

Effect of Propolis Extract as a Natural Preservative on the Microbial Content of Kareish Cheese

Amany M. El-Deeb¹ and Sabrien A. Omar²

¹Food Technology Research Institute, Agriculture Research center, Giza, Egypt.

²Dept. of Microbiol., Fac. of Agric. Mansoura Univ., Mansoura, Egypt.



ABSTRACT

The present study aimed to utilize propolis extract as natural preservative and attractive healthy ingredients on the microbial content of kareish cheese during storage. Effect of two extracts of propolis (ethanolic and water extract) were used at different concentrations on the growth of three bacterial strains, *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and *Bifidobacterium bifidum* being used as starter. The affect of these extracts on some spoilage contaminants of dairy microorganisms in milk medium were also studied. The resultant kareish cheeses from different treatments (with ethanol and water propolis extracts) were analyzed for phenolic compounds, antioxidant activity, microbiological and sensory properties when fresh and during storage (28 day) at 5±1°C. The counts of *Str. thermophilus* and *Lb. bulgaricus* in milk with different concentrations of propolis extracts were significantly higher than those in control. Ethanolic extraction of propolis (EEP) and water extraction of propolis (WEP) at 600 and 1000 mg /100ml displayed a bactericidal effect with all of the tested spoilage microorganisms. The addition of propolis extract had a negligible effect on the content of cheese moisture, and slight decrease in total protein of cheese was observed along the storage period. High acidity of kareish cheese observed with increasing the concentration of added propolis extracts. Addition of different concentrations (6 and 10%) of propolis extracts (ethanolic and water) increased of the phenolic compounds, flavonoids and antioxidant activity with the increase of propolis extracts. The counts of *Lb. bulgaricus* and *Str. thermophilus* in kareish cheese treatments were significantly ($p \leq 0.05$) higher than these in the control. However, counts of mesophilic (MBC) and psychrophilic (PBC), coliform bacteria and moulds & yeasts in kareish cheese treatments were not detected until 21 days. Kareish cheeses made with water propolis extract (T3 and T4) were of higher sensory evaluation and with the best acceptability during storage period, compared with cheese made by ethanolic propolis extract (T1 and T2).

Keywords: Kareish cheese, propolis extract, benefits bacteria, spoilage microorganisms, physicochemical, phenolic compounds, microbiological and sensory evaluation.

INTRODUCTION

Kareish cheese is one of the most traditional cheese consumed by Egyptians, a fat free, and characterized with its lower price. It is recommended for persons suffering from obesity, cholesterol and heart diseases. Kareish cheese contains high protein content and excellent source of calcium, phosphorous, and water-soluble vitamins. It contains high moisture content, and is not pickled after processing, it must be consumed fresh. Its maximum shelf-life does not exceed 12 days at 5 °C (Abou-Dawood and Gomai, 1977, Aman, 1994; El Bagoury and Mosaad, 2002, Fahmi, 1960 and Osman *et al.*, 2010).

Spoilage organisms in kareish cheese are various pathogenes such as *Salmonella*, *Listeria spp*, coliforms, *Staphylococcus aureus* and yeasts and molds may also be found in milk and dairy products (De Buysier *et al.*, 2001 and Reys *et al.*, 2002)

Therefore, there is a great effort exerted to improve the kareish cheese quality, and to increase its shelf life. However, the food industry is now facing challenge to reduce the use of synthetic antimicrobial chemical compounds, so there is a growing demand for using 'natural' additives (Burt, 2004).

Propolis is a resinous material collected by bees from plant. It acts as an antimicrobial, antifungal, antiviral against certain spp. of bacteria, fungi and virus. Flavonoids, aromatic acids, diterpenic acids and phenolic

are the principal compounds responsible for the biological activities of propolis. The above mentioned compounds could have an activating or inhibiting effect on growth and metabolism of bacteria. (Kujumgiev *et al.*, 1999, Bankova, 2005, Lu *et al.* 2005; Orsi *et al.*, 2005, Satoshi *et al.* , 2005; Vargas-Sanchez *et al.*, 2014 and Boubakeur *et al.*, 2015).

The aim of this study was to develop kareish cheese with healthy benefits by using ethanolic extract and water extract of propolis natural preservatives at different concentrations as a functional food during cold storage at 5±1 °C for 28days.

MATERIALS AND METHODS

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 until processing. Skim milk powder (SMP) produced by Australian Dairy products, Pty Ltd., Australia was used. Fresh buffalo skim milk used in kareish cheese making was obtained from the herd of the Faculty of Agriculture, Cairo University, Egypt. Guar Gum was obtained from Danisco Ingredients (Juelsminde, Denmark) by Misr Food Additives Company (MIFAD), Egypt. Commercial table salt obtained from local market.

