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Effect of Using Echinacea Extract on the Shelf Life of Labneh

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Abstract

Labneh is a favorable product by a variety of customer groups due to its pleasant texture and nutritional value, where the antioxidant and antimicrobial properties of *Echinacea purpurea* L extract (EPE) were investigated and its impact on improving the quality the labneh. Antioxidant was determined (32.01mg TE/g) by (DPPH), total phenols (30.72mg GAE/g) and flavonoids (16.53 mg CE/g). Antimicrobial properties of EPE against gram positive, gram negative bacteria mold and yeast was determined at concentration 10, 20 and 50 mg/ mL. The most effective concentration was 50 mg/mL. Phenolic compounds were determined for EPE and results indicated that caffeic acid gave the highest value. EPE was added to labneh treatments (L1, L2 and L3) at concentrations 1, 1.5 and 2 % respectively, compared with control sample during storage time for 21 days. Physico-chemical properties were studied during storage, and were with significant differences between all treatments. Total antioxidant and microbial analysis of treatments was determined and results indicated that the highest value was for L3 with significant differences between all treatments during storage period. Mold and yeast were not detected at fresh treatments and the highest value of mold and yeast was for control sample and the lowest value was for L3 at the end of storage period. Gave the treatment L3 the best results for improve the shelf life of labneh compared with other treatments. Sensory evaluation revealed acceptable all treatments at fresh time, after 14 days till the end of storage period, treatment L3 had the highest acceptability and control sample had the lowest acceptability.

Keywords: *Echinacea* purpurea; Antioxidant;Antimicrobial; Labneh; shelf life.

1. Introduction

Fermented dairy products have different properties and marked by numerous nutritional, healthy and biological benefits that lead to maintain and promote human health [1]. Labneh is a widely used product in the Middle East, especially in In Arab Countries, and it is a traditional fermented milk product that is consumed. Labneh is a fermented dairy product produced by removing yogurt's water-soluble components to create a semi-solid substance called labneh. In labneh the total solid content becomes 23–25% and the fat content reaches a range of 9–11 %. There are some problems that cause corruption labneh or reduce the life of ordinary labneh; even it was stored at low temperatures. This could be as a result of improper product handling or storage, which are both known to cause sanitary issues with cotton bags used in manufacturing [2].

Recently, there has been a growth in interesting of consumers health by utilizing the functional and medicinal health properties of herbs and spices Phytochemicals are abundant in order to maintain and improve health, nutrition, and immunity, particularly during the Covid-19 pandemic era. Fortification of dairy products with various herbs and spices has gained popularity as a way to take advantage of their therapeutic and functional properties. Milk and other dairy products are well-liked complementary delivery systems for providing customers with the functional, nutritional, and other health [3].

Echinacea purpurea L. (*E. purpurea*) is one of most fundamental medicinal plants respect to the Asteraceae family. Drugs made from echinacea have been used successfully to control the immune system. Syrups with an echinacea base are particularly effective in pediatric medicine. Echinacea is a

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potential immunomodulatory agent for COVID-19 [4].

Several chemical substances are present in this plant with biology-related activities including antioxidant and antimicrobial effects, have also been established. Methanol extract of *Echinacea purpurea L.* (*E. purpurea*) has antioxidant activities. Crude methanolic extract had antimicrobial effect against six microbial strains included bacteria, fungi and yeast like *Escherichia coli* (G-), *Streptococcus faecalis* (G+), *Aspergillus oryzae*, *Candida albicans*, *Saccharomyces cerevisiae* and *Alternaia solani*. Yeast (*Saccharomyces cerevisiae*) was most sensitive to *E. purpurea extracts*, with an average value of 15.0 mm at concentration 100 ppm extract. [5]**.** Sumac Extract [6].

EPE was superior compared with synthetic antioxidants in preservatives of cake in control of molds and lipid oxidation throughout 60 days of storage at 25^{o} C. Cake that contained 1000 ppm of EPE had the lowest PV (Peroxide Value) and had more antioxidant activity than cake that contained 200 ppm of BHA ($p \leq 0.01$). EPE was able to slow down the oxidation rate of the cake. The best antibacterial activity was demonstrated by EPE at 1500 and 2000 ppm [7].

