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Utilization of Propolis Extract as A Natural Preservative in Raw Milk

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ABSTRACT

Natural antimicrobials have been considered of more importance due to their increase concerns among chemical preservatives among consumers. The effect on the quality of raw milk sample by different concentrations of propolis (5, 10 and20%) in water extract (WEP), stored at 30°C and 5±1°C was evaluated. pH value, titratable acidity and microbiological examination were detected. The addition of 2% water extract of propolis (20% extract) to raw milk resulted in acceptability of the milk up to 12 and 48 hours at 30 and5±1℃, respectively. Total bacterial count, coliform, molds and yeasts gradually decreased with the addition of more concentration of water extract of propolis (5, 10 and20%), compared with the control. The effect of 1,2 and 3% of water extract of propolis on the characteristics of yoghurt during storage(14 day) at 5±1°C were studied. Titratable acidity of T1 and T2 treatments increased, compared with the control. T3(3% water extract of propolis) resulted in the highest value of total phenolic compounds, flavonoids and antioxidant activity .Sensory evaluation revealed that yoghurt samples fortified with 1 and 2% of water extract of propolis resulted in the best treatments until the end of storage period.

Keywords: Raw milk, propolis, physiochemical, microbiological examination, phenolic compounds, and sensory evaluation.

INTRODUCTION

Milk is considered to be as a complete food, containing high quantities of proteins, vitamins, and minerals. Natural milk (preservative-free) is perishable, and is usually of a relatively short lifetime, as it offers an ideal environment for the microorganisms to grow. Preservative-free milk can be of a conswiderable effect in spreading certain pathogenic bacteria causing salmonellosis, brucellosis, listeriosis, and tuberculosis. Unlike some other foods and drinks, the addition of preservatives to prolong the shelf life of milk is prohibited. For this reason, preservatives present in milk are considered as contaminants. With the increasing demand for dairy products and the necessity for reducing losses in industrial production due to poor quality, the requirement for high quality milk has increased. Numerous efforts were conducted to find out natural antimicrobial substitutes to prevent bacterial and fungal growth in foods and dairy products. Recently, due to the great consumer awareness, using natural preservatives became very popular due to the great consumer awareness to inhibit the growth of undesirable microorganisms in food. Such antimicrobials could be directly added into the product formulation, coated on its surface or incorporated into the packaging material. Propolis is a product, collected by honey bees from plant exudates, which gained popularity as an alternative medicine and as a substitute of antimicrobial substances used in the preservation of food and dairy products. Propolis is the substance responsible for neutralizing any bacteria, fungi or virus that enters the hive. It contains approximately 55% resinous compounds and balms, 30% beeswax, 10% aromatic essential oils, 5% bee pollen and about 150 compounds. Propolis were successfully used in treating numerous of human diseases such as the cardiovascular, blood systems disorder, infections of the respiratory system, dental care, dermatology, cancer treatment, immune system, digestive tract disorders and liver protection (Kolankaya *et al.,* 2002, Greenaway *et al.,*1996, Wilbey,1996, Najafi *et al.,* 2007; Sforcin, 2007 and Fuca *et al.*2013).

Nonethanolic propolis extracts compounds characterized with higher pharmacological activity, compared to ethanolic extracts. Propolis extract in water is also characterized with its higher effectivness, as compared to ethanolic extract of propolis. Furthermore, the derivatives water extract propilis and its polyphenolic compounds significantly decrease the growth and proliferation of tumour cells. A total of forty-four compounds have been identified in commercial Egyptian propolis (Volpert, and Elstner 1993, Orsolic and Basic 2003 and Farre *et al.,*2004).

Propolis is also characterized with a wide range of biological and pharmacological activities against bacterial and fungal infections in the bee hives (Bankova *et al.,*2000). It has potential to uncover new biologically active compounds with important pharmacological effects, especially antibacterial, antiviral, anti-inflammatory, antitumor, antioxidant, anticancer substances and new bioactive molecules (Hegazi *et al.,*1997, Kimoto *et al.,*1998 , Hegazi and Abd El Hady(2002). Furthermore, another example of propolis preservation properties, its antifungal activity in different fruit juices. However, they additionally concluded that due to its strong aromatic flavor, it should be added in small amounts, so as not to affect the organoleptic qualities of the product (Koc , 2007).

The present study was aimed to investigate the effects of water extract of propolis (WEP) as a natural preservative of raw milk and in maintaining its health promoting effects.