Table 1. Chemical composition (%) of buffalo skim milk used in manufacture of kareish cheese

Item	fat	Moisture	protein	Carbohydrate*	Ash	Acidity	pH
Buffalo skim milk	0.4	89.27	4.40	4.97	0.96	0.16	6.67

By difference *

Freeze-dried culture DVS of *Lb. delbrueckii ssp. bulgaricus* , *Streptococcus thermophilus* and *B. bifidum* DI were obtained (from Chr. Hansen Laboratory,

Copenhagen, Denmark). Pure bacterial strains of LAB and *B. bifidum* DI were prepared separately as mother cultures in autoclaved (121 °C/10 min) reconstituted

skim milk powder (10%w/v) in conical flasks using 0.02% (w/v) inoculums after cooling to 37 °C for 16h. Cultures were prepared 24 h before use. Three bottles of sterilized skim-milk were inoculated with loopfuls of freshly-prepared cultures of *Lb. delbrueckii ssp. bulgaricus*, *Streptococcus thermophilus* and *B. bifidum* DI. The bottles were then incubated at 37°C for 12 h (Haddadin *et al.*, 2007), followed by storage at 4±1° C until used.

Three spoilage bacterial species; *Staphylococcus aureus*, *Bacillus cereus* and *E. coli* were grown on nutrient agar at 37 °C, and maintained at 4°C. Two spoilage fungal species; *Rodotorula glutinis* (yeast) and *Aspergillus oryzae* were cultured in potato dextrose agar at 28°C and maintained at 4°C. All cultures were kindly provided by Microbiology Dept. Faculty of Agric., Mansoura University, Egypt. Bacterial inocula were prepared by growing the cells in nutrient broth at 37 °C for 24 h. Cell suspensions were diluted with peptone water to provide initial cell counts of 10⁵-10⁷ CFU/ ml, fungal inoculum was prepared as spore suspension in peptone water to provide 10⁵ spore/ml.

Ethanol extraction of propolis (EEP) was prepared according the method mentioned by (Abd El Hady and Hegazi, 2002). Water extraction of propolis (WEP) was prepared with the same technique, with the exception of using water instead of alcohol for dissolving the propolis.

As with the effect of propolis extracts on LAB and *B. bifidum*, different concentrations of propolis extracts (namely 0, 300,600 and 1000 mg) were added to 100 ml reconstituted skim milk (10%w/v), followed by pasteurization at 63°C for 30 min. Duplicate conical flasks of each propolis concentration were inoculated with *Streptococcus thermophilus*, *Lb. delbrueckii ssp. bulgaricus* and *B. bifidum* DI (0.5 ml aliquots of a culture in skim-milk), and incubated at 37°C for 16 h in ;duplicate flasks of the control milk were treated similarly. After incubation, serial dilutions were made in sterile peptone water (15 g/l) and aliquots (0.1 ml) were spread onto pre-poured plates of selective media for each strain (M17 agar was used to enumerate *Streptococcus thermophilus*; *Lb. delbrueckii ssp. bulgaricus* on MRS agar ,while MRS agar supplemented with 0.05% (w/v) L-Cys-HCl (Merck) for *B. bifidum* DI count at 37±1°C for 48h under anaerobic condition. The results were recorded as colony-forming units (cfu) per ml of milk. (Haddadin *et al.*, 2008)

For examining the effect of propolis extracts on some spoilage microorganisms, different concentrations of propolis extracts (namely 0, 300,600 and 1000 mg) were added to 100 ml skim milk, carefully mixed, followed by pasteurization at 63°C for 30 min in screw-cap bottles. Control samples of milk without propolis were prepared. Duplicate bottles of each propolis concentration were inoculated with *E. coli*, *S. aureus*, *B. cereus* at 37°C, *Rodotorula glutinis* and *A. oryzae* and incubated at 25°C for 24 h and 3-5 days (for fungi) (0.5 ml aliquots of a culture in skim-milk) duplicate bottles of the control milk were treated similarly. After incubation, serial dilutions were made in sterile peptone water and were spread onto pre-poured plates of specific

media for each microbe; the results were recorded as colony-forming units (cfu) per ml of milk.

Kareish cheese was manufactured according to the method adopted by Fahmi (1960). Five batches of kareish cheese were made, first batch was served as control (C) being made from fresh buffalo skim milk without propolis extract. The other batches were fortified with propolis extracts (ethanolic and water) at the rate of 6 and 10 %. All treatments were heated at 75 °C for 15 sec, before the addition of extracts, and immediately cooled to 40°C. Propolis extracts (in four treatments) and starter culture (*Lb. delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus* (1:1) were added at level of 2% in control and all treatments for coagulation. After complete coagulation, the curd was separately transferred into gauze for wheying off in 24h. With 1% salt was dispersed on curd, then cut and stored in plastic bags contained pasteurized salted whey (3% salt). The resultant cheese was analyzed when fresh and after 7, 14, 21 and 28 day during storage at refrigerator (5± 1°C). All treatments were of three replicates.

The pH value of cheese was measured using pH meter (HANNA 8417). Titratable acidity (TA) as described by Ling (1963), moisture, protein, ash and soluble nitrogen contents were determined according to AOAC (2000). All chemical measurements were prepared in triplicates. Phenolic compounds and antioxidant activity were measured according to Li *et al.*, (2009).

M 17 agar was used to enumerate *Streptococcus thermophilus*, while MRS agar used for the enumeration of *Lb. delbrueckii ssp. bulgaricus* in Karish cheese

Skim milk agar was used to determine the total counts of mesophilic (MBC) and psychrotrophic (PBC) bacteria. Incubation was carried out at 30°C for 48 hours in MBC and at 6.5°C for 10 days in PBC. Coliform group was determined using MacConkey agar. Sorbitol MacConky agar was used for *E. coli*, and *Salmonella* & *Shigella* agar (SS) were used for *Salmonella typhimurium*.

