii* spp. *bulgaricus* and *Streptococcus thermophilus* (1:1) were obtained from Chr. Hansen's Laboratories, Copenhagen, Denmark. *Bifidobacterium angulatum* DSM 20098, *Lactobacillus rhamnosus* DSM 20245 and *Lactobacillus gasseri* ATCC 33323 were obtained from Institute of Microbiology, Federal Research Center for Nutrition and Food, Kiel, Germany.

### Preparation of yoghurt

Fresh mixture (1:1) of cow and buffalo milks was standardized to ~3% fat, heated to 85°C for 30 min, immediately cooled to 42°C [3] and divided into seven portions as represented in Table 1. All treatments were filled into plastic cups (~100 ml), and incubated at 42°C until the pH reached ~4.6. Yoghurt from different treatments was kept refrigerated at 4 ± 1°C and analyzed for its textural profiles, syneresis and microstructure.

### Texture profile analysis

Texture profile analysis (TPA) test was performed by using a TA XT2 texture analyzer (Stable Micro Systems Ltd, Godalming, and Surrey, UK). An SMS P/0.5 probe was used to measure the TPA of the samples at room temperature, which was done in 3 repetitions. During the pretest, compression, and relaxation of a sample, the speed of the probe was 1.0 mm/s, while the speed of obtaining the data was 200 pps. The thickness of the samples was set at 5 cm and 30% of the original depth was compressed during the first stage [25].

### Whey separation (syneresis)

Syneresis was determined according to the method described by Dannenberg and Kessler [26].

### Microstructural analysis

The microstructure of yoghurt was analyzed by scanning electron microscopy (SEM) technique (Joel, JSM-6460LV Scanning Electron Microscope, Oxford, Instruments). The samples were prepared according to the following condition (fixation in 2.8% glutaraldehyde, dehydration in different ethanol solution percentages, extraction with chloroform, dehydrated in absolute ethanol for 24 h, dried using a "Critical Point Dryer" (CPD 030, BALTEC, Liechtenstein) and coating with gold (BALTEC, SCD 005, Sputter coater) as described by Sandoval-Castilla et al. [27]. The voltage used for SEM watching was 25 kV.

No.	Treatments	Formula
I	Control (C)	3% yoghurt starter
II	Treatment 1 (T1)	1.5% yoghurt starter + 1.5% <i>Lb. rhamnosus</i> + 0.6% RJ*
III	Treatment 2 (T2)	1.5% yoghurt starter + 1.5% <i>Lb. gasseri</i> + 0.6% RJ
IV	Treatment 3 (T3)	1.5% yoghurt starter + 1.5% <i>Bif. angulatum</i> + 0.6% RJ
V	Treatment 4 (T4)	1.5% yoghurt starter + 1.5% <i>Lb. rhamnosus</i> + 0.8% BPG**
VI	Treatment 5 (T5)	1.5% yoghurt starter + 1.5% <i>Lb. gasseri</i> + 0.8% BPG
VII	Treatment 6 (T6)	1.5% yoghurt starter + 1.5% <i>Bif. angulatum</i> + 0.8% BPG

\* RJ: Royal jelly; \*\*BPG: Bee pollen grains

Table 1: Different treatments of produced yoghurt.

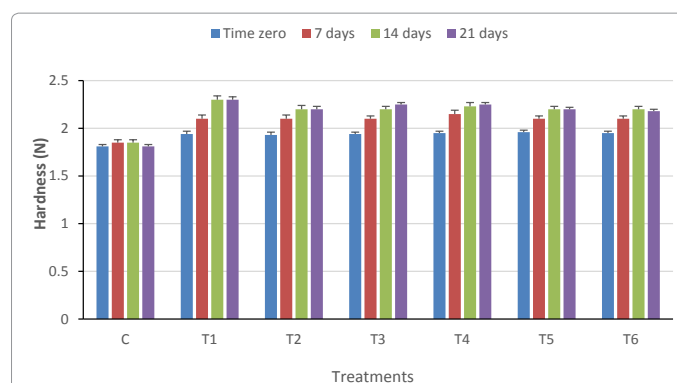


Figure 1: Hardness of produced yoghurt treatments during storage period at 4 ± 1°C.

