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Influence of Incorporating Honey and Royal Jelly on The Quality of Yoghurt During Storage

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THE PRESENT study was carried out to investigate the effect of adding different concentrations of bee honey (BH) and / or royal jelly (RJ) on the viability of yoghurt starter culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*) and monitoring the quality of yoghurt during storage at 5 ± 1°C. The study included two parts; the first part concerned the influence of adding different concentrations of each of BH and RJ on the viability of starter cultures (*in vitro*), incubation time and overall acceptability of the resultant yoghurt. Based on the preliminary results, the second part tested the effect of best added concentrations of both BH and / or RJ on certain chemical, rheological, microbiological and organoleptic properties of the resultant yoghurt, compared with the control. Significant differences ($P \leq 0.05$) in the viability of starter cultures, incubation time and overall acceptability were observed by adding different concentrations of BH or RJ, compared with the control. The increase in incubation time and the decrease in viability of yoghurt starter were proportional to the concentrations of BH and RJ. Moreover, BH and RJ improved significantly the viability of starter culture and acceptability of yoghurt till 4% and 0.6%, respectively. The variations in the mean values of ash, total solids and total nitrogen content of yoghurt samples were found to be significant ($P \leq 0.05$) during storage. However, there was insignificant difference between treatments in the fat content, while there was significant decrease in pH, total reducing sugars and diacetyl content during storage. As expected, the addition of BH and / or RJ increased the mineral content in the resultant yoghurt, compared to the control. Yoghurt samples contained 0.6% RJ had the lowest count of yeasts and moulds compared to the other treatments. Rheological properties of the resultant yoghurt were significantly affected by incorporation of RJ with or without BH. Generally, samples made with adding 4 % BH + 0.2 % RJ, for sweetened yoghurt, or 0.6 % RJ, resulted in the best quality of yoghurt during storage at 5 ± 1°C for 9 days.

Recently, there has been an increasing interest in the use of natural food additives and incorporation of health-promoting substances into diet. The food industry can contribute to a more healthy and balanced diet through improvements in the nutritional quality of products (Roodenburg *et al*., 2008).Yoghurt is one of the most popular fermented milk products worldwide because it has many health benefits such as improving lactose intolerance, reducing risk of certain cancers, anticholesterolaemic effects, prevention of genital and urinary tract infections (Savadogo *et al*., 2006) and other health attributes associated with probiotic bacteria (Mckinley, 2005).

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In this concern, implementing a special milk product is a target to individuals who need certain food supplements or healthy substances. Of these substances bee products such as honey and royal jelly are among some of the most remarkable and versatile nutrients. Bee products are emerging as truly exceptional in their ability to protect against disease and aging.

Honey is a very complicated food substance. Major components in honey, on average, are water (17.2%), fructose (38.2%), glucose (31.3%), sucrose (1.3%), maltose (7.3%), polysaccharides (1.5%), free acids (0.43%) such as gluconic, formic, oxalic acids...etc., ash (0.169%) and nitrogen (0.041%). Minor substances (1-3%) which are responsible for important honey properties include minerals, vitamins, hormones, enzymes, antioxidants and other unidentified components (FAO, 1996 and Baltrušaityte *et al*., 2007). The use of honey as a therapeutic substance has been rediscovered by the medical profession in more recent times, and it is gaining acceptance as an antibacterial agent for the treatment of peptic ulcers, gastritis, upper gastrointestinal dyspepsia including doudenitis and ulceration and gastroenteritis in infants (Molan, 1992 and 1999). Some studies have demonstrated its effect against foodborne pathogens and food spoilage organisms (Mundo *et al*., 2004 and Taormina *et al*., 2001).

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 foodborne pathogenic bacteria (Attalla *et al.,* 2007). FAO (1996) have reported that about 100 - 300 mg of royal jelly is the most commonly recommended daily dosage for human use.

The principal constituents of royal jelly are water (65%), protein (12%), sugars (15%), lipids (5%) and mineral salts (Stocker, *et al.*, 2005). Although, they occur with notable variations, the composition of royal jelly remains relatively constant when comparing different colonies, bee races and time (Otani *et al.,* 1985). 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 (Howe *et al.,* 1985). A number of enzymes are also present including glucose oxidase. An insulin-like substance has been identified by Kramer *et al*. (1977 and 1982). Royal jelly contains thiamine, riboflavin, pantothenic acid, pyridoxine, niacin, folic acid, inositol and biotin and lipids (Vecchi et *al*., 1988). A surprising and completely new finding by Stocker *et al*. (2005) that royal jelly, as a form of lactation on the insect level, shows the same homeostatic adjustment as mammalian and human milk.

Few researches have focused on the effect of fortifying yoghurt with honey (Varga, 2006; El–Baz & Zommara, 2007 and Abd El-Rahman & Salama, 2008). Also, data on

the capability of yoghurt starter organism to use honey to grow is relatively sparse in the scientific literature (Chick *et al.*, 2001 and Ustunol & Gandhi, 2001). This work aimed to supplement yoghurt with natural, nutritional, palatable and available bee products (honey and royal jelly) and to study the effect of adding these materials on some quality characteristics and probable changes of yoghurt during storage at 5 ± 1°C for 9 days.

