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Effect of Adding Propolis on Quality Standards of Raw Milk and Yoghurt

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Abstract

Food additives spread around the world may have potentially harmful effects, Propolis is considered a natural additive that meets the increasing demand for natural antioxidants and antimicrobials in place of synthetic preservatives. Different concentrations of water extract of Propolis (WEP) were assessed, the pH and microbiological quality of raw milk were evaluated, as well as the quality characteristics and bioactives in manufactured yoghurt. WEP 20% was the best concentration compared to WEP 5% and 10% and exhibited an acceptable pH value of milk for 48 hours. The addition of increased concentrations of WEP 20% (1, 2, and 3%) resulted in a significant decrease and gradual reduction of the total bacterial, coliform, yeast, and mold counts compared to the control group. Propolis-supplemented yoghurt had higher pH values than the control group. Yoghurt groups treated with 1% and 2% WEP achieved the highest scores and significantly different ($P<0.05$) with control and 3% WEP groups in sensory examination until the end of the storage period. Furthermore, the counts of yeast and mold progressively decreased with the addition of higher concentrations of WEP throughout the storage period as 2%, 3% WEP groups were significantly different ($P<0.05$) with control and 1% WEP groups. The total phenolic, flavonoid content and antioxidant activity of yoghurt treated with WEP were improved and significantly different ($P<0.05$) compared to the untreated group. In conclusion, the raw milk and yoghurt preserved with propolis improved the quality of milk and increased bioactivity and nutritional benefits of yoghurt by elevating its antioxidant capacity. As a consequence, the produced yoghurt in our study proved that it is an acceptable product with functional, probiotic potential and has health-promoting properties that might be commercialized.

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Introduction

Propolis contains a significant concentration of physiologically active ingredients that have potent antioxidant effects.[1] Many chemicals are known to be involved in propolis antioxidant properties, including kaempferol and phenethyl caffeate, as well as flavonoids and phenolic compounds.[2] Propolis antioxidant activities may be connected to flavonoids and phenolic compounds.[3] Propolis flavonoids and phenolic compounds have the ability to scavenge free radicals and bind heavy metal ions and biological polymers, making them potential antioxidant molecules. Furthermore, propolis phenethyl ester of caffeic acid inhibits the production of reactive oxidative.[4] Moreover, caffeic acid's phenethyl ester acts as a superoxide radical scavenger and lipid peroxidation inhibitor.[5] Propolis has been investigated for its antimicrobial action since the late 1940s, it has been used to fight a wide range of bacteria, fungi, and viruses, which has shown varying efficacy against various species.[6,7]

During the current COVID-19 pandemic, propolis has attracted significant scientific interest due to its anti-inflammatory and immunoregulatory benefits and its potent antiviral action towards pathogens that induce severe syndromes, particularly those carried on by corona viruses.[8,9]

Bees collect propolis which is dark sticky resinous material from trees, leaf buds, twigs, and trunk wounds, including *Populous spp*. of *Castanea sativa* and *Aesculus hippocastanum*. Propolis is mixed with beeswax when it is carried back to the colony, which is used to seal and sterilize the colony nest by worker "hive" bees.[10,11]

It contains about 55% resinous components and balms, 30% beeswax, 10% fragrant essential oils, and 5% bee pollen[12] as well as 300 chemicals.[13] Since ancient times, propolis has long been utilized in folk medicine to keep people healthy.[14] Propolis has a wide range of biological qualities, including hepatoprotective, immunostimulant, antiviral, antifungal, antibacterial, local anesthetic, anti-inflammatory, antioxidant, and cytostatic properties.[15]

Natural (preservative-free) milk is perishable and has a short shelf-life since it provides a perfect environment for microorganisms to proliferate. Milk without preservatives can have a significant impact on the propagation of harmful microorganisms that cause salmonellosis, brucellosis, tuberculosis, and listeriosis as a few of the most common infections in humans. The demand for good quality milk has increased as the demand for dairy products has grown to reduce the industrial losses owing to low-quality milk. To inhibit microbiological growth in dairy products, multiple efforts were made to identify natural antibacterial alternatives. Natural preservatives have become increasingly popular because of increased consumer knowledge to inhibit the proliferation of unwanted microbes in food. Antimicrobials might be in the product composition, product surface, or packing substances.[16]

Despite its acidity, yoghurt promotes the growth of spoilage microorganisms, such as yeast and mold.[17] Aside from yeast and molds, an excessive amount of lactic acid bacteria (LAB) can induce changes in organoleptic properties, such as acidity, which can shorten the shelf life of yoghurt. The proliferation of these bacteria during storage might result in undesirable sensory properties, gas production, color changes, emulsion cracking, and pH reductions.[18] Supplementing yoghurt with propolis as a natural preservative was effective in improving the quality and sensory scores (color and appearance, body and texture, flavor, taste) of yoghurt.[16]

Propolis antibacterial, antifungal, and antioxidant properties, as well as its availability in food and/or food additives, which is generally recognized as safe[19,20] makes it a good contender for usage in new food products as a natural ingredient preservative. Also, apiary products are a strong source of antioxidants, they produce a high content when they are added to yoghurt and milk, which increases the polyphenol content and antioxidant activity.[21]

In comparison to the ethanolic extract of propolis, the water extract of propolis (WEP) is distinguished by its increased efficacy. Furthermore, the polyphenolic components in WEP and its derivatives greatly reduce tumor cell growth and proliferation. In commercial Egyptian propolis, a total of 44 compounds have been discovered.[22,23]

Consumers have recently begun to believe that natural preservatives are preferable to synthetic

preservatives in terms of efficacy and safety, although synthetic preservatives have been linked to carcinogenic and teratogenic effects.[24] Propolis in food has various health benefits for consumers, as well as ensuring product safety, microbiological stability, and food quality throughout storage.[25] All foods originating from animals, including meat products, are particularly vulnerable to microbial contamination. Propolis is used to preserve food quality and microbiological stability while it is being stored.[26] Propolis contains a substance called artepillin C that has significant effects as fruit preservation and potent antifungal action.[27]

The aim of the present research was to examine the effects of WEP as natural preservation on the quality of raw milk and quality characteristics, plus the bioactive properties of manufactured yoghurt.

