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Organic Propolis Extract as a Natural Fortifier and Preservative for Milk

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ABSTRACT

Natural materials that can be used as preservatives in the food sector have become very important. Propolis is getting a lot of attention due to its antioxidant and antibacterial properties. The objectives of this paper was to see if organic propolis extract could be used as a natural preservative and fortifier in raw and pasteurised milk storage. Propolis extracts were employed at two different concentrations (0.5 and 1 %) to test their impact. T.S., pH, Titratable acidity, colour, Total flavonoid, and total phenolic compounds were all measured in the samples. Total antioxidant capacity, microbiological, and sensory qualities in fresh and stored at 5±1°C for 14 days. Result showed that addition of propolis extract had a negligible effect on T.S, color and sensory properties. However there was increased in the phenolic compounds, flavonoids and antioxidant capacity with the increase of propolis extracts. Acidity increased during storage in control of raw milk samples however in other samples with propolis acidity increased slowly. Control of raw milk coagulation after 5 days while samples with propolis, coagulation occurred after 10 days. In the case of pasteurized milk acidity increased slowly and coagulation occurred after 14 days. The counts of bacteria increased slowly in sample with propolis compared with control, coliform bacteria and molds & yeast not detected until 14 days.

Keywords: Milk, pasteurization, polyphenolic compounds, Color



INTRODUCTION

Raw milk and dairy products manufactured from unpasteurized milk (including raw and thermised milk), such as cheese, butter, cream, ice cream, and kefir, have seen a growth in consumer demand. (McLauchlin, *et al.* 2020)

Milk is a complete food since it contains a lot of proteins, vitamins, and minerals. Natural (preservative-free) milk is perishable and has a limited shelf life because it provides a perfect habitat for germs to thrive. Milk without preservatives has a significant impact on the spread of pathogenic microorganisms that cause salmonellosis, brucellosis, listeriosis, and TB. Unlike certain other foods and beverages, adding preservatives to milk to lengthen its shelf life is illegal. As a result, preservatives found in milk are regarded as pollutants. The need for good quality milk has increased as the demand for dairy products has grown, as has the need to reduce losses in industrial output owing to low quality. (El-Deeb, 2017)

Numerous attempts have been made to discover natural antimicrobial alternatives to inhibit bacterial and fungal development in meals and dairy products. Using natural preservatives to suppress the growth of harmful bacteria in food has become highly popular as a result of increased consumer knowledge. Antimicrobials of this type might be added directly to the product composition, coated on its surface, or included into the packaging material. (Willis, *et al.* 2017)

Raw milk spoils in just a few days, even when stored at refrigerated temperatures. On the other hand, pasteurized (high temperature system, short time; HTST) and chilled milk has a shelf life of 7 to 28 days (Meunier-Goddik and

Sandra 2002), the shelf life of pasteurized milk varies from 3 to 8 days due to low quality of the raw material and an unreliable cold chain that exposes the product to abuse during distribution and commercialization. Bacterial spoilage is the greatest limiting factor in extending the shelf life of conventional HTST pasteurized milk beyond 14 days. The growth and metabolism of microorganisms shorten the shelf-life of milk, causing undesirable changes in aroma and taste properties that affect consumer acceptance of the products (Fromm and Boor 2004).

Propolis, a intensify gathered by bees from plant secretions, need picked up Notoriety as an elective pharmaceutical and an elective will antibacterial chemicals utilized within nourishment Furthermore dairy protection. Propolis will be the concoction answerable for neutralizing At whatever bacteria, growths alternately infections that enter those hive. It is composed of approximately 55% resinous compounds and balsam, 30% beeswax, 10% aromatic essential oils, 5% bee pollen and more than 150 chemicals.

Propolis has been influentially worn to appetizing a marque of worldly diseases, exclusive of cardiovascular trial, confidential practices troubles, respiratory infections, dental trouble oneself, dermatology, complaint medicate, demonstrate customs, digestive tract disease and liver protection. (Najafi *et al.*, 2007; Sforcin, 2007 and Fuca *et al.*, 2013).