Materials and Methods

The present study was performed at the Dairy Processing Pilot Plant, Dairy Sci. Dept., Fac. Agric., Fayoum Univ., Egypt. Fresh buffalo's milk was obtained from the herd of the Military Farm, Demo Region, Fayoum Governorate, Egypt. Starter cultures containing lyophilized strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* (*Str. thermophilus* and *Lb. bulgaricus*; 1:1) were obtained from Chr. Hansen's Lab., Copenhagen, Denmark. Bee honey (BH); clover, *Trifolium alexandrinum,* honey was obtained during June, 2009, from the apiary of the Fac. Agric., Fayoum Univ. Honey was produced by colonies headed with first hybrid Carniolan queen bees, *Apis mellifera carnica*, and situated in wooden standard Langstroth's hives. After harvesting and ripening honey by ordinarily beekeeping practices, honey samples were packed and kept in transparent plastic jars away from direct light at room temperature until use. Royal jelly (RJ) was produced from another set of colonies, under the same conditions, by dequeening colonies for 1 day. After three days of dequeening, royal jelly was gathered from naturally conducted queen cells, samples were packed in opaque plastic vials, and kept frozen at 0°C until use. No chemical treatments or extraordinary practices were done to the tested bee colonies during the production period. Chemical composition and pH values of raw materials used for manufacture of the tested yoghurt is presented in Table 1.

Lyophilized starter cultures were propagated in fresh sterile cow skim milk (9.8% total solids) 6-7 successive transfers of mother cultures were done before use, and prepared bulk culture was kept at fridge and used within 48 hr.

TABLE 1. Chemical composition and pH values of raw materials used for manufacture of yoghurt.

Raw Materials	Moisture (%)	Fat (%)	T.N (%)	T.R.S (%)	Ash (%)	pH	Minerals (ppm)							
							Ca	P	Fe	Zn	Mn	Ni	Mg	K
Milk	84.893	3.00	0.797	5.38	0.929	6.65	1339.60	267.83	7.18	3.90	0.058	3.37	42.50	465.22
Honey	18.000	0.04	0.042	70.00	0.414	3.60	252.00	337.72	46.19	10.43	3.01	9.97	42.76	1013.20
Royal Jelly	68.229	6.80	2.380	7.10	1.191	3.40	79.68	251.14	19.05	5.14	1.24	3.78	37.25	481.00

T.N: Total nitrogen T.R.S: Total reducing sugars ppm: Part per million

Two experiments were designed to examine the effects of BH and RJ on the viability of starter cultures and quality of resultant yoghurt during storage at 5±1°C for 9 days. In the first experiment**;** different concentrations of BH or RJ were first placed in Petri-dishes to examine their effects on the viability of the starter cultures (*Str. thermophilus* and *Lb. Bulgaricus*) separately. Then, M17 and MRS agar media were poured for the first and second microbes, respectively. These plates were incubated at 37°C for 48 and 72hr, respectively. Also, in the first experiment yoghurt was produced by adding different concentrations of each BH or RJ as shown in Fig. 1. The incubation time (time required for yoghurt to reach the coagulation pH of 4.7 at 42 ± 1°C) and overall acceptability of fresh yoghurt treatments were recorded. While in the second experiment, according to the results obtained from experiment 1, suitable concentrations of BH and RJ were added to milk, yoghurt was prepared as illustrated in Fig. 1, where, 40 kg milk were divided into four equal portions. The first portion served as control (without any additives), the second had 4 % BH, the third had 4 % BH plus 0.2 % RJ and the fourth had 0.6 % RJ(w/v), respectively. The resulting yoghurt samples were analyzed for the tested parameters when fresh and after 3, 6 and 9 days of storage at 5 ± 1°C except moisture, total nitrogen, fat, ash, total reducing sugars and diacetyl contents which were determined when fresh and after 9 days period, the minerals content and rheological properties (viscosity, syneresis and curd tension) were measured in fresh samples only.

All experiments were repeated in triplicates, each analysis was done twicely and the averages were calculated.

The counts of *Str. thermophilus* and *Lb. bulgaricus* were carried out by using M17 and MRS agar media, respectively as described in Oxoid (1990)**.** Coliform bacteria, yeasts and moulds were enumerated on MacConkey's agar and potato dextrose agar medium, respectively as described in the American Public Health Association (APHA, 1994).

Moisture contents in milk, RJ and yoghurt samples were carried out according to the AOAC (2000), while in BH, the method reported by FAO (1986) was applied using Abbe refractometer; reading of refractive index was converted, after correction of temperature. Fat and ash contents were estimated according to AOAC (2000). Total reducing sugars were determined immediately after coagulation and the rate of its decrease, as a function of activity of starter during incubation time, was calculated. Total reducing sugars (as lactose) in milk and yoghurt samples were determined as described by Lawrance (1968), whereas in BH and RJ were determined colorimetrically (as glucose) using the phenol-sulfuric method outlined by Dubois *et al.* (1956). The method of Dave and Shah (1997) was used to determine titratable acidity (as % lactic acid). For all samples, total nitrogen was determined by the macro-Kjeldahl method as reported in AOAC (2000). The pH values were measured using a pH-meter (CG71 Schott – Gerate GMBHD 6238). Mineral contents were determined in ash according to AOAC (2000) as follows; atomic absorption spectrophotometer (ZEISS, AAS 5, Germany) was used to measure Fe, Zn, Mn, Ni, Ca and Mg contents, while K was determined using a flame photometer (JENWAY,PFP7,7837,UK) whereas, P content was determined according to the method described by Morrison (1964). Diacetyl was estimated in yoghurt samples as described by Krampitz (1957).