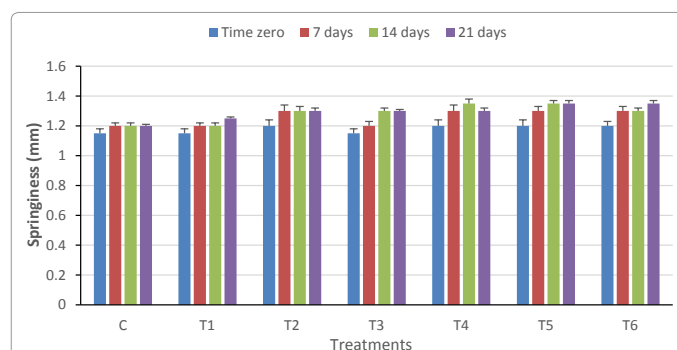


Figure 2: Springiness value of produced yoghurt treatments during storage period at 4 ± 1°C.

### Statistical analysis

The statistical analysis was carried out using statistical program (MSTAT-C 1989) with multi-function utility regarding to the experimental design under significance level of 0.05 for the whole results. Multiple comparisons applying LSD were carried out according to Finney [28].

## Results and Discussion

### Textural characteristics of yoghurt

Texture profile analyses simulate the conditions of a product in the mouth by compressing. The texture of different treated yoghurts was evaluated using a texture analyzer by means of the following attributes: hardness (HD), springiness (SP), cohesiveness (CO), gumminess (GU), and chewiness (CH), respectively. Results in part demonstrate that, except for the cohesiveness significantly improved the textural characteristic of yogurts.

**Hardness:** Hardness is described to the maximum force required for the first compression of food between the molars; also, it is a critical parameter for evaluation of textural attributes. The data in Figure 1, demonstrated the highest hardness was measured in T5 yoghurt (1.96 ± 0.02 N), while the lowest one was observed in control sample yogurt (1.81 ± 0.02 N). During the cold storage of yoghurt samples, the hardness was gradual increase in all samples incorporated (RJ) or BPG) compared the control sample up to 21 days. The increased hardness is related to an improvement of the textural of the yoghurt and makes the yoghurt less susceptible to rearrangements within its network and consequently less susceptible to shrinkage and serum expulsion [29]. These findings are in agreement with Metry and Owayss [30].

**Springiness:** Springiness is originally called “Elasticity” and it is important parameters of yoghurts. As shown in Figure 2, no significant differences ( $P>0.05$ ) were detected between yoghurt contained (RJ or BPG) and control sample at time zero. The springiness was increased during the storage period up to 21 days. The greater springiness may be related to stronger gel structures, due to increased charged groups on the amino acids groups a function of whey protein denaturation [31].