The greater efficiency of propolis extract in water is well known. In addition. Tumor cell growth and proliferation are greatly reduced by propolis and its polyphenolic components. In commercial Egyptian propolis, a total of 44 compounds have been discovered. (Orsolich and Basic 2003 and Farre *et al.*, 2004).

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Propolis has a wide range of biological and pharmacological properties that protect beehives from bacterial and fungal diseases (Bankova et al., 2000).

It has the potential to discover novel bioactive chemicals with significant pharmacological effects, such as antibacterial, antiviral, anti-inflammatory, antitumor, antioxidant and anticancer substances, as well as new bioactive molecules. Hegazi and Abd El Hady (Hegazi and Abd El Hady, 2002). Additionally, propolis has antifungal properties and can be found in various juices. Due to its strong aromatic odor, propolis should be used in small amounts to avoid affecting the organoleptic properties of the product (Koc, 2007).

The present study was aimed to investigate the effect of extract of propolis (EP) as a natural preservative on the shelf life of raw milk and pasteurized milk .

MATERIALS AND METHODS

Milk samples: All fresh cow's milk samples were collected from the herd at the Department of Animal production, Faculty of Agriculture, Minia University.

Propolis: Crude organic propolis, collected by honeybees (*Apis mellifera* L.) from New Valley, Dakhla region was supplied by Plant Protection Department at the Faculty of Agriculture, Minia University. Crude propolis covered with aluminium foil was stored at 10°C in dark conditions before it was used in extraction.

Preparation of Propolis water extract:

crude propolis (1.5 g) was combined with 10 mL of ethanol/water (1:1) and swirled for 1 day at room temperature using a magnet stirrer, as described by Paviani et al. (2009). Filtration was used to remove the insoluble fraction, with the filtrates being frozen at -10°C overnight and then filtered again to lower the wax content of the extracts. At a temperature of 40°C, the alcohol was evaporated in a rotary.

Preparation of milk samples:

The experiment was divided into two parts, the first part being a cow's milk, which was heated at 72 °C for 15 seconds, before the addition of extracted immediately cooled to 40°C. Pasteurized milk was supplemented with (0.5%, 1.0%,) of organic propolis extract the other part was raw cow's milk supplemented with (0.5%, 1.0%,) of propolis extract. Then all samples were kept at 5°C.

Physicochemical characteristics

Total Soluble Solids (TSS%); pH value; Ash content and Moisture content were determined according to the methods of AOAC, (2002).

Titrateable acidity: Titrateable acidity was determined according to Adekunte et al. (2010).

Determination of color:

Color qualities were estimated by a coloring distinction meter (model shading Tec-PCM, USA) (Francis, 1983).

Total Flavonoid Calculation

The total flavonoid content was determined using catechin as a reference using the aluminum chloride method (Abu Bakar et al. 2009).

Total Antioxidant Capacity: Total Antioxidant Capacity was determined according to (Kanika et al 2015)

Total phenolics content (TPC):

TPC was measured using a Folin- Ciocalteu assay according to Musa et al. (2011).

Microbiological tests Standard plate count agar was used for total bacterial count. MRS agar was used for total lactic acid bacterial count (The enumeration of yeasts , molds and *E. coli* was detected using Macconocky agar (APHA, 1994).

Sensory analysis:

The sensory properties of the milk samples were tested by 10 trained panelists using sensory scores: 10 for texture, 10 for colour, 20 for flavour and 40 for acceptability. (Amerine et al., 1965)

Statistical analysis

Data gathered were subjected to two-way Analysis of Variance (ANOVA) to decide the general impact of treatments, on physicochemical and quality attributes of samples. The differences were separated using the Least Significant Difference (LSD) [(Motulsky, 1999)].

RESULTS AND DISCUSSION

The sensory properties of milk samples were tested by 10 trained members before starting the research. Propolis extract was added in concentrations (0.5, 1, 1.5, and 2 %) in order to determine the highest concentration that can be used from the extract and to prevent the appearance of the bitter taste of propolis in milk and organoleptic accepted by consumers . The addition of Propolis extract concentration (0.5 and 1%) gave a better and closer to the taste and color of the pasteurized milk, so these concentrations were used with raw milk.

