



ANTIMICROBIAL, PHYSICOCHEMICAL AND SENSORY CHARACTERISTICS OF HONEY TREATED CREAM

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Abstract

The study includes the inhibitory activity of natural honey (medicinal plants, clover, cedder and eucalyptus) honey on the some genus of gram positive and gram negative bacteria including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella* spp., *Serratia* spp. The results showed that there is a clear and different effect of honey on the bacteria under study, where eucalyptus honey had the greatest effect against all tested bacteria (*Pseudomonas aeruginosa*, *Klebsiella* spp., *Serratia* spp., *Staphylococcus aureus* with the zone of inhibition (10.3, 13.2, 16.2, 28.4) mm respectively followed by cedder honey with (9.7, 12.9, 13.4, 19.5) mm respectively. Based on these results, eucalyptus honey was selected for use in the subsequent study. Cream was processed by the addition of different concentrations (0, 5, 10, 15 and 20) % of eucalyptus honey and kept under refrigeration (6 ± 1)°C for up to 15 days. Incorporation of bee honey in cream formulation led to substantial improvement in the sensory properties, it was noted that honey impact flavor, color, sweet taste, texture and Spread ability characteristics to the cream samples treated with honey compared to the other samples and keeping qualities of the cream. Honey can help upscale the nutritive value of such cream sample and enhance consumer preference. These results indicate that the bee honey can be used as natural preservative to enhance the shelf life of cream at refrigerator temperature for 15 days.

Key words: cream, bee honey, antimicrobial, Physicochemical properties, sensory evaluation.

Introduction

Honey is considered to be an excellent natural product, from the most complex mixtures containing a high percentage of sugars (80-85%) and 15-20% water as well as minerals, proteins, vitamins, organic acids, flavonoids, phenolic acids and enzymes (Kucuk *et al.*, 2007; Truchado *et al.*, 2009). The honey pH ranges from 3.2 to 4.0, honey acidity was attributed to organic acids resulting from enzymatic action in the ripening nectar (Kucuk *et al.*, 2007; Gomes *et al.*, 2010). The high sugar concentration of honey and also the low honey pH can be responsible for the antibacterial activity (Yatsunami and Echigo, 1984; Mundo, 2004).

Honey has antimicrobial properties that discourage the growth or persistence of many microorganisms. The antibacterial property of honey has long been recognized in vivo and in vitro (Aljadi and Yusoff, 2003). It has an indirect antimicrobial action, it can fight microbial infection through its immune activating, anti-inflammatory and prebiotic activity (Al-Waili *et al.*, 2011). The direct antimicrobial action of honey is inhibits the growth of bacteria and fungi especially against gram-positive bacteria (Bogdanov, 1997; Molan, 1997). Both bacterial and bactericidal effects of honey have been studied by Molan, 1992 and Molan, 1997 against many bacterial strains, many of which are pathogenic such as *Bacillus anthracis*, *Corynebacterium*

diphtheria, *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella pneumonia*, *Proteus* sp., *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*, *Salmonella* sp., *Serrata marcescens*, *Shigella* sp., *Staphylococcus aureus*, *Streptococcus pyogenes*, *Vibrio cholerae*. The antimicrobial properties of honey were largely attributed to H₂O₂ and non-peroxide compounds present in honey (Weston, 2000). Antibacterial activity of non-peroxide in honey was associated with the proteinaceous compounds, sugar concentration, and antioxidants found in honey (Basualdo *et al.*, 2007; Truchado *et al.*, 2009).

