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Nutrient Composition and Microbiological Quality of Three Unifloral Honeys with Emphasis on Processing of Honey Probiotic Youghurt

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Abstract: Physicochemical and microbiological quality of 25 citrus, clover and cotton unifloral honeys along with identification of the predominant bacterial flora were assayed. Results of phyisicochemical parameters such as water content (w), total sugar (Ts), Total nitrogen (TN), acidity as citric acid and pH values were determined for all examined honeys. Color was determined and the average of values for L*, a* and b* were 10.42, 0.77 and 9.90 for clover honey 8.57, 1.72 and 6.78 for cotton seed honey and 11.54, 3.11 and 11.09 for citrus honey, respectively. Citrus honey had a low microbial load compared to clover and cotton honeys. No coliforms were detected in all the three types of honey examined. While all tested samples of cotton floral honey were contaminated with yeast and moulds. The honey bacterial flora includes strains of *Bacillus butyricum, B. subtilis, Enterococcus faecium, lactobacillus acidophilus, L. casei, L. plantarum, Lactococcus lacits, Lact, cremoris and Micrococcus luteus.*Honey yoghurt stored at 10°C for 5 days contained ~10^8 cfu/ml from the probiotic bacteria used regardless the percentage of honey added.

Key words: Honey · Microbiological quality · Egypt

INTRODUCTION

Honey is a natural gathered energetic food modified and secreted by honey *bee Apis medllifera* [1]. The ancient Egyptians who using honey as a delicious food had pointed out to its use as a healing substance. Such concept is referred to in Surat El-Nahl, the Hoely Quraan [2]. Honey has a unique nutritive composition including carbohydrates water and more than 180 substances as amino acids, vitamins, minerals and enzymes[3-5].

Honey is a product with minimal types and level of microorganisms. However, it can carry spores of yeast, mold and bacteria, which may persist in it [6].

Honey can be used as an ingredient in food or as part of a nutraceutical, drug and cosmetic. Therefore counts and types of microorganisms in honey need to be definitely recognized. The various bacterial and geographical origins play an important role on the characteristic of honey. In Egypt, where different crops are cultivated, three distinct unifloral types of honey could be identified, i.e, citurs, clover and cotton honeys. A complet analysis of citurs was recorded [7]. However, other types of unifolral honeys need to be studied as the literature in this concern are scarce.

Recently, there is a worldwide increasing demand by consumers to natural foods and foods claimed to enhance human health. Honey has a priority in this concern as it contains oligosarccharides (known as a bifidogenic factor) beside a wide range of other valuable nutrients. Its use to formulate both probiotic dairy and other food products is a subject for current research work [8]. Therefore, the present investigation was planned to study the nutritive composition, microbiological qualities, identification of bacterial isolates of citrus, clover and cotton uniforal honeys locally produced and to suggest a recipe of functional food containing honey namely, yoghurt.

MATERIALS AND METHODS

Honey: 25 samples of honey from Fayoum province were subjected for study. They include 8 (28%). 7 (28%) and 10 (44%) citrus, clover and cotton unifioral honey, respectively. Sample (about 1 kg) were transported in Sterilized glass bottles for analysis.

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Milk Raw: Cow's milk containing (3% fat) was obtained from a nearly farm.

Starter Cultures: Strains of *lactobacillus bulgaricus and Streptococcus thermophilus* (1:1 ratio) in 2% v/v were used for preparation of yoghurt. These cultures were obtained from Hansen (Denmark).

Probiotic Lactic Acid Bacteria: *L. acidophilus* and *Bifidobacterium bifidum* were kindly donated by the local collection of the Dairy Microbiology Laboratory. National Research Center.

Microbiological Media: The following media were prepared and used for each parameter as described by the manufacturers. Plate count agar, reinforced clostridial agar and Malt agar (Oxoid) for aerobic. Anaerobic and mold and yeast counts, respectively. Violet red bile agar MRS and aesculin azide agar (Difco) for coliforms lactobacilli and faecal streptococci counts, respectively.