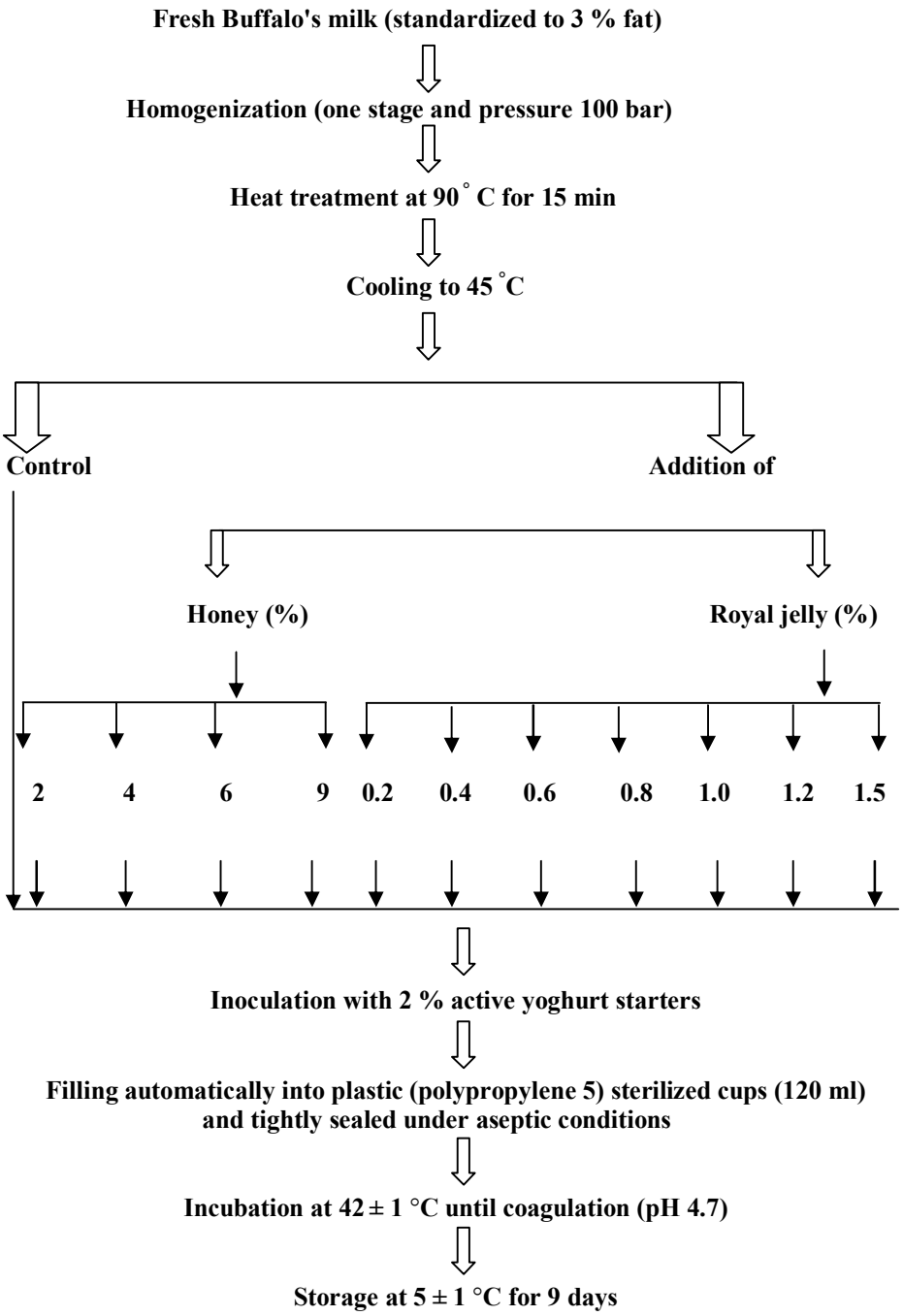

Fig. 1. Flow chart showing different treatments of manufactured yoghurt.

Viscosity was measured after 24 hr at 25 ^{o}C ± 0.5 at 50 rpm using RV spindle No.3 (DV-II + Viscometer Brookfield Engineering Labs. Inc. Middle Boro, MA02346, USA). The method of Dannenberg and Kessler (1988) was used to measure syneresis (the resistance to wheying off); the amount of whey which drained off was collected after 3 hr at 10 ^{0}C (in a cooling incubator) and taken as an index for syneresis (ml / 100 g yoghurt sample). Curd tension was determined as described by EL–Dieb (1995).

Fresh and stored (for 3, 6 and 9 days) yoghurt samples were judged for flavor (45 points), body & texture (40 points) and color & appearance (15 points) by fifteen experienced judges of the staff members of Dairy Sci. Dept. and Food Sci. Dept., Fac. Agric., Fayoum Univ. The score card was designed as described by Bodyfelt *et al.* (1988).

For the statistical analysis, the experimental design used was randomized complete blocks with three replications. Except for the values in Table 2, all the rest of the values were factorially analyzed. All data were subjected to statistical analysis according to Snedecor and Cochran (1980). Comparisons of the means were carried out using the least significant difference (LSD) at P≤ 0.05 level.

TABLE 2. Effect of different concentrations of honey and royal jelly on the viability of starter cultures, incubation time and overall acceptability of fresh yoghurt (Mean ± SD).