Counts of *S. aureus* of cheese samples were estimated by Baird parker Agar medium at 37°C for 48 h. Potato dextrose agar was used for counting molds and yeasts at 25°C for 5 days (APHA, 1992).

Cheese samples were sensory scored by 8–10 panelists including the staff members of Dairy Research Department, Food Technology Research Institute, agricultural Research Center according to their consistency in attending as mentioned by Nelson and Trout (1964) for flavor (50 points), body and texture (35 points) and appearance (15 points). 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

The effect of different concentration of two extracts of propolis (ethanolic and water extracts) on the growth of three bacterial strains, namely *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and *Bifidobacterium bifidum* over

16h at 37°C is presented in (Table 2). Data showed that the counts of *Str. thermophilus* and *Lb. bulgaricus* in milk with different concentrations were significantly higher than in the control. Data also showed that the counts increased by increasing of the concentrations of propolis extracts. Enhancement of the growth was also demonstrated by Abd El Hady and Hegazi (2002) and Huang *et al.*, (2014). The positive effect of popolis might be attributed to the flavonoid content, which acts as antioxidants by chelating with free radicals (Boubakeur *et al.*, 2015).

On the other hand, the count of *Bif. bifidum* in milk in the presence of 300 and 600 mg/100 ml milk of each propolis extracts are not of significantly effect, compared to control. However, the two extracts of propolis at concentration 1000 mg/100ml milk had adverse effect on the count of *Bif. bifidum*. These results could be relevant for those using propolis as a medicine, and they are similar with those reported by Haddadin *et al.*, (2008). Probiotics, especially, bifidobacteria, grow poorly in milk, compared to the traditional yoghurt bacteria due to the lack of proteolytic and glycolytic activities, and also due to the higher nutritional demands of some nutrients (Mohammadi and Mortazavian, 2011).

Table 2. Effect of ethanolic and water extracts of propolis on the growth of *Str. thermophilus*, *Lb. bulgaricus* and *B. bifidum* (log.cfu/ml).

Strains	<i>Str. thermophilus</i>		<i>Lb. bulgaricus</i>		<i>B. bifidum</i>	
	EEP	WEP	EEP	WEP	EEP	WEP
C	6.43 ^b	6.73 ^b	7.17 ^b	7.66 ^c	6.22 ^{ab}	6.49 ^a
300	6.86 ^b	7.26 ^a	8.31 ^a	8.22 ^{bc}	6.37 ^a	6.70 ^a
600	7.41 ^a	7.48 ^a	8.53 ^a	8.51 ^a	6.86 ^a	6.87 ^a
1000	7.60 ^a	7.75 ^a	8.92 ^a	8.90 ^a	5.68 ^b	5.47 ^b
LSD	0.0076	0.0394	0.0021	0.0104	0.0237	0.0026

a, b, c: means with the same letter in the same column between different concentrates are not significantly different (p<0.05).

The count of *S. aureus*, *E. coli*, *B. cereus*, *R. glutinis* and *A. oryzae* in sterilized milk medium supplemented with 300, 600 and 1000 mg/100 ml of each EEP and WEP at 37°C are presented in Table (3).

Table 3. Effect of ethanolic and water extracts of propolis on some spoilage microorganisms (log.cfu/ml) in milk medium.

Treatment & concentrations mg/100ml	Microorganisms				
	<i>S. aureus</i>	<i>E. coli</i>	<i>B. cereus</i>	<i>R. glutinis</i>	<i>A. oryzae</i>
Control	5.15 ^a	5.25 ^a	5.44 ^a	7.25 ^a	7.26 ^a
EEP 300	3.51 ^b	4.84 ^b	4.30 ^b	5.56 ^b	5.67 ^b
600	ND ^c	ND ^c	ND ^c	ND ^c	ND ^c
1000	ND ^c	ND ^c	ND ^c	ND ^c	ND ^c
LSD	0.0127	0.1028	0.0761	0.0201	0.0081
Control	5.15 ^a	5.25 ^a	5.44 ^a	7.25 ^a	7.26 ^a
WEP 300	3.77 ^b	4.89 ^b	4.57 ^b	5.67 ^b	5.56 ^b
600	ND ^c	ND ^c	ND ^c	ND ^c	ND ^c
1000	ND ^c	ND ^c	ND ^c	ND ^c	ND ^c
LSD	0.0204	0.0141	0.0046	0.0275	0.0124

a, b, c: means with the same letter in the same column between different concentrates are not significantly different (p<0.05).

The results reveal that at concentration 300 mg of each EEP and WEP, the log cfu significantly reduced from the initial counts, compared with control These results were observed with all of the tested spoilage microorganisms. The obtained results also illustrate that

EEP and WEP at concentration 300 mg may have bacteriostatic effect. Whereas, by increasing the concentration of each them up to 600 and 1000 mg, the growth completely inhibited. These results indicated that EEP and WEP at 600 and 1000 mg displayed a bactericidal effect. The previous results are in a good agreement with those of Grange and Davey, (1990) and Temiz *et al.*, (2011). Confirmatory to the obtained results are also observed by Hanaa *et al.*, 2013 and Kubiliene *et al.*, (2015), who noticed that the propolis are antimicrobial activity. According to the obtained results, propolis could be considered as an ideal natural preservative.