Color Evaluation: Honey color differences were determined using a spectrocolorimeter with the CIE lab. Color scale this color assessment system is based on the Hunter L* a* and b* coordinates L* representing lightness and darkness + a* - redness a* - greenness-b* and yellowness and b* - bludenss (Hunter, Labscan XE-Reston VA, USA). The instrument was standardized against a white tile of Hunter Lab Color standard (LXNO 16379) X = 77.26, y = 91.94 and z = 8814 before each measurement.

Physicochemical Composition: Moisture (w), total nitrogen (TN), ash, total sugar (TS), pH, total acidity (mg/100g) of honey were determined according to the methods of [9] together with diastase activity [10].

Microbiological Parameters: These were including aerobic, anaerobic, coloiform, yeast and mould counts, also, the counts of lactobacilli and faecal streptococci were determined [11].

Lactic Culture Preparation and Honey Yoghurt: Cultures of *S. thermophilus*, *L. delbreuckii* ssp. Bulgaricus, *L. acidophilus* and *Bif. Bifidum* were propagated on suitable media and used to inoculate milk prepared for processing of probiotic yoghurt as follows:

Cow's milk of 3% fat and 8.5% solids not fat (SNF) was fortified with 1 % skim milk powder and stirred to ensure complete dissolving, then, the quantity was distributed in 4 equal portions in one liter flasks and 0.10,20 and 30% citrus honey (w/v) were added to each portion and pasteurized at 90°C for 5 min using a laboratory water bath, cooled to 40°C and inoculated with 2% (v/v) of a cocktail containing the four aforementioned strains of lactic cultures.

Inoculated milk was dispensed in plastic cups of 100 ml volume incubated at 37°C for about 4 hrs or until a firm clot was obtained. Yoghurt was then cooled to 5°C and kept as such during the period of investigation. Bacterial counts on M17 and MRS media were done on yoghurt samples when fresh (0 time), 3 days and 5 days storage at 5°C. pH values were measured. The organoleptic properties of honey yoghurt were evaluated by a panel of judges of stuff members at Dairying Department.

Identification of Selected Bacterial Isolates: Selected randomly hundred bacterial isolates from plates of aerobic counts were picked, purified and identified using API kits (Bio-merieux. France). The appropriate APL kits were used according to the results of microscopic examination, Gram staining and catalase activity of the isolates which were carried out firstly [12].

Statistical Analysis: The data was statistically analyzed for variance and the least significant difference (LSD) as described by Richard and Gouri[13].

RESULTS

Physicochemical Composition: Table 1 shows the results of physicochemical parameters of the 3 unifloral honey samples studies. While, Fig. 1 compares their mean for water content (w), total sugars (TS), total nitrogen (TN), acidity (A), pH and ash, respectively. Table 2 show the reflectance of honey clover,cotton andcitrus.

Microbiological Quality: Table 3 showing the log contents of aerobic, anaerobic,coliform, yeasts, mold,lactobacilli,faecal streptococci counts for the three types of honey under

Identification of Bacterial Isolates from Honey: Fig. 2 showed the results which includes 45 isolates cocci, Gram positive and catalase negative; 25 rods,

Table 1: Physicochemical characterization and composition of different honeys honey (Mean±SE)

Type of honey	W	TS	TN	Acidity	pH	Ash	Diastase
clover	15.87±0.06	81.22 ±0.07	0.69±0.03	2.89±0.03	4.49±0.08	0.29±0.11	++
Citrus	0.49±0.076	15.89±0.074	81.21±0.027	0.058±0.038	2.78±0.021	4.08±0.045	+
Cotton	16.02±0.027	80.48±0.71	0.063±0.012	2.93±.23	4.51±0.71	0.24±0.74	+