Materials and Methods

Preparation of Propolis

Crude Propolis (honey bee glue) samples were collected using Propolis traps from propolis samples honeybees Research Department Apiaries at Plant Protection Research Institute, ARC, Egypt. Impurities (wood, straw, pieces, and insects) were manually removed, which were then finely ground in a waring blender before being placed in a dark glass container at room temperature till use.[28] In the current experiment, various amounts of 5, 10, and 20 g of finely ground propolis were utilized to determine the optimal amount of propolis to keep milk pH for a protracted period of time, and then 100 ml of deionized water was added then shaken by vortex mixer (Labnet International, Inc, Edison, NJ USA) for 2 hours at 65 °C. The solutions were cooled to ambient temperature before being centrifuged (Remi Centrifuge, Bombay, India) for 5 minutes at 1,500 rpm. In a dark bottle, the supernatant was preserved until it was utilized.[29]

Preparation of Raw Milk

1,700 ml raw cow milk (Holstein breed, 3.6% fat, and 3% protein) samples were obtained from the Faculty of Agriculture, Kafrelshiekh University, Egypt. All samples were kept in a cool box on melting ice and transported within 4 hours of collection to the laboratory. In the next step, samples were divided into 4 equal groups (425ml/ group), including control (milk sample without propol is), milk with WEP 5%, milk with WEP 10%, and milk with WEP 20%. All groups were saved in the refrigerator at 5 ± 1°C. The pH analyses were performed from the initial time to 72 hours with intervals of 12 hours.

From the previous experiment, the ideal quantity of propolis to maintain milk pH for an extended length of time (WEP 20%) was added in different concentrations (1, 2 and 3%) to raw cow's milk in addition to a control group (Total milk amount: 1,000 ml; 250 ml/ group) to determine pH and microbiological examinations throughout 72 hours of storage period in a refrigerator at 5 ± 1°C (with interval of 24 hours) to determine the overall quality of the milk.

Preparation of Yoghurt

A yoghurt culture, Direct Vat Set (DVS) containing *Lactobacillus delbrueckiissp. bulgaricus* and Streptococcus thermophilus in a 1:1 ratio was provided by Chr. Hansen's Lab. in Copenhagen, Denmark.

For yoghurt production, fresh raw cow milk (4,000 ml) (Holstein breed, 3.6% fat, and 3% protein) was obtained from the Faculty of Agriculture, Kafrelshiekh University, Egypt. The milk was heated for 10 minutes at 80°C, then quickly cooled down to 40°C. It was then reheated to 40°C, followed by inoculation of 2% starter culture (L. *delbrueckii* ssp. *bulgaricus* and S. *thermophilus* in a 1:1 ratio) and division of milk into 4 equal parts. The first part was presented as a control, while the second was treated with 1% WEP (propolis 20%), the third with 2% WEP (propolis 20%), and the fourth with 3% WEP (propolis 20%). For clotting, all milk parts were incubated for 3-4 hours at 42°C, after which the yoghurt cups were cooled down to 15-20°C before being refrigerated at 5 ± 1°C.[30]

Sensory evaluation, pH, and microbiological examinations were performed over the course of the storage period every 3 days until 15 days of storage in the refrigerator.

Chemical Examination

Determination of pH

pH value was determined by an electrical pH meter (Adwa pH meter AD11, Romania).

Determination of Total Phenolic

The total phenolic, total flavonoid content, and antioxidant activity were analyzed at National Research Centre (NRC), Dokki, Egypt. According to the Folin-Ciocalteu method, total phenolic compounds were identified and represented as µg of Gallic Acid Equivalent (GAE) per gram of sample (µg GAE/g).[31]

Determination of Total Flavonoids

The total flavonoids content was measured using the aluminum chloride ($AlCl_3$) colorimetric technique and represented as µg of Catechin Equivalent per gram of sample (µg CE/g).[32] 50 µl of 5% $NaNO_2$ was mixed with 100 µl of the appropriate extracts. After 5 min, 500 µl of a 10% $AlCl_3$ solution was added to form a flavanoid−aluminum complex.

After 7 min, 250 µl of 1 M NaOH was added, and the mixture was centrifuged for 10 min. The absorbance of the supernatant was measured at 510 nm against the blank containing the extraction solvent instead of a sample.

Determination of Antioxidant Activity

Antioxidant activity (radical DPPH scavenging activity) was measured according to Hwang and co-worker[33] using the stable 1,1-Diphenyl-2-picrylhydrazyl (DPPH).