MATERIALS AND METHODS

Buffaloes' milk samples were obtained from local market, Giza. Egypt; its composition was: TS was 16.5% and Fat was 6.5 %. Fresh buffaloes' skim milk was obtained from the herd of the Faculty of Agriculture, Cairo University for yoghurt making (0.5% fat and 8.75% SNF).

Propolis used in this work was obtained from Plant Protection Department at the Faculty of Agriculture, Mansoura University. Propolis was kept at room temperature in the dark bottle until processing.

Yoghurt culture Direct Vat Set(DVS) of *Lb. delbrueckii ssp. bulgaricus* and *Streptococcus*

thermophilus in the ratio (1:1) were obtained from Chr. Hansen's Lab., Copenhagen, Denmark. The cultures were propagated in sterilized skim milk, and incubated at 37^0C for 16 hrs.

5, 10 ,20 and 40 g of fine ground propolis were mixed with 100ml deionized water and shaking at 65ºC for 2hours. It was cooled to room temperature, and centrifuged at 1500 rpm for 5 min. the supernatant was kept in a dark bottle until used (Said *et al.,*2006).

Organoleptic tests were done by experienced taste panelists including the staff members of Dairy Research Department, Food Technology Research Institute, agricultural Research Center.

For making the yoghurt, fresh buffaloes' skim milk was heated at 80^0C for 10 min and cooled rapidly to 4^0C, reheated to40^0C, inoculated with 2% of starter culture, and divided into 4 equal portions. The first was served as a control, while the second was mixed with 1% water extract of propolis, the third was mixed with 2% water extract of propolis, and the fourth was mixed with 3% water extract of propolis. All treatments were incubated at 42^0C for 3-4 hr. for coagulation, then the yoghurt cups were cooled to 15-20ºC, and transferred to the refrigerator (5±1ºC).The chemical and sensory evaluation were carried out in the fresh yoghurt and after7 and 14 days of storage. The pH value was measured using pH meter (HANNA 8417), the titratable acidity (TA) as described by Ling (1963). All chemical measurements were prepared in triplicates. Phenolic compounds, flavonoids and antioxidant activity in yoghurt samples were extracted according to the method of Li *et al.* (2009). The concentration of phenolic compounds in the extracts was determined by the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965), using gallic acid as a standard analysis were carried out in triplicate and calculated from a calibration curve obtained with gallic acid.

Microbiological analysis were carried out in all samples by detecting the total bacterial count (TBC), coliform and moulds & yeasts according to American public health association (APHA, 1992).

Sensory evaluation of yoghurt samples was conducted by panellists. The panellists were asked to evaluate the colour and appearance, aroma, body & texture, taste and overall acceptability when fresh and after 7and 14 days of storage (Ranadheera *et al*., 2012).

Data were statistically analyzed using SPSS (Ver.11) software program ANOVA with two independent factors at significant level of 0.05 (Steel *et al*., 1997). Multiple comparisons were carried out applying the least significant difference (LSD).

RESULTS AND DISCUSSION

Resuls in Table (1) illustrate the phenolic compounds , flavonids and antioxidant activites of water extracts of propolis (WEP). The total phenolic content in water extract was11.18 ± 0.511 mg/g ,while(El Sohaimy and Masry,2014) reported higher total phenolic content in Egyptain than Chinese propolis extracts. The limiting factors affecting the concentration of phenolic compounds are the type of solvents, extract temperature, stirring and the origin and source of the propolis (Hegazi *et al.,* 2014). It could also be found that the propolis extract in water was more effective, compared with the ethanolic extract. No significant differences were detected in the total phenolic compounds in nonethanolic solvent, compared with those found in ethanolic extract. Propolis nonethanolic extracts have antioxidant activity resulting mostly from ferulic and caffeic acids (Volpert and Elstner (1993 and Kubiliene *et al.,*2015). The antioxidant activity of propolis might be due to the ability of phenolic compounds to donate hydrogen ions, which can prevent the oxidation and deterioration of food substances during storage. The high antioxidant activity of propolis makes it a good natural antioxidant that can use as a natural preservative and/or food additives to prevent deterioration (El Sohaimy and Masry,2014).