Echinacea is commonly recognized as purple coneflower, one of the most popular herbs all over the world. Echinacea plants have an amazing variety of active compounds, such as caffeic acid, alkamides, phenolic acids, rosmarinic acid, polyacetylenes, and many others.

The present investigation was carried out to investigate the effect of using of Echinacea extract on the shelf life and improved the quality of labneh as natural preservative which make it useful for industry and in response to the rising demand for a variety of high-quality milk products.

2. MATERIAL & METHODS

2.1. Preparation of *Echinacea purpurea* L. extract

Mix 1.0 g of *Echinacea purpurea* L. powder was taken then, added 20 ml ethanol (80%) and magnetic stirring is used for extraction at room temperature for 6h. Extract was then dried using a rotary evaporator and stored at 4 °C for other experiments [8]. The strains using in this study were brought from the cultures in National Research Centre. These tests done under bio-safety cabinet class.

2.2. Manufacture of Labneh

Fresh buffalo milk was heated to 90°C for 20 minutes, then cooled to 40 °C to inoculate with 3% of the yoghurt starter culture (*Streptococcus salivarius sub sp. thermophilus* and *L. bulgaricus delbruckii* sub sp. *bulgaricus*1:1) it was entirely coagulated up to pH 4.6. In order to allow the whey to drain, the resulting coagulant was combined and placed into cheese cloth bags, which were then hung in a refrigerator set at 6 °C. Then 0.5 % NaCl was added and thoroughly combined. The resulting labneh was divided into treatments one of them made without EPE as control, and EPE was added to three portions at ratio of 1.0, 1.5 and 2 % (L1, L2 and L3, respectively).The samples were taken at 0, 7, 14, 21 days for chemical, microbial and organoleptic properties analysis.

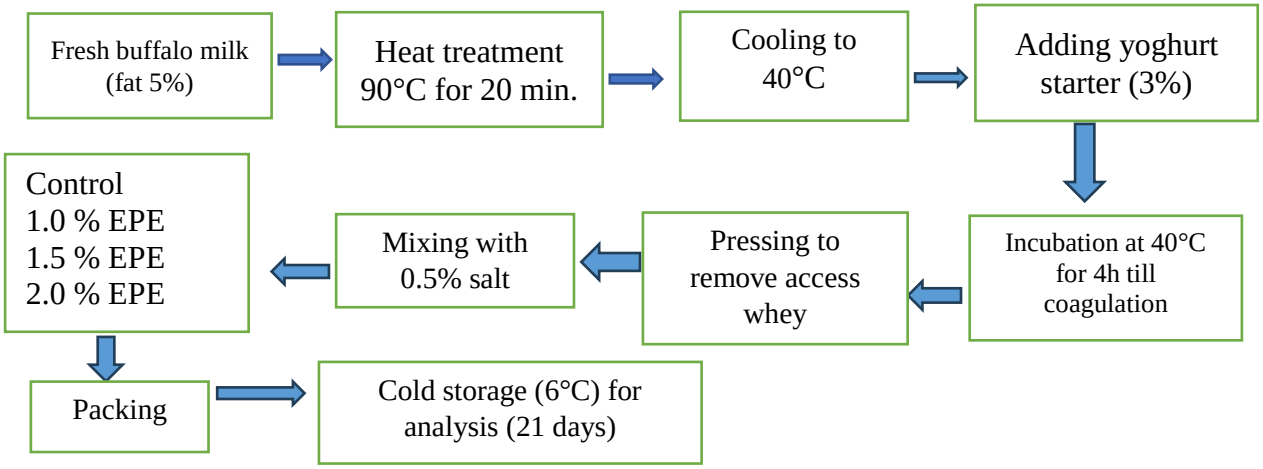

Fig. 1. Diagram for the manufacture of labneh.

