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Effect of Libyan Honeybee Propolis as a Natural Preservative for the Laboratory Soft Cheese (The laboratory cheese)

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Abstract:— *In the current study the effect of adding aqueous extract of propolis on the organoleptic properties, chemical and microbiological quality attributes of soft cheese (the laboratory soft cheese) were examined. Propolis aqueous extracts (zero, 10mℓ and 20mℓ/L) pasteurized milk were added before the manufacture of soft cheese, stored in Refrigerator at 5± 2°C for 30 days with periodical testing every 7 days. No remarkable differences were noted in organoleptic properties for both control and treated samples. In the contrary, development in acidity of control samples was more progressive than other samples were the higher propolis concentration the lower acid development. Moisture content was slightly higher in cheese with higher propolis concentration. Moreover, the most marked effect on microbial population in cheese was recorded in the coliform count. It is recommended to add propolis aqueous solution (10%) to milk in ratio of 20mℓ/liter pasteurized milk to prolong the shelf-life and improving the safety of soft cheese.*

***Keywords*: Libyan Propolis, Natural Preservative, Laboratory Soft Cheese.**

INTRODUCTION

Propolis" bee glue" is a mixture of bees wax and resins collected by honey bees from various plant sources (substances exuded from wounds in plants, lipophilic materials on leaves and leaf buds, gums, resins, and lattices) and mixing salivary enzymes (β-glucosidase) [1]. Bee mixes the original propolis with bee wax and β-glycosidase they secrete it during propolis collection [1] and [2]. This truly therapeutic substance not only guards beehives against intruders, but it can also fend off and treat all kinds of unwanted health conditions [3]. Propolis has actually been used medicinally by humans since ancient times, it is intensively used in the food industry as a supplement and as a folk medicine and by the cosmetic [3] and [4]. Approximately 420 different compounds have been classified so far in propolis, giving it wide variety in many important pharmacological properties such as antimicrobial, antioxidative, anti-tumor, anti-inflammatory, antifungal, antiparasitic activities [5], [6], [7] and [8]. Propolis were successfully used in treating numerous of human diseases such as the cardiovascular, blood systems disorder, infections of the respiratory system, dental care, dermatology, cancer treatment, immune system, digestive tract disorders and liver protection [9], [10] and [11]. In addition, propolis reduces fungal toxins production "aflatoxins and ochratoxins" [12], [13], [14] and [15]. The strong antimicrobial activity of propolis is due to Flavonoids [16]. At least 38 flavonoids have been found in propolis [17]. A large number of studies have shown an inhibitory effect of bee propolis on a variety of microorganisms [8], [18]. Moreover, propolis is also used in human nutrition due to its content of amino acids and vitamins (A, B_1, B_2, B_6, C and E) [19]. Propolis is stable product; keep its antibiotic activity, even when stored for one year or longer. It is

used as a preservative in food products due to their antioxidant and antimicrobial activities and thus may actually prolong the shelf-life of some food products [19]. The present study was aimed to describe the use of the natural preservative "propolis" to prolong its shelf-life of soft cheese making in consideration not Altering its organoleptic properties.

MATERIAL AND METHODS

Sampling and preparation of propolis

300 gm of propolis was collected. The propolis was made by honey bees from plants around bee hives in Zawia area, northwest of Libya. A stock of 10% propolis aqueous solution was prepared from previously grind propolis, soaked in distilled water with periodical shaking for 7 days. The extract was filtered through Zees filter for obtained sterile aqueous propolis solution (10%).

Cheese manufacturing

The laboratory soft cheese was made at the local laboratories from cow milk (13% solids as refract meter degree) 0.02% calcium chloride and 6% sodium chlorid were added. Aqueous propolis extract (10%) was added at the rate of (zero, 10mℓ and 20mℓ/liter of milk), Calf

rennet powder (chr. Hansen's-Denmark) was added to milk at the rate of 5g/100kg distributed in yoghurt plastic cups (150 mℓ), sealed and incubated at 45ºC for two hours and then transferred to the refrigerator at 5ºC ± 2ºC and stored for 30 days; triplicates were made from each treatment. Samples were obtained for organoleptic properties, chemical and microbiological analysis at zero time (just after manufacture), 7, 15, 21 and 30 days.

Examination of cheese

Organoleptic examination

Cheese samples were examined by 19 panelists using cheese score card [20] including score guide for flavor (50 points), body and texture (35 points) and appearance and colour (15 points). The test was performed once at zero time for ensuring acceptability of the product after adding propolis extract.

Chemical analysis

Cheese samples were examined for its moisture % and titratable acidity [21], while pH was determined using pH meter model SA 720 (Orion, USA) after efficient mixing using distilled water.

Microbiological analysis

Cheese samples were prepared according to method recommended by [22], examined for their total colony count, total yeast and mould count as well as coliform count according to [23].

RESULTS AND DISCUSSION

Organoleptic properties

The finding of the experiment in table (1) illustrate the Mean organoleptic scores for control and treated samples just after manufacture. Concerning organoleptic, it is definitely clear from table (1) that there were no marked differences between control and treated samples even in higher concentrations. There were no change in odour, taste or colour of the product produced. These results were agree with those previously reported by [13], [14] and [15].

Chemical analysis

Results in table (2) show that acidity of different propolis concentrations % of laboratory cheese during storage period for control and treated samples with propolis**.** Results in table (3) show that pH of laboratory cheese during storage period for control treated samples with different propolis concentrations. These findings came in accordance with those previously mentioned by many researchers such as [9], [11], [13] and [14].