Table1. Effect of propolis extracts on the physicochemical properties of pasteurized milk (PM) and raw milk (RM).

Pasteurization milk (PM)			
Treatments	Moisture%	TS%	Ash%
Control	87.61	12.52	0.72
P 0.5%	87.58	12.41	0.71
P 1%	87.48	12.38	0.70
LSD 0.5	0.169	0.169	0.008
Raw milk (RM)			
Control	87.56	12.45	0.72
P 0.5%	87.37	12.4	0.71
P 1%	87.2	12.36	0.71
LSD 0.5	0.486	0.405	0.006

The chemical compositions of pasteurized milk (PM) and raw milk (RM) treated with propolis aqueous extract are shown in Table (1). Clearly, the addition of propolis extract had negligible effects on moisture and TS. The ash content of the RM and PM treatments decreased slightly compared to the control. These data are in good agreement with El-Deeb and Omar , (2017) reported that cheese treatments prepared using ethanolic extract of propolis (EEP) and water extracts of propolis, there were minor variations in moisture, total solids, and ash content.

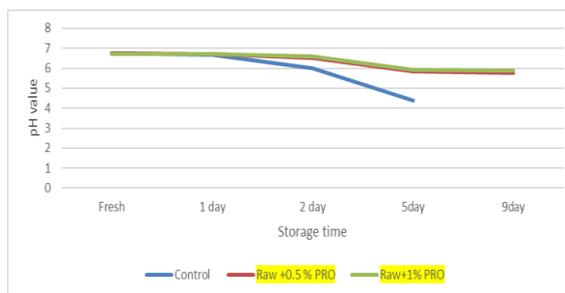


Fig. 1. Effect of propolis extracts on the pH of Raw milk (RM)

The variations in pH values and titratable acidity values of several variants of milk samples after storage at 51°C using two concentrations of propolis extracts are shown in Fig (1,2,3,4) When the milk was fresh, the data revealed that there were increased disparities in pH values across all treatments, including the control milk. Furthermore, throughout the 15-day storage period at 51 °C, the pH values of all milk steadily fell. The findings of this study accord with those of Janhoj *et al* (2008). According to some, the reduction in pH levels during storage might be linked to the breakdown of lactose and protein components.

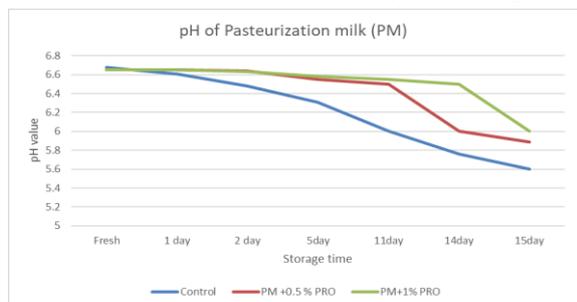


Fig. 2. Effect of propolis extracts on the pH of Pasteurized milk (PM)

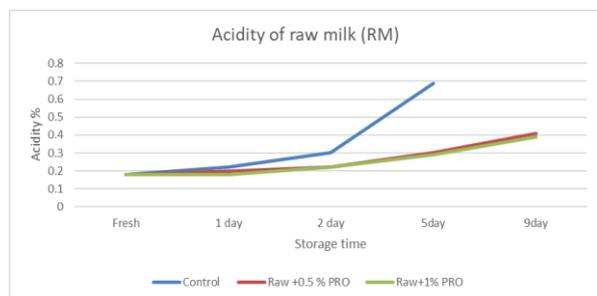


Fig. 3. Effect of propolis extracts on acidity of raw milk (RM).

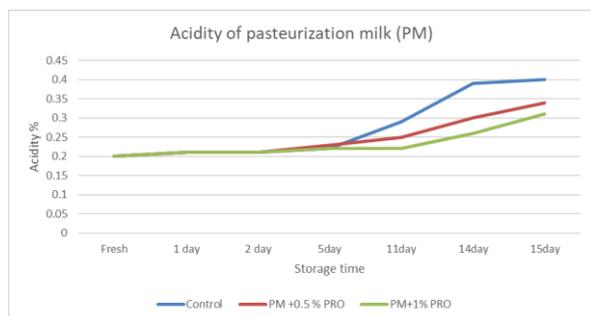


Fig. 4. Effect of propolis extracts on acidity of pasteurized milk (PM).