2.3. The profile of phenolic acids

The extract (1g) was added to 20 ml of 2M NaOH in a quick-fit cone flask, washed with N2, and the stopper restored at room temperature. The extract was incubated in 6M HCl for 4 hours, with a pH adjustment of 2. The supernatant was obtained after centrifuging the extract at 5000 rpm for 10 minutes. Two extractions of phenolic substances were performed using a 50 mL combination of ethyl ether and ethyl acetate at a ratio of 1:5. The samples had been reconstituted in 2 ml of methanol following the separation and 45 °C evaporation of the organic phase. HPLC analysis was carried out using a liquid chromatograph from Agilent Technologies' 1100 series that has an auto sampler and a diode-array detector. The analytical column was an Eclipse XDBC18 (150X 4.6 m; 5m) with a C18 guard column (Phenomenex, Torrance, CA). The mobile phase was composed of acetonitrile (solvent A) and 2 % acetic acid in water (v/v). The gradient programmer was as follows: 100 % B to 85 % B in 30 minutes, 85 % B to 50 % B in 20 minutes, 50 % B to 0 % B in 5 minutes, and 0 % B to 100 % B in 5 minutes. The flow rate was maintained at 0.8 ml/min for a total of 70 minutes.

The peak of benzoic acid and cinnamic acid derivatives were simultaneously evaluated at 280 and 320 nm with a 50l injection volume. Before injection, extract was filtered using an Acrodisc 0.45 m syringe filter from the Gelman Laboratory in Michigan. Utilizing consistent retention times and UV spectra, the peaks were located and compared to the standards.

2.4. Total phenolic content

The total phenolic content was calculated using the Folin-Ciocalteau method according to [9]. The samples (0.5 ml) were introduced to the reagent Folin- Ciocalteau (5 ml, of a sample diluted 1:10 with distilled water) for 5 minutes, and then aqueous sodium carbonate (4ml and 1M) was added. The absorbance of the reaction mixture at 765nm was measured using a UV-Vis spectrophotometer (model - Systronics 2202). Gallic acid served as the standard. The values were computed as mg/100g of gallic acid equivalents.

2.5. Total flavonoids content:

The amount of flavonoids in the EPE was measured. Total flavonoid concentrations of samples were expressed as mg of catechin equivalent per gramme of extract (mg CE/g) using catechin as a reference. The measurement of total flavonoid in the extract was as following: Briefly, 10 ml volumetric flask containing 4ml of distilled water was added to an aliquot (0.50ml) of each EPE. 0.3 of 5 % $NaNO_2$ was introduced to this flask. Add 0.3 ml of 10 % $AlCl_3$ after 5 minutes of incubation. 2ml of 1M NaOH was added at the six-minute mark, and then distilled water was added until the mark. An orangey-yellow color was created. Using a UV-vis spectrophotometer, the absorbance at 510nm was determined after 10 min of incubation. The extract was examined three times. Equation was used to calculate the extract's total flavonoid content.

TFC = the total content of flavonoids compounds, mg/g plant extract in CE.

C = concentration of catechin obtained from the curve (mg/L).

V = the volume of the sample solution (L).

m = mass of extract in grams.

DF = Dilution factor

2.6. Antimicrobial activity of EPE

Antimicrobial activity was carried out according to the methods described by [10 and 11]**.** For antibacterial properties, the examined microorganisms were added to tubes of Tryptic soy broth, and they were then cultured at 37° C for four hours. These cultures' turbidity was adjusted using 0.5 McFarland. On the surface of solid nutrition agar plates, sterile cotton swabs were used to create a homogenous bacterial lawn. In order to make discs 6mm that were impregnated with various concentrations of Echinacea extract (10, 20 and 50 mg/mL), Whatman filter paper no. 1 was utilized. The impregnated discs were placed on the surface of nutrient agar plates with streaks. The triplicate plates were inverted and incubated for 16 to 18 hours at 35 degrees Celsius.