Type of addition	Ratio (%)	Counts of starter cultures (log cfu/g)		Incubation Time (hours)	Overall acceptability (100 degree)*
		Str. thermophilus	*Lb. bulgaricus*		
Control	Zero	8.64± 0.73 ^{b}	7.87 ± 0.46 ^{b}	3.30 ± 0.05 ^{e}	96 ± 2.03^{ab}
Royal Jelly	0.2	8.75 ± 0.52 ^{b}	8.12 ± 1.03 ^{ab}	3.23 ± 0.02 ^{ef}	96 ± 2.03 ^{ab}
	0.4	8.83 ± 0.74 ^{b}	8.39 ± 0.64 ^{a}	3.22 ± 0.07 ^{ef}	95 ± 2.08 ^{ab}
	0.6	8.89 ± 0.74 ^{b}	8.60 ± 0.56 ^{a}	3.02± 0.12 ^{e}	95 ± 2.08 ^{ab}
	0.8	7.48± 0.82 ^{c}	6.94 ± 0.15 ^{c}	3.85 ± 0.05 ^{d}	90 ± 3.06 ^{b}
	1.0	4.82 ± 0.63 ^{d}	5.45 ± 0.60 ^{cd}	5.49 ± 0.05 ^{c}	82 ± 5.86^{c}
	1.2	4.68 ± 0.33 ^{d}	5.22 ± 0.11 ^{cd}	6.20 ± 0.05 ^{b}	77 ± 1.73^{c}
	1.5	3.88 ± 0.64 ^{e}	3.41 ± 0.74 ^{e}	7.52 ± 0.07 ^{a}	71 ± 6.03^{d}
Honey	2.0	8.50 ± 0.38 ^{b}	8.86 ± 0.26^{a}	3.23± 0.03 ^{ef}	96 ± 1.73^{b}
	4.0	9.98 ± 0.61^{a}	9.20 ± 0.84^{a}	3.15 ± 0.05 ^{f}	98 ± 1.00^{a}
	6.0	7.95 ± 0.03 ^{c}	7.83 ± 0.46^{b}	5.20 ± 0.02 ^{d}	99 ± 1.00^{a}
	9.0	6.20 ± 0.61^{d}	4.83 ± 0.32^{d}	7.18± 0.12 ^{a}	91 ± 3.06^{c}

SD: Standard deviation cfu/g: colony forming units per gram yoghurt.
* Total score of sensory acceptability.
Values marked with the different alphabetical letter (s) within a comparable group of means are statistically different using revised LSD test at P ≤ 0.05.

Results and Discussion

Viability of starter organisms, incubation time and overall acceptability of yoghurt as affected by adding different concentrations of honey or royal jelly

To select the suitable concentrations of both BH and RJ during the manufacture of yoghurt, preliminary experiments were carried out to examine their incorporating effect on the viability of yoghurt starter organisms *(in vitro)*, fermentation time (incubation time) of milk and overall acceptability of the resultant yoghurt.

Significant differences ($P \leq 0.05$) were found in the viability of starter cultures, incubation time and overall acceptability by adding different concentrations of BH or RJ, compared with control. The addition of BH or RJ to *Str. thermophilus* and *Lb. bulgaricus* showed significant effect on their numbers (Table 2). RJ enhanced the growth of both *Str. thermophilus* and *Lb. bulgaricus* up to 0.6 % and 0.4 %, respectively. Then, the counts of starter strains decreased and did not comply with the minimum count of 10^6 cfu / ml with increase of RJ (at 1.0 % or more). Also, the log viable count of *Lb. bulgaricus* was slightly less than that of *Str. thermophilus* at the same concentrations. On the other hand, the viability of yoghurt starter was improved when the concentration of honey was 4 %, which could be attributed to the high mineral and sugar contents in honey (Table 1). These results are in accordance with those reported by Kajiwara *et al.* (2002). In contrast, Varga (2006) demonstrated that the presence of honey at 1.0% to 5.0% (w/v) did not significantly influence the viability of *Str. thermophilus* and *Lb. bulgaricus*. Otherwise, the counts decreased with the increase of BH concentration, which might be due to its antimicrobial characteristics such as the presence of organic acids and hydrogen peroxide (Mundo *et al*., 2004). The differences in incubation time were insignificant ($P \leq 0.05$) up to 0.6 % RJ and 2% BH. However, a significant increase in incubation time was observed by increasing the concentrations of RJ and BH up to 1.5% and 9%, respectively compared with the control. This could be attributed to the exceeded concentrations of RJ and BH, which led to the reduction in the activity of the yoghurt starters. Moreover, the results obtained also showed that the overall sensorial acceptability of the resultant yoghurt samples insignificantly increased ($P \leq 0.05$) with either RJ or BH until 6.0 % and 0.6%, respectively, but tended to decrease significantly ($P \leq 0.05$) by increasing the concentrations of RJ or BH. This could be attributed to taste deviations (increased sweetness in case of BH and sour taste in case of RJ), as well as undesirable texture, especially, at higher concentrations, which lead to be refused by judgments. The obtained preliminary results also indicated that yoghurt manufactured by adding 0.6% RJ or 4.0% BH gave the best quality.

Chemical composition of yoghurt from different treatments during storage at 5 ± 1°C

The chemical composition of yoghurt samples produced from different treatments during storage at 5 ± 1°C for 9 days are illustrated in Table 3. According to the results obtained, the variations in the moisture content of yoghurt samples were found to be insignificant ($P \leq 0.05$) during storage, but it was significant among treatments. The presence of BH or RJ had insignificant influence on fat content in the resultant yoghurt, while cold storage significantly ($P \leq 0.05$) increased the mean values of fat, total nitrogen and ash contents, as a result of water loss. However, total reducing sugar and diacetyl content significantly decreased. These findings are in agreement with

those of Essawy *et al.* (2005) and Abd El-Rahman and Salama (2008). As expected the reducing sugars before fermentation were higher in T_2 and T_3 than T_1 and T_4, which were 5.72, 5.79, 5.30 and 5.32 %, respectively. This increase was attributed to the high content of reducing sugars in the honey (Table 1). The % decrease of reducing sugars (during the incubation time) in yoghurt samples containing honey (T_2) was more than that of other treatments which recorded; 16.98%, 26.57 %, 20.21%, and 18.42% for T_1, T_2, T_3, and T_4, respectively. This might be due to the high sugars content in honey (Table 1) and high activity of yoghurt starters as shown in Table 4. Also, the results showed that further significant reduction of reducing sugars content was observed in all treatments during storage. This decrease was due to their fermentation by starter cultures. The decrease rates during storage were; 38.64%, 48.81%, 49.35%, and 33.41% for T_1, T_2, T_3 and T_4 and, respectively. Obviously, diacetyl values decreased significantly ($P \leq 0.05$) during storage in all yoghurt samples. Similar results were obtained by Salama *et al*. (2002). It could also be noticed that adding BH and /or RJ had significant effect on diacetyl content, compared to control (T_1).