Table 1. Mean organoleptic scores for control and treated samples just after manufacture

Score items	Propolis 10% (ml/1 Liter milk)		
	0	10	20
Flavor (50 points)	45	45	44
Body and texture (35 points)	31	32	32
Appearance and colour (15 points)	13	13	13
Total Score (100 points)	89	91	89

0 = non treated samples

Table 2. Acidity of different propolis concentration % of laboratory cheese during storage period for control and treated samples

Storage period (Days)	Propolis 10% (ml/1 Liter milk)		
	0	10 ml	20 ml
Fresh	0.13 %	0.13 %	0.15 %
7 days	0.20 %	0.21 %	0.20 %
15 days	0.35 %	0.32 %	0.33 %
21 days	0.39 %	0.39 %	0.35 %
30 days	0.50 %	0.45 %	0.42 %

0 = non treated samples

Table 3. pH of laboratory cheese during storage period for control treated samples with different propolis concentrations

Storage period (Days)	Propolis 10% (ml/1 Liter milk)		
	0	10 ml	20 ml
Fresh	6.75	6.75	6.75
7	6.40	6.50	6.50
15	6.25	6.10	6.15
21	5.30	5.85	5.80
30	4.90	5.55	5.75

0 = non treated samples

Results clearly indicate that, the pH and titratable acidity were affected with the addition of propolis extract (Tables 2&3). At zero time pH and acidity were nearly the same. With storage, data showed resulted in a gradual decrease of pH with an increase of titratable acidity in control and all treatments during the storage period. The increase in acidity and decrease in pH value of cheese from different treatments were adversely proportional to propolis concentrations. This increase and difference in acid production may be due to the effect of propolis on the acid producing microorganisms [24]. The findings in table (4) show that Moisture of laboratory cheese during storage period for control treated samples with different propolis concentrations. It is obvious from the results in table (4) that the addition of propolis extract had a negligible effect on the composition of cheese moisture content of the resultant cheese. The storage of cheese had noticeable effect on properties, composition, acidity and pH, changes. The same findings were reported by [13] and [14].

Table 4. Moisture of laboratory cheese during storage period for control and treated samples with different propolis concentrations

Storage period (Days)	Propolis 10%ml/1 Liter milk		
	0	10 ml	20 ml
Fresh	64.35 %	66.45 %	67.25 %
7 days	62.75 %	64.55 %	65.35 %
15 days	61.25 %	63.85 %	64.65 %
21 days	60.45 %	62.75 %	64.45 %
30 days	59.65 %	61.35 %	62.55 %

Results in table (5) show the effect of different ratios of aqueous propolis extract 10% upon different microbial populations of laboratory cheese during storage.

Table 5. Effect of different ratios of aqueous propolis extract 10% upon different microbial populations of laboratory cheese during storage

Count Storage/days	Total colony Count			Total yeast and mauld Count/gram			Coliform count (MPN/g)*		
	0 control	10 ml	20 ml	0 control	10 ml	20 ml	0 control	10 ml	20 ml
Zero time	55×10^5	60×10^5	120×10^4	20×10^8	30×10^4	15×10^4	250×13^5	240×10^3	225×10^3
7 days	70×10^6	50×10^6	80×10^3	25×10^5	40×10^4	30×10^3	40×10^4	40×10^3	30×10^2
15 days	230×10^7	80×10^5	30×10^3	105×10^5	20×10^4	45×10^2	230×10^4	90×10^2	(zero)
21 days	40×10^8	50×10^5	50×10^2	40×10^6	20×10^2	25×10^2	280×10^4	40×10^2	(zero)
30 days	90×10^8	40×10^4	20×10^2	108×10^6	280	230	55×10^5	25×10^2	(zero)

It is clear that from table (5), the total bacterial count increased with storage time for control samples. On the contrary the count was reduced in all treated samples with propolis in different concentrations. the higher the propolis concentration, the faster the bacterial reduction. " Ref. [24] found that, the flavonoids had positive effect on the growth of some kinds of bacteria as they act as prebiotics. These results agreed with [15] and [25]. This is due to the destructive effect on some bacterial population and inhibitory effect on the other bacteria [24]. The effect of propolis on fungal count was more pronounced. Reduction was dramatic in laboratory soft cheese treated with high propolis concentration. Lower propolis concentration reduced the yeast and mould count proportionally according to its concentrations. Control laboratory soft cheese should continuous increase in count during the whole period of storage with visible growth after 10 days post manufacturing. These results substantiated the use of propolis as antifungal agent for protecting cheese along its storage period. These results were agree with those previously reported by [5], [26], [27] and [28]. Propolis is not only protecting against fungi but also against its toxin production [12] and [28].

Table (5) demonstrate the effect of different propolis concentrations on coliform count (MPN/gram). Propolis has inhibitory to destructive effect on different members of coliforms [12] and [29]. Complete reduction was obtained using the higher propolis concentration after 15 days and prolonged for 25 days when using 10 ml propolis per 10% liter milk. These findings came in accordance with those previously mentioned by many researchers, who noticed the antibacterial and antifungal effect against Gram positive bacteria [5], [18], [27], [28], [30] and [31].

CONCLUSION

Findings obtained from the current study confirm that the supplementation of milk with propolis aqueous solution (10%) as a natural preservative was identified as the best in improving the quality and microbial safety. propolis seems to be prefect antibacterial and antifungal agent, with no toxic effects. In addition it had no effect on organoleptic properties of cheese. It is recommended to use propolis extracts in preserving laboratory soft cheese for longer time and in the mean time improve the safety of the product.

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