Honey has been used in wound treatment for more than 2,000 years. The honey has recently been shown to have inhibitory effect on 60 types of aerobic and anaerobic, gram positive and negative bacteria. It has also been shown to have antifungal effects for yeast, *Aspergillus* and *Penicillium*. And the acquisition of many microbes resistant to the types of antibiotics is currently one of the main reasons for the reappraisal of the therapeutic uses of bee honey, studies have shown that honey have high activity against both Gram-positive and Gram-negative bacteria in vitro and support their use in the treatment of infections not responding to antibiotics (Kwakman *et al.*, 2010; Vandamme *et al.*, 2013) and used as food, component in pharmaceuticals and osmectics (Pardo, 2005). According to the health properties of honey, the thrust of this study was to

evaluate the efficacy of honey as a biological preservative of cream; adding honey to cream can obviate the body needs to many nutrients and in long time can improve the society health level. The purpose of this study is to add honey to cream and to analyze the interest level of consumers to this product.

Objectives: The effect of bee honey on safety and storability of half and half cream. And the effect of bee honey on physicochemical and sensory properties of cream.

Materials and Methods

Test Bacteria

Four isolates of pathogenic bacteria were obtained from the postgraduate laboratories of Food Science Department-Agriculture College - Baghdad University, studied bacteria including the species of *Staphylococcus aureus*, *Serratia sp.*, *Klebsiella sp.*, *Pseudomonas aeruginosa*. Some microscopic and confirmed tests were performed to identify its qualities and purity.

Honey Samples

The four types of natural honey samples used for this work were taken from different beekeeping places locations in Baghdad city; these are (medicinal plants honey, clover honey, cedder honey, eucalyptus honey). They were used as found comprising 100% concentration each.

Preparation of Cream

Raw cream with 28-35% fat was manufactured in the dairy factory of Agriculture College - Baghdad University. Honey was added to the cream after pasteurization directly with good mixing until the distribution of honey in the cream in a homogeneous manner. Honey concentration of (0, 5, 10, 15, 20) % have been used in cream treatments. These percentages have been defined after the first sensory testing and by the assessors' evaluations. The industrial antioxidant at a concentration of 200 ppm was added to other samples of the cream and was considered a comparison treatment. The experimental treatments included:

- T1: control treatment (0%honey)
- T2: cream + 5% honey
- T3: cream + 10% honey
- T4: cream + 15% honey
- T5: cream + 20% honey
- T6: cream + 200 ppm BHA

In the last step, this manufactured cream is packaged and maintained in 1 ± 6 °C for 15 days during which the Physicochemical tests were carried out immediately after processing (0 day) while sensory tests

were assessed after processing and at intervals of 5, 10 and 15 days post storage.

Microbiological Tests

Measurement of Antibacterial Activity

DISC diffusion assay was used to determined antibacterial activity of honey samples as described by Patton *et al.* (2006). Suspensions of the studied pathogenic bacteria were prepared by the reported turbidity standard McFarland 0.5 procedures then 100 μ L of the suspension was inoculated onto Muller Hinton agar by streaking plate method. Eight millimeter diameter-filter paper was saturated with honey samples of medicinal plants honey, clover honey, cedder honey and eucalyptus honey. Nutrient agar plates were separately flooded with different test bacteria already in sterile nutrient broth by culturing at 37°C for 24 hr. Muller-Hinton agar plates were drained and allowed to dry at 37°C for 30 min before placing various honey samples disks onto the surface of the Mueller Hinton agar plates (5 disks per dish) the plates were incubated at 37°C for 24 hr. The diameter of the zones of inhibition was measured and recorded.

Physicochemical tests

Fat measurement was done by the mentioned Babcock method according to the Eckles *et al.* (1997). pH cream was measured in this study by used SUNTEX Sp-701 pH meter, the protein, moisture and ash contents were determined following the procedure described by A.O.A.C. (2000).

Sensory Evaluation

The sensory evaluation of the cream samples was carried out by a number of member sensory panel of Food Science Department, College of Agriculture, University of Baghdad semi-trained according to the procedure of TROUT and NELSON (1964), the panelists evaluated the cream samples of the different treatments for Which included properties: taste and flavor and gave 45 degrees, texture 30 degrees and Spread ability 10 degrees and color and external appearance 15 degrees. Sensory evaluation was performed at ages 0, 5, 10 and 15 days of refrigerated storage a temperature of 1 ± 6 °C.

