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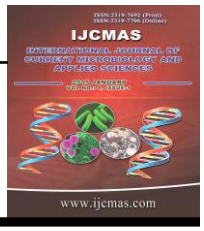

Original Research Article

Characteristics of Bioyoghurt Fortified With Fennel Honey

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Keywords

Bioyoghurt, Fennel honey, Antioxidant, Phenolic compounds, Antimicrobial activity, Storage period

A B S T R A C T

Bioyoghurt was made by adding different concentrations of fennel honey 5, 10 and 15% to milk, using 3% probiotic ABT-5 cultures(*Lactobacillus acidophilus, Bifidobacterium bifidum* and *Streptococcus thermophilus*) and held at 6±2°C for 14 days. The titratable acidity and total solids of control and bioyoghurt were increased with increasing fennel honey concentrations and storage period. Also, as the storage period progressed, the fat, total protein and ash contents of yoghurt gradually decreased for control and all treatments. The total plate and *B. bifidum* counts were increased in all treatments till the end of storage period and *Str. thermophilus* were increased up to the end of storage period. Bioyoghurt made with 5% fennel honey gained the highest of overall scores.

Introduction

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 and other health attributes associated with probiotic bacteria (Mckinley, 2005 and Savadogo *et al*., 2006).

In recent years, there has been increasing interest in the use of natural and healthy food additives and incorporating health-promoting substances into the diet due to its healthy and natural image (Chen *et al*., 2000). Honey is a natural, sweet, syrupy fluid collected by bees from nectar of flowers. The pleasant aroma and taste of this viscous liquid ranging in color from pale yellow to dark amber varies according to geographical and seasonal conditions.

The high content of sugars, small amounts of amino acids, lipids, along with some vitamins and minerals imparts its high nutritional value. Honey has good medicinal and antimicrobial properties and is used in different cuisines (Allia and Thakur, 2012).

Honey may serve as a natural food preservative due to its antimicrobial properties. Honey in combination with milk

provides an excellent nutritional value and it is recommended for use for children as a main source of nutrition (Klain and Massiom, 1969 and Chen *et al.*, 2000).

Honey is considered as an intermediate moisture food, generally contains a low microbial load and has a long shelf life. The water activity of honey reserves the food stuff (Scott, 1957). It has been observed that molds, yeasts and bacterial spores can be present in honey at low levels, but vegetative bacteria generally are not found (Snowdon and Cliver, 1996).

Yoghurt, Kefir, and similar fermented milk products are on the way to becoming major nutraceuticals aimed at treating a variety of disease conditions (Katz, 2001).

Consumption of yoghurt inhibited the growth of certain types of tumor, and it has been suggested that some factor in the cell walls of the bacteria could be responsible for the effect (Gilliland, 1979).

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 (Kajiwara *et al.*, 2002).

Also, there has been continued interest in incorporating honey into foods due to its healthy' and natural image (Tamime and Robinson, 1985). The high osmolarity and acidity of honey are among the physical characteristics that contribute to its antibacterial activity.

Hydrogen peroxide, volatiles, organic acids, flavonoids, beeswax, nectar, pollen and propolis are important chemical factors that provide antibacterial properties to honey (Molan, 1992).

Finally, honey could be used as a sweetener and prebiotic in order to improve fermentative aptitudes of bifidobacteria in desirable flavor mix probiotic product and with a relatively stable shelf life (Riazi and Ziar, 2012).

The phenolic compounds present in honey are flavonoids and phenolic acids which can also serve as markers for determining the botanical origin of honey. The phenolic acids were divided into two subclasses, substituted benzoic acids and cinnamic acids while the flavoids were divided into three classes with structural similarity namely flavonols, flavones and flavanones. The phenolic compounds contribute to the beneficial properties of honey due to their anti-oxidant nature (Estevinho *et al.*, 2008).

The present study was undertaken to develop bioyoghurt using fennel honey as a sweetener; natural prebiotic and probiotic bacteria and to evaluate the chemical, microbiological and sensory properties of bioyoghurt held at6±2°C for two weeks of storage.

Materials and Methods

Materials

Buffalo's milk was obtained from Collection Milk Centre in Arab EL Awamer, Assuit, Egypt.

ABT-5 cultures (*Lactobacillus acidophilus, Bifidobacterium bifidum* and *Streptococcus thermophilus*) were obtained from Chr. Hansen; Arpajon, France.