Phenolic compounds, flavonoids and total antioxidant activity of (ethanolic and water) propolis extracts are illustrated in Table (4). Data showed that the phenolic compounds and flavonoids of ethanolic propolis were higher than in water propolis extract. Biologically active substances mostly have low solubility in water, and the amount of phenolic compounds in water extracts is 10-fold lower than in ethanolic extracts (Mello *et al.*,2010 and Moura *et al.*,2009) . While,(Volpert and Elstner 1993) found that the propolis extract in water suppresses is of more effective in suppressing the generation of free radicals than ethanolic extract agree with (Orsolic and Basic 2003 and Nagai *et al.*,(2003).

Table 4. Phenolic compounds, flavonoids and total antioxidant activity of ethanolic and water extracts of propolis.

Material	phenolic compounds (mg/g)	Flavonids (mg/g)	Antioxidant activity (%)
EPE	13.64 ± 0.440 ^a	10.57 ± 0.605 ^a	67.12 ± 0.288 ^b
WPE	11.18 ± 0.511 ^b	7.716 ± 0.587 ^b	70.44 ± 0.327 ^a
LSD	0.0810	0.3510	0.3012

a, b: means with the same letter in the same column between different concentrates are not significantly different (p<0.05).

Chemical composition of kareish cheese treatments made with ethanolic extract of propolis (EEP) and water extracts of propolis is shown in Table (5). It is obvious that the addition of propolis extract had a negligible effect on the moisture contents of cheese, and slight decrease in moisture and total protein of cheese treatments along the storage period. These data in agreed with Abd El-Aziz *et al.*, (2012). The slight differences of moisture in T2 and T4 might be due to the increase of the concentration of ethanolic and water propolis extracts, which came in agreement with Moawad *et al.*, (2001).Ash contents of kareish treatments showed slightly increase, compared with control when fresh and during the storage period. These results are in agreement with that reported by Ismail *et al.*, (2006) and Todaro *et al.*, (2013).

Soluble nitrogen content of kareish cheese varied significantly and increased directly with the storage period according to the microbial activity and due to the effect of propolis extract on lactic acid bacteria, especially, in T3 and T4 treatments. These results were in agreement with Moawad *et al.*, (2001) and Mahmoud *et al.*, (2013).

The changes in pH values and the titratable acidity values during storage at (5± 1°C) of different variants of Karish cheese using different concentrations of propolis extracts (ethanolic and water) are given in Fig. 1. Data showed that little differences between all

treatments including the control cheeses in pH values when fresh. In addition, it could be noticed that the pH values of all kareish cheeses gradually decreased during the storage period at (5± 1°C) for 28 days. The decrease in pH values during storage could be related to the hydrolysis occurred in lactose and protein contents. The results of the present study are in agreement with Magdoub *et al.*, (1995) and Janhøj *et al.*, (2008).

Table 5. Chemical composition (%) of Kareish cheese as affected by different concentrates of ethanolic and water extracts of propolis during storage period.

Treatments& Storage preiod	Moisture	Protein	Ash	S.N
Control				
Fresh	74.61 ^{Ba}	16.94 ^{Aa}	3.65 ^{ABc}	0.43 ^{Ae}
7day	74.14 ^{Bb}	16.81 ^{Ab}	3.70 ^{ABbc}	0.58 ^{Bd}
14day	73.80 ^{Bb}	16.54 ^{Ac}	3.77 ^{Ca}	0.79 ^{Bc}
21day	72.81 ^{BCc}	15.91 ^{Abe}	3.79 ^{Ca}	0.90 ^{ABb}
28day	72.15 ^{Bd}	15.29 ^{Cd}	3.84 ^{BCa}	0.98 ^{Ba}
T1				
Fresh	74.72 ^{Ba}	16.92 ^{Aa}	3.73 ^{Ae}	0.42 ^{Ae}
7day	73.81 ^{Cb}	16.81 ^{Aa}	3.77 ^{ABd}	0.56 ^{Bd}
14day	73.28 ^{BCc}	16.63 ^{Ab}	3.83 ^{ABc}	0.75 ^{BCc}
21day	72.83 ^{BCd}	16.23 ^{Ac}	3.90 ^{Bb}	0.86 ^{Bb}
28day	72.29 ^{Be}	16.01 ^{Ad}	3.96 ^{Ba}	0.97 ^{Ba}
T2				
Fresh	75.36 ^{Aa}	16.73 ^{Aa}	3.75 ^{Ae}	0.38 ^{Be}
7day	75.15 ^{Aa}	16.52 ^{ABb}	3.82 ^{Ad}	0.52 ^{BCd}
14day	74.55 ^{Ab}	16.32 ^{ABc}	3.92 ^{Ac}	0.67 ^{Cc}
21day	74.20 ^{Ac}	15.94 ^{ABd}	4.02 ^{ABb}	0.78 ^{Cb}
28day	73.68 ^{Ac}	15.65 ^{BCc}	4.14 ^{ABa}	0.93 ^{Ca}
T3				
Fresh	74.64 ^{Ba}	16.58 ^{ABb}	3.68 ^{ABe}	0.42 ^{Ae}
7day	74.41 ^{Ba}	16.37 ^{Bb}	3.74 ^{ABd}	0.61 ^{Ad}
14day	73.90 ^{Bb}	16.14 ^{Bc}	3.82 ^{ABc}	0.79 ^{Bc}
21day	73.50 ^{Bc}	15.89 ^{Bd}	3.91 ^{Bb}	0.93 ^{Ab}
28day	73.22 ^{ABc}	15.58 ^{Cde}	4.11 ^{ABa}	1.04 ^{Aa}
T4				
Fresh	75.67 ^{Aa}	16.76 ^{Aa}	3.79 ^{Ac}	0.44 ^{Ae}
7day	75.26 ^{Ab}	16.56 ^{ABa}	3.87 ^{Abc}	0.59 ^{ABd}
14day	74.66 ^{Ac}	16.13 ^{Bb}	3.96 ^{Ab}	0.82 ^{Ac}
21day	74.33 ^{Ad}	15.72 ^{Bc}	4.17 ^{Aa}	0.94 ^{Ab}
28day	73.94 ^{Ae}	15.35 ^{Dd}	4.22 ^{Aa}	1.06 ^{Aa}