The antifungal assay was carried out utilizing the disc diffusion technique on Potato Dextrose Agar (PDA) media, [11]. Tested fungi was transferred to a test tube A loopful of the growing, containing 10 ml of 0.01% tween 80 solutions to create the spore suspension. Using a glass rod, 100 mL of the spore suspension was applied to the Potato Dextrose Agar plates that had solidified, and the plates were then left to dry for 30 minutes. The dry plates' surface was covered with the impregnated discs. The plates were turned over and let to sit at 28°C for 24-48 hours.

Following incubation, the inhibition zones' diameters were also assessed. Using a ruler held on the back of the upside-down petri plates, zones are measured to the closest millimeter. Three replicates were averaged and the results were expressed as mean ±SD according to [10].

2.7.Determination of physicochemical properties of labneh

The physicochemical properties of labneh include TS, protein, fat, ash, pH and titratable acidity % of the labneh samples was measured according to [12].

2.8. Radical scavenging of DPPH

The capacity of scavenging free radicals was calculated in the example of DPPH, the reaction volume was 3.0 mL with a final concentration of 50 M. using method of [13], by the effective DPPH (1,1-Diphenyl-2-picryl). At 60 minutes, the absorbance at 517nm (A) was measured in comparison to a pure methanol blank. The following equation was used to determine the percentage of the DPPH free radical that was inhibited:

Inhibition (%) = 100 × (A control−A sample)/A control

A calibration curve was created to determine the antioxidant activity. With ascorbic acid, and expressed as mg of Trolox equivalent (TE) per gram of sample.

2.9. Microbiological analyses

2.9.1. Aerobic mesophilic Count

Total viable counting was performed using the plate count agar medium as recommended by [14] and [15]. The plates were examined for total count (cfu/g) after two days of aerobic incubation at 35°C.

2.9.2. Total count of *Str.thermophilus* and *L. bulgaricus*.

In this case, 10 grams of the sample were suspended in 90 mL of sterile peptone to determine the counts of *Str. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in order. A selective medium for bacteria using M17 agar, which was then incubated at 37 °C for 48 hours. MRS agar (pH 5.4 by glacial acetic acid, Merck) was applied to the count of *L. delbrueckii* subsp. *bulgaricus* then incubated at 37°C for 72 hr in an anaerobic jar by anaerobic kits [16].

2.9.3. Mold and Yeast Count

Molds and yeasts were examined using Potato dextrose agar. Dilutions were created and then transferred to sterilized plates (about 1 mL per plate). The plates were examined for yeast and fungal population (cfu/g) after 3-5 days of incubation at 30 °C [15].

2.10. Sensory evaluation of labneh with echnaciae extract

Sensory evaluated of labneh samples was determined. The panelists gave labneh ratings of 0 to 60 points for flavor, 0 to 30 points for body and texture, and 0 to 10 points for appearance. [17].

2.11. Statistical analysis

SPSS 20 for Microsoft Windows was used to examine the data. At the P (0.05) level of significance, Duncan's multiple-range test was used to examine the statistical data

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3. RESULT AND DISSECTION

3.1. Phytochemical properties of echnaciae extract

Table1. Showed phytochemical properties of echnaciae extract (antioxidant activity DPPH, total phenol and total flavonoids). The DPPH value of echnaciea extract was 32.01 (mg TE /g). Total phenols of echnaciae extract value were 30.72 mg GAE/g and total flavonoids value was 16.53 mg CE/g. These results were in agreement with [18] who found that total phenols of echnaciea extract was 60.2 (mg GAE/g) and total flavonoids value was 32.3 mg RE/g. It is may be due to differences between varieties of echnaciae and difference of extraction method.