Fennel honey was obtained from Emtnan Company, Assuit, Egypt.

Methods

Manufacture of Fortified Bioyoghurt

Milk was heated to 90ºC for 5 min., and then cooled immediately to 40±1ºC. The milk was inoculated at a rate of 3% with ABT-5 then the milk was divided into four parts. The first part was served as a control and the other three parts were fortified with honey at different levels 5, 10 and 15%. Inoculated milk was dispensed in plastic cups of 100 ml volume and incubated at the fermentation temperature (40°C) until the pH decreased to 4.6. Fermentation was stopped by rapid cooling to 6±1°C. Yoghurt samples were analyzed when fresh, 3, 7and 14 days of storage (Tamime and Robinson, 1999).

Chemical Analysis

The titratable acidity was determined according to AOAC (2000). The protein and moisture of Bioyoghurt were estimated from the crude nitrogen content of the samples determined by the Kjeldahl and oven-drying methods, respectively (IDF, 1993) and the fat contents were determined by using Gerber and Soxelt according to the methods of (Ling,1963)and (IDF,1986), respectively. Total sugars had been estimated according to Anthron method (Hedge and Hofreiter, 1962) using spectrophotometer (Model Uviline 9400) at 620 nm. Antioxidant compounds (Vitamins A, C and D) were determined by High Performance Liquid Chromatography (HPLC), Agilent Packared (1200 series) equipped with auto sampling injector solvent degasser ultraviolet (UV) detector and quarter HP pump (1200 series), according to Jayaprakasha *et al.* (2003). Also, the concentrations of phenolic compounds were estimated by Jayaprakasha *et al.* (2003). Ash contents were determined in muffle furnace at 550˚C over night according to AOAC (2000).

Trace elements content had been estimated according to the method by James (1995) in Analytical Chemistry of Foods. The dilutions were applied to the atomic absorption spectrophotometer to estimate the levels of investigated elements (ppm) using atomic absorption spectrophotometer (AA-630-02 Shimadzu-Japan).

Microbiological Analysis

The standard plate count technique was used to enumerate the total bacterial count of bioyoghurt. Appropriate dilutions of bioyoghurt samples were plated in duplicate on an agar medium (IDF, 1991). *Streptococcus thermophillus* and *Bifidobacterium bifidum* were enumerated by the method as described by IDF (1997) and Dave &Shah (1996), respectively. Yeasts & molds and coliform bacteria were enumerated according to the methods of IDF (1985a and b) respectively. Plates containing 20-200 colonies were counted, and the results expressed as colony forming units per gram (cfu g^{-1}) of sample (IDF, 1991).

Sensory Analysis

Yoghurt was judged when fresh, and after 3, 7 and 14 days of storage by ten panelists of staff member ofFood Technology Research Section and some normal consumers, using the scores sheet according to Badawi *et al.*(2008).

Statistical Analysis

Results were evaluated statistically using the software program of the SAS system (SAS, 1999). Differences between means were determined by Duncan’s multiple range test

at a level of 0.05 probability (Steel & Torrie, 1980).

Result and Discussion

The Chemical Composition of Honey

The chemical parameters of the fennel honey are illustrated in Table (1) and it is clear that the acidity was 47.35 meq/kg, fat content 0.13%, protein 2.45%, moisture 26.37% total solids 73.62%, ash 0.43 % and total sugars 70.37 %. These results are in parallel with those of Buba *et al.*(2013) and Dinkov (2014). While, Ghadge *et al.*(2008) reported that carbohydrate 82.80%, moisture 17.20 fat 0.30% and protein content 0.20% and ash 0.17%, for Bulgarian fennel honey bee. The acidic pH may be due to varying acids and minerals in the honey samples and storage period of product.

Table.2 shows the levels of some trace elements content of fennel honey, the most abundant metal in honey was K (117.95 ppm). Other major metals present in honey were Ca (34.00), Mg (18.70), Na (10.15), Fe (0.60) Zn (0.57) and Cu (0.57ppm) were present in intermediate quantities (Pohl, 2009). These results are in agreement with those of Fernandez- Torres (2005) and Liberato and Morais (2013).

The levels of some antioxidant content of fennel honey are shown in Table (3), vitamin C was the most abundant in honey. These results are lower than those of Chua *et al.* (2013), but higher than obtained by Bogdanov (2012) and Buba *et al.* (2013). On the other hand, both of Vit D and A were found in a lower values.