Table 1. Phenolic compounds, flavonids and total antioxidant activity of water extracts of propolis20% (WEP)

Material	phenolic compounds(mg/g)	Flavonoids (mg/g)	Antioxidant activity (%)
WEP	11.18 ± 0.511	7.716 ± 0.587	70.44 ± 0.327

Results in Table (2) show that milk samples fortified by 5, 10 and 20% of water extract characterized with excellent color and odor, compared with control. In the same Table, data observed that unacceptable color and odor of milk samples fortified by 40% of water extract of propolis (2ml/100ml milk) were observed. The addition of 0.5 percent betel leaf extract (v/v) to raw milk was found to remain the acceptablity up to 11 hours of storage. Milk samples stored in calabash containers were of excellent taste and odor while fresh and after 2 days two, compared to milk samples stored in plastic containers (Sivakumar and Dhanalakshmi (2016) Yemane *et al.,*2016).

Table 2. Color and odor quality affected by different concentrations of water extract of propolis (WEP) in raw milk sample

Parameter	2ml extract / 100 ml milk				
	Control	**5%**	**10%**	**20%**	**40%**
Color	Excellent	Excellent	Exellent	Excellent	Unacceptable
Odor	Excellent	Excellent	Excellent	Excellent	Unacceptable

Excellent: 9-10 Good: 8-9 Unacceptable: Less than 6

The changes in pH and the titratable acidity of raw milk samples during storage at (30 and 5±1ºC) in the presence of different concentrations of water extract of propolis (WEP) (5,10 and 20%) as a natural preservative are given in (Table3). The pH of control(A) decreased from 6.72 to 4.32 and from 6.80 to 5.41 at the end of storage period at 30 and 5±1ºC, respectively. While treatments B, C and D fortified by using different

concentrations of water extract of propolis (WEP) (5,10 and 20%), the pH decreased ($P \leq 0.05$) ,especially with high concentrate of propolis extract (D) from 6.78 to 6.03 and from 6.77 to 6.37 at the end of storage period at 30 and 5±1ºC, respectively. The mode of action of a natural preservative is inhibition of microbial growth, oxidation and certain enzymatic reactions occurring in milk. Acidity results estimated by titration are presented in Table (3). Acidity of the control sample (A) significantly increased ($P < 0.05$) during storage, 0.153 to 0.762% and 0.153 to 0.235% after 24h. and 72h. at 30 and 5±1ºC, respectively. It is well known that the acidity in milk is developed due to the breakdown of milk sugar (lactose) into lactic acid by the fermentative effect of acid producing bacteria. Water extract of propolis treated milk samples (B, C and D) decreased by the addition of (2ml of propolis extract /100ml milk) ($P \leq 0.05$), compared with control ,especially in the presence of high concentration of propolis extract (D). Treated milk samples were of 0.684, 0.464, 0.275 and 0.248, 0.244, 0.187 % acidity after 24h. and 72h. at 30and 5±1ºC, respectively. An increase in the titratable acidity and decrease in the pH of the milk samples with added 0.5%, 0.75% and 1% level of tulsi leaves extract (Sivakumar, 2017). These results are in agreement with Abbas and Osman,1998.

Table 3. Changes in the PH values and Titratable acidity (%) of raw milk samples as affected by different concentrations of water propolis (WEP) addition