Table 1. Phytochemical properties of echnaciae extract

Antioxidant activity DPPH (mg TE/g)	Total phenol (mg GAE/g)	Total Flavonoids (mg CE/g)
32.01	30.72	16.53

TE = Trolox. GAE=Gallic acid equivalent. CE= catechin equivalent

3.2. Antimicrobial activity of different concentration of EPE

Table (2) showed antimicrobial effects of EPE at concentration (10, 20 and 50 mg/mL). The results showed that extracts had an obviously growth inhibition on gram-positive pathogenic bacteria (*Bacillus cereus* and *Staphylococcus aureus*). The antibacterial activity concentration (10, 20 and 50 mg/ mL) of the ethanolic extract of EPE against *Bacillus cereus* ranged from 9-12 mg/mL and ranged from 8.30-9.70 mg/mL for *Staphylococcus.* The antibacterial activity concentration of the ethanolic extract of EPE against *Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa* ranged from 8-9.70, 8.30-10.70 and 8.30-11.70 mg/mL, respectively. The antibacterial activity concentration of the ethanolic extract of EPE against *Aspergillus flavus, Aspergillus carbonarius, Fusarium proliferatum* and *Penicillium verrucosum* ranged from 8.33-11.67, 9-13.33, 8.33-12.33 and 8.67-12.67. The effect of EPE on *Candida albicans* (yeast) ranged from 8.70- 10.30. According to reports, *Echinacea purpurea* has demonstrated strong immunoregulatory, anti-inflammatory, and antioxidant abilities and there were not negative effects in the clinical testing stages. These results are in agreement with [5] who studied effect of extract on six microbial strains included bacteria, fungi and yeast i.e.: *Alternaia solani, Escherichia coli (G-), Streptococcus faecalis (G+), Aspergillus oryzae* ,*Candida albicans* and *Saccharomyces cerevisiae*. EPE, with an average value of 15.0 mm at concentration of 100 ppm extract, had the greatest impact on yeast (*Saccharomyces cerevisiae*). [6] who found that different pathogenic strains including *B. cereus, S. aureus*, and *S. typhimurium* were used to inoculate the manufactured cheese, and adding sumac extract caused a decrease of about five log cycle orders of magnitude during the entire experiment; however, the addition of sumac extract and probiotic bacteria caused the disappearance of the pathogen after 21 days for *S. aureus*. [7] resulted that EPE at concentrate 1500 and 2000 ppm showed the best antifungal on the cake antimicrobial activity ($p<0.01$). [18] studied antimicrobial effect of classical extract (ethanol extract) and ultrasound extraction for echninacea and found that the inhibition zone's dimensions for all microorganisms were higher for extracts obtained by classical extraction extracts by ultrasound extraction. The differences were in significant differences for of *E. coli, B. subtilis, C. albicans* and *S.cerevisiae.*Results indicated that there were not growth inhibition zones for *A. niger* for the two extracts.

Table.2 Antimicrobial activity of Echinacea extract

Type of pathogenic microbes		Inhibition zone in mm developed by different concentration of Echinacea extract (Mean ± SD)		
	Tested organisms	**10 mg/ml**	**20 mg/ml**	**50 mg/ml**
Gram-positive bacteria	*Bacillus cereus*	9.00 ± 1.00	9.67 ± 0.58	12.00± 1.00
	Staphylococcus aureus	8.30 ± 0.6	9.00 ± 0.0	9.70 ± 0.58
Gram-negative bacteria	*Escherichia coli*	8.00 ± 0.0	8.67 ± 0.58	9.70± 0.58
	Salmonella typhi	8.30 ± 0.6	9.00 ±0.0	10.70 ± 0.58
	Pseudomonas aeruginosa	8.30 ± 0.6	9.33 ± 0.58	11.70 ± 0.58
Fungi	*Aspergillus flavus*	8.33 ±0.5	9.67 ± 0.58	11.67 ± 0.58
	Aspergillus carbonarius	9.00 ± 0.0	11.33 ± 0.58	13.33 ± 0.58
	Fusarium proliferatum	8.33 ±0.6	10.33 ± 0.58	12.33 ± 0.58
	Penicillium verrucosum	8.67 ±0.6	10.67 ± 0.57	12.67 ± 0.57
Yeast	*Candida albicans*	8.70 ± 0.6	9.33 ± 0.56	10.30 ± 0.58