The hydrogen atom or electron donation abilities of the samples and some pure compounds were measured from a light purple-colored DPPH methanol solution. One milliliter of various concentrations (100~1,000 µg/ml) of each extract in 10% ethanol was added to a 1 mL DPPH radical solution in methanol (DPPH concentration, 0.2 mM). The mixture was shaken, and allowed to stand for 30 min, the absorbance was measured at 515 nm. Percent inhibition of the DPPH free radical was calculated by the following formula: $\text{Inhibition}(\%) = 100 \times (A_{control} - A_{sample})/_{Acontrol}$

Where $A_{control}$ is the absorbance of the control reaction, and A_{sample} is the absorbance with the test compound. Ascorbic acid was used as a control.

The antioxidant activity was determined using a calibration curve prepared with Trolox acid, and expressed as µg of Trolox Equivalent (TE) per gram of sample (µg TE/g).

Microbiological Examination

Total bacterial count (TBC), as well as coliform count, total yeast and mold counts were examined for all samples.[34]

Total Bacterial Count

One ml of raw milk sample was transferred into a sterile test tube containing 9 ml of peptone water, (biolab, Cairo, Egypt). After mixing, the sample was serially diluted up to $1{:}10^{-7}$ and aliquots (1 ml) were pour plated using 15-20 ml standard plate count agar (Oxoid, UK) and mixed thoroughly. The plates were then incubated at 30 °C for 48 h. Colony counts were accomplished using a colony counter.

Coliform Count

Presumptive test for total coliforms was done by lauryl sulfate tryptose broth (LST) (Himedia, India) inoculated with 1 ml of previously prepared dilutions. Tubes were supplied with inverted Durham tubes for gas detection, and then incubated for 24-48 h at 37°C. All LST tubes showing both turbidity and gas within 48 h were recorded and the Most Probable Number (MPN) was obtained from MPN food tables for the recorded 3 tubes dilutions.

Total Yeast and Mold Count

Total yeast and mold counts, from previously prepared serial dilutions, 1 ml taken and added into the center of sterile plates and 15 ml of Sabarouds Dextrose Agar (SDA) (Himedia, India) were poured into each plate, all plates incubated at 25°C for 5 days.

Sensory Evaluation

Panelists from The Food Hygiene Department at the Animal Health Research Institute (Agriculture Research Center) conducted the sensory evaluations of yoghurt samples. The panelists were requested to evaluate the product fresh state as well as every 3 days during 15 days of refrigerator storage with the assessment of the product color and appearance, body and texture, flavor, taste, and general acceptability.[35]

Statistical Analysis

All measurements were analyzed by using Statistical Package for the Social Sciences (SPSS) 22.0 (IBM Corp., Armonk, NY, USA. All parametric data was represented as mean ± standard errors (SE) which was analyzed by one-way analysis of variance

(ANOVA), followed the least significant differences (LSD) test. Mean comparisons between the groups were considered significant at (P<0.05).

Results and Discussion

Table 1 shows the variations in pH of raw cow milk samples throughout the storage period (72 hours) at 5 ± 1°C with different concentrations of WEP (5, 10, and 20%) as a natural food preservative. From the initial time to 72 hours of the storage period, the pH levels of the treated groups declined significantly, where WEP 5 % had the lowest pH in the treated groups and WEP 20% had the highest rate (P<0.05).

The pH of the control group had the lowest pH value compared to the treated groups, where it decreased from 6.70 ± 0.06 to 5.67 ± 0.03 at the termination of the storing period (72 hours at 5 ± 1°C), whereas the pH of WEP 5, 10, and 20% groups reduced from 6.73 ± 0.03 at the initial time to 5.73 ± 0.03, 5.77 ± 0.03 and 6.07 ± 0.03, respectively, at the termination of the storing period (72 hours at 5 ± 1°C).

Table 1: Variation in pH value (Mean ± SE) of raw cow milk that affected by various concentrations of WEP through refrigeration

Time (hours)/ Groups	Control	WEP (pro polis 5%)	WEP (pro polis 10%)	WEP (pro polis 20%)
initial time	6.70 ± 0.06^{a}	6.73 ± 0.03^{a}	6.73 ± 0.03^{a}	6.73 ± 0.03^{a}
12 hr	6.57 ± 0.03^{b}	6.70 ± 0.06^{a}	6.73± 0.03^{a}	6.73 ± 0.03^{a}
24 hr	6.50 ± 0.06^{b}	6.57 ± 0.03^{ab}	6.67 ± 0.03^{a}	6.67 ± 0.03^{a}
36 hr	6.23 ± 0.07^{b}	6.27 ± 0.03^{b}	6.43 ± 0.03^{a}	6.53± 0.03^{a}
48hr	6.07 ± 0.03^{b}	6.17 ± 0.03^{b}	6.17 ± 0.03^{b}	6.37±0.03^{a}
60 hr	5.70 ± 0.06^{c}	5.97 ± 0.03^{b}	5.97 ± 0.03^{b}	6.17 ± 0.03^{a}
72 hr	5.67 ± 0.03^{b}	5.73 ± 0.03^{b}	5.77 ± 0.03^{b}	6.07 ± 0.03^{a}

Different superscript letters (a,b and c)in the same row differ significantly at a level of P<0.05
WEP: Water extract of propolis

The normal pH values of raw cow milk range are between 6.3and 6.8[36,37] So, the raw cow milk samples in the control group were acceptable for up to 24 hours at 5 ± 1°C, but the treated group with WEP 20% remained acceptable for up to 48 hours.