The variations in milk's titratable acidity followed the opposite pattern as the pH. The findings show that milk acidity was significantly influenced, with an increase in acidity as the percentage of added propolis extracts was reduced. When comparing raw milk with propolis to raw milk without propolis, the control acidity rose dramatically as storage time increased, and the similar pattern was observed in pasteurised milk. Generally the addition of propolis reduced the evolution of increasing acidity, reduced pH and lengthen shelf life of all samples compared to control. Raw milk added propolis coagulated after 10 days however pasteurized milk coagulated after 15 days. Propolis had a everywhere parade of native and pharmacological activities compare bacterial and fungal infections in the beehives (Bankova *et al.*,2000) .

Table 2. Effect of propolis extracts on the color attributes of pasteurization milk (PM) and raw milk (RM)

Tret	Raw milk (RM)				
	L	A	B	ΔE	Chroma
Control	92.07	7.765	5.275	-	9.39
P 0.5%	91.06	7.524	5.18	0.74	9.13
P 1%	90.08	7.165	5.13	1.51	8.82
LSD 0.5	0.172	0.041	0.136	-	-
Pasteurization milk (PM)					
Control	91.97	7.56	5.16	-	9.15
P 0.5%	90.28	7.48	5.11	1.179	9.06
P 1%	90.11	7.12	5.02	1.365	8.71
LSD 0.5	0.119	0.432	0.023	-	-

The color characteristics (L, a, b, ΔE, and chroma) for pasteurized milk (PM) and raw milk (RM) are reported in Table (2). The results indicated that raw milk (RM) control color values were 92.07, -7.77, 5.28 and 9.39 for "L", "a", "b" and chroma, respectively. These values decreased after adding propolis extract and became 91.06, -7.52, 5.18 and 9.13 for 0.5% propolis extract, 90.08, -7.17, 5.13 and 8.82 for 1% propolis extract. However ΔE values were increased with adding propolis extract found to be 0.74 and 1.51 for 0.5 and 1% propolis, respectively .

The same trend was found when propolis extract was added to pasteurize milk (PM). The results showed that pasteurized milk (PM) control had the color values of 91.97, -7.56, 5.16 and 9.15 for "L", "a", "b" and chroma, respectively. These values decreased with the increment propolis extract and became 90.28, -7.48, 5.11 and 9.06 for 0.5% propolis extract, 90.11, -7.12, 5.02 and 8.71 for 1% propolis extract. While ΔE values were increase with adding propolis extract the value was found to be 1.18 and 1.37 for 0.5 and 1% propolis.

Color variations in dairy products can be caused by technological procedures, as well as storage conditions and time. Higher levels of lightness (cream, milk) are caused by homogenization, whereas heating procedures can induce either an increase or a reduction in parameter L*. Denaturation of B-lactoglobulin and its conjugation to J-casein resulted in an increase in whiteness. (Grigioni and colleagues, 2010).

Color variations in food products are commonly described using the Hunter colour parameters (L), (A), and (B). However, it was suggested that hue angle and chroma be used as more realistic colour metrics. Color variations can alternatively be stated as a single numerical number, ΔE. The magnitude of the entire colour difference is defined by this value. (Abdel-Aleem, *et al.* 2019)

Table (3) shows the phenolic components of milk samples as impacted by varying quantities of propolis extract (EP). Data indicated that milk samples with different concentrations of propolis extract (0.5 and 1 %) had an increment of the phenolic compounds, this increment ranged between (29.16 – 39.53 mg/100g) for PM and of ranged of RM between (35.23 - 43.28 mg/100g) and this compounds decreased during storage . Phenolic substances are bioactive components, and phenols are organic compounds containing an aromatic ring that is chemically linked to one or more hydrogenated substituents when matching functional derivatives are present. [Pasupuleti *et al* 2017, Wagh *et al* 2013]. phenolic components, such as flavonoids, are widely found in propolis, and diverse phenolic compounds may contribute to the functional qualities of bee products, such as antioxidant, antibacterial,

antiviral, antiinflammatory, antifungal, wound healing, cardioprotective, and other activities. [Pasupuleti et al 2017, Wagh et al 2013, El-Deeb 2017– Djais et al 2020].