Statistical analysis

A global experiment was applied with a complete random design to study the effect of treatment and storage periods in the degrees of sensory evaluation. The mean differences between the mean and the least significant difference (LSD) were measured using SAS (2012).

Results and Discussion

Microbiological Tests

In vitro antibacterial activity of four types of local Iraqi honey 100% (w/w) concentration (medical plants, clover, cedder and eucalyptus) honey was tested against Gram-positive and Gram-negative bacteria (*Staphylococcus aureus*, *Serratia* spp., *Klebsiella* spp., *Pseudomonas aeruginosa*). The results indicated that eucalyptus honey was the significantly ($P < 0.05$) effective against these bacteria and the diameter of the inhibition zones was (28.4, 16.2, 13.2, 10.3) mm respectively, followed by cedder honey, which gave diameters of inhibition zones (19.0, 13.4, 12.9, 9.7) mm respectively (Table 1), this is may be due to the different chemical composition of different honey species. This result in agreement with the data reported by Al- talibi *et al.* (2012), the difference in the type and concentration

of honey leads to a different degree of bacterial growth inhibition, these authors observed that Iraqi eucalyptus honey 100% (w/w) has anti-bacterial activity with growth inhibition zones ranged from 6.7 to 15.2 mm against *Serratia marceccens*, *Proteus mirabilis*, *E. coli*. Eucalyptus honey was higher effectiveness than Ramanuz honey for the same concentration, the unequal activity between the two types of honey may be due to their differences in the amount of flavanoids and phenolic acids (D'Arcy, 2005). Dark honeys contain more minerals than the lighter ones (Anupama *et al.*, 2003). Dark honeys contain more minerals than the lighter ones, good correlation between *P* fund color scale and inhibition zone ($R^2 = 0.65 - 0.68$) mm for the *Staphylococcus aureus* ATCC 24213, *Micrococcus luteus* ATCC 49732 and *Escherichia coli* ATCC 25922, was noticed (El-Shahawi and Al-Hindi, 2014).

Table 1: Antimicrobial activities of un-diluted (100 %) honey samples vs. tested bacteria

inhibition zone diameter (mm)					
Species	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella</i> spp.	<i>Serratia</i> spp	<i>Staphylococcus aureus</i>	LSD value
honey samples					
Medical plants	3.1	0	6.0	20.1	3.176 *
Clover	6.4	0	10.2	15.8	2.066 *
Cedder	9.7	12.9	13.4	19.5	2.703 *
Eucalyptus	10.3	13.2	16.2	28.40	4.052 *
LSD value	2.863 *	2.735 *	3.068 *	3.921 *	---

* The numbers in the table represent a rate for repeaters. (0) means no antibacterial activity or inhibition.

The results of *in vitro* susceptibility of raw available honey having varying degree of antibacterial activities against Gram-positive and negative bacteria. Our results resembles that of others (French *et al.*, 2005; Agbaje *et al.*, 2006; Basson and Grobler, 2008) and agreed with the work of El-Amari and Ben-Gweirif (2010) who found that honey inhibited the growth of *S. aureus*, *E. coli*, *Shigella* spp. and *Pseudomonas* spp., the high inhibitory effect of the honey samples was 100% showed higher inhibition zone (36 mm) on *E. coli* isolated resistance to Ampicillin, than honey samples 75, 50 and 25% Which gave inhibition zones with diameters 30, 29 and 28 mm respectively (AL-Akili *et al.*, 2014; Osho and Bello, 2010). While Sheriff *et al.* (2012) pointed out the absence of inhibitory activity of both natural and traditional un- diluted honey on all types of tested bacteria, but after dilution of both type of honey at a ratio of 1:2 the results showed different effect for each one, the effect of 1:2 natural honey on *E. coli*, *Proteus mirabilis*, *Serratia marceccens* were 41, 30, 25 mm respectively. The largest effect of the natural honey was showed to be on *Serratia marceccens* at dilution 1:4 and the zone of inhibition was 35 mm.