3.3. Phenolic compounds of echnaciea extract by HPLC

Table (3) showed phenolic compounds of echnaciea extract by HPLC and showed that the highest compound was caffeic acid (1985.229 µg/ g) followed by ferulic acid (273.941 µg/g) and gallic acid (132.467 µg/ g) and the lowest compound was Sinapic acid (3.354 µg/ g). Catechin, rosmarinic acid, apegnin-7-glycoside, quercetin, Kaempferol and Chrysin non detected. These results were in agreement with [19] who found that echnaciea extract contained Caftaric acid (4659µg/g) for *E. purpurea* root/aerial with *H. canadensis* L. and Chlorogenic acid (32 µg/g). [20] discovered that the main component of *E. pallida* and *E. purpurea* leaf and flower water extracts is caffeic acid, while methanol extract of *E. pallida* flowers has the highest phenolic concentration. Caffeic acid derivatives have biological properties of natural phenolic compound include anticancer [21and 23], anti-inflammatory [24], antiviral [25] and antioxidant [21 and 26].

Table.3 Phenolic compounds of echnaciea extract by HPLC

Compound	Result (conc µg/g)
Gallic acid	132.647
Protocatechuic acid	13.345
p-hydroxybenzoic acid	37.769
Catechin	ND
Chlorogenic acid	28.216
Caffeic acid	1985.229
Syringic acid	9.304
Vanillic acid	13.590
Ferulic acid	273.941
Sinapic acid	3.354
p-coumaric acid	21.521
Rutin	40.670
rosmarinic acid	ND
apegnin-7-glycoside	ND
Cinnamic acid	14.081
quercetin	ND
Kaempferol	ND
Chrysin	ND

3.4. Physico-chemical composition of labneh with echnaciea extract during storage

Table (4) showed chemical composition of labneh with echnaciea extract during storage at 0,7,14 and 21 days. The results revealed that the maximum TS content was 30.79 % recorded for fresh control treatment compared with other treatments (L1, L2 and L3) of labneh with concentration 1, 1.5 and 2 % echnaciea extract respectively. TS values increased during storage period for all treatments. It may be due to expelling moisture during storage. Protein and ash decreased with increasing the ratio of echnaciae extract. Fat value increased with echnaciae extract. It may be due to fat content of echnaciea extract. At the end of storage, all treatments values increased to their maximum levels with significant differences. Ash content labneh samples ranged between 0.76-0.92% for treatment (L3) and control treatment, respectively at starting of storage and reached to 0.94 and 0.77% for treatment (L3) and control treatment, respectively at the end of storage (21 days). These results were in agreement with [27] who studied the physicochemical properties of labneh prepared by addition of 0.3, 0.5, and 0.7 % herb sage (powder).

Fig.2. showed pH values of labneh with different concentrations echnaciea extract during storage. The addition of an echnaciea extract had an effect on pH values compared to the control treatment in fresh time and during storage period, and in general the pH values decreased in all storage periods for 21 days with significant differences for all treatments. These results were in agreement with [28] who found that adding dill and caraway essential oils to labneh had

slight effect on titratible acidity contents and values were increased in all samples during storage.

Fig.3. showed the results of acidity for labneh with different concentrations from echnaciae extract. On the contrary pH, there is a significant difference in acidity between treatments, and the results indicated that acidity decreased with increasing the concentration of EPE at fresh samples comparison to control treatment. The control treatment is recorded 1.53 % followed by other treatments 1.49, 1.47, 1.43 % for L1, L2, and L3, respectively, and in general acidity increased during storage periods (21 days). The acidity for treatments recorded 1.62, 1.58, 1.54 and 1.5 % for control, L1, L2 and L3 treatments, respectively in the end of storage period. It may be due to increasing of lactic acid which is produced from bacteria or extract during storage. The change in the acidity content of labneh is an important factor improving and developing labneh quality, since it affects the shelf life and the acceptability of labneh products [29].

Table.4. Physico-chemical composition of labneh with echnaciea extract during storage.

