# YEMEN BEE PROPOLIS AS A NATURAL PRESERVATIVE FOR LABNEH

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## Abstract

The effect of adding aqueous extract of propolis (bee glue) on the microbiological, chemical and organoleptic properties attributes of Labneh were studied.

Propolis aqueous extract 10% (5, 10, 15 and 20ml/Kg of curd) was added before packaging, and then storing it in a refrigerator at 5 ± 1ºC for 30 days with a periodical testing every 5 days. The lactic acid bacteria (LAB) counts increased up to the 15 days of a storage period then decreased up to the end of the storage period. Moreover, (LAB) and Lipolytic bacteria counts were affected by the added propolis aqueous extract, while molds and yeasts were not detected in the fresh and stored Labneh in all treatments except the control and Labneh containing 5 and 10 ml/kg of curd. Also, coliforms and proteolytic bacteria were not detected in the fresh and stored Labneh in all treatments.

No remarkable differences were recorded in organoleptic properties for both control and treated samples. However, development in acidity of the control samples was more progressive than the other treated samples; the higher propolis concentration, the lower acid development. Moisture percentage content was slightly higher in Labneh with higher concentrations. Total solids, fat, soluble nitrogen, total nitrogen of Labneh gradually increased with storage to the maximum at the end of storage.

It is recommended to add Propolis aqueous solution 10% to curd of Labneh in a ratio of 15 ml/kg of curd to prolong the shelf life and improve the safety of Labneh.

**Key words** : propolis , Labneh , preservative , microbial groups , shelf life.

## Introduction

Labneh is a concentrated Yoghurt, which originated in the Middle East area. it is usually made of using milk inoculated with lactic acid bacteria then concentrated the curd in cloth bags (Hamad and Al-sheikh, 1989). It is a white to creamy paste that has a smooth texture with a taste crossing between sour cream and cottage cheese and a characteristic sharp flavor that is largely modulated by diacetyl produced during fermentation (Tamime *et al*., 1999). The traditional method of producing Labneh consists of straining the whole milk yoghurt in a cheese cloth bag to the desired total solid level. Industrially, an excess liquid is removed from the traditional yoghurt by mechanical separators. The product is usually packaged in airtight translucent plastic containers, and displayed under refrigeration (~ 5 to 7 ºC) in retail outlets.

The shelf life of traditional Labneh is short even if it is stored under refrigeration, this may be due to the sanitary problems usually associated with cloth bags used and uncontrolled hygienic handling, which increase microbial contamination (El-Samargy, 1997). The cloth bag could be the main source for *staphylococci*, yeasts, and moulds, while the presence of coliform bacteria may mainly due to un-sufficient heat treatment of Labneh during processing (Abou-Donia *et al*., 1992). The high microbial load of Labneh coupled with packaging, storaging conditions would result in the formation of off-flavors and an undesirable physico-chemical change that eventually lead to product failure (Muir and Banks, 2000). The stated shelf life of Labneh, in Egypt ranges between 14 and 21 days and is largely based on commercial experience. Therefore, there is a need to develop ways to improve keeping quality and shelf life of Labneh. This would improve the market ability of the product.

Propolis (bee glue) is a resinous hive product, collected by bees. It is used in medicine of different nations as early as 3000 BC. It has antimicrobial, antifungal, antiviral, immunostimulant and antioxidant activities (Bratter *et al*., 1999 and Koo *et al*., 2000).

The strong antimicrobial activity of Propolis is due to flavonoids (Grange and Davey, 1990). At least 38 flavonoids have been found in Propolis (Greenaway *et al*., 1990). A large number of studies have shown an inhibitory effect of Propolis on a variety of microorganisms (Kujumgiev *et al*., 1999). Moreover, Propolis is also used in the nutrition due to its content of amino acids and vitamins (A, B1, B2, B6, C and E) as reported by Ghisalberti, (1979).

Propolis is stable product which maintains its antibiotic activity, even when it is stored for a year or longer. It 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.

In Egypt (El-deib *et al*., 1997) used Propolis for the surface of Ras cheese and found that propolis layer was completely protective against mould and bacterial growth. It has also been used as an antioxidant in meat products in addition to its protective action against different microbial populations (Dessouki *et al*., 1980).

The present study describes the use of the natural preservative (i.e., Propolis) to prolong shelf life of Labneh making in consideration not altering its organoleptic properties.