Results in Table (1) revealed that the *S. aureus* was the most sensitive bacteria to different types of honeys, while *Pseudomonas aeruginosa* was the most resistant pathogen. In general, the Gram+ bacteria were more sensitive to the honey phenolics compounds extracts than the Gram- bacteria. Our results were in agreement with the data observed by Rauha *et al.* (2000); Mundo *et al.* (2004); Agbaje *et al.* (2006); Al-talibi *et al.* (2012), these authors also observed that *S. aureus* was the most sensitive bacteria to manuka and pasture honeys. The highest activity of polypehnic mixtures isolated from nine Slovakian honey samples (evaluated by using minimum inhibitory concentration (MIC) method) was observed in a case of *S. aureus* (32- 64) g.mL⁻¹ and *P. aeruginosa* (64 -128) g.mL⁻¹ (Kačániová *et al.*, 2011).

Results of the inhibitory activity of raw honey against pathogens have been presented by Taormina *et al.* (2001) and Basualdo *et al.* (2007) which are similar to the results obtained in this work, that have been carried out in different experimental conditions. The antimicrobial activity of honey is highly complex due to the involvement of multiple compounds such as high sugar concentration, hydrogen peroxide and

nonperoxide factors high osmolarity, and the low pH (Taormina *et al.*, 2001). The antimicrobial capacity of phenolic compounds, in a general way, is well known (Rauha *et al.*, 2000). Recently evidence has been provided that methylglyoxal and the antimicrobial peptide bee defensin-1 found at high levels in Manuka honey were identified as important antibacterial compounds responsible for its activity (Adams *et al.*, 2009; Fidaleo, *et al.*, 2011; Kwakman and Zaat, 2012).

According to the results obtained in this study (*in vitro*), eucalyptus honey was selected for use in subsequent studies as an antimicrobial and antioxidant to prolong the shelf life of the local cream, as well as the

study of the effect of adding honey on the sensory characteristics of the cream locally manufactured.

Physiochemical Tests

The effect of bee honey on the proximate composition of cream is shown in Table (2). The moisture content of the honey cream treatments was lower than control treatment (T1). Obviously as the level of bee honey added to cream increased the moisture content of the cream decreased. The result was consistent with the report of Belewu and Morakinyo (2009), the 15% honey treated cheese sample was highest in dry matter content followed by the 10% honey treated cheese sample and the least was the sorghum treated cheese sample.

Table 2: Physiochemical tests results for Untreated and Honey treated cream

Treatments	Moisture	Protein	Fat	pH	Ash
T1	62.09	41.81	30.0	6.6	0.41
T2	60.99	51.09	28.1	6.1	0.43
T3	60.63	51.91	27.89	5.9	0.47
T4	59.03	53.57	27.92	5.8	0.48
T5	58.23	55.25	27.25	5.6	0.53

Treatments: (T1: 0 % honey (untreated cream), T2: 5 % honey cream, T3: 10 % honey cream, T4: 15 % honey cream, T5: 20 % honey cream).

This was also supported by the result of Antony *et al.* (2006) and Mohammed (2006) who found reduction in the moisture content of meat products treated with honey, the low moisture content of the product containing the honey could be due to the effect of low water activity and high osmotic pressure of the bee honey. It was also observed that products containing honey dry-out more slowly and have a lesser tendency to crack (Molan, 1992).

Results in Table (2) revealed that, the increased in protein content of honey treated cream, the T5 had the highest protein content and the lowest content was related to the T1 (0 % honey), while T2 and T3 treatments had similar protein contents approximately. This result was supported by Belewu and Morakinyo (2009) who showed that the 15% honey treated cheese sample significantly increased the cheese protein content. This finding is consistent with the results of many researchers (Haskim *et al.*, 1999; Antony *et al.*, 2006; Mohammed, 2006) who reported that the addition of honey to meat and turkey products increased the protein content, this may be due probably to the enzyme present in the honey (Dawson and Mathew, 1998).