The natural preservation function of propolis by preventing microbial growth, some enzymatic processes, and oxidation might occur in milk, which may be the cause of the pH increase.[38] The pH values of raw cow milk during refrigerated storage influenced by different percentages (1, 2, and 3%) of WEP 20% are shown in Table 2. At the initial time, the pH values of milk samples in the control group and propolis treatments were 6.60 ± 0.058, but after 72 hours of storage, the pH values of the control group were considerably lower than other groups (P<0.05). The pH of milk groups supplemented with 1, 2, and 3% WEP had higher pH values than the control group (unacceptable) at the end of the storage period (72 hours), and milk groups supplemented with 2 and 3% WEP had an acceptable pH more than groups supplied with 1% WEP.

Total bacterial, coliform, yeast and mold counts were examined in milk groups during 72 hours of storage at 5 ± 1°C. They were evaluated in relation to different concentrations (1, 2, and 3%) of propolis water extract 20% (Table 2). The initial time of TBC values in control, 1% WEP (20%), 2% WEP (20%), and 3% WEP (20%) were 4.99 ± 4.62, 4.91 ± 4.53, 4.85 ± 4.46 and 4.79 ± 4.39 log10 CFU/ml, respectively. While at the end of the storage period (72 hours) all treated groups with different concentrations of propolis were significantly lower in TBC (P<0.05) (3.91 ± 1.76, 3.86 ±1.76, and 3.62 ± 1.94 log10 CFU/ml, respectively than the control group (6.05 ± 5.68 CFU/ml), and were within the acceptable limit (5 x 10^{4} CFU/ml) according to EC guidelines.[39]

The total bacterial count serves as a measure for the sanitation of milk production and handling. In fact, the microbial load in healthy animal milk was minimal (< 1,000 bacteria/ml), but when milk is left at room temperature, it might increase by 100 times or more.[40] In the present study, after 24 hours, the TBC of the control milk group increased significantly (P< 0.05) and was higher than the other WEP treated groups, exceeding the maximum acceptable standard of the EC (5 x104 CFU/ml).[39] The microbiological counts in food fortified with propolis were shown to be influenced, as 2% and 3% WEP supplementation in raw milk decreased total bacteria, El-Deeb.[16] indicating propolis antibacterial action.

Coliform count values in control and treated milk groups were > 3.041(MPN/ml) at the initial time, whereas at the end of the storage period (72 hours), the control group had the highest coliform count (> 4.041 MPN/ml) when compared to other groups supplemented with different concentrations of propolis (1% WEP, 2% WEP, and 3% WEP) that coliform count were 2.556, 2.041, and 2.322 MPN/ml, respectively and obviously, groups supplemented with 2% WEP and 3% WEP was more efficient in reduction of coliform count when comparing to the other group at the end of storage period.

Table 2: Variation in pH value and microbiological examination (Mean±SE) of raw cow milk affected by the different percentages of WEP20%through refrigeration

Time(hours) / Groups	Control	1% WEP (propolis 20%)	2% WEP (propolis 20%)	3% WEP (propolis20%)
pH				
initial time	6.60 ± 0.06^{a}	6.60 ± 0.06^{a}	6.60 ± 0.06^{a}	6.60 ± 0.06^{a}
24hr	6.50 ± 0.06^{a}	6.60 ± 0.06^{a}	6.60 ± 0.06^{a}	6.60 ± 0.06^{a}
48hr	5.80 ± 0.06^{b}	6.23 ± 0.09^{a}	6.27 ± 0.07^{a}	6.33 ± 0.03^{a}
72hr	5.37 ± 0.03^{b}	6.17 ± 0.07^{a}	6.23 ± 0.09^{a}	6.20± 0.06^{a}
Microbiological examination (log10 CFU/ml)				
Total bacterial count (CFU/ml)				
initial time	4.99± 4.62^{a}	4.99 ± 4.53^{a}	4.85 ± 4.46^{a}	4.79 ± 4.39^{a}
24 hr	6.00 ± 5.67^{a}	4.30 ± 3.76^{b}	4.08 ± 2.76^{b}	3.92 ± 2.52^{b}
48 hr	6.04 ± 5.67^{a}	3.98 ± 1.82^{b}	3.92 ± 1.52^{b}	3.79 ± 1.76^{b}
72 hr	6.05 ± 5.68^{a}	3.99 ± 1.76^{b}	3.88 ± 1.76^{b}	3.62 ± 1.94^{b}
Coliform count (MPN/ml)				
initial time	> 3.04	> 3.04	> 3.04	> 3.04
24 hr	>3.04	<3.04	<2.66	<2.46
48 hr	>4.04	<2.88	<2.54	<2.36
72 hr	>4.04	<2.56	<2.04	<2.32
Total yeast and mold count (CFU/ml)				
initial time	3.84 ± 1.76a	3.45 ± 1.76^{d}	3.65 ± 1.76^{c}	3.68 ± 1.76^{b}
24 hr	3.76 ± 1.76^{a}	3.49± 1.52^{b}	3.46 ± 1.76^{c}	3.39 ± 1.52^{d}
48 hr	4.15 ± 2.76^{a}	3.34 ± 1.94^{b}	3.26 ± 1.52^{bc}	2.99 ± 1.52^{c}
72 hr	4.34 ± 2.76^{a}	3.10 ± 1.52^{b}	2.85 ± 1.76^{b}	2.67 ± 1.82^{b}

Different superscript letters (a,b,c) in the same row differ significantly at level of P< 0.05
WEP: Water extract of propolis

The initial count of total yeast and mold count were 3.84 ± 1.76, 3.45 ± 1.76, 3.65± 1.76 and 3.68 ± 1.76 log10 CFU/ml in control, 1% WEP, 2% WEP, and 3% WEP groups, respectively and increased to 4.34 ± 2.76, 3.10 ± 1.52, 2.85 ± 1.76 and 2.67 ± 1.82 log10 CFU/ml at the end of storage period. The reduction

in yeast and mold counts was more obvious in milk groups supplemented with high propolis content, where at 48 hours of storage, milk group of 3% WEP was significantly different from control and 1% WEP groups, but no significant difference with 2% WEP while at the end of storage period all supplemented groups with WEP were differ significantly with control group, sousing propolis concentrations below a particular threshold proportionately reduced yeast and mold counts.