A, B, C: Means with same letter among treatments in the same storage period are not significantly different.

a, b, c: Means with same letter for same treatment during storage periods are not significantly different

T1: kareish cheese with 6% EEP T3: kareish cheese with 6% WPE

T2: kareish cheese with 10% EEP T4: kareish cheese with 10% WPE

The changes in titratable acidity of cheese followed an opposite trend to pH. The obtained results indicate that cheese acidity was not greatly affected, while there was increase in acidity values of cheese with increasing the concentration of added propolis extracts. Then the cheese acidity greatly increased as the storage time prolonged. It was also noticed that after 28days of storage there were slight differences between the acid values of the control and the supplemented with propolis cheeses (Fig.1). The cheese acidity was affected with the storage period more than with adding propolis extracts (ethanolic and water). Similar results were also found by Moawad *et al.*, 2001 and Staffolo *et al.*, (2004)..

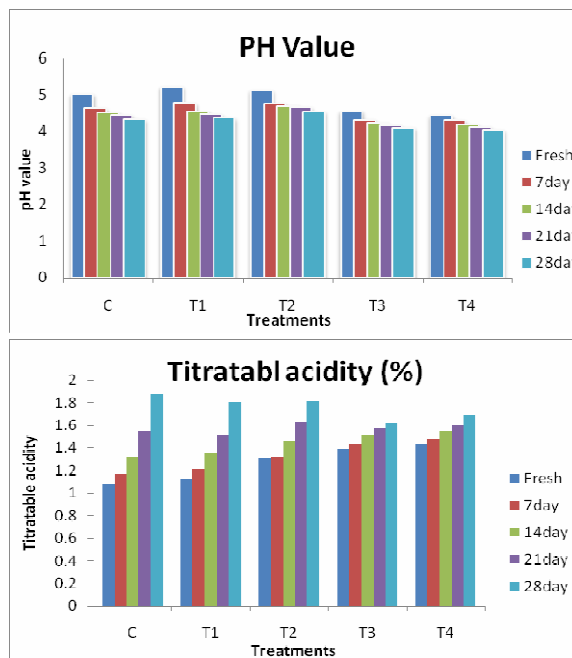


Fig 1. Changes in pH values and acidity (%) of kareish cheese treatments as affected by different concentrates of ethanolic and water extracts of propolis during storage period.

Table 6. Phenolic compounds, flavonids and total antioxidant activity of kareish cheese treatments.

Treatments	phenolic compounds (mg/g)	Flavonids (mg/g)	Antioxidant activity(%)
Raw milk	0.77 ± 0.219	0.39 ± 0.506	46.56 ± 0.150
Treatments			
Control	0.69 ± 0.461	0.34 ± 0.250	42.64 ± 0.000
T1	1.38 ± 0.089	0.94 ± 0.416	48.74 ± 1.209
T2	2.02 ± 0.352	1.35 ± 0.212	51.23 ± 2.027
T3	1.24 ± 1.409	0.76 ± 0.225	49.78 ± 0.209
T4	1.43 ± 0.256	1.06 ± 0.457	52.94 ± 0.031
LSD	0.1415	0.1524	0.1201

T1: kareish cheese with 6% EEP T3: kareish cheese with 6% WPE

T2: kareish cheese with 10% EEP T4: kareish cheese with 10% WPE

The effect of adding different concentrations of propolis extracts on phenolic compounds, flavonids and antioxidant activity of kareish cheese at fresh are illustrated in Table (6). As generally kareish cheese with adding different concentrations (6 and 10%) of propolis extracts (ethanolic and water) increased of the phenolic compounds, flavonids and antioxidant activity with the increase ratio of propolis extracts. Adding different propolis extracts increased the antioxidant capacity of dairy beverages. On the other hand, pasteurization didn't affects on the antioxidant capacity of beverages with added ethanolic propolis extract. (Cottica *et al.*, 2015).