The fat content of the honey cream treatment was lower than untreated cream. There was no obvious difference in fat content between different honey treatments (table 2). This was in agreement with study of

Tajik and Jalali (20014), the fat percentage in all ginger-honey cream formulations was stable, therefore there is no significant difference between these formulations. And supported by the results of Belewu and Morakinyo (2009) that honey treated cheese samples showed no significant variation in the crude fat content.

Results showed that the untreated cream (T1) pH is higher than all the honey cream treatments (Table 2). The untreated cream pH that was equal to 6.6 did have clear difference with T2 (5% honey), T3 (10%honey) and T4 (15% honey), and the lowest pH was related to the T5 (20 % honey). There was a slight difference in ash content between both treated and un treated cream treatments, this was supported by the results of Belewu and Morakinyo (2009) that the ash content of the all honey treated cheese samples with 5 and 10% honey, showed no significant variation ($p > 0.05$), that due probably to the poor content of ash in honey (Mouteria, 2006).

Sensory Evaluation

Data in Table (3) showed the means of sensory properties revealed significant differences ($p \leq 0.05$) between the scores given by panelists for color and appearance, taste and flavor, texture, and Spread ability of the honey cream treatments and un-treated cream sample during storage periods.

The results in Table (3) shows that honey cream treatments with 5, 10, 15 and 20 % bee honey has a slight effect (no significant $p \leq 0.05$) on the color and appearance of the cream at 0, 5 days. But after 10 days of storage there was a significantly decrease in the degrees given to the un-treated cream treatment (T1) because of the fungal growth appeared in this treatment, While honey cream treatments T2, T3, T4 and T5 have been obtained high scores of 10.2, 10.5, 10.7 and 10.9 respectively at 15 days (Fig.1). This may be attributed to the positive effect of honey in reducing the counts of contaminated microorganisms in the cream. The various honey colors are basically all nuances of yellow amber like different dilutions of caramel sugar (Antony *et al*, 2002). Belewu and Morakinyo (2009) found that the color of honey-treated cheese samples differed significantly from un-treated samples due to the addition of honey. Thus, the color of honey-processed cheese samples could enhance consumer preference. The effect of the storage period on the color and appearance of the cream as shown on Fig. (2). Obviously there was no significant difference ($P > 0.05$) of all honey cream treatments.

In terms of taste and flavor, honey cream treatments got a higher rating by panelists. The addition of honey to the cream was accepted by consumers, honey cream was more acceptable than un-treated and BHA creams (Fig. 3). The sweet taste/flavor of the honey treated cream samples can be due to the addition of honey. However, the flavor and aroma of honey are usually derived from plant origin. The result herein was consistent with the report of Tajik and Jalali (2014), the flavored cream (formulation L) with 5% ginger powder and 30% honey, was the best formulas of flavored cream and more acceptable than traditional creams according to the assessors. The carbohydrates found in honey have the ability to improve the intensity of desirable flavors and reduce the intensity of others. Honey enhances sweetness Intensity, decreases sourness, and decreases the bitterness (Bogdanov, 2011). The effect of the storage period on the taste and flavor of honey cream treatments is shown in Figure (4), the highest score was found at day 0 where the honey creams were stored for 5, 10 and 15 days. The taste and flavor values decreased but the decrease was insignificant ($P > 0.05$).

Table 3 : The sensory evaluation of cream treatments during cold storage at $6 \pm 1^\circ\text{C}$