As can be seen from Table 4, the presence of BH slightly increased the titratable acidity (T.A) in fresh samples, compared with other treatments. As expected, the mean value of T.A of fresh samples was significantly lower than those of stored samples which is due to the partial fermentation of lactose, whereas the pH values had opposite trend to those shown by T.A. Both BH and RJ had significant effect ($P \leq 0.05$) on pH values of yoghurt samples.

Microbiological analysis of yoghurt from different treatments when fresh and during storage

The mean counts for viability of yoghurt starters as affected by BH and RJ are shown in Table 4. The counts of *Str. thermophilus* and *Lb. bulgaricus* significantly decreased during storage period in all treatments. This might be due to the decrease of pH during storage. These results are in accordance with those given by Lankaputhra *et al.* (1996) and Saccaro *et al.* (2009). The incorporation of BH and / or RJ enhanced the growth of yoghurt starters. The obtained findings are similar to those reported by Kajiwara *et al.* (2002) and Zommara *et al.* (2004) who reported that honey acts as a prebiotic because it contains fructose and oligosaccharides which might be the primary components contributing to enhance the growth and promoting of lactic and acetic acids production by *Bifidobacterium ssp*. and yoghurt starter. Otherwise, these results are in contrast to those of Roumyan *et al*. (1996), who found a considerable inhibition in the growth of *Lb. bulgaricus* when testing the influence of honey added to the starter organisms in Bulgarian yoghurt.

In contrast the effect of cold storage on the viability of yoghurt starter was more pronounced than the effect of BH and/or RJ. The viable counts of *Str. thermophilus* and *Lb. bulgaricus* remained above 10^6 cfu / g in yoghurt treatments (T_4, T_2 and T_3) compared with control (T_1) until the end of storage period, the viable counts of yoghurt starter in T_1 samples did not comply with the minimum count of 10^6 cfu / g at the end of storage (9 days). In this respect, yoghurt must contain viable starter counts at the time of consumption ranging between 10^6- 10^8cfu / g as minimum values to produce the health benefits of these microorganisms (Anonymous, 2004).

TABLE 3. Effect of honey and/ or royal jelly on chemical composition of yoghurt from different treatments during storage at 5 ± 1°C.

Storage periods (days)	Treatments				Treatment effect (Mean ± SD)
	Moisture (%)				
	T_1	T_2	T_3	T_4	
Fresh	83.95^{a}	82.40^{a}	82.28^{a}	83.40^{a}	83.01 ± 0.80^{A}
9	83.73^{a}	82.34^{a}	82.26^{a}	83.35^{a}	82.92 ± 0.73^{A}
Mean ± SD	83.84± 0.16^{A}	82.37 ± 0.04^{C}	82.27 ± 0.01^{CD}	83.37 ± 0.03^{B}	
	Total nitrogen (%)				
	T_1	T_2	T_3	T_4	
Fresh	0.814^{b}	0.805^{bcd}	0.800^{d}	0.817^{ab}	0.809 ± 0.01^{B}
9	0.822^{a}	0.811^{bc}	0.813^{bc}	0.825^{a}	0.818 ± 0.01^{A}
Mean ± SD	0.818 ± 0.01^{A}	0.808 ± 0.01^{B}	0.807 ± 0.01^{BC}	0.821 ± 0.01^{A}	
	Fat (%)				
	T_1	T_2	T_3	T_4	
Fresh	3.17^{a}	3.10^{a}	3.10^{a}	3.17^{a}	3.13 ± 0.04^{A}
9	3.27^{a}	3.25^{a}	3.28^{a}	3.27^{a}	3.27 ± 0.01^{B}
Mean ± SD	3.22 ± 0.07^{A}	3.18 ± 0.11^{A}	3.19 ± 0.13^{A}	3.22 ± 0.07^{A}	
	Ash (%)				
	T_1	T_2	T_3	T_4	
Fresh	0.937^{a}	0.907^{a}	0.893^{a}	0.927^{a}	0.916 ± 0.02^{A}
9	0.987^{a}	0.936^{a}	0.916^{a}	0.971^{a}	0.952 ± 0.3^{B}
Mean ± SD	0.962 ± 0.03^{A}	0.922± 0.02^{BC}	0.905 ± 0.02^{C}	0.949 ± 0.03^{AB}	
	Total reducing sugars (%)				
	T_1	T_2	T_3	T_4	
Fresh	4.40^{ab}	4.20^{b}	4.62^{a}	4.34^{b}	4.39± 0.17^{A}
9	2.70^{c}	2.15^{d}	2.34^{d}	2.89^{c}	2.52 ± 0.34^{B}
Mean ± SD	3.55 ± 1.20^{A}	3.18 ± 1.45^{B}	3.48 ± 1.61^{A}	3.61 ± 1.02^{A}	
	Diacetyl (ppm)				
	T_1	T_2	T_3	T_4	
Fresh	24.10^{d}	30.94^{c}	38.88^{a}	33.52^{b}	31.86 ± 6.14^{A}
9	18.18^{f}	21.96^{e}	24.91^{d}	20.68^{e}	21.43 ± 2.80^{B}
Mean ± SD	21.14± 4.19^{D}	26.45 ± 6.35^{BC}	31.90 ± 9.88^{A}	27.10± 9.08^{B}	

Values marked with the same letter (s) are not significantly different using revised LSD test at $P \leq 0.05$.