Table (7) showed the effect of adding different concentrations of EEP and WEP on the count of *Lb. bulgaricus* and *Str. thermophilus* in fresh kareish cheese and during storage periods at 5 ± 1°C for 28 days. Data show that the counts of *Lb. bulgaricus* and *Str. thermophilus* in EEP or WEP supplement kareish cheese

were significantly ($p \leq 0.05$) higher than in the control cheese. The increase in counts could be attributed to the presence of propolis extracts. These results are similar to those reported by Saddiq and Danial, (2014). The counts of *Str. thermophilus* and *Lb. bulgaricus* in kareish cheese samples (T1) with the addition of EEP were higher than these detected in all treatments after 21days storage.

Prolonging the storage period resulted in an increase in counts of two bacterial strains until 14 days in cheese control, while the increase in cheese with added EEP and WEP continued until 21 days. These results are similar to those obtained by Haddadin *et al.*, 2008 and El-Bialy (2016).

Table 7. Effect of EEP and WEP on growth of *Str. thermophilus* and *Lb. bulgaricus* in Kareish cheese treatments during storage period.

Storage Period Days	Treatments				
	C	T1	T2	T3	T4
			<i>Str. thermophilus</i>		
Fresh	5.87 ^b	6.29 ^c	6.19 ^e	6.32 ^c	6.15 ^c
7	5.91 ^b	6.71 ^d	6.50 ^d	6.59 ^d	6.30 ^d
14	6.22 ^a	6.95 ^c	6.91 ^c	6.84 ^c	6.85 ^c
21	5.97 ^b	7.43 ^a	7.40 ^a	7.40 ^a	7.25 ^a
28	5.32 ^c	7.09 ^b	7.06 ^b	7.06 ^b	7.02 ^b
LSD	0.0204	0.0363	0.0247	0.0168	0.0016
			<i>Lb. bulgaricus</i>		
Fresh	6.90 ^c	7.09 ^e	6.54 ^c	6.25 ^d	6.44 ^d
7	7.25 ^b	7.49 ^d	6.91 ^d	6.54 ^c	6.76 ^c
14	7.63 ^a	7.81 ^c	7.25 ^b	6.84 ^b	6.97 ^b
21	7.05 ^c	8.23 ^a	7.64 ^a	7.10 ^a	7.31 ^a
28	6.64 ^d	8.01 ^b	7.07 ^c	6.84 ^b	6.83 ^c
LSD	0.0248	0.0405	0.0113	0.0323	0.0211

Different small letters within each treatment during storage on the same column are differing significantly at $p \leq 0.05$.

The effect of adding different concentrations of EEP or WEP on the counts of mesophilic and psychrophilic (MBC, PBC), total coliform, *E. coli*, *S. aureus*, *Salmonella* sp. and M&Y in Kareish cheese during storage periods at $5 \pm 1^\circ\text{C}$ for 28 days is presented in Table (8). The obtained results showed that MBC and PBC were not detected in control of kareish cheese when fresh or during storage until 7 days. By increasing the storage period the counts of MBC or PBC increased until 28 days. MBC and PBC in the treated kareish cheese were not detected until 14 and 21 days, respectively. This might be due to the post contamination during storage. Also, the obtained results reveal that the water extract of propolis (WEP) at 10% was more effective than other treatments.

Counts of coliform detected in the control cheese was 3.63 log cfu/g and 4.18 log cfu/g after 14 and 28 days of storage, respectively. The treated cheese with propolis extracts resulted in the inhibition of coliform group and lowered the maximum growth level in the cheese at the end of storage period. Kareish cheese samples made by the addition of 10% EEP was found completely free from coliforms.

Salmonella spp. and *S. aureus* were not detected in all cheese treatments when fresh or during the storage period. This might be due to the antimicrobial substances formed by lactic acid bacteria against the majority of other bacteria, from one side (Jacobsen *et al.*, 1999), and the high hygienic condition during making and storage of cheese, from othe side.

Moulds and yeasts count detected in the control cheese after 7 days of storage. While the treated kareish cheese with propolis extracts lead to the inhibition and retardation of moulds and yeasts growth, as they were not detected in treated cheeses until 21 days.

From the achieved results, it is clear that the addition of water extract of propolis at concentration of 10% is relatively more effective than other concentrations. These results are confirmed with those

observed by Cowan, (1999), Temiz *et al.*, (2011), Abou Dawood (2002) and Ismail *et al.*, (2006)

Sensory evaluation of dairy products is of importance of the potential preference of the consumer. Sensory properties of Kareish cheese were affected by different concentrates of propolis extracts when fresh and during the storage period at ($5 \pm 1^\circ\text{C}$) for 28 days (Table(9)). Significant differences ($p \leq 0.05$) were found between cheeses, where the type of propolis extracts (ethanolic or water) were the principle factors influencing the sensory properties of the treated cheeses. Also, the score for flavor and texture were affected by the level of ethanol and water propolis extracts. kareish cheeses made with water propolis extract (T3 and T4) were more accepted by the panelists, as compared with cheese made by ethanolic propolis extract (T1 and T2), which characterised by higher acid flavour and of non-acceptance. In addition, the score of fresh samples indicated that kareish cheese treatments (T4) with 10% water extracts of propolis (WEP) gained the highest score, compared with other treatments, followed by the control. These results are in agreement with Moawad *et al.*, 2001 and Metwalli, 2011). On the other hand, the addition of higher concentration of ethanolic extract of propolis (EEP) in kareish cheese(T2) decreased the flavor, texture and appearance score, compared with other treatments.