Table 3. Effect of propolis extracts on the TPC content mg/100g in pasteurized milk (PM) and raw milk (RM)

Pasteurization milk (PM)				
Tret	Storage period			
	0	5	10	14
Control	0.80	0.60	0.51	0.39
P 0.5%	29.16	28.00	26.99	23.69
P 1%	39.53	35.08	31.61	18.26
LSD 0.5	2.880			
Raw milk (RM)				
Control	1.17	0.91	-	-
P 0.5%	35.23	35.07	32.96	-
P 1%	43.28	39.26	35.86	-
LSD 0.5	7.205			

Table (4) shows the effects of varying quantities of aqueous extract of propolis (EP) on the flavonoids in milk samples . The results revealed that adding different quantities of propolis extract (0.5 and 1 percent) to milk samples enhanced the amount of Flavonoids components. PM increments varied from 11.01 to 33.33 mg/100g, whereas raw milk increments ranged from 18.57 to 47.62 mg/100g.

Table 4. Effect of propolis extracts on the TFC content mg/100g in Pasteurized milk (PM) and raw milk (RM)

Pasteurization milk (PM)				
Tret	Storage period			
	0	5	10	14
Control	2.29	1.29	1.02	0.56
P 0.5%	11.01	9.65	8.91	6.25
P 1%	33.33	31.97	21.07	19.25
LSD 0.5	0.902			
Raw milk (RM)				
Control	2.40	1.21	-	-
P 0.5%	18.57	14.29	9.01	-
P 1%	47.62	41.71	34.39	-
LSD 0.5	1.451			

Table (5) shows the tinnic content of milk samples as a function of propolis extract concentrations . Data showed that milk samples with adding of propolis extract rate (0.5 and 1 %) were in the range between(11.26 ,29.65 mg/100g) for Pasteurized milk ,whears they were in to rang between(17.02 , 32.92 mg/100g) in raw milk and this compounds decrease during storage

Table 5. Effect of propolis extracts on the Tinnic content mg/100g in Pasteurized milk (PM) and raw milk (RM)

Pasteurized milk (PM)				
Tret	Storage period			
	0	5	10	14
Control	2.84	2.13	1.01	0.89
P 0.5%	11.26	9.78	8.92	6.25
P 1%	29.65	23.86	16.17	13.46
LSD 0.5	1.786			
Raw milk (RM)				
Control	3.04	2.23	-	-
P 0.5%	17.02	10.96	9.20	-
P 1%	32.92	23.83	20.27	-
LSD 0.5	4.057			

Antioxidant capacity of milk samples as affected by propolis (EP) are given in Table (6). Data showed that milk samples with adding different propolis (0.5 and 1 %) increase the antioxidant capacity, this from 92.8 to 112.1(mg/100g) for PM respectively whereas these valu were 149.5 and 162.8

(mg/100g) when raw milk use .These compounds decreased during storage .Phenolic compounds are bioactive compounds, propolis is recognised for its antioxidant properties and high levels of polyphenolic chemicals. The antioxidant potential of dairy beverages was improved by adding propolis extracts. propolis extract polyphenolic components are likely more resistant to heat treatments, which protects other antioxidant constituents in dairy products (de Groot, 2013).