W= water content, TS= total sugars, TN= total nitrogen, A= acidity

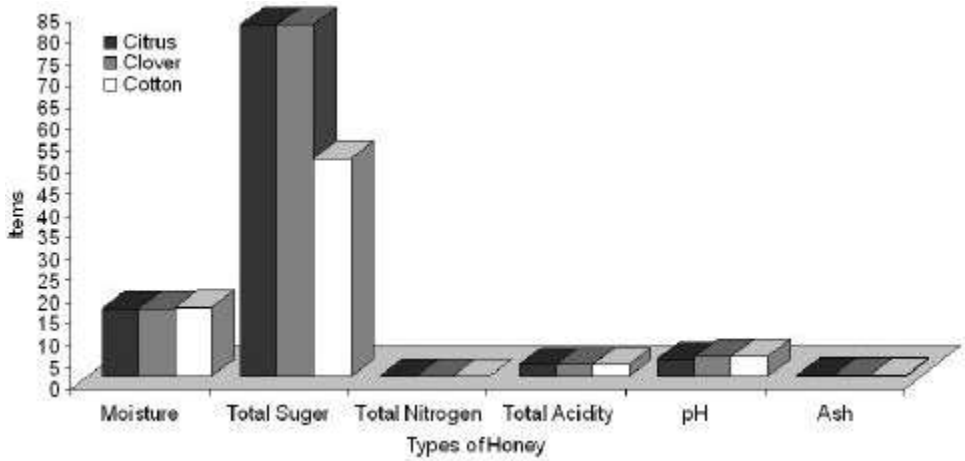

Fig. 1: Composition of mean physicochemical composition for clover, citrus and cotton uniflora honey samples

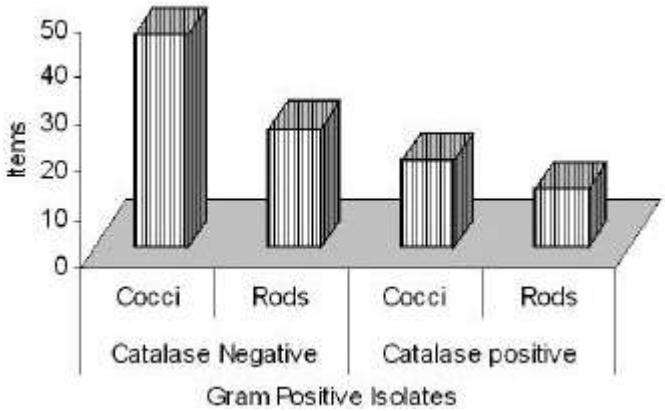

Fig. 2: Results of identification of 100 bacterial isolates to the Genus level

Table 2: Color reflectance of honey clover, cotton and citrus

Sample	L*	a*	b*	DE
Clover				
1	10.55	0.68	9.81	82.94
2	10.09	0.65	9.87	82.88
1	10.07	-0.75	6.60	82.83
4	10.45	0.67	9.99	82.82
7	10.82	0.88	9.98	82.44
10	10.23	0.66	9.90	85.71
14	10.74	1.16	10.18	82.56
Mean	10.42	0.77	9.90	
Cotton				
3	8.55	0.38	7.11	82.94
4	8.58	0.61	9.88	82.54
5	8.64	0.39	6.81	83.28
2	8.35	4.17	6.81	84.71
3	8.22	1.99	5.61	84.65

Table 2: Continued

9	8.37	5.56	7.64	84.86
10	9.09	-1.14	4.68	83.68
5	8.65	0.40	6.73	84.27
6	8.80	0.25	6.11	72.68
11	8.44	3.83	6.88	79.57
12	8.64	0.23	6.42	80.03
Mean	8.57	1.72	6.78	
Citrus				
1	11.01	0.26	9.90	82.24
2	11.33	4.50	11.37	82.28
3	11.70	3.45	11.74	81.90
4	11.19	-0.68	9.43	81.99
5	11.46	1.90	10.80	81.95
6	11.69	8.16	12.31	82.28
7	12.46	2.86	12.13	81.19
Mean	11.54	3.11	11.09	