Statistical analysis for total score values of kareish cheese treatments cleared that treatments T3 and T4 were significantly different than control. This was noticed among fresh and stored samples. Storage of kareish up to 28 days decreased the quality of all treatments including the control. Treatments containing water propolis extract remained as the best acceptable product, followed by control treatment, while treatments containing ethanolic propolis extract (T1, T2) came last and were of less preferable to panelists at the end of storage. Ethanolic extract of propolis (EEP) has some disadvantages such as a strong taste and adverse reactions or intolerance to the alcohol (Mello *et al.*,2010 and Matsui *et al.*,2004).

Table 8. Microbiological analysis of kareish cheese treatments with WEP and EEP extracts at different concentrations during cold storage

Treatments	Storage Period (days)	MBC	PBC	Total coliform	<i>E.coli</i>	<i>Salmonella sp.</i>	<i>S. aureus</i>	M&Y
C	Fresh	ND ^c	ND ^d	ND ^d	ND	ND	ND	ND ^d
	7	ND ^c	ND ^d	ND ^d	ND	ND	ND	ND ^d
	14	4.80 ^b	3.61 ^c	3.63 ^c	ND	ND	ND	3.27 ^c
	21	5.37 ^{ab}	4.27 ^b	4.27 ^b	ND	ND	ND	3.27 ^b
	28	5.76 ^a	4.82 ^a	4.18 ^a	ND	ND	ND	3.94 ^a
	LSD	0.0075	0.0155	0.0108	-----	-----	-----	0.0194
T1	Fresh	ND ^c	ND ^b	ND ^b	ND	ND	ND	ND ^b
	7	ND ^c	ND ^b	ND ^b	ND	ND	ND	ND ^b
	14	ND ^c	ND ^b	ND ^b	ND	ND	ND	ND ^b
	21	3.52 ^b	ND ^b	ND ^b	ND	ND	ND	ND ^b
	28	4.28 ^a	3.37 ^a	2.34 ^a	ND	ND	ND	4.33 ^a
	LSD	1.0218	0.0192	0.1009	----	-----	-----	0.0276
T2	Fresh	ND ^b	ND	ND	ND	ND	ND	ND ^b
	7	ND ^b	ND	ND	ND	ND	ND	ND ^b
	14	ND ^b	ND	ND	ND	ND	ND	ND ^b
	21	ND ^b	ND	ND	ND	ND	ND	ND ^b
	28	4.24 ^a	ND	ND	ND	ND	ND	4.33 ^a
	LSD	0.0040	-----	----	----	-----	-----	0.0576
T3	Fresh	ND ^c	ND ^b	ND ^b	ND	ND	ND	ND ^b
	7	ND ^c	ND ^b	ND ^b	ND	ND	ND	ND ^b
	14	ND ^c	ND ^b	ND ^b	ND	ND	ND	ND ^b
	21	3.43 ^b	ND ^b	ND ^b	ND	ND	ND	ND ^b
	28	4.52 ^a	3.76 ^a	3.26 ^a	ND	ND	ND	4.27 ^a
	LSD	0.0739	0.0807	0.1091	-----	-----	-----	0.1052
T4	Fresh	ND ^b	ND	ND ^b	ND	ND	ND	ND ^b
	7	ND ^b	ND	ND ^b	ND	ND	ND	ND ^b
	14	ND ^b	ND	ND ^b	ND	ND	ND	ND ^b
	21	ND ^b	ND	ND ^b	ND	ND	ND	ND ^b
	28	4.26 ^a	ND	3.13 ^a	ND	ND	ND	3.86 ^a
	LSD	0.1173	-----	0.0937	----	-----	-----	0.1040

Different small letters within each treatment during storage on the same column are differ significantly at $p \leq 0.05$

Table 9. Sensory evaluation of kareish cheese treatments as affected by ethanolic extract of propolis (EEP) and water extracts of propolis (WEP) during storage.