Composition (%)	Treatments C	L_1	L_2	L_3	SE
		0 time			
TS	30.79^{a} ±0.44	30.65^{b}±0.36	30.54^{c}±0.30	30.40^{d}±0.22	0.08
Protein	11.35^{a}±0.01	11.29^{b}±0.01	11.25^{c}±0.01	11.15^{d}±0.01	0.02
Fat	14.32^{d}±0.05	14.52^{c}±0.05	14.75^{b}±0.04	14.82^{a}±0.05	0.03
Ash	0.92^{a}±0.05	0.85^{b}±0.01	0.80^{b}±0.01	0.76^{c}±0.05	0.03
		7 Days			
TS	30.92^{a}±0.51	30.68^{b}±0.37	30.56^{c}±0.30	30.44^{d}±0.01	0.09
Protein	11.40^{a}±0.18	11.31^{b}±0.17	11.27^{c}±0.16	11.18^{d}±0.05	0.03
Fat	14.38^{d}±0.05	14.55^{c}±0.05	14.81^{b}±0.04	14.86^{a}±0.05	0.02
Ash	0.92^{a}±0.05	0.85^{b}±0.05	0.80^{b}±0.01	0.77^{d}±0.05	0.03
		14 Days			
TS	31.06^{a}±0.01	30.71^{b}±0.05	30.59^{c}±0.01	30.48^{d}±0.04	0.10
Protein	11.45^{a}±0.05	11.34^{b}±0.05	11.29^{c}±0.06	11.21^{d}±0.05	0.02
Fat	14.43^{d}±0.05	14.56^{c}±0.06	14.83^{b}±0.05	14.92^{a}±0.05	0.03
Ash	0.93^{a}±0.05	0.85^{b}±0.05	0.80^{b}±0.01	0.77^{d}±0.05	0.03
		21 Days			
TS	31.19^{a}±0.05	30.75^{b}±0.04	30.61^{c}±0.05	30.52^{d}±0.03	0.10
Protein	11.49^{a}±0.11	11.35^{b}±0.02	11.25^{c}±0.02	11.23^{d}±0.03	0.02
Fat	14.49^{d}±0.05	14.57^{c}±0.05	14.78^{b}±0.04	14.94^{a}±0.04	0.02
Ash	0.94^{a}±0.05	0.85^{b}±0.05	0.80^{c}±0.01	0.77^{d}±0.01	0.03

C= Control. L1= Labneh with 1% echnaciae extract. L2= Labneh with 1.5% echnaciae extract. L3= Labneh with 2% echnaciae extract. a-d The different superscript letter have a significant difference in the same row. (Duncan's test $P<0.05$)

3.5. Antioxidant activity as DPPH Scavenging of labneh treatments

In the DPPH assay, plant extract capacity to serve as a hydrogen atom or electron donor in the transition of DPPH into its reduced form, DPPH was examined. The levels of antioxidant activity of different labneh treatments (DPPH scavenging activity) during storage period (21 days) at 0,7,14, and 21 days in Fig.4. Data showed that increasing of DPPH with increasing the levels of echnaciae extract. It may be due to increasing DPPH values of echnaciae extract. These results were in agreement with [30]**.** Results indicated that L3 value was 0.33 (mgTE/ g) at the beginning of storage and decreased during storage to 0.24 (mgTE/ g) while, fresh control sample was 0.20 (mgTE/g) and decrease to 0.15 (mgTE/ g) at 21 days. Data showed that there was decreasing of DPPH values during storage period (21 days).