Time (Hour)	Treatments at 30ºC							
	pH Value				Acidity(%)			
	A	B	C	D	A	B	C	D
0h	6.78± 0.010	6.79± 0.032	6.77± 0.026	6.78± 0.005	0.153± 0.001^{b}	0.153± 0.032^{b}	0.155± 0.005^{a}	0.156± 0.003^{a}
3	6.72± 0.026	6.74± 0.022	6.69± 0.026	6.75± 0.132	0.173± 0.016^{a}	0.168± 0.022^{b}	0.166± 0.040^{bc}	0.160± 0.003^{c}
6h	6.33± 0.109^{d}	6.43± 0.040^{c}	6.54± 0.023^{b}	6.73± 0.025^{a}	0.125± 0.012^{a}	0.205± 0.017^{b}	0.187± 0.042^{c}	0.165± 0.044^{d}
9h	5.84± 0.010^{d}	5.94± 0.005^{c}	6.34± 0.027^{b}	6.66± 0.011^{a}	0.375± 0.005^{a}	0.311± 0.005^{b}	0.244± 0.009^{c}	0.169± 0.034^{a}
12h	5.45± 0.004^{d}	5.77± 0.067^{c}	5.94± 0.028^{b}	6.58± 0.010^{a}	0.443± 0.018^{a}	0.384± 0.041^{b}	0.294± 0.010^{c}	0.172± 0.023^{d}
15h	5.12± 0.027^{d}	5.44± 0.034^{c}	5.66± 0.023^{b}	6.45± 0.008^{a}	0.564± 0.002^{a}	0.456± 0.040^{b}	0.345± 0.007^{c}	0.191± 0.008^{d}
18h	4.90± 0.010^{d}	5.12± 0.005^{c}	5.25± 0.020^{b}	6.27± 0.025^{a}	0.685± 0.003^{a}	0.536± 0.104^{b}	0.487± 0.031^{c}	0.234± 0.030^{d}
21h	4.74± 0.036^{d}	4.85± 0.009^{c}	5.08± 0.031^{b}	6.10± 0.034^{a}	0.703± 0.013^{a}	0.610± 0.015^{b}	0.525± 0.023^{c}	0.258± 0.001^{d}
24h	4.32± 0.041^{d}	4.53± 0.024^{c}	4.74± 0.012^{b}	6.03± 0.010^{a}	0.762± 0.022^{a}	0.684± 0.007^{b}	0.464± 0.016^{c}	0.275± 0.042^{d}
	Treatments at 5±1 ºC							
	A	B	C	D	A	B	C	D
0h	6.80± 0.010	6.80± 0.032	6.78± 0.005	6.77± 0.026	0.153± 0.001^{b}	0.155± 0.005^{a}	0.155± 0.005^{a}	0.156± 0.003^{a}
12h	6.70± 0.005	6.73± 0.024	6.73± 0.025	6.76± 0.013	0.161± 0.001	0.160± 0.022	0.160± 0.012	0.158± 0.030
24h	6.62± 0.032^{b}	6.67± 0.013^{ab}	6.68± 0.032^{a}	6.72± 0.012^{a}	0.174 ± 0.011^{a}	0.173± 0.016^{ab}	0.168± 0.024^{b}	0.158± 0.017^{c}
36h	6.42± 0.044^{c}	6.48± 0.005^{b}	6.53± 0.021^{b}	6.64± 0.003^{a}	0.188± 0.019^{a}	0.185± 0.019^{a}	0.179± 0.016^{b}	0.165± 0.040^{c}
48h	6.23± 0.045^{c}	6.27± 0.004^{c}	6.36± 0.024^{b}	6.53± 0.016^{a}	0.200± 0.001^{a}	0.195± 0.012^{ab}	0.192± 0.015^{b}	0.172± 0.042^{c}
60h	5.95± 0.005^{d}	6.04± 0.007^{c}	6.12± 0.018^{b}	6.44± 0.007^{a}	0.232± 0.042^{a}	0.224± 0.011^{ab}	0.221± 0.014^{b}	0.181± 0.023^{c}
72h	5.41± 0.021^{d}	5.54± 0.031^{c}	5.75± 0.019^{b}	6.37± 0.030^{a}	0.235± 0.005^{a}	0.248± 0.001^{ab}	0.244± 0.013^{b}	0.187± 0.010^{c}

Mean values within each row followed by different letters in the superscript (a,b,c, …) are significantly different at $P \leq 0.05$.
A: Control B,C,D: WEP(5,10 and 20%)

Data show that the control raw milk samples was acceptable up to 6 hours and 24 hours at 30 and5±1ºC. While, in the presence of 2% water extract of propolis to the raw milk sample remained acceptable up to 12 hours and 48 hours at 30 and5±1ºC. However,

when using 0.5% of water extract of betel leaves to the raw milk resulted in acceptable up to 11 hours of storage (Sivakumar and Dhanalakshmi, 2016).

The total bacteria, coliform, moulds and yeasts counts were determined as affected by using water extract of propolis (2ml/100ml milk) during storage at 24h. and 72h. at 30 and 5±1ºC (Table4). Data showed that the highest total bacterial count obtained in untreated milk samples (control) during storage was 5.80 to 8.26 and from 5.81 to 6.57 (log cfu/ml) at 30 and 5±1ºC, respectively. While, the total bacterial count obtained in milk fortified with water extract of propolis decreased from 5.81 to3.34 and from 5.81 to 3.35 (log cfu/ml) at 30 and 5±1ºC, respectively. The same trend was observed in moulds & yeasts and coliform bacterial count. The presence of propolis was found to be active as an antibacterial and antifungal agents. These findings

came in accordance with those previously mentioned by several investigators, who noticed the antibacterial and antifungal effect against Gram positive bacteria, and those of Gram negative staining property *Escherichia coli* and *Staph. aureus* (Abd El Hady and Hegazi, 2002, Grange and Davey, 1990, Kujumgiev *et al* 1999, Bankova, 2005, Elalfy *et al.,* 2011, Yemane *et al.,*2016 and Sivakumar, 2017).