Storage perio (days)	C	T1	Treatments T2	T3	T4
			Flavor(50)		
Fresh	40.33 ^{Ea}	45.00 ^{Ca}	48.33 ^{Aa}	42.33 ^{Da}	46.30 ^{Ba}
7	44.70 ^{Bab}	40.33 ^{Cab}	35.01 ^{Db}	44.33 ^{Bb}	47.67 ^{Ab}
14	44.00 ^{Bb}	37.00 ^{Dbc}	36.00 ^{DEab}	41.00 ^{Cc}	46.33 ^{Ac}
21	41.02 ^{Bc}	34.67 ^{Cc}	33.12 ^{CDbc}	41.67 ^{Bc}	45.33 ^{Ad}
28	35.04 ^{Cd}	30.00 ^{Dd}	28.33 ^{Ec}	41.67 ^{Bc}	43.67 ^{Ae}
			Body & Texture (35)		
Fresh	33.70 ^{ABa}	30.33 ^{BCa}	31.00 ^{Ba}	33.00 ^{ABa}	34.00 ^{Aa}
7	32.72 ^{Aa}	26.33 ^{BCb}	28.67 ^{Bab}	32.00 ^{Aab}	32.67 ^{Aab}
14	30.33 ^{ABa}	24.00 ^{Dbc}	26.00 ^{Cbc}	29.33 ^{Bbc}	31.33 ^{Aabc}
21	26.04 ^{BCb}	21.33 ^{CDcd}	22.33 ^{Ccd}	27.67 ^{Bcd}	29.67 ^{Abc}
28	24.15 ^{BCb}	18.25 ^{CDd}	19.03 ^{Cd}	25.00 ^{Bd}	27.67 ^{Ac}
			Appearance (15)		
Fresh	14.00 ^{Aa}	12.00 ^{BCa}	12.33 ^{Ba}	12.67 ^{Ba}	13.67 ^{ABa}
7	14.00 ^{Aa}	11.02 ^{Ca}	8.07 ^{Db}	12.00 ^{Ba}	13.33 ^{ABa}
14	12.12 ^{ABa}	10.33 ^{Ba}	7.33 ^{Cb}	10.03 ^{BCab}	13.33 ^{Aa}
21	11.30 ^{ABab}	9.25 ^{Bab}	6.33 ^{Db}	8.33 ^{Cb}	12.06 ^{Aab}
28	9.11 ^{Bb}	7.31 ^{Db}	6.03 ^{Eb}	8.33 ^{Cb}	10.25 ^{Ab}
			Total(100)		
Fresh	96.33 ^{ABa}	83.67 ^{CDa}	84.00 ^{Ca}	92.67 ^{Ba}	96.67 ^{Aa}
7	92.67 ^{Ba}	77.33 ^{Db}	76.33 ^{DEb}	89.00 ^{Ca}	95.03 ^{Aab}
14	86.33 ^{Bb}	71.67 ^{Dc}	69.00 ^{Ec}	84.05 ^{Cb}	90.67 ^{Abc}
21	76.67 ^{Cc}	65.33 ^{Dd}	62.13 ^{Ed}	80.33 ^{Bb}	87.00 ^{Acd}
28	67.67 ^{Cd}	55.33 ^{De}	53.67 ^{Ee}	74.33 ^{Bc}	82.33 ^{Ad}

A, B, C: Means with same letter among treatments in the same storage period are not significantly different.

a, b, c: Means with same letter for same treatment during storage periods are not significantly different

CONCLUSION

The present study confirmed that adding of 6 and 10% of water extracts of propolis (WEP) to kareish cheese can be recommended as natural and safe sources of phenolic compound, high acceptability and antimicrobial agents during storage periods.

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تأثير مستخلص البروبوليس كمادة حافظة طبيعية علي المحتوي الميكروبي في الجبن القريش أماني محمد الديب¹ وصابرين أحمد عمر²

¹معهد بحوث تكنولوجيا الأغذية-مركز البحوث الزراعية – الجيزة – مصر

²قسم الميكروبيولوجي- كلية الزراعة – جامعة المنصورة – المنصورة – مصر

تهدف الدراسة إلى استخدام مستخلص البروبوليس كمادة حافظة طبيعية لإنتاج جبن قريش ذو قيمة غذائية وصحية عالية. تم استخدام نوعين من مستخلص البروبوليس (الكحولي والمائي) بتركيزات مختلفة ودراسة تأثيرهم علي نشاط بكتيريا *Streptococcus thermophilus*, *Lactobacillus bulgaricus* و *Bifidobacterium bifidum* وبعض الميكروبات الملوثة لمنتجات الألبان في بيئة لين سائلة. كذلك تم تحليل الجبن القريش المصنع بالمعاملات المختلفة (١٠ و ٦%) لكل من البروبوليس الكحولي والمائي لدراسة الخواص الكيميائية، المواد الفينولية، مضادات الأكسدة، الخواص الميكروبيولوجية والحسية بعد التصنيع وأثناء فترات التخزين المختلفة حتي ٢٨ يوم عند ٥ ± ٥°م. أظهرت النتائج أن العد الكلي لبكتيريا أي أعداد ميكروبية علي بيئات العد للميكروبات الملوثة تحت الدراسة عند تركيزات ٦٠٠، ١٠٠٠، ١٠٠/مجم ١٠٠ مل لين. وأوضحت نتائج التحليل الكيمائي أن تأثير مستخلص البروبوليس ضعيف علي محتوى الرطوبة أو البروتين، حيث أن هناك نقص طفيف في معاملات الجبن القريش أثناء التخزين. بينما أظهرت نتائج الحموضة، والمواد الفينولية ومضادات الأكسدة ارتفاعاً في القيم بزيادة نسبة المستخلص المضافة (٦ و ١٠%). أيضاً أظهرت النتائج أن العد الكلي لبكتيريا *Streptococcus thermophilus*, *Lactobacillus bulgaricus* في معاملات الجبن القريش زيادة معنوية، بينما لم تظهر أي اعداد من البكتيريا الميزوفيلية، السيكروفيلية، بكتيريا الكوليفورم والخمائر والفطريات حتي ٢١ يوم من التخزين. وأشارت نتائج التحكيم الحسي أن المعاملات T3 و T4 المضاف لها مستخلص البروبوليس المائي كانت أعلى درجات التحكيم، بالإضافة إلي تحسين الخواص الحسية طوال فترة التخزين مقارنة بالكنترول والمعاملات T1 و T2 المضاف لها مستخلص البروبوليس الكحولي. لذلك توصي الدراسة بانتاج الجبن القريش بإضافة مستخلص البروبوليس المائي.