The number of yeast and mold in the control milk group increased substantially, suggesting that propolis might be used as an antifungal agent to preserve milk during storage. These findings matched those previously published by El-Alfy *et al.*,[41] Also, according to El-Deeb.,[16] the yeast and mold count reduced from 4.775 (log 10 CFU/ml) in the control group to 2.105 (log10 CFU/ml) in the supplemented milk group with 2% WEP. As well, an ethanolic extract from propolis suppressed the growth of yeast and mold after 23 days in freshly squeezed pomegranate juice.[42]

Table 3 indicates the variations in total phenolics, flavonoid components, and antioxidant activity. The total phenolic content of propolis water extract (WEP 20%) was 639.735 ± 0.688 µg GAE/g. El Sohaimy and his team[43] observed that Egyptian and Chinese propolis had lesser phenolic compounds, with 137.52 ± 0.003 and 123.08 ± 0.005 µg GAE/g, respectively, while El-Sayed and co-worker[44] found greater phenolic components in ethanolic propolis extract (2,315.2 ± 0.67 µg/g).

It was also established that the water-based propolis extract was more efficient than ethanolic extract.[16] The type of solvents used, the temperature at which the propolis extract is extracted, stirring, as well as the propolis origin and source are all agents that limit the concentration of phenolic components.[45]

In regards of flavonoids, the present research found that the total flavonoid concentration in WEP 20% was 332.84 ± 0.22 µg CE/g. El-Sayed and co-worker[44] demonstrated that ethanolic propolis extract had a higher total flavonoid concentration (1,578.1 ± 1.98 µg /g). The amount of flavonoids found in propolis is related to the vegetation collected by honey bees.[46]

The antioxidant activity of propolis water extract (WEP 20%) was 1,029.71 ± 0.71 µg TE/g. Propolis antioxidant effect may be due to the phenolic components 'ability to transfer hydrogen ions, which aids in food preservation by preventing oxidation and deterioration. Propolis is a good natural antioxidant with a high antioxidant activity that can be utilized as a natural food preservative to keep food fresh.[43] Cabral *et al.*,[47] discovered a link between antioxidant property and phenolic component content in propolis, specifically in terms of flavonoid concentration[48] and microbial stability.[49]

Polyphenolic compounds such as cinnamic acid, rosmanol, benzoic acid, cinnamyl, p-coumaric acid, chlorogenic acid, aromatic acids, naringenin, quercetin, and their esters have been detected in abundance in several varieties of propolis in previous studies.[50] Propolis antioxidant activity was attributed primarily to phenolics and flavonoids. Propolis therapeutic value is due to its antioxidant properties, which are mostly derived from polyphenols.[51] Andrade *et al.*,[52] stated that phenolic and flavonoid compounds are the major ingredients resulting in a variety of biological actions, such as immune potentiation, chemoprevention, and antitumor actions, therefore, using propolis is beneficial to the consumer's health when used in yoghurt manufacturing. Compared to commonly use dietary antioxidants like BHA, BHT, and TBHQ, which have been linked to toxicity issues,[53] propolis demonstrated better lipid peroxidation prevention and free radical sequestration.

The amount of phenolic components, flavonoids, and antioxidant activities increased as the concentration of propolis extracts increased when different concentrations of propolis water extract (1, 2, and 3%) were added to manufacturing yoghurt ($P<0.05$). The current study findings were comparable to El-Deeb.,[16] who found an increase in phenolic components, flavonoid components, and antioxidant activities in yoghurt groups as the concentration of propolis extracts increased.

Adding propolis extracts to dairy beverages increased their antioxidant capacity. The polyphenolic components in propolis extracts are likely higher heat resistant and protect the antioxidant compounds in milk products.[54]

Table 3: Changes in total phenolic, flavonoids components, and antioxidant activity (Mean ± SE) of WEP 20% and freshly manufactured yoghurt groups affected by the different percentages of WEP 20%

Groups	Phenolic components (µg GAE/g)	Flavonoids components (µg CE/g)	Antioxidant activity by DPPH (µg TE/g)
WEP 20%	64.74 ± 0.69	332.84 ± 0.22	1,029.71 ± 0.71
Fresh manufactured yoghurt			
Control	18.65 ± 0.03^{d}	4.63 ± 0.00^{d}	34.63 ± 0.03^{d}
1% WEP (propolis 20%)	30.44 ± 0.50^{c}	5.48 ± 0.09^{c}	39.29 ± 0.41^{c}
2% WEP (propolis 20%)	56.09 ± 0.50^{b}	16.00 ± 0.43^{b}	53.98 ± 0.41^{b}
3% WEP (propolis 20%)	111.74 ± 0.50^{a}	26.75 ± 0.00^{a}	62.45 ± 0.87^{a}

Different superscript letters in the same column differ significantly at level of P<0.05
WEP: Water Extract of Propolis