Table 4. Microbiological examination (log cfu/ml)of raw milk samples as affected by 20% water extract of propolis (WEP) addition

Time (Hour)	Treatments at 30ºC					
	Total bacterial count/ml		Mould and yeast/ml		Coliform count/ml	
	A	D	D	A	A	D
0h	5.807	5.611	4.118	4.005	5.123	5.003
3h	6.112	5.012	4.432	3.611	5.380	4.601
6h	6.357	4.841	4.605	3.455	5.658	4.320
9h	6.771	4.502	4.790	3.225	5.891	4.245
12h	7.015	4.271	5.121	2.986	6.174	4.121
15h	7.466	4.077	5.384	2.791	6.361	3.695
18h	7.810	3.892	5.619	2.306	6.521	3.815
21h	8.144	3.547	5.781	2.435	6.647	3.671
24h	8.262	3.340	5.826	2.185	6.705	3.507
			Treatments at 5±1ºC			
	A	D	A	D	A	D
0h	5.807	5.611	4.018	4.005	5.123	5.003
12h	5.961	5.105	4.113	3.722	5.178	4.436
24h	6.085	4.776	4.287	3.371	5.315	3.719
36h	6.147	4.308	4.365	2.930	5.367	3.232
48h	6.255	4.131	4.502	2.501	5.421	2.886
60h	6.415	3.805	4.681	2.372	4.537	2.531
72h	6.573	3.351	4.775	2.105	4.702	2.052

A: Control D: 2% of Water extract of propolis

Coagulation time of the yoghurt made by with adding different levels of water extract of propolis(1,2 and 3%) is given in Table (5).

Table 5. Coagulation time of yoghurt samples as affected by different values of water extract of propolis (WEP)

Treatments	Control	T1	T2	T3
Coagulation time H : min	3:05	2.45	2:55	3:15

control : 0 Water extract of propolis T1: 1% Water extract of propolis
T2: 2% Water extract of propolis T3: 3% Water extract of propolis

It could be seen that the treatment fortified by 1% of water extract of propolis (T1) recorded the lowest coagulation time (2,45 H.min), followed by T2 which recorded(2,55 H.min). On the other hand, control andT3 treatment recorded long coagulation time (3.05and 3.15H.min). The variation of coagulation time could be attributed to the effect of water extract of propolis being added on the activity of lactic acid bacteria, and on the ability of producing acid which led to slow rate of acid development, and prolonged the time of coagulation with level of water extract of propolis (T3 treatment). Similar results were obtained by Olasupo *et al.,*(1996) and Elalfy *et al.,*(2011)

The effect of adding different levels of water extract of propolis(1,2 and 3%) on acidity and pH of yoghurt samples made from buffalo skim milk during storage period at 5±1 ºC are given in Table (6). Data show that the addition 1% and 2% (T1 and T2 treatments) a gradual increase of titratable acidity in compared to control. While, T3 treatment(3% of WEP) recorded the lowest value in acidity with other treatments. Also, data showed resulted in a gradual decrease of pH with an increase of titratable acidity in control and all treatments during the storage period. Boubakeur *et al*., (2015) found that the flavonoids had positive effect on the growth of *Streptococcus thermophilus* and *Lactobacillus rhamnosus* as they act as prebiotics. These results agreed with Varga 2006 and Vijayalakshmi *et al.,*2010).