Table 6. Effect of propolis extracts on the total antioxidant capacity content mg/100g as ascorbic acid in pasteurized milk (PM) and Raw milk (RM)

Pasteurized milk (PM)				
Tret	Storage period			
	0	5	10	14
Control	45.7	43.3	39.3	36.7
P 0.5%	92.8	63.2	51.5	45.1
P 1%	112.1	73.2	66.8	60.5
LSD 0.5	1.432			
Raw milk (RM)				
Control	52.2	48.8	-	-
P 0.5%	149.5	126.4	83.9	-
P 1%	162.8	155.8	118.7	-
LSD 0.5	9.516			

Table (7) reveals that treated samples with propolis had a lower microbial load than controls, and all milk samples were free of coliform bacteria, yeasts, and moulds. Propolis' antibacterial action is attributed to its phenolic components, primarily flavonoids, since simple phenols, phenolic acids, and polyphenols are active antimicrobial agents (Cowan, 1999; Temiz et al., 2011). Propolis has long been utilised as a folk medicine treatment, in apitherapy, as a component of biocosmetics and health foods, and for a variety of other applications (Bankova et al., 2000; Banskota et al., 2001; El-Bassiony et al., 2012).

Table 7. Microbiological analysis of pasteurized milk (PM) and raw milk (RM) treatments with propolis extracts at storage

samples	Bacteria	Mold and yeast	E.coli	salmonella
Pasteurized milk (PM)				
PM	Fresh	52×10 ²		
	5day	90×10 ²		
	10 day	300×10 ²		
	15day	500×10 ²		
Control	Fresh	50×10 ²		
	5day	60×10 ²		
	10 day	100×10 ²		ND
	15day	170×10 ²		
PM+1/2 % PRO	Fresh	48×10 ²		
	5day	57×10 ²		
	10 day	100×10 ²		
	15day	100×10 ²		
Row milk				
RM control	Fresh	300×10 ²		
	5day	500×10 ²		
	10 day	Coagulate		
RM+1/2 % PRO	Fresh	200×10 ²		
	5day	350×10 ²		ND
	10 day	390×10 ²		
RM +1% PRO	Fresh	150×10 ²		
	5day	300×10 ²		
	10 day	330×10 ²		

CONCLUSION

The current study showed that adding 0.5 and 1% of propolis extracts to milk can increase the shelf life of raw and pasteurized milk, therefore it is recommended to be used as natural and sources for preserving milk and fortifying it with biologically active compounds and antimicrobial agents during storage of milk.

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تأثير مستخلص البروبوليس العضوي على خصائص اللبن المبستر والخام

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أصبحت المواد الطبيعية التي يمكن استخدامها كمواد حافظة في قطاع الأغذية مهمة للغاية. يحظى البروبوليس باهتمام كبير بسبب خصائصه المضادة للأكسدة والمضاد لنمو البكتيريا. الهدف من الدراسة الحالية استخدام مستخلص البروبوليس العضوي كمادة حافظة طبيعية ومعزز لللبن الخام والمبستر أثناء التخزين. تم استخدام مستخلصات البروبوليس بتركيزتين مختلفتين (٠,٥ و ١٪). تم تحليل اللبن الناتج من المعاملة (مستخلص البروبوليس) وتقدير TS ، ودرجة الحموضة ، والحموضة المعيارية ، واللون ، والفلافونويد الكلي ، والمركبات الفينولية الكلية ، والسعة الإجمالية لمضادات الأكسدة ، والميكروبيولوجية. وخصائصه الحسية طازجة وأثناء التخزين (١٤ يوماً) عند ٥ ± ١ درجة مئوية. أظهرت النتائج أن إضافة مستخلص البروبوليس كان له تأثير ضئيل على T.S ، خواص حسية اللون ، ومع ذلك زادت المركبات الفينولية والفلافونويد والمواد المضادة للأكسدة الكلية مع زيادة إضافة مستخلصات البروبوليس. أما بالنسبة للحموضة تزداد ببطي في معاملات مستخلص البروبوليس العضوي ، كما أنه في اللبن الخام في الكنترول حدث تجبن بعد مرور ٥ أيام بينما في العينات المضاف إليها بروباليز حدث تجبن بعد ١٠ أيام أما بالنسبة لللبن المبستر لم يحدث تجبن لكل العينات وعند تقدير العدد الكلي للبكتريا وجد زيادة ببطي في العينات المضاف إليها البروبوليس العضوي مقارنة بالكنترول ولم يظهر أي نمو للفطريات والخمائر و بكتريا القولون والسالمونيلا في جميع العينات