Capital letter (s) indicates differences between main effects, and small letter (s) indicates differences within interaction of each character.

SD: Standard deviation. ppm: Part per million

T_1: Control (without additives) T_2: 4% honey T_3: 4% honey + 0.2 % royal jelly T_4: 0.6 % royal jelly

TABLE 4. Effect of honey and/or royal jelly on titratable acidity, pH and microbiological analysis of yoghurt from different treatments during storage at 5 ± 1°C.

Treatments	Storage periods (days)				Treatment effect (Mean ± SD)
	Titratable acidity (%)				
	Fresh	3	6	9	
T_1	0.77[a]	0.87[a]	1.04[a]	1.12[a]	0.95 ± 0.16 [A]
T_2	0.80[a]	0.86[a]	0.90[a]	1.23[a]	0.95 ± 0.16 [A]
T_3	0.78[a]	0.87[a]	0.96[a]	1.03[a]	0.91 ± 0.10 [B]
T_4	0.77[a]	0.86[a]	0.94[a]	1.09[a]	0.91 ± 0.10 [B]
Mean ± SD	0.78 ± 0.01 [D]	0.86 ± 0.01 [C]	0.96 ± 0.06 [B]	1.12 ± 0.08 [A]	
pH values					
T_1	4.50[a]	4.37[a]	4.15[a]	3.93[a]	4.24 ± 0.25 [A]
T_2	4.48[a]	4.28[a]	4.07[a]	3.73[a]	4.14 ± 0.32 [B]
T_3	4.57[a]	4.32[a]	4.22[a]	4.03[a]	4.28 ± 0.22 [A]
T_4	4.57[a]	4.30[a]	4.02[a]	3.95[a]	4.21 ± 0.28 [AB]
Mean ± SD	4.53 ± 0.05 [A]	4.32 ± 0.04 [B]	4.11± 0.09 [C]	3.91 ± 0.13 [D]	
***Lb. bulgaricus* (log cfu/g)**					
T_1	8.14[a]	7.65[a]	7.10[a]	5.95[a]	7.21 ± 0.94 [B]
T_2	8.92[a]	8.20[a]	7.07[a]	6.41[a]	7.65 ± 1.12 [A]
T_3	9.45[a]	8.74[a]	7.52[a]	6.78[a]	7.87 ± 1.44 [A]
T_4	9.29[a]	7.98[a]	7.39[a]	6.49[a]	7.79 ± 1.17 [A]
Mean ± SD	8.95 ± 0.58 [A]	8.14 ± 0.46 [B]	7.02 ± 0.36 [C]	6.39 ± 0.34 [D]	
***Str. thermophilus* (log cfu/g)**					
T_1	8.35[b]	8.24[b]	7.52[c]	5.89[f]	7.50± 1.14 [B]
T_2	9.23[a]	8.35[b]	7.58[c]	6.01[ef]	7.79 ± 1.36 [A]
T_3	9.45[a]	8.23[b]	6.73[d]	6.37[def]	7.69 ± 1.42 [AB]
T_4	9.33[a]	8.54[b]	7.41[c]	6.50[de]	7.94 ± 1.24 [A]
Mean ± SD	9.09 ± 0.50 [A]	8.34 ± 0.14 [B]	7.31 ± 0.39 [C]	6.19 ± 0.29 [D]	

Cfu: colony forming units / g yoghurt.
SD: Standard deviation
Values marked with the same letter (s) are not significantly different using revised LSD test at $P \leq 0.05$.
Capital letter (s) indicates differences between main effects, and small letter (s) indicates differences within interaction of each character.
T_1: Control (without additives) T_2: 4% honey T_3: 4% honey + 0.2 % royal jelly T_4: 0.6 % royal jelly

The results obtained also illustrated that no growth of either yeasts or moulds could be detected in all fresh samples but, was detected in T_1 , T_2 , T_3 and T_4 after 6, 3, 3 & 9 days with; 11, 52, 18 and 70 cfu / g, recorded, respectively. The addition of BH led to an increase in yeast and mould counts, which were higher in T_2 (4% BH) than T_3 (4 % BH + 0.2 % RJ). This could be attributed to mixed BH into the heated yoghurt milk after cooling to incubation temperature in order to avoid losing some of honey's heat–labile bioactive substances. Such results agree with those of Jakobsen and Naryhus (1996), who mentioned that unprocessed honey often contains yeasts and moulds, they noted that yeast and perhaps mould spoilage may be a major problem in fermented milks when products are supplemented with honey, which is a source of infection and also provides nutrients for yeast growth and fermentation. There was a significant difference in the counts of yeasts and moulds among treatments and control. The counts of yeast and mould in stored yoghurt samples (after 9 days) were; 230, 890, 277 and 70 cfu/g for T_1, T_2 T_3 and T_4, respectively. The lowest counts were with T_4, which might be due to the growth inhibitory effect of RJ on the food spoilage organisms. In this concern, the results reported by Blum *et al*. (1959) demonstrated that RJ had fungicidal effect. Also, *in vitro* studies have confirmed that l0-hydroxydecanoic acid (the main fatty acid in royal jelly) has antibiotic activity and the antibiotic effectiveness is thermostable, *i.e.* is not destroyed by moderate heating (Yatsunami and Echigo, 1985). Coliforms were not detected in all fresh and stored yoghurt treatments. This could be attributed to hygienic conditions during manufacture and the role of yoghurt starter in inhibiting coliforms as they produce a range of antimicrobial compounds. Similar results were obtained by Hosny (2002).