**Materials and Methods**

The fresh milk of cow was obtained from the herd of the college of Agriculture, Minufiya University, Egypt.

The yoghurt starter containing: *streptococcus salivarus subsp thermophillus* EMCC1143 and *Lactobacillus delbreckii subsp. Bulgaricus* EMCC1102 were obtained from Cairo Mircen (Ain shams University, Egypt).

Propolis samples were collected from the hybrid honey bee colonies at Ibb governorate, Yemen.

**Preparation of Propolis**: A stoke solution of 10% Propolis aqueous solution was prepared from Propolis which was previously ground, soaked in distilled water with shaking for 7 days. The extract was filtered through Ziess filter to obtain the sterile aqueous propolis solution 10% (Moawad *et al*., 2001).

**Preparation of Labneh**

Labneh was made of the standardized cow milk by the traditional method as described by (El-Samargy *et al*., 1988). Labneh curd was divided into five batches. The first was served as a control and to the other four batches Propolis aqueous solution 10% was added at the rate of (5, 10, 15 and 20 ml/kg) of curd and mixed with each part re-divided into equal portions ~ 100 gm, packed in a plastic container, and stored at 5 $^{o}$C ± 2 for 30 days.

Labneh samples were analyzed microbiologically and chemically every 5 days. All the treatments were carried out in triplicates.

**Microbiological analysis**

Lactic acid bacterial count (LAB), yeast, mould and coliform count were determined according to the American Public Health Association 1978.

**Chemical analysis**

Labneh samples were analyzed for total solids, fat, total and soluble nitrogen, titratable acidity values according to ( Ling 1963), and total volatile fatty acids (TVFA), (Kisikowski 1982).

**Sensory evaluation**

Labneh samples were evaluated for (flavor, appearance body, texture) by 15 panelists of the staff members of Dairy Science and Technology Department Minufiya University. Samples were presented to judges in plastic cups in random order and judges were provided with rinse water, plastic spoons (Ismail *et al*., 2006).

**Statistical analysis**

Randomized block design and 2*3 factorial design were used to analyze the data. Duncan's test was used to make the multiple comparisons (Steel and torrie 1980). Significant differences were determined at ($P \leq 0.05$).

## Results and Discussion

**Microbial analysis**

The data listed in Table (1) revealed that, LAB counts increased with an advanced storage period in all treatments. The count was reduced in all Labneh treated samples with propolis extract added. This is due to the destructive effect on some bacterial population and the inhibitory effect on the other bacteria (Smith *et al*., 1986 and Meresta and Meresta., 1985).

One of the most important parameter to determine the quality and shelf life of Labneh is the count of yeast and mould. In addition to quality deterioration, the microbiological counts have been used as indices for the end of shelf life of dairy products (Muir *et al*., 2000). Moulds and yeasts were not detected in the fresh Labneh for all treatments. However, moulds and yeasts appeared in the samples of the control Labneh, Labneh with 5 ml/kg propolis after 15 days and in Labneh with 10 ml/kg after 20 days. These results substantiated the use propolis as antifungal agent protecting Labneh along its storage period. Propolis is used as protective agent in Ras cheese (El-deib *et al*., 1997). Propolis is not only protecting against fungi but also against its toxin production (Pepdljnfak *et al*., 1982).

Table (1): The effect of different ratios of the aqueous propolis extract 10% upon different microbial of Labneh during storage.

| Labneh samples | Lactic acid bacterial count CFU $10^6$/gm | | | | | | | Yeast and Moulds count CFU $10^6$/gm | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Storage period (days) | | | | | | | Storage period (days) | | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 | 30 | 0 | 5 | 10 | 15 | 20 | 25 | 30 |
| C* | 143 | 166 | 178 | Spo. | Spo. | Spo. | Spo. | ND | ND | 2 | Spo. | Spo. | Spo. | Spo. |
| $T_1$ | 139 | 146 | 163 | 169 | Spo. | Spo. | Spo. | ND | ND | ND | 4 | Spo. | Spo. | Spo. |
| $T_2$ | 135 | 139 | 148 | 156 | 169 | Spo. | Spo. | ND | ND | ND | ND | 8 | Spo. | Spo. |
| $T_3$ | 135 | 139 | 144 | 151 | 162 | 177 | 182 | ND | ND | ND | ND | ND | ND | 10 |
| $T_4$ | 131 | 136 | 138 | 143 | 152 | 163 | 175 | ND | ND | ND | ND | ND | ND | ND |

Each value in the Table is the mean of three replicates
Spo. : Spoiled
C* : Control Labneh made from cow milk containing 4% fat.
$T_1$, $T_2$, $T_3$ and $T_4$ Labneh made of cow milk containing 4% fat with 10% propolis added at rate of 5, 10, 15, and 20 ml/kg of curd.
ND : Not detected.