Table 3: Microbiological quality of honey samples

		Log counts cfu/g					
No	Type of honey	Aerobic	Anaerobic	Coliform	Yeast and Mould	Lactobacilli	Faecal Streptococci
1	Cotton Unifloral Honey	6.57	Nil	Nil	3	3.3	5.56
2		4.69	Nil	Nil	4.3	1.47	3.49
3		5.39	Nil	Nil	2.6	1.69	4.83
4		4.30	1	Nil	2.47	Nil	3.47
5		5.73	Nil	Nil	1.47	3.8	4.85
6		4.80	2	Nil	2.3	Nil	2.84
7		6.43	2.3	Nil	3.0	Nil	2.30
8		5.47	2.47	Nil	6.69	3.3	6.0
9		7.17	2.3	Nil	2.69	3.3	6.0
10		5.47	3.69	Nil	2.77	3.1	6.3
11		7.00	2.39	Nil	2.77	3.1	6.3
1	Clover Unifloral Honey	4.17	3.08	Nil	0	Nil	3.3
2		4.32	2.97	Nil	2.97	Nil	Nil
3		3.30	Nil	Nil	0	Nil	Nil
4		3.69	3.0	Nil	0	Nil	Nil
5		4.6	2.5	Nil	0	Nil	Nil
6		1.69	Nil	Nil	0	Nil	2.0
7		1.47	Nil	Nil	0	Nil	3.0
1	Citrus Unifloral Honey	2.54	Nil	Nil	0	Nil	2.17
2		1.9	Nil	Nil	0	Nil	2.0
3		2.3	Nil	Nil	0	Nil	2.17
4		6.6	3.07	Nil	2.27	3.0	5.69
5		Nil	Nil	Nil	0	Nil	Nil
6		Nil	Nil	Nil	0	Nil	Nil
7		Nil	Nil	Nil	0	Nil	Nil

Table 4: Identification of 100 isoltes to the species level

Genus	Species	No.	Total
Bacillus	*B. butylicum*	4	12
	B. butyricum	2	
	B. subtilis	6	
Enterococcus	*Ent. faecium*	27	27
Lactobacillus	*L. acidophilus*	12	25
	L. casei	7	
	L. plantanum	6	
Lactococcus	*L. lactis*	9	18
	L. cremaris	9	
Micrococcus	*M. luteus*	18	18
	Total		100

Table 5: Analysis of honey fortified yoghurt during storage at 10°C

	Treatment							
	Control		10 percent		20 percent		30 percent	
Time	M_{17}	MRS	M_{17}	MRS	M_{17}	MRS	M_{17}	MRS
Cfu/g								
1st day	$6x10^{10}$	$80x10^{9}$	$14x10^{10}$	$12x10^{10}$	$11x10^{8}$	$12x10^{10}$	$6x10^{9}$	$7x10^{7}$
3rd day	$15x10^{8}$	$36x10^{9}$	$20x10^{7}$	$31x10^{8}$	$10x10^{7}$	$25x10^{8}$	$14x10^{8}$	$41x10^{7}$
5th day	$21x10^{7}$	$31x10^{8}$	$28x10^{8}$	$75x10^{7}$	$30x10^{6}$	$13x10^{8}$	$17x10^{7}$	$22x10^{6}$

Gram positive and catalase positive. Further identification was performed using API kits and accordingly as shown by Table 4.

Processing of Honey Fortified Yoghurt: Table 5 shows the results of bacteriological analysis of yoghurt fortified using 10, 20 and 30 % honey (w/v) and control yoghurt (without addition of honey).

DISCUSSIONS

Concerning the physicochemical parameters of the 3 studied unifloral honey samples. For water content the data were nearly the same and also for TS, TN and acidity. The trend is different for the pH and ash figures.. Citrus honey had a higher ash content compared to the other two other studied honey types and a lower pH value than those for clover and cotton honeys. It was also clear that most samples were diastase positive which means that these samples were extracted using no or low heating. Accordingly, the positive samples should be regarded as crude honeys which may contain the natural founded vitamins, enzymes and amino acids in their crude form which are considered more valuable and beneficial for human nutrition. The results obtained during the current study were similar to those of [11, 12]. Ash content of citrus honey is greatly higher than those for the other two types of honey samples examined. Honey is a source of minerals such as potassium, phosphorus, calcium and magnesium. It could be concluded, based on the present findings, that citrus honey may be considered as an excellent source of minerals.