Mineral content and rheological properties of yoghurt from different treatments

The influence of BH and RJ on the mineral contents of resulting yoghurt samples is illustrated in Table 5. It could be noticed that incorporation of BH and/or RJ to yoghurt leads to an increase in its content of minerals, considering that BH and RJ are rich sources of minerals (Table 1). The results showed that the viscosity of yoghurt samples containing BH were slightly increased, which might be due to the high content of solids in BH (Table 1) which led to an increase in both the viscosity and curd tension in the resulting yoghurt samples. In contrast, the addition of RJ slightly decreased the viscosity and curd tension of resultant yoghurt which consequently increased the syneresis. T_4 had the highest value of syneresis (45.00 ml), while T_2 had the lowest value (38.67 ml).

TABLE 5. Effect of honey and/or royal jelly on mineral contents and rheological properties of yoghurt from different treatments.

Treatment	Mineral content (ppm)								Rheological properties		
	Ca	Mg	K	P	Fe	Zn	Mn	Ni	Syneresis (ml/100g)	Curd tension (gm)	Viscosity (CP)
T_1	1327.8	42.69	451.73	262.83	7.52	4.67	0.085	3.25	40.33	45.73	1310
T_2	1388.5	47.75	493.47	310.12	9.24	5.42	0.139	4.22	38.67	54.40	1345
T_3	1563.3	58.29	512.97	360.32	44.96	6.89	0.751	4.98	41.00	46.00	1333
T_4	1315.5	44.31	559.09	292.40	31.47	4.72	0.289	3.64	45.00	41.27	1085

ppm: Part per million CP: Centi poise
T_1: Control (without additives) T_2: 4% honey T_3: 4% honey + 0.2 % royal jelly T_4: 0.6 % royal jelly

Organoleptic properties of yoghurt from different treatments when fresh and during storage

The results of sensory evaluation were significantly affected by treatments ($P \leq 0.05$) and storage at 5±1°C for 9 days (Table 6). Fresh yoghurt samples had the highest score, but it was significantly decreased during storage period. Addition of BH significantly improved the flavor of resultant yoghurt compared to other treatments. Addition of honey has the ability to decrease the sourness of fermented milk; this function of honey can serve to increase consumer acceptability of acidic products such as yoghurt (Roumyan *et al*., 1996). Also, these findings are in agreement with Varga (2006) who found that the addition of honey up to 3% improved the sensory quality of the resultant yoghurt without having a detrimental effect on characteristic of lactic acid bacteria. In contrast, this result was in disagreement with those obtained by Bandyopadhyay *et al*. (2008) in carrot and honey-fortified milk product. Resultant fresh yoghurt samples produced from T_3 gained the highest scores ($P \leq 0.05$) for overall acceptability, while yoghurt sample produced from T_4 gained very close scores to T_1 (control). Yoghurt flavor and texture were more affected ($p \leq 0.05$) in fresh yoghurt samples containing BH and/or RJ and during storage period, while the judgments did not show any significant differences in color and appearance score by incorporation of BH and/or RJ during storage compared with control samples.

Conclusion

From the foregoing results it could be concluded that, yoghurt can be successfully made using 4% bee honey + 0.2% royal jelly, also 0.6% royal jelly, separately, gave the best acceptability and nutritional quality of the resultant yoghurt during storage at 5 ±1°C for 9 days.

TABLE 6. Effect of honey and/or royal jelly on organoleptic properties of yoghurt from different treatments during storage at 5 ± 1°C.

Treatments	Flavor (45)				Treatment effect (Mean ± SD)
	Storage periods (days)				
	Fresh	3	6	9	
T_1	43^{abc}	43^{abc}	40^{def}	36^{g}	41 ± 3.2^{A}
T_2	44^{a}	44^{a}	39^{ef}	32^{h}	40 ± 6.0^{B}
T_3	44^{a}	44^{a}	41^{bcd}	35^{g}	41 ± 4.6^{A}
T_4	43^{ab}	42^{bc}	41^{bcd}	39^{f}	41 ± 2.0^{A}
Mean ± SD	44 ± 1.0^{A}	43 ± 1.3^{A}	40 ± 1.1^{B}	36 ± 2.8^{C}	
Body & Texture (40)					
T_1	38^{a}	38^{a}	36^{a}	35^{a}	37 ± 1.6^{A}
T_2	39^{a}	39^{a}	36^{a}	35^{a}	37 ± 1.6^{A}
T_3	39^{a}	39^{a}	37^{a}	36^{a}	38 ± 1.7^{A}
T_4	38^{a}	36^{a}	36^{a}	34^{a}	36 ± 1.8^{B}
Mean ± SD	39 ± 0.6^{A}	38 ± 1.3^{A}	36 ± 0.6^{B}	35 ± 0.7^{C}	
Color & appearance (15)					
T_1	15^{a}	15^{a}	15^{a}	14^{a}	14 ± 0.3^{A}
T_2	15^{a}	15^{a}	14^{a}	14^{a}	14 ± 0.3^{A}
T_3	15^{a}	14^{a}	13^{a}	14^{a}	14 ± 0.3^{A}
T_4	15^{a}	14^{a}	14^{a}	14^{a}	14 ± 0.3^{A}
Mean ± SD	15 ± 0.0^{A}	14 ± 0.4^{AB}	14 ± 0.4^{AB}	14 ± 0.4^{C}	
Total (100)					
T_1	96^{cd}	96^{cd}	91^{ef}	85^{h}	92 ± 5.0^{A}
T_2	98^{a}	98^{a}	89^{fg}	81^{i}	92 ± 5.2^{A}
T_3	98^{a}	97^{abc}	91^{ef}	85^{h}	93 ± 6.5^{A}
T_4	96^{cd}	96^{cd}	91^{ef}	87^{gh}	92 ± 4.1^{A}
Mean ± SD	97 ± 1.6^{A}	96 ± 2.5^{A}	91 ± 1.0^{B}	84 ± 2.6^{C}	

SD: Standard deviation

Values marked with the same letter (s) are not significantly different using revised LSD test at $P \leq 0.05$.