Coliforms not detected in fresh Labneh and during storage period in all resultant Labneh, which indicate the good hygienic condition followed in its production.

**Chemical Composition:**

The data presented in Tables (2) and (3) showed that no great differences were observed with the different concentration of Propolis treatments and control when fresh.

The second important quality criterion that determines the acceptability and shelf life of Labneh is the titratable acidity (TA). The present data clearly indicated that TA values increased significantly ($p \leq 0.05$) in all treatments during the storage period. This increase and difference in acid production may be due to the effect of Propolis on the acid producing microorganisms. In addition, TA values of Labneh were affected with different amount of Propolis extract added.

Table (2) showed the changes in the total solids (TS) and fat/dry matter of Labneh during the storage period. It was clear that, no significant differences ($p \leq 0.05$) were observed in the TS and the fat/dry matter of Labneh between the five treatments of propolis and the control either when fresh or during the storage period. TS and fat were increased during storage due to the moisture loss.

The SN/TN ratio, of Labneh from different treatments Table (3) showed a significant increase through the storage period. However, the SN/TN ratio was affected with the amount of propolis used. This may be due to the effect of propolis extract on undesirable organisms especially in $T_3$ and $T_4$. Concerning the total volatile fatty acids (TVFA), the results indicated that the TVFA were affected by the concentration of Propolis. The lowest value was recorded when 20 ml/kg of curd was added. In general, during the storage period TVFA value were gradually increased in all the treatments in the variable rates, and they had no significant

effects ($p \leq 0.05$) in the fresh Labneh but had significant effect ($p \leq 0.05$) during the storage period.

Table (2): The effect of using Propolis extract on some chemical properties of Labneh during the storage period (average of 3 replicates)

| Properties | The storage period/days | Treatments | | | | |
|---|---|---|---|---|---|---|
| | | C | $T_1$ | $T_2$ | T3 | $T_4$ |
| Total Solids % | 0 | 23.56[a] | 23.49[a] | 23.31[a] | 23.26[a] | 23.15[a] |
| | 5 | 23.73[a] | 23.64[a] | 23.42[a] | 23.39[a] | 23.27[a] |
| | 10 | 23.91[a] | 23.72[a] | 23.56[a] | 23.44[a] | 23.38[a] |
| | 15 | Spo. | 23.86[a] | 23.69[a] | 23.56[a] | 23.45[a] |
| | 20 | Spo. | Spo. | 23.76[a] | 23.71[a] | 23.53[a] |
| | 25 | Spo. | Spo. | Spo. | 23.83[a] | 23.66[a] |
| | 30 | Spo. | Spo. | Spo. | 23.91[a] | 23.76[a] |
| Fat/Dry Matter % | 0 | 29.53[a] | 29.42[a] | 29.40[a] | 29.37[a] | 29.31[a] |
| | 5 | 29.61[a] | 29.48[a] | 29.45[a] | 29.41[a] | 29.36[a] |
| | 10 | 29.65[a] | 29.52[a] | 29.50[a] | 29.46[a] | 29.42[a] |
| | 15 | Spo. | 29.55[a] | 29.53[a] | 29.51[a] | 29.45[a] |
| | 20 | Spo. | Spo. | 29.58[a] | 29.53[a] | 29.46[a] |
| | 25 | Spo. | Spo. | Spo. | 29.56[a] | 29.51[a] |
| | 30 | Spo. | Spo. | Spo. | 29.61[a] | 29.55[a] |
| SN/TN % | 0 | 13.36 | 13.31 | 13.29 | 13.30 | 13.28 |
| | 5 | 13.50 | 13.39 | 13.35 | 13.32 | 13.30 |
| | 10 | 13.76[a] | 13.53[b] | 13.42[bc] | 13.38[bc] | 13.33[c] |
| | 15 | Spo. | 13.68[a] | 13.56[b] | 13.43[c] | 13.39[c] |
| | 20 | Spo. | Spo. | 13.62[a] | 13.51[b] | 13.42[c] |
| | 25 | Spo. | Spo. | Spo. | 13.56[a] | 13.47[b] |
| | 30 | Spo. | Spo. | Spo. | 13.67[a] | 13.56[b] |

Each different letters (in the same row) means that the multiple comparisons are different from each other. Letter (a) is the highest mean followed by b, c……etc.
0 : See Table (1).

