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].
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, Mohstedor, Benha University, Toukh, Kaliobia, Egypt. Bee pollen grains (BPG) and royal jelly (RJ) was obtained from Institute of Microbiology, Federal Research Center for Nutrition and Food, Kiel, Germany.

The tested yoghurt is presented in Table 1.

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. rRhannusos+0.8% BPG.
- T5: 1.5% yoghurt starters+1.5% Lb. gasser+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 coliform 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 Bif. angulatum, Lb. rhamnosus and Lb. gasser were carried out as described by Martin et al. [25], Saxelin et al. [26] and Mattijsic et al. [27] respectively.

Curd tension of yoghurt was measured as described by Kammerlehenner et al. [28]. The quantity of whey which has separated from yoghurt samples after 2 h at 5°C (syneresis) of control and yoghurt were 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].

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.

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.

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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 ≤ 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 ≤ 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 ≤ 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 ≤ 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]. Coliform 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 ≤ 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
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.

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).

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].

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, addition 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.
### 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.

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<th>Body and texture (40 points)</th>
<th>Appearance (15 points)</th>
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<td></td>
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</tr>
<tr>
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<td>36.0 cc</td>
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<td>91.5 eb</td>
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<td>36.0 bc</td>
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<td>93.0 bb</td>
</tr>
<tr>
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<td>42.0 cb</td>
<td>36.0 bc</td>
<td>15.0 aa</td>
<td>93.0 cb</td>
</tr>
<tr>
<td>T4</td>
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<td>36.5 ac</td>
<td>14.5 ba</td>
<td>92.5 db</td>
</tr>
<tr>
<td>T5</td>
<td>41.0 eb</td>
<td>36.5 ac</td>
<td>14.5 ba</td>
<td>92.0 db</td>
</tr>
<tr>
<td>T6</td>
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<td>36.5 ac</td>
<td>14.5 ba</td>
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<tr>
<td>C</td>
<td>43.0 fa</td>
<td>38.0 ca</td>
<td>15.0 ca</td>
<td>96.0 ea</td>
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<tr>
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Conclusion

The results of this study showed that, yoghurt can be successfully made using probiotic (Bif. 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 ≤ 0.05) between all functional yoghurt. Addition of RJ and BPG increased greatly but variably the Ca, P, 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.

References