Table.4 shows that the amount of Oleuropein, Cinnamic, Ellagic, Benzoic, Ferulic, Vanillic, Chlorogenic, Catecol, Catechein, and Protocatchuic were detected. Also Coumarin, Salycilic, Caffeine, Caffeic and Hydroxy tyrosol were not detected in honey samples. These results are in agreement with those of Martos *et al.*(1997) and Aljadi and Kamaruddin (2004). Since different plants contain different phenolic compounds and show variation in their total phenolic content (Zheng and Wang, 2001), the significant variation between Gelam and Coconut honeys in their total phenolic contents is due to the variation in their floral sources. Gelam honey has been shown to contain some phenolic acids, such as gallic, ferulic, caffeic, benzoic, and cinnamic acids, whereas coconut honey contains gallic, caffeic, and benzoic acids, besides some of other unknown phenolic compounds (Aljadi and Kamaruddin, 2004).

The Chemical Composition of Bioyoghurt

Table.5 shows that the titratable acidity content of the developed bioyoghurt was affected by the percentage of added fennel honey and storage period. The titratable acidity content of bioyoghurt was increased significantly ($p<0.05$) with the progressing of storage period in all treatments. On the other hand, the titratable acidity content of bioyoghurt increased with the increasing of the herbs honey concentration. These results are in harmony with those of O'Neil *et al.*(1979), Ghadge *et. al.* (2008) and Rashid and Thakur (2012). They showed that the acidity of fortified yoghurt increases as the concentration of fortification increased, because honey contains organic acids which increase the acidity. That means, acidity of ingredients affects acidity of yoghurt.

The mean value of titratable acidity of fresh samples was lower significantly ($p<0.05$) than those of stored samples which are due to the partial fermentation of lactose; also the acidity goes on increasing with the

progress of the storage period from 0.78 to 0.98% in fresh control and from 1.59% to 1.98 in 15% honey bioyoghurt at the end of storage period.

These results are in compatible with those of Chick *et al.* (2001) who found that honey was not inhibitory to *S. thermophilus*, *L. delbrueckii* ssp. *bulgaricus*, or *B. bifidum* at a level of 5% with honey enhancing lactic acid production by bifidobacteria.

The mean values of fat content in honey bioyoghurt increased as well as the increasing of honey. The mean values of fat content tend to slightly decreased with the progress of the storage period. These results are in agreement with those of Wedad and Owayss (2009) who observed that the presence of bee honey had insignificant influence on fat content in the resultant yoghurt. On the other hand, O, Neil *et al.*(1979), Ghadge *et al.* (2008) and Rashid and Thakur (2012) showed that there was a decreasing in fat percentage with increase in concentration honey in yoghurt.

The mean values of protein content increased insignificantly ($p<0.05$) with the increasing in the percentage of fennel honey, because honey contains low protein content 2.45%, these finding is nearly coincided with the results of Rashid and Thakur (2012). The highest mean protein percentage score was recorded in the honey bioyoghurt 15% at the end of storage period.

Generally, the total solids increased with the increasing in the percentage of fennel honey because honey contains higher amounts of total solids (Rashid and Thakur, 2012).

The highest mean in total solid percentage score was recorded in 15% honey bioyoghurt, and increased at the end of storage period. These results are in partly agreement with those of Wedad and Owayss (2009) who showed that the variations in the moisture content of yoghurt samples were found to be insignificant during storage, but it was significant among treatments.

The mean values of ash content decreased significantly ($p<0.05$) with the increasing of honey, because fennel honey contains low ash content (0.43%). The highest mean ash content score was recorded in control bioyoghurt and during storage period was decreased gradually till the end of storage. The variations in the ash content of yoghurt samples were found to be insignificant ($P \leq 0.05$) during storage. These results are in harmony with those of Wedad and Owayss (2009).

Data in Table (7) show that the total plate count, *Str. thermophilus* and *B. bifidum* counts of bioyoghurt were affected by the addition of honey and storage periods. Mostly the total viable bacterial count increased gradually till the end of the storage period. However, the total bacterial counts of bioyoghurt containing 10% honey were higher than that of the control in fresh and at the end of the storage period. Similar results are obtained by Stijeptic (2012) who found that yoghurt enriched with combination of whey protein concentrate and 4% honey had the highest value of lactic acid during the storage period. These results are in accordance with those of Abd-Elsalam *et al.* (2011) and Dzomba *et al.* (2013) and they found that the total plate count for all treatments increased through the first two weeks of storage period, then declined at the third weeks, the control samples showed the lowest count during the storage period, whereas the treatments with the highest count was that of supplemented with honey and probiotic bacteria.