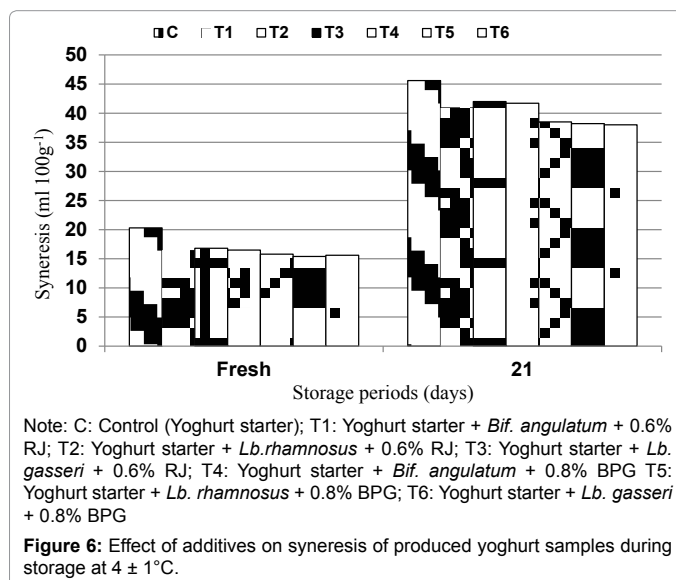
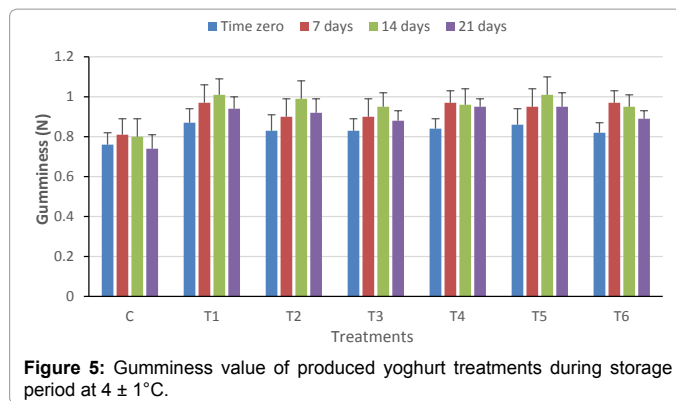
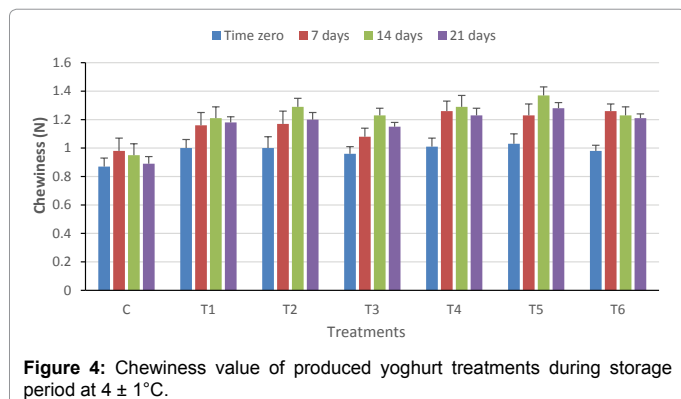
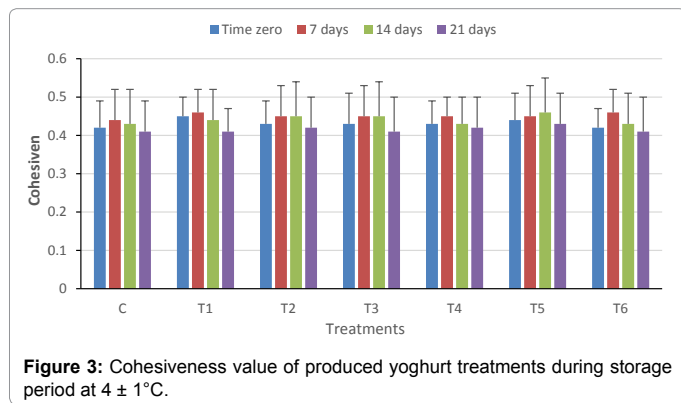
**Cohesiveness:** Cohesiveness known as the extent of deformation before rupture, therefore, cohesiveness values are a direct function of the work needed to overcome the internal bonds of the material. Cohesiveness indicates structural integrity and is often discussed in terms of the bond strength; whereas springiness reflects the structural integrity of yoghurt. All probiotic yoghurt treatments were no significant differences ( $P>0.05$ ) of cohesiveness value during storage period (Figure 3). Cohesive yoghurts may be pulled into threads or strings and may have more stickiness in the mouth, influencing the consistency and texture negatively [32].

**Gumminess:** Gumminess is expressed as the energy required disintegrating a semi solid food product to a state ready for swallowing. The progressive significant increase in gumminess with increase ( $P<0.05$ ) in storage period until 21 days could be attributed to the progressive increase in the hardness and cohesiveness of all treatments (Figure 4).