Mean protein content of the three honey types were 0.39, 0.34 and 0.34 g/ 100g for clover, citrus and cotton honeys, respectively (total nitrogen x 5.6). Honey is not intended as a protenaceous food but it contains a series of free amino acids necessary for health promoting effect of honey [12].

The moisture and temperature conditions during extraction of honey (as shown by diastase test) are influencing growth of microorganisms in it, low water content has long been used to control spoilage of honey [14].

In the current study, the mean 7 reading of L*, a* and b* values was 10.42, 0.77 and 9.90 in honey clove, respectively. While the mean 11 reading of L*, a* and B* values was 8.57, 1.72 and 6.78 in honey cotton, respectively. On the other hand the mean 7 readings of L*, a* and 6* values was 11.54, 3.11 and 11.09, in honey citrus, respectively.It should be mentioned that the L* values indicate lightness, while a* indicates redness and b* indicates yellowness of color as measured by the Hunter Lab apparatus.

Higher aerobic colony counts were observed for cotton samples over those for clover or citrus honeys. Moreover, citrus honey samples proved to contain no bacteria detected. Accordingly, it appears that the bacteriological quality of citrus honey is the best. The same finding seems standing for the other microbiological parameters also. This may be attributed to the presence of certain antimicrobial components in citrus honey which are not contained by clover or cotton honeys. The presence of different antibacterial substances in honey was previously discussed [2,6,15].

The need for microbiological data on honey will increase as new technologies for honey develop. Microorganisms in honey may influence quality or safety. Primary source at microbial contamination are likely to include pollen, the digestive tracts of honey bees, dust, air, earth and nectar and sources which are very difficult to control.. secondary (after harvest) sources that influence any food product are also sources of contamination for honey. These include air, food handless, corss-contamination, equipment and buildings. While the secondary sources of contamination could be controlled, the remaining primary counterparts are out of any control. All samples of cotton honey contained yeast and mould counts ranging from log 1.47 to log 6.69 while only one samples from either clover or citrus honey was positive and with a relatively low log count of 2.97 and 2.27, respectively. The same explanation for aerobic and anaerobic counts of honey standing also in this case. All samples were negative for coliforms, which mean that the sanitary conditions during extraction and handling honey in the apiaries are quite efficient. All samples of cotton honey contained a relatively high log counts for faecal streptococci then clover and citrus honeys. The same situation is also correct for lactobacilli. This could be explained that cotton honey samples may contain various oligosaccharides responsible for enhanced lactic acid bacteria during ripening of honey in the hives itself [6,12].

The current results revealed that honey contains some bacteria namely those of the genera lactobacillus, Streptococcus, Micrococcus and Bacillus. The source of such bacteria originated primary from the activity of the bees themselves or secondary during extraction of the honey.

It was worthy mention that the lactic cultures added to the milk in the beginning of processing of honey fortified yogurt (0 time) had approximately 10^6 cfu/ml live

lactic acid bacteria. The lactic cultures added to the yoghurt milk had propagated steadily in control and the three treatments as observed from counts after the first day of yoghurt storage. Apart from the incubation temperature used during processing of yoghurt (40-42°C). which is known to permit luxuriant bacterial growth, it could be concluded that honey is not a barrier that could completely inhibit the growth of lactic acid bacteria in the concentrations used in this study. However, one can observe that the counts of LAB tend to slightly decrease as the concentration of honey increased. After five days storage of yoghurt, it appears that the counts of LAB tends to gradually decrease in all treatments and the control. However, the counts remained live in the product still high enough to comply the requirement needed by health authorities for probiotic yoghurt.

Honey is becoming an increasingly popular additive in yoghurt and other dairy products [16]. It contains fructooligosaccharides (FOS) which act as prebiotics in the gut therefore honey could be of great value in the concept of functional foods [17]. Incorporation of LAB in dairy foods fortified with honey is a controversial issue and needs to be thoroughly investigated. This point may be studied in future.

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