**Organoleptic Properties:**

Table (4) gives the average scores for organoleptic properties of Labneh as affected by the amount of Propolis added. The results suggest that the addition of propolis extract improved the organoleptic quality of Labneh. The quality of Labneh slightly decreased during storage, but retained acceptable properties.

After 10, 15, 20 days the samples C, $T_1$, $T_2$ deteriorated during storage, respectively. This result was in line with the finding of (Khadragy 1988) who found that the fresh Labneh made from buffalo's milk gained more score points than 10 days old of Labneh. Labneh made with propolis extract (10%) with (15 and 20 ml/kg) got higher scores and more acceptable as compared with control. $T_1$ and $T_2$ used during the storage period. It was observed an increase in the shelf life for 30 days at 5 $^{o}$C ± 2 with an accepted and clean acid flavor with out signs of yeast spoilage. Also, the concentration of Propolis extract had no significant effect ($p \leq 0.05$) Table (4) on fresh and after 5 days of storage and had significant effect on the rest of the storage period.

It could be concluded that Propolis extract seems to be a perfect antibacterial and antifungal agent, with no toxic effects and it can be successfully

used to increase the shelf life of Labneh for 30 days at 5 ± 1°C and in the mean time improves the safety of the product.

Table (3): Change in Titratable acidity and Total volatile fatty acids during storage of Labneh for control and treated samples with different amount of propolis extract (average of 3 replicates).

| Properties | The storage period/days | Treatments | | | | |
|---|---|---|---|---|---|---|
| | | C** | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
| TA% | 0 | 1.18[a] | 1.18[a] | 1.18[a] | 1.18[a] | 1.18[a] |
| | 5 | 1.29[a] | 1.25[b] | 1.22[c] | 1.22[c] | 1.20[c] |
| | 10 | 1.37[a] | 1.32[b] | 1.25[c] | 1.23[d] | 1.23[d] |
| | 15 | Spo. | 1.38[a] | 1.29[b] | 1.25[c] | 1.25[c] |
| | 20 | Spo. | Spo. | 1.32[a] | 1.28[b] | 1.28[b] |
| | 25 | Spo. | Spo. | Spo. | 1.36[a] | 1.31[b] |
| | 30 | Spo. | Spo. | Spo. | 1.39[a] | 1.33[b] |
| TVFA ml NaOH 0.1N/100gm Labneh | 0 | 6.63[a] | 6.65[a] | 6.70[a] | 6.67[a] | 6.65[a] |
| | 5 | 6.75[a] | 6.71[b] | 6.69[a] | 6.69[a] | 6.67[a] |
| | 10 | 7.53[a] | 7.42[b] | 7.18[c] | 7.01[d] | 6.88[e] |
| | 15 | Spo. | 7.49[a] | 7.31[b] | 7.18[c] | 7.02[d] |
| | 20 | Spo. | Spo. | 7.39[a] | 7.24[b] | 7.14[c] |
| | 25 | Spo. | Spo. | Spo. | 7.31[a] | 7.21[b] |
| | 30 | Spo. | Spo. | Spo. | 7.38[a] | 7.27[b] |

0: See Table (1).

Each different letters (in the same row) means that the multiple comparisons are different from each other. Letter (a) is the highest mean followed by b, c……etc.

Table (4): Sensory evaluation of Labneh during the storage period, for control and the treated samples with different propolis amount of Propolis extract (average of 3 replicates).