Counts of *Str. thermophillus* bacteria increased gradually in all treatments up to the end of storage period except the control samples up to the 7 days of storage period. These results might be due to the effect of cold storage and acidity development on bacterial growth. These results are in agreement with those of Abd- Elsalam *et al.* (2011) they showed that counts of *Str. thermophillus and Lb. bulgaricus* increased up to the second week of storage period, and then decreased gradually in all treatments up to the end of storage period. However, Rashid and Thakur (2012) reported that the highest mean viability of *Str. thermophillus* and *Lb. bulgaricus* were recorded in 10% honey yoghurt. Also, Varga (2006) mentioned that the presence of honey at 1.0% to 5.0% (w/v) did not significantly influence the viability of microorganisms (i.e., *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus*) in yoghurt held at 4 °C for 14 days. Counts of *Bifidobacteria* increased gradually in all honey bioyoghurt samples up to the end of storage period. The count decreased with the increase of honey concentration. It is clear that the 5% fennel honey enhancement the growth of *Bifidobacteria* compared with plain, 10 and 15% fennel honey bioyoghurt.

The counts of yeasts and moulds were not detected till the 7 days of storage period; this might be due to the severe of heat treatments, the microbial load of added honey and antimicrobial effects of fennel honey. These results are in agreement with those of El-Nagar & Shenana (1998) and El-Nagar and Brennan (2001).

On the other hand, the coliform bacteria counts were not detected in both fresh and at the end of storage period in all treatments; this might be due to the severe of heat treatments of milk and the role of lactic acid bacteria in preservation of the products which associated with their ability to produce a range of antimicrobial compounds. These results are in agreement with those of El-Nagar and Brennan (2001), Tammam *et al.* (2011) and Rashid and Thakur (2012).

Table.1 Chemical composition of fennel honey samples

Acidity meq/kg	Moisture	Total solids	Total sugars	Total protein	Fat	Ash
47.35	26.37	73.62	70.37	2.45	0.13	0.43

Table.2 Some elements content (ppm) fennel honey samples

Mg	**Na**	**K**	**Mn**	**Fe**	**Ca**	**Zn**	**Cu**
18.70	10.15	117.95	0.026	0.60	34.00	0.57	0.57

Table.3 Some vitamins content (ppm) of fennel honey samples

Vitamin A	Vitamin D	Vitamin C
0.842	1.20	40.32

Table.4 Phenols content (ppm) of fennel honey samples

Phenolic compounds	ppm	Phenolic compounds	ppm
Oleuropein	1.68	Vanillic	2.43
Cinnamic	4.72	Caffeic	ND
Coumarin	ND*	Chlorogenic	29.08
Ellagic	0.012	Catecol	71.82
Benzoic	90.95	Catechein	0.005
Salycilic	ND	Protocatchuic	23.45
Ferulic	4.16	Hydroxy tyrosol	ND
Caffeine	ND		
*ND: Not Detected			

Table.5 Influence of storage period on the chemical composition of bioyoghurt fortified with fennel honey

Components (%).	Treatments	Storage periods (days)				Mean
		Fresh	3	7	14	
Titratable Acidity	Control	0.78	0.84	0.90	0.98	0.88^{d}
	T1	1.31	1.37	1.55	1.60	1.46^{c}
	T2	1.50	1.67	1.75	1.88	1.70^{b}
	T3	1.59	1.70	1.81	1.98	1.77^{a}
	Mean	1.30^{d}	1.40^{c}	1.50^{b}	1.61^{a}	
Fat	Control	3.92	3.80	3.82	3.78	3.83^{d}
	T1	4.07	4.03	4.03	3.90	4.01^{c}
	T2	4.13	4.10	4.07	4.00	4.08^{b}
	T3	4.20	4.17	4.13	4.10	4.15^{a}
	Mean	4.08^{a}	4.04^{b}	4.01^{b}	3.95^{c}	
Total Protein	Control	4.19	4.06	3.90	3.80	3.98^{c}
	T1	4.58	4.24	4.10	4.14	4.26^{b}
	T2	4.80	4.50	4.36	4.20	4.46^{b}
	T3	5.24	5.00	4.97	4.30	4.88^{a}
	Mean	4.70^{a}	4.45^{b}	4.33^{bc}	4.11^{c}	
Control. Plain bioyoghurt T1. Bioyoghurt 5% fennel honey T2. Bioyoghurt 10% fennel honey T3. Bioyoghurt 15% fennel honey						