Capital letter (s) indicates differences between main effects, and small letter (s) indicates differences within interaction of each character.

T_1: Control (without additives) T_2: 4% honey T_3: 4% honey + 0.2 % royal jelly T_4: 0.6 % royal jelly

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تأثير إضافة عسل النحل وغذاء الملكات على جودة الزبادى أثناء التخزين

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يهدف هذا البحث إلى دراسة تأثير إضافة نسب مختلفة من عسل النحل وغذاء الملكات على حيوية بكتيريا بادىء الزبادى و زمن التحضين وخواص الزبادى الناتج ولذلك تم تلقيح بكتيريا البادئ (كلا على حده فى أطباق بترى) مع نسب مختلفة من العسل و غذاء الملكات (كلا على حده) والتحضين، و حساب أعداد بكتيريا البادئ الناتج مع استخدام البيئات القياسية لتنميتها، وبناءً على النتائج الأولية تم اختيار أفضل نسبة إضافة لكل من العسل و غذاء الملكات وإضافتها إلى اللبن المستخدم لتصنيع الزبادى بالطريقة التقليدية كما تم تصنيع معاملة بدون إضافات للمقارنة (كنترول) . تم تحليل الزبادى الناتج من المعاملات المختلفة طازجا وخلال فترة التخزين (٩أيام على درجة حرارة ٥ ± ١°م) تحليلاً كيميائياً و حسياً وميكروبيولوجياً وتقدير بعض الخواص الريولوجية ، كما تم تحليل النتائج المتحصل عليها إحصائيا .

و قد أظهرت هذه النتائج الآتي :
لم توجد فروق معنوية فى أعداد بكتيريا البادىء وزمن التحضين بين المعاملات التى أضيف إليها غذاء الملكات أو العسل بنسب منخفضة مقارنة بعينة الكنترول ، بينما كانت هناك زيادة معنوية لهذا المحتوى البكتيرى بزيادة تركيز المادة المضافة إلى ٠,٦ % غذاء ملكات أو ٤ % عسل وترتب على ذلك حدوث انخفاض معنوى لزمن التحضين .

ومن ناحية أخرى فإن إضافة العسل بنسبة ٦ % أعطى أعلى قبول حسى لدى بعض المحكمين و لكن هذه النسبة كانت مصحوبة بزمن تجبن أطول و حيوية أقل لبكتريا البادىء. لذلك (طبقا لنتائج الجزء الأول من الدراسة) تم اختيار ثلاث نسب هى: ٤ % عسل ، و ٠,٦ % غذاء ملكات ، و٤% عسل + ٠,٢ % غذاء ملكات أضيفت للبن المعد لتصنيع الزبادى. وقد دلت النتائج على وجود زيادة فى النسبة المئوية للحموضة حتى نهاية فترة التخزين بينما لوحظ انخفاض فى قيم رقم الأيدروجين و اللاكتوز أثناء التخزين . كما أدى إضافة كل من العسل وغذاء الملكات إلى زيادة محتوى الزبادى من المعادن بالمقارنة بعينة الكنترول. ومن ناحية أخرى أدت إضافة العسل إلى زيادة اللزوجه والجذب الخثرى وإنخفاض معدل إنفصال الشرش من العينات مقارنة بالكنترول ، كما أدت هذه الإضافات إلى زيادة أعداد بكتريا البادىء بالعينات الطازجة بالمقارنة بعينة الكنترول ثم حدث انخفاض فى هذه الأعداد حتى نهاية فترة التخزين و لكنها لم تقل عن الحد الأدنى الموصى به لوجود بكتيريا البادئ (١٠ ٦ مستعمرة / جرام) فيما عدا عينة المقارنة التى سجلت عدداً أقل من ذلك فى نهاية مدة التخزين ، وقد بدأ ظهور أعداد قليلة من الفطريات و الخمائر أثناء التخزين و خاصة فى تلك العينات المحتوية على العسل فقط بينما سجلت أقل الأعداد بالعينات المحتوية على غذاء الملكات فقط ، كما أظهرت نتائج التقييم الحسى أن إضافة عسل النحل يؤدى إلى حدوث زيادة معنوية فى مجموع قيم الخواص الحسية للزبادى الطازج بالمقارنة بالكنترول ولم تؤثر هذه الإضافات معنوياً على لون ومظهر العينات، وقد حدث انخفاض معنوى فى متوسط قيم نكهة وقوام العينات عند اليوم السادس من التخزين و خاصة تلك المحتوية على العسل فقط .

لذلك توصى هذه الدراسة بإضافة ٤ % عسل النحل + ٠,٢ % غذاء ملكات النحل فى حالة تصنيع الزبادى المحلى، أو إضافة ٠,٦ % غذاء ملكات النحل عند تصنيع الزبادى الغير محلى، للحصول على أعلى صفات جودة للمنتج الطازج و أثناء التخزين على درجة حرارة ٥ ± ١°م لمدة ٩ أيام .