**Chewiness:** Chewiness is expressed as the energy required chewing a solid food product to a state where it is ready for swallowing. Chewiness was increased during storage period until 21 days could be attributed to the progressive significant increase ( $P<0.05$ ) in the springiness and gumminess of all samples (Figure 5).

### Syneresis of yoghurt

The impact of RJ and BPG on the syneresis contents of yoghurt

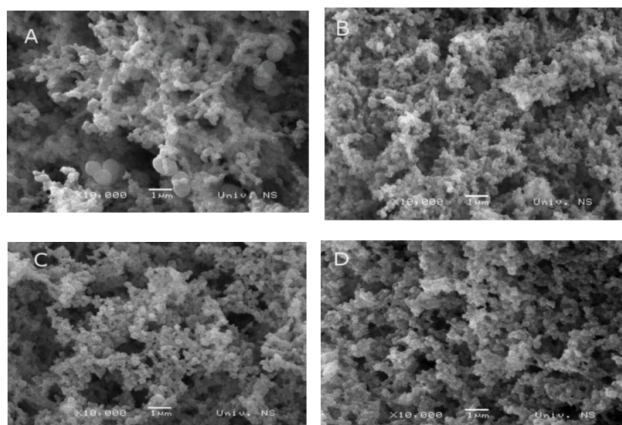


treatments is illustrated in Figure 6. There is an inverse relationship between the levels of total solids and syneresis. The addition of RJ (T1, T2, T3) and BPG (T4, T5, T6) was significantly ( $P<0.05$ ) decreased the syneresis value, compared to control sample (C). However, the control sample had the highest value of syneresis, while T5 had the lowest value. These results are in accordance with those given by Metry and Oways [30].

### Microstructures analysis of yoghurt

The microstructures of cow/buffalo milk yoghurt with RJ and BPG in (T1, T2 and T4) are shown in Figure 7. The SEM micrograph illustrated that the casein micelles appeared relatively uniformly distributed and were similar in size (Figure 7A). Figures 7B-7D showed that the appearance of casein micelles was more uniformly distributed and similar in size. These differ were probably due to the interactions between casein micelles and RJ or BPG through mainly hydrophobic interaction leading to the formation of casein matrix.

The casein micelles are play the major role in acid coagulation ( $\sim\text{pH}$  4.6) of milk, based on a reduction in surface charge (zeta potential) from the originally high net negative charges in milk to near no net charge. As well, the solubilization of a colloidal calcium phosphate which is a structural unit within micelles also occurs during acidification. Therefore, the hydrophobic interactions increase and results in the formation of a three-dimensional network of casein micelles linked together in chains, clusters and strands [33].



Note: C: Control (Yoghurt starter); T1: Yoghurt starter + *Bif. angulatum* + 0.6% RJ; T2: Yoghurt starter + *Lb. rhamnosus* + 0.6% RJ T4: Yoghurt starter + *Bif. angulatum* + 0.8% BPG

**Figure 7:** Microstructure (SEM) of yoghurt prepared without any addition (control, C1) (A), and with the addition of T1 (B), T2 (C) and T4 (D).

The SEM analysis for the microstructure of probiotic yoghurt showed that when RJ and/or BPG were added to the yoghurt a relatively more comprehensive network was formed thus resulting in improved consistency and water holding capacity of the cow/buffalo' milk yoghurt. The obtained results were in an agreement with those reported by Wang et al. [9].

## Conclusion

In the current study, the impact of natural compounds such as royal jelly and bee pollen grains on yoghurt characteristics were evaluated. The yoghurt samples contained RJ and/or BPG were appeared stable during cold storage up on 21 days. No significant differences ( $P > 0.05$ ) were observed in springiness and cohesiveness between sample incorporated RJ and/or BPG and control. However, the syneresis was significantly ( $P < 0.05$ ) decreased in yoghurt with RJ and/or BPG compared to control. The SEM micrograph demonstrated the samples contained RJ and/or BPG have the casein micelles relatively uniformly distributed and were similar in size.

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