Table 4 displayed the yoghurt coagulation time of groups treated with various percentages of WEP (1, 2, and 3%). The yoghurt group with 1% WEP had the fastest clotting time (2.43 hours/minutes) and then the 2% WEP (2.55 hours/minutes). Furthermore, control and 3% WEP had a long clotting time (3.15 and 3.05 hours/minutes, respectively). This variance might be related to the influence of adding propolis water extract on the growth of starter culture since Boubakeur *et al.*,[38] discovered that flavonoids, which act as probiotics, had a beneficial influence on the improvement of *Lactobacillus rhamnosus* and *Streptococcus thermophilus.*

Table 4: Yoghurt coagulation time of groups affected by the different percentages of WEP 20%

Groups	Control (propolis 20%)	1% WEP (propolis 20%)	2%WEP (propolis 20%)	3%WEP (propolis 20%)
Coagulation time(hours)	3.15	2.43	2.55	3.05

WEP: Water Extract of Propolis

Sensory evaluation (quantitative and/or descriptive) is commonly used to evaluate the flavor, texture, appearance, and other aspects of food products as just a function of processing factors.[55] Table 5 summarizes the data of sensory property assessments for the control and manufactured yoghurt groups. The results showed that throughout the storage period, the control yoghurt group got the lowest score; on the other hand, as the concentrations of WEP increased, the sensory score of the produced yoghurt groups decreased. All qualities in all groups exhibited considerably decreased likeability during the storage duration of 15 days at 5 ± 1°C.

Over the duration of 15 days of storage, the yoghurt groups containing 1 and 2% WEP got the highest color and appearance scores, whereas the yoghurt group containing 3% WEP obtained the lowest. The yoghurt groups containing 2% WEP, followed by 1% WEP, obtained the top scores for body and texture, taste, and overall acceptability and no significant difference between them in flavor character during the storage period of 15 days at 5 ± 1°C, while the yoghurt group containing 3% WEP received the lowest scores throughout the storage period. These findings were similar to those of El-Deeb.,[16] who found that propolis use in milk and yoghurt at higher concentrations (3%) had a negative

impact on acceptance and the highest sensory scores were obtained at concentrations of 1 and 2% WEP (20% extract). Propolis has a distinct and pungent odor, as well as its inclusion in food formulas may cause a change in color and, more importantly, a disagreeable odor.[56]

Sensory evaluation results showed that manufactured yoghurt with 2% WEP provided the best sensory ranking at the termination of the storage time, compared to the other treatments, followed by 1% WEP.

Table 5: Variation in sensory evaluation (Mean±SE) of the manufactured yoghurt groups affected by different concentrations of WEP 20% through refrigeration

Groups/Storage time (days)	Control	1% WEP (propolis 20%)	2%WEP (propolis 20%)	3%WEP (propolis20%)
Color and appearance (n=9)				
Initial time	8.51 ± 0.02^{b}	8.72 ± 0.03^{a}	8.67 ± 0.02^{a}	7.87 ± 0.09^{c}
3rd	8.45 ± 0.03^{b}	8.70 ± 0.03^{a}	8.65 ± 0.02^{a}	7.82 ± 0.07^{c}
6th	7.53 ± 0.15^{b}	8.50 ± 0.06^{a}	8.37 ± 0.09^{a}	7.40 ± 0.12^{b}
9th	6.98 ± 0.01^{c}	8.20 ± 0.06^{a}	8.03 ± 0.04^{b}	6.99 ± 0.01^{c}
12th	6.28 ± 0.04^{c}	7.90 ± 0.06^{a}	8.00 ± 0.06^{a}	6.70 ± 0.10^{b}
15th	6.07 ± 0.04^{b}	7.70 ± 0.06^{a}	7.90 ± 0.06^{a}	6.17 ± 0.09^{b}
Body and texture (n=9)				
initial time	7.93 ±0.09^{b}	8.57 ± 0.12^{a}	8.67 ± 0.12^{a}	8.43 ± 0.09^{a}
3rd	7.70 ±0.06^{c}	8.43 ± 0.15^{ab}	8.53 ± 0.09^{a}	8.17 ± 0.09^{b}
6th	6.97 ±0.09^{b}	8.10 ± 0.06^{a}	8.30 ± 0.06^{a}	8.13 ± 0.09^{a}
9th	6.83 ±0.12^{c}	7.90 ± 0.06^{b}	8.20 ± 0.06^{a}	8.07 ± 0.07^{ab}
12th	6.53 ±0.15^{c}	7.73 ± 0.09^{b}	8.17 ± 0.03^{a}	7.93 ± 0.03^{ab}
15th	6.30 ±0.06^{c}	7.63 ± 0.09^{b}	8.13 ± 0.03^{a}	7.80 ± 0.06^{b}
Flavor (n=9)				
initial time	8.63 ± 0.03^{a}	8.73 ± 0.09^{a}	8.70 ± 0.06^{a}	7.40 ± 0.36^{b}
3rd	8.37 ± 0.09^{a}	8.63 ± 0.09^{a}	8.60 ± 0.15^{a}	6.83 ± 0.52^{b}
6th	7.90 ± 0.06^{b}	8.53 ± 0.09^{a}	8.50 ± 0.15^{a}	6.23 ± 0.15^{c}
9th	7.70 ± 0.06^{b}	8.43 ± 0.09^{a}	8.40 ± 0.15^{a}	6.17 ± 0.12^{c}
12th	6.53 ± 0.18^{b}	8.13 ± 0.03^{a}	8.10 ± 0.10^{a}	6.07 ± 0.12^{c}
15th	6.27 ± 0.15^{b}	8.03 ± 0.03^{a}	8.00 ± 0.06^{a}	6.03 ± 0.07^{b}
Taste (n=9)				
initial time	8.50 ± 0.12^{a}	8.60 ± 0.12^{a}	8.53 ± 0.09^{a}	6.80 ± 0.06^{b}
3rd	8.33 ± 0.09^{a}	8.50 ± 0.12^{a}	8.43 ± 0.09^{a}	6.67 ± 0.07^{b}
6th	8.00 ± 0.06^{b}	8.40 ± 0.12^{a}	8.33 ± 0.09^{a}	6.53 ± 0.09^{c}
9th	7.83 ± 0.09^{b}	8.07 ± 0.03^{ab}	8.27 ± 0.07^{a}	6.40 ± 0.12^{c}
12th	7.33 ± 0.09^{b}	7.53 ± 0.19^{b}	8.20 ± 0.10^{a}	6.23 ± 0.07^{c}
15th	6.83 ± 0.09^{c}	7.33 ± 0.24^{b}	8.13 ± 0.09^{a}	6.10 ± 0.06^{d}
Overall acceptability (n=9)				
initial time	8.60 ± 0.06^{a}	8.70 ± 0.06^{a}	8.37 ± 0.03^{b}	7.00 ± 0.06^{c}
3rd	8.30 ± 0.06^{b}	8.57 ± 0.03^{a}	8.27 ± 0.03^{b}	6.80 ± 0.06^{c}
6th	7.87 ± 0.09^{b}	8.20 ± 0.06^{a}	8.17 ± 0.03^{a}	6.60 ± 0.06^{c}
9th	7.50 ± 0.06^{b}	8.07 ± 0.03^{a}	8.13 ± 0.03^{a}	6.47 ± 0.07^{c}
12th	6.83 ± 0.03^{c}	7.90 ± 0.06^{b}	8.07 ± 0.03^{a}	6.37 ± 0.07^{d}
15th	6.30 ± 0.06^{c}	7.67 ± 0.09^{b}	8.03 ± 0.03^{a}	6.27 ± 0.07^{c}