Table 6. Changes in the titratable acidity (%) and PH values of yoghurt samples as affected by different ratio of water extract of propolis (WEP) addition during storage

Storage (days)	C	T_1	T_2	T_3
Titratable acidity (%)				
Fresh	0.65 ± 0.01	0.67 ± 0.10	0.70 ± 0.06	0.64 ± 0.13
7	0.82 ± 0.09	0.85 ± 0.07	0.88 ± 0.03	0.80 ± 0.09
14	0.95 ± 0.13	0.99 ± 0.02	1.10 ± 0.06	0.93 ± 0.05
PH Values				
Fresh	4.52 ± 0.21	4.65 ± 0.05	4.61 ± 0.27	4.56 ± 0.29
7	4.39 ± 0.18	4.47 ± 0.16	4.44 ± 0.12	4.40 ± 0.13
14	4.25 ± 0.25	4.31 ± 0.09	4.29 ± 0.25	4.35 ± 0.08

control : 0 Water extract of propolis T1: 1% Water extract of propolis
T2: 2% Water extract of propolis T3: 3% Water extract of propolis

Phenolic compounds, flavonoids and antioxidant activity of yoghurt samples as influenced by different levels of water extract of propolis (WEP) are given in Table (7). Propolis is known to have antioxidant capacity thanks to its high concentration of polyphenolic compounds. Data showed that yoghurt samples with adding different concentrations of water extract of propolis (1,2 and 3%) characterized with an

increase of the phenolic compounds, flavonids and antioxidant activites, with the increase of propolis extracts concentration. Adding different propolis extracts increased the antioxidant capacity of dairy beverages. Polyphenolic compounds in propolis extract are probably more resistant to heat treatments, which provides protection to other antioxidant ingredients in the dairy products (Cottica *et al.,* 2015). Significant relationship was established between the total phenols and flavones and flavonols in either aqueous or methanolic extracts at the $P<0.01$ level. Miguel *et al.*,(2014).

Sensory evaluation of food products is an important indicator of potential consumer preference. The prepared yoghurt as shown in Table (8) showed that increasing levels of water extract of propolis negatively influenced the sensory scores of some properties of yoghurt.

Table7. Changes in phenolic compounds, flavonoids and antioxidant activity of the fresh yoghurt samples as affected by different ratio of water extract of propolis (WEP)

Treatments	Phenolic compounds (mg/100g)	Flavonoids (mg/100g)	Antioxidant activity(%)
C	0.639± 0.450^{d}	0.441± 0.848^{d}	59.11± 0.800^{d}
T1	0.750± 0.452^{c}	0.508± 0.401^{c}	60.54± 0.350^{c}
T2	0.865± 0.577^{b}	0.584± 0.513^{b}	62.38± 0.352^{b}
T3	0.972± 0.904^{a}	0.666± 0.500^{a}	63.65± 0.370^{a}
LSD	0.0251	0.0617	0.1524

Mean values within each row followed by different letters in the superscript (a,b,c…) are significantly different at $P \leq 0.05$.

Table 8. Sensory properties of the produced yoghurt as affected by as affected by different ratio of water extract of propolis (WEP) during storage.

Storage period (days)	Treatments C	T_1	T_2	T_3
Color and appearance (9)				
Fresh	8.51 ± 0.500^{a}	8.74 ± 0.300^{a}	8.53 ± 0.252^{a}	7.07 ± 0.404^{b}
7	7.24 ± 0.872^{b}	8.11 ± 0.306^{a}	8.07 ±0.252^{a}	6.03 ± 0.351^{c}
14	6.00 ± 0.400^{b}	7.80 ± 0.230^{a}	7.67 ± 0.503^{a}	6.07 ± 0.404^{b}
Aroma (9)				
Fresh	8.07 ± 0.404^{a}	8.47 ± 0.451^{a}	8.47 ± 0.351^{a}	6.33 ± 0.351^{b}
7	7.67 ± 0.306^{b}	8.30 ± 0.300^{a}	8.20 ± 0.120^{ab}	6.033 ± 0.351^{c}
14	6.50 ± 0.300^{b}	8.03 ± 0.351^{a}	8.03 ± 0.451^{a}	6.10 ± 0.361^{b}
Body & Texture (9)				
Fresh	7.07 ± 0.306^{b}	8.43 ± 0.104^{a}	8.47 ± 0.446^{a}	6.60 ± 0.2006^{b}
7	6.77 ± 0.252^{b}	8.30 ± 0.300^{a}	8.23 ± 0.252a	6.43 ± 0.404^{b}
14	6.23 ± 0.252^{b}	7.81 ± 0.404^{a}	8.40 ± 0.361^{a}	6.30 ± 0.300^{b}
Taste (9)				
Fresh	8.03 ± 0.351^{a}	8.47 ± 0.416^{a}	8.37 ± 0.351^{a}	6.37 ±0.351^{b}
7	7.77 ± 0.252^{b}	8.13 ± 0.306^{a}	8.07 ± 0.115^{ab}	6.20 ± 0.200^{c}
14	6.30 ± 0.300^{b}	7.75 ± 0.400^{a}	7.71 ± 0.306^{a}	6.03 ± 0.351^{b}
Overall Acceptability (9)				
Fresh	8.17 ± 0.289^{a}	8.600 ± 0.361^{a}	8.34 ±0.137^{a}	6.47 ± 0.252^{b}
7	7.33 ± 0.352^{b}	8.43 ± 0.306^{a}	8.00 ± 0.300^{a}	6.37 ± 0.351^{c}
14	6.03 ± 0.351^{b}	7.84 ± 0.351^{a}	7.71 ± 0.351^{a}	6.33 ± 0.351^{b}
Total Scores (45)				
Fresh	40.30 ± 0.100^{b}	42.16 ± 0.346^{a}	41.87 ± 0.050^{ab}	32.57 ± 0.154^{c}
7	36.27 ± 0.260^{b}	42.33 ± 0.203^{a}	41.60 ± 0.237^{a}	31.54 ± 0.115^{c}
14	31.14 ± 0.205^{b}	39.07 ± 0.250^{a}	39.43 ± 0.250^{a}	30.40 ±0.054^{b}