Table.6 Influence of storage period on the chemical composition of bioyoghurt fortified with fennel honey

Components (%).	Treatments	Storage periods (days)				Mean
		Fresh	3	7	14	
Total Solids	Control	9.33	10.17	11.68	13.12	11.08[d]
	T1	12.59	13.12	14.14	14.95	13.70[c]
	T2	13.247	13.48	14.95	15.04	14.18[b]
	T3	16.97	16.98	17.71	17.93	17.40[a]
	Mean	13.03[d]	13.44[c]	14.62[b]	15.26[a]	
Ash	Control	0.89	0.86	0.86	0.75	0.84[a]
	T1	0.75	0.66	0.63	0.60	0.66[b]
	T2	0.69	0.61	0.60	0.57	0.62[c]
	T3	0.59	0.60	0.60	0.57	0.59[d]
	Mean	0.73[a]	0.68[b]	0.67[c]	0.62[d]	

Table.7 Influence of storage period on the bacteriological analysis of bio yoghurt fortified with fennel honey

Microbiological Properties (cfu x10^6/gm)	Treatments	Storage periods (days)			
		Fresh	3	7	14
Total plate count	Control	60	150	170	174
	T1	119	180	210	216
	T2	110	159	202	191
	T3	100	140	140	170
Str. thermophillus count	Control	16	35	47	43
	T1	40	44	41	45
	T2	20	34	35	38
	T3	20	33	30	36
Bifidobacteria count	Control	7	38	33	35
	T1	24	39	45	54
	T2	21	32	40	44
	T3	10	31	35	38
Yeasts &*Moulds count*	Control	ND*	ND	31	31
	T1	ND	ND	68	50
	T2	ND	ND	57	42
	T3	ND	ND	35	30

Table.8 Influence of storage period on the properties of fennel bioyoghurt

Sensory Properties	Treatments	Storage period (days)			
		Fresh	3	7	14
Flavour (45)	Control	33	30	28	26
	T1	43	42	41	40
	T2	41	41	41	40
	T3	43	42	40	38
Body and Texture (30)	Control	29	28	26	10
	T1	29	29	28	26
	T2	24	24	24	23
	T3	23	23	22	23
Appearance (15)	Control	14	12	11	5
	T1	13	13	13	11
	T2	13	13	13	11
	T3	10	12	11	12
Acidity (10)	Control	8	7	6	5
	T1	9	6	5	5
	T2	7	6	5	5
	T3	5	5	5	5
Overall Scores (100)	Control	84	77	71	46
	T1	94	90	87	82
	T2	85	84	83	79
	T3	81	82	78	78

Furthermore, the aerobic & anaerobic bacteria counts were not detected in all treatments except 15% honey bioyoghurt at the third day to the end of storage period, this might be due to the microbial load of honey added and the post contamination after manufacture and at the end of storage period in all treatments; this might be due to the severe of heat treatments of milk and the role of lactic acid bacteria in preservation of the products which associated with their ability to produce a range of antimicrobial compounds. Nearly the same results are recorded by Abdel Fattah (2006) who mentioned that the aerobic bacteria counts were not detected till the fifth day of storage period. Fennel honey bioyoghurt containing 5, 10 and 15% gained higher scores for flavour than that control in fresh and during the storage period till 14 days. However, bioyoghurt with 5 and 10% fennel honey gained the highest scores for flavour at the end of storage period (Table 8).

Also, Table (8) shows that bioyoghurt with 5% fennel honey gained higher scores for body & texture appearance during the storage period. However, bioyoghurt containing 5% of fennel honey recorded the highest values for overall sensory attributes as compared to other treatments at the end of storage period followed by 10% fennel honey.

Bioyoghurt of acceptable chemical, microbiological and sensory properties can be prepared using 3% ABT-5 cultures and 5% fennel honey. The fresh and stored (for 7 days) product recorded high values for all sensory attributes.

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