Storage period (day) \ Treatments		Color and Appearance (15)	Taste and Flavor (45)	Textures (30)	Spread ability (10)
T1	0	15.00	41.55	28.35	9.00
	5	13.00	36.80	23.6	8.80
	10	7.76	28.20	21.4	7.60
	15	6.43	22.40	20.1	5.70
T2	0	15.00	43.00	28.9	9.4
	5	14.76	41.33	27.00	9.20
	10	12.00	39.00	25.60	8.60
	15	10.2	37.83	25.00	7.60
T3	0	14.00	42.80	28.80	9.60
	5	13.83	41.00	27.60	9.40
	10	11.50	39.44	26.00	8.80
	15	10.50	38.49	25.80	7.80
T4	0	13.90	42.00	27.60	9.00
	5	13.20	40.43	27.40	9.00
	10	12.40	38.00	27.1	8.50
	15	10.70	38.61	26.70	7.30
T5	0	13.50	40.00	27.30	8.40
	5	13.00	38.00	27.30	8.30
	10	13.00	36.60	27.10	8.00
	15	10.090	37.65	27.90	7.80
T6	0	15.00	41.00	27.90	8.10
	5	13.00	37.33	24.00	7.80
	10	8.93	34.00	23.00	7.00
	15	7.25	30.80	21.60	6.50
LSD value		4.138 *	3.922 *	3.069	2.018 *

* The numbers in the table represent a rate for 5 repeaters.

Treatments: (T1: 0 % honey (untreated cream), T2: 5 % honey cream, T3: 10 % honey cream, T4: 15 % honeycream, T5: 20% honey cream).

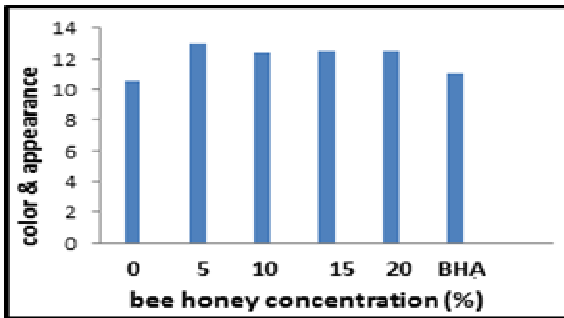


Fig. 1 : Effect of the bee honey concentration on the color and appearance of cream treatments

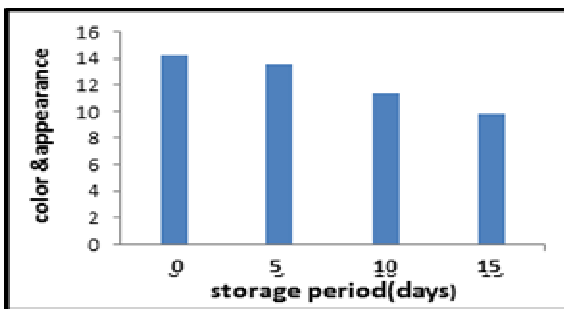


Fig 2: Effect of the storage period on the color and appearance of honey treated cream

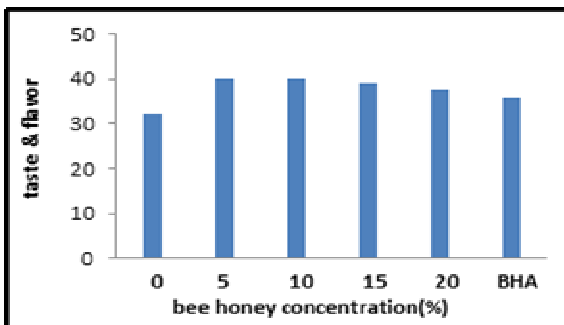


Fig. 3: Effect of the bee honey concentration on the taste and flavor of cream treatments

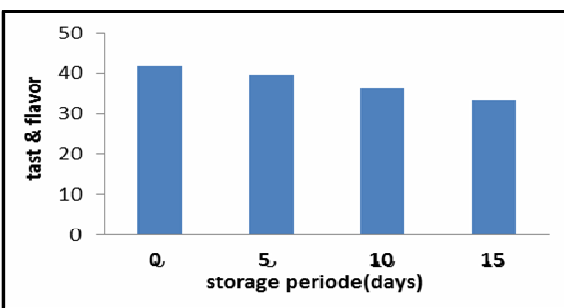


Fig. 4 : Effect of the storage period on the taste and flavor of honey treated cream

The results showed that the texture and Spread ability characteristics of the honey treated cream samples were positively affected by the addition of honey compared to the un-treated and BHA cream (Fig. 5,7). The grades given by the panelists for the honey cream treatments during the period 5, 10, 15 days for texture and at 15 day for Spread ability were significantly superior to those given for un-treated samples and BHA treatment (Table 3). When the effect of the storage period on the texture and the Spread ability properties of honey-treated cream treatments table (6), (8) were examined, the general trend was that the measured parameters estimates had decreased insignificantly ($P>0.05$) with the increase in the storage period.