Mean values within each row followed by different letters in the superscript (a,b,c…) are significantly different at $P \leq 0.05$.

Yoghurt samples containing 1and 2% water extract of propolis (T1 and T2) gained higher scores for aroma, body& texture , taste and overall acceptability than control in fresh and during the storage period till 14 days at 5±1 ºC. However yoghurt samples with 3% water extract of propolis (T3) recorded the lowest scores for all parameter at fresh and during of storage period (Table 8). Also, yoghurt containing 1% of water extract of propolis (T1) recorded the highest values for overall sensory attributes as compared to other treatments at the end of storage period followed by T2 (2% of water extract of propolis). These results were similar to those observed by Metry and Owayss 2009, Cottica *et al.,* 2015, and Bakr *et al.,* 2015)

CONCLUSION

Results obtained in the present study confirm that the supplementation of raw milk with 2% water extract of propolis as a natural preservative (20% extract) was identified as the best in improving the quality and microbial safety. Also the yoghurt supplemented with 1 and 2% showed the highest sensory scores, compared with the control. This method of preservation could be used to encourage the dairy farming by making possible the collection of more milk of high quality, which in turn is prerequisite for increased manufacture of high quality yoghurt.

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استخدام مستخلص البروبوليس كمادة حافظة طبيعية في اللبن الخام

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تهدف الدراسة إلي استخدام مستخلص البروبوليس المائي كمادة حافظة طبيعية في اللبن الخام والابتعاد عن أضرار المواد الحافظة الكيميائية. تم تقييم حفظ اللبن باستخدام تركيزات مختلفة (٥و١٠ و٢٠%) من مستخلص البروبليس المائي بتقييم pH والحموضة والفحص الميكروبيولوجي أثناء التخزين علي درجات ٣٠ و ٥±١ م . أظهرت نتائج الحموضة أن إضافة ٢% من المستخلص البروبليسي المائي(استخلاص بنسبة ٢٠%) إلي اللبن الخام أدي إلي إطالة فترة الحفظ إلي١٢ ساعة و٤٨ ساعة علي درجات ٣٠ و ٥±١ م مقارنة بالكنترول. وأكدت نتائج الفحص الميكروبيولوجي انخفاض تدريجي في العد الكلي البكتيري ومجموعة الكوليفورم والفطريات والخمائر مع ارتفاع قيمة المستخلص البروبوليسي المضافة (٥و١٠ و٢٠%) مقارنة بالكنترول. تم أيضاً دراسة تأثير إضافة ١و٢و٣% من مستخلص البروبوليس المائي علي خواص الزبادي أثناء التخزين ١٤ يوم علي ٥±١ م .وأوضحت النتائج إرتفاع معدل الحموضة في المعاملات T1 و T2 مقارنة بالكنترول يقابله انخفاض في ال pH. كذلك أظهرت المعاملة T3 (٣% إضافة من المستخلص) إرتفاع قيم المواد الفينولية والفلافونيد ومضادات الاكسدة . وأوضحت نتائج التحكيم الحسي أن معاملات الزبادي المدعمة ب١و٢% من مستخلص البروبوليس المائي(T1 و T2) سجلت أعلي قيم الخواص الحسية حتي نهاية فترة التخزين.