Total scores (n = 45)				
initial time	42.17 ± 0.13[a]	43.32 ±0.03[a]	42.94 ± 0.06[a]	37.50 ± 0.30[b]
3rd	41.15 ± 0.14[a]	42.83 ±0.05[a]	42.48 ± 0.07[a]	36.29 ± 0.31[b]
6th	38.27 ± 0.19[b]	41.73 ±0.08[a]	41.67 ± 0.05[a]	34.89 ± 0.35[c]
9th	36.84 ± 0.20[b]	40.67 ±0.09[a]	41.03 ± 0.06[a]	34.10 ± 0.34[b]
12th	33.50 ± 0.18[b]	38.96 ±0.10[a]	40.54 ± 0.04[a]	33.30 ± 0.33[b]
15th	31.77 ± 0.13[b]	38.36 ±0.11[a]	40.19 ± 0.04[a]	32.37 ± 0.33[b]

Different superscript letters in the same row differ significantly at level of P< 0.05
WEP: Water Extract of Propolis, n=9: the evaluation score for each characteristic

The differences in pH values of the yoghurt groups supplemented with different concentrations of (1, 2, and 3%) WEP 20% were given in Table 6. All yoghurt groups had approximately the same pH at the initial time and thereafter gradually declined until day 15 of the storage period (P< 0.05), where 2% WEP was considerably higher (4.37 ± 0.03), followed by 1% WEP (4.30 ± 0.06), and 3% (4.20 ± 0.06), while control group had the lowest pH (4.17 ± 0.07). Propolis concentrations were adversely proportional to the reduction in pH values of yoghurt in different treatments.

Since *Lactobacillus acidophilus* is recognized as homofermentative bacteria, Batista *et al.*,[57] reported that lowered pH is typical in yoghurt during refrigerated storage as a result of its metabolism, which results in the production of lactic acid from lactose fermentation. Furthermore, one of the most significant components of yoghurt flavor is acidity, with a pH of around 4.4 being optimum.

As seen in previous studies, supplementation of propolis increased the pH of yoghurt as well as other milk products and reduced the acidic taste[58,59,16]

The effect of different propolis concentrations on coliform count log10/MPN/gm in yoghurt samples supplemented by varying percentages of WEP 20% through refrigerated storing is shown in Table 6. From fresh to the end of the storage period, no coliform bacteria were detected in any of the control and treated groups; this could be related to the extreme heat treatments used on milk, in addition to the significance of lactic acid bacteria in product preservation that is linked to their ability to develop antimicrobial substances. These findings are in accordance with some studies.[60.61]

Total yeast and mold count are regarded as an indicator of yoghurt spoilage; the count of yeast and molds is among the most essential factors in determining a product's shelf life and quality. Microbiological counts have been considered markers indicating the end of shelf life of dairy products and quality impairment.[62]

The initial yeast and mold count for all yoghurt groups (control, 1% WEP, 2% WEP and 3% WEP) were 3.78 ± 2.76, 3.76 ± 1.76, 3.52 ± 1.76, and 3.24 ± 1.52 log 10 CFU/gm, respectively as reported in Table 6. There is a significant difference in total yeast and mold count between control and treated groups on day 9 where 3% WEP showed the best treatment (1.87 ± 0.33 log10 CFU/gm) followed by 2% WEP and 1% WEP (1.91 ± 0.58 and 1.96 ± 0.33 log10 CFU/gm, respectively) while control group (untreated) had markedly higher count (2.82 ± 0.52 log10 CFU/gm, P< 0.05).