| The storage period/days | Properties | Treatments | | | | |
|---|---|---|---|---|---|---|
| | | C** | $T_1$ | $T_2$ | T3 | $T_4$ |
| 0 | Flavor (50) | 45 | 45 | 45 | 45 | 45 |
| | Body & Texture (35) | 31 | 30 | 30 | 30 | 30 |
| | Appearance (15) | 14 | 14 | 14 | 14 | 14 |
| | Total (100) | 90[a] | 89[a] | 89[a] | 89[a] | 89[a] |
| 5 | Flavor (50) | 42 | 43 | 43 | 43 | 42 |
| | Body & Texture (35) | 30 | 30 | 30 | 30 | 30 |
| | Appearance (15) | 14 | 14 | 14 | 14 | 14 |
| | Total (100) | 86[a] | 87[a] | 87[a] | 87[a] | 86[a] |
| 10 | Flavor (50) | 40 | 42 | 42 | 40 | 40 |
| | Body & Texture (35) | 30 | 29 | 29 | 30 | 30 |
| | Appearance (15) | 13 | 13 | 13 | 14 | 13 |
| | Total (100) | 83[a] | 84[a] | 84[a] | 84[a] | 83[a] |
| 15 | | Spo | 40 | 40 | 40 | 40 |
| | | | 28 | 29 | 29 | 29 |
| | | | 13 | 13 | 13 | 13 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Total (100) | | $81^{b}$ | $82^{ab}$ | $82^{ab}$ | $84^{a}$ |
| 20 | | Spo. | Spo. | 40 | 40 | 40 |
| | | | | 29 | 29 | 29 |
| | | | | 13 | 13 | 13 |
| | Total (100) | Spo. | Spo. | $82^{a}$ | $82^{a}$ | $82^{a}$ |
| 25 | | Spo. | Spo. | Spo. | 40 | 40 |
| | | | | | 28 | 29 |
| | | | | | 13 | 12 |
| | Total (100) | Spo. | Spo. | Spo. | $81^{a}$ | $81^{a}$ |
| 30 | | Spo. | Spo. | Spo. | 40 | 40 |
| | | | | | 28 | 28 |
| | | | | | 13 | 12 |
| | Total (100) | | | | $81^{a}$ | $80^{a}$ |

0: See Table (1).
Each different letters (in the same row) means that the multiple comparisons are different from each other. Letter (a) is the highest mean followed by b, c……etc.

# استخدام صمغ النحل اليمني
# كمادة حافظة طبيعية في صناعة اللبنة

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## الملخص

تعتبر اللبنة من المنتجات اللبنية سريعة الفساد لذا اهتمت هذه الدراسة بإطالة مدة حفظها حيث تم دراسة تأثير إضافة أربعة أحجام ( 5, 10, 15 و 20 مل) لكل كيلو جرام خثرة من المستخلص المائي لصمغ النحل والذي تركيزه 10٪ على الخواص الميكروبية . الكيميائية والحسية للبنة المصنعة بالطريقة التقليدية. وقد تم حفظ اللبنة على 5 $^0$م لمدة 30 يوم وأخذت عينات عند صفر , 5 , 10 , 15 , 20 ,25 و 30 يوم وذلك لتحليلها ميكروبيا . كيميائيا وحسيا. وقد أوضحت النتائج المتحصل عليها تأثر عدد بكتيريا حامض اللاكتيك بزيادة معدل إضافة المستخلص المائي لصمغ النحل. أما الخمائر والفطريات فلم تظهر في العينات الطازجة وخلال التخزين ظهرت في المقارنة (CK) بعد 10 أيام وفي حالة إضافة 5 مل من المستخلص المائي لصمغ النحل ظهرت بعد 15 يوم و وفي حالة إضافة 10 مل من المستخلص المائي لصمغ النحل ظهرت بعد 20 يوم بينما في حالة التركيزات الأعلى لم تظهر الخمائر والفطريات وحتى نهاية فترة التخزين. الحموضة وكذلك الأحماض الدهنية الطيارة زادت بتقدم فترة التخزين وكذلك نسبة النتروجين الذائب إلى النتروجين الكلي. بينما لم تتأثر نسبة الدهن إلى المادة الجافة أثناء التخزين. تأثرت الحموضة وكذلك نسبة الأحماض الدهنية الطيارة بإضافة المستخلص المائي لصمغ النحل حيث انخفضت بزيادة معدل الإضافة. حصلت اللبنة المصنعة بإضافة المستخلص المائي لصمغ النحل وكذلك المقارنة على نفس درجات التحكيم الحسي.

أظهرت النتائج إمكان استخدام المستخلص المائي لصمغ النحل كمادة حافظة طبيعية حيث أمكن بنجاح زيادة مدة حفظ اللبنة المخزنة على 5 $^0$م لمدة 30 يوم بينما المقارنة تلف بعد 10 أيام. لذا نوصي بإضافة المستخلص المائي لصمغ النحل والذي تركيزه 10٪ بمعدل 15 مل لكل كيلوجرام خثرة وذلك لإطالة مدة حفظ اللبنة.

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