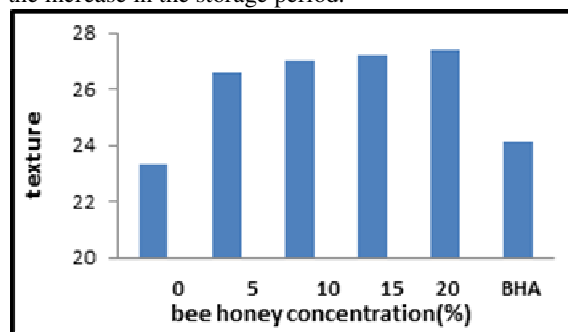


Fig. 5: Effect of the bee honey concentration on the texture of cream treatments of honey treated cream

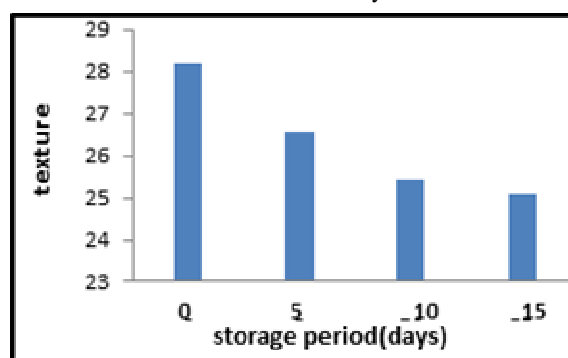


Fig. 6: Effect of the storage period on the texture

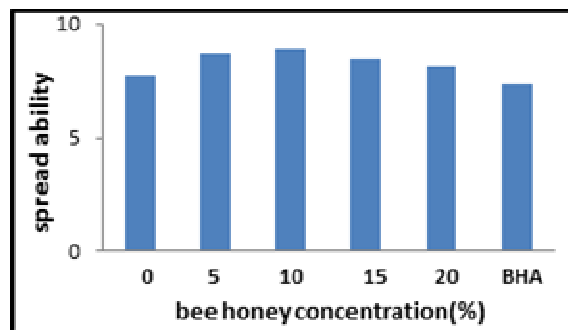


Fig. 7: Effect of the bee honey concentration on the spread ability of cream treatments

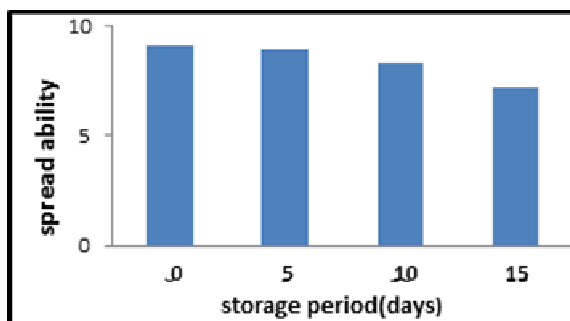


Fig. 8: Effect of the storage period on the Spread ability of honey treated cream

Conclusion

The result of this study revealed that eucalyptus honey had the greatest effect against all tested bacteria (*Pseudomonas aeruginosa*, *Klebsiella spp.*, *Serratia spp.*, and *Staphylococcus aureus*). Giving these results, honey may be useful for inhibiting microbial growth in cream and other milk products that are less stable. According to it we could improve the quality and the shelf life of cream. On the other hand, it was noted that honey impact flavor, color, sweet taste, texture and Spread ability characteristics to the cream samples treated with honey compared to the un-treated samples. Honey can help upscale the value (nutritive and sensory) of such cream sample and enhance consumer preference.

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