On day 15of the storing period, yeast and mold counts were markedly lower (P< 0.05) in yoghurt groups supplemented with 2% and 3% WEP (1.68 ± 0.58 and 1.57 ± 0.33 (log10 CFU/gm, respectively) than 1% WEP and control group (2.16 ± 0.52 and 3.67 ± 1.52 log10 CFU/gm, respectively).

It is obvious that lower propolis concentrations result in a commensurate decrease in yeast and mold counts, according to their concentrations. These findings backed up the use of propolis in yoghurt storage as an antifungal agent.

Raw milk supplemented with 2% WEP (20% extract) was the most effective natural preservative for increasing quality and microbiological safety.[16]

Table 6: Variations in pH levels and microbiological examination (Mean ± SE) of yoghurt groups affected by different concentrations of WEP 20% through refrigeration

Groups	Control	1% WEP (pro polis 20%)	2%WEP (pro polis 20%)	3%WEP (pro polis 20%)
Storage time(days)	**pH**			
initial time	5.10 ± 0.06^{a}	5.00 ± 0.06^{ab}	5.00 ± 0.06^{ab}	4.90 ± 0.06^{b}
3rd	4.57 ± 0.12^{a}	4.60 ± 0.1^{a}	4.63 ± 0.09^{a}	4.57 ± 0.12^{a}
6th	4.30 ± 0.06^{a}	4.40 ± 0.06^{a}	4.43 ± 0.03^{a}	4.33 ± 0.03^{a}
9th	4.23 ± 0.03^{b}	4.43 ± 0.07^{a}	4.40 ± 0.06^{ab}	4.27 ± 0.07^{ab}
12th	4.20 ± 0.06^{c}	4.33 ± 0.03^{ab}	4.43 ± 0.03^{a}	4.23 ± 0.03^{bc}
15th	4.17 ± 0.07^{b}	4.30 ± 0.06^{ab}	4.37 ± 0.03^{a}	4.20 ± 0.06^{ab}
Microbiological examination (log10 CFU/gm)				
Coliform count (MPN/gm)				
initial time	< 3.0 MPN/g	< 3.0 MPN/g	< 3.0 MPN/g	< 3.0 MPN/g
3rd	< 3.0	< 3.0	< 3.0	< 3.0
6th	< 3.0	< 3.0	< 3.0	< 3.0
9th	< 3.0	< 3.0	< 3.0	< 3.0
12th	< 3.0	< 3.0	< 3.0	< 3.0
15th	< 3.0	< 3.0	< 3.0	< 3.0
Total yeast and mold count (CFU/gm)				
initial time	3.78 ± 2.76^{a}	3.76 ± 1.76^{a}	3.52 ± 1.76^{b}	3.24 ± 1.52^{c}
3rd	2.96 ± 0.95^{a}	2.40 ± 0.76^{b}	2.17± 0.52^{c}	2.10 ± 0.52^{d}
6th	2.68 ± 0.76^{a}	2.00 ± 0.58^{b}	1.96 ± 0.33^{bc}	1.94 ± 0.58^{c}
9th	2.82 ± 0.52^{a}	1.96 ± 0.33^{b}	1.91 ± 0.58^{c}	1.87 ± 0.33^{d}
12th	3.15 ± 1.76^{a}	1.81 ± 0.58^{b}	1.76 ± 0.33^{b}	1.61 ± 0.58^{b}
15th	3.66 ± 1.52^{a}	2.16 ± 0.52^{b}	1.68 ± 0.58^{c}	1.57± 0.33^{c}

Different superscript letters in the same row differ significantly at a level of P< 0.05
WEP: Water Extract of Propolis

Propolis is commonly utilized in different food formulas such as meat, seafood, dairy, juice, oils, and fruits to extend the shelf-life, reduce lipid oxidation, and give health benefits to consumers.[54,63]

Antibacterial and antifungal activities have been discovered in propolis. Cottica *et al.*,[54] stated that adding ethanol and propolis water extracts to dairy drinks resulted in the maximum antioxidant activity and the least amount of aldehyde formation during light storage. Moreover, some researchers have found that propolis may be used to manufacture yoghurt, except for a few minor sensory impairments.[64]

Substituting Brazilian red propolis for potassium sorbate at a concentration of 0.05% yielded increased phenol and flavonoid content as well as enhanced antioxidant activity in yoghurt manufacturing.[65]

Propolis at a level of 5-10% can also be used to increase kareish cheese quality and extend its she lf life by 30 days with improving taste.66 Also, adding 6-10% water extracts of propolis (WEP) to kareish cheese can be advised as a safe and natural source of phenolic compounds, as well as high acceptance and antibacterial agents throughout storage periods.[67] Furthermore, Saleh *et al.*,[68] confirmed that 5% of propolis could be used to increase the quality and extend shelf life of Tallaga cheese while giving a pleasant flavor.

Conclusion

The raw milk preserved with propolis proved to be an effective natural product in this research, as it improved the quality and microbiological safety of the milk. The water extract of propolis used in the yoghurt preparation had high phenol and flavonoid content, as well as superior antioxidant activity. It also had a high ability to suppress microbial growth. As a result, the propolis-preserved yoghurt developed in this study has proven to be a potent natural product, as the natural antioxidant has health-promoting properties as well as being an excellent natural preservative. The yoghurt treated with 2% water extract of propolis had the highest sensory scores. As a consequence, the produced yoghurt demonstrated that it is a novel product with a functional and probiotic potential that might be commercialized.

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Conflict of interest

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