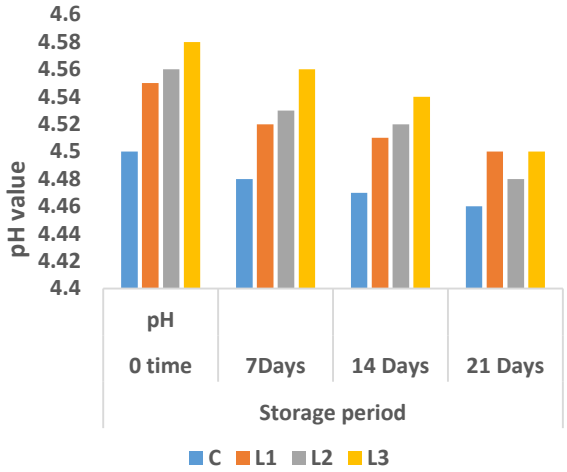


Fig. 2. pH values of labneh with echnaciea extract during storage. Where: C= Control sample. L_1= Labneh with 1% echnaciae extract. L_2= Labneh with 1.5% echnaciae extract. L_3= Labneh with 2% echnaciae extract

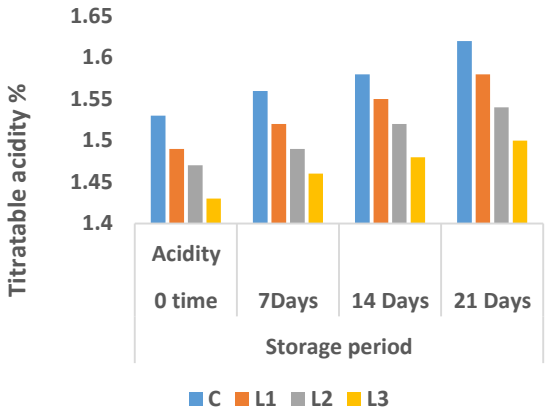


Fig.3. Acidity values of labneh with echnaciea extract during storage. Where: C= Control sample. L_1= Labneh with 1% echnaciae extract. L_2= Labneh with1.5% echnaciae extract. L_3= Labneh with 2% echnaciae extract

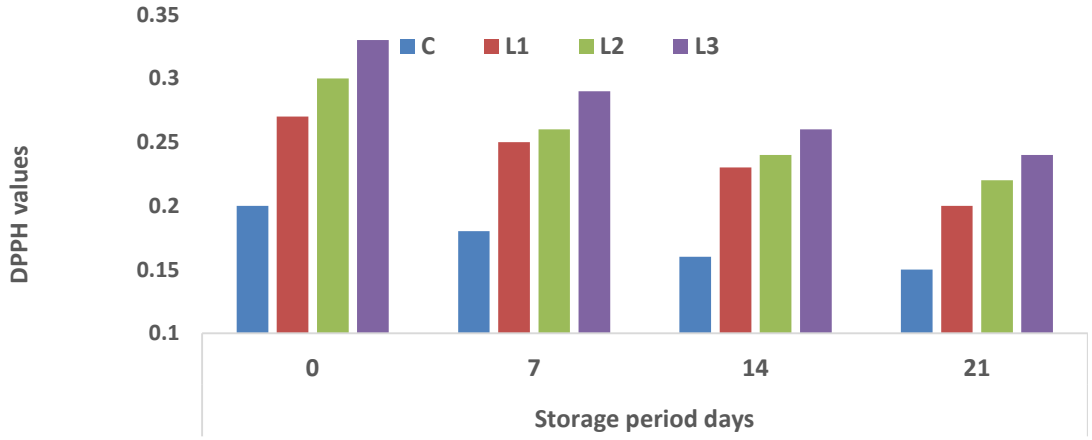


Fig.4 Antioxidant activity as DPPH scavenging of labneh treatments during storage period. Where: C= Control sample. L_1= Labneh with 1% echnaciae extract. L_2= Labneh with 1.5% echnaciae extract. L_3= Labneh with 2% echnaciae extract

3.6. The microbiological characteristics of labneh with echnaciea extract

The microbiological characteristics of control labneh and produced with echnaciea extract are displayed in Table (5). The effect of adding different concentrations of echnaciea extract on aerobic mesophilic count, *Str.thermophilus*, *L.bulgaricus* and molds and yeasts in labneh during storage periods at 4^{o}C for 21 days is presented in Table (5). The mold and yeasts count detected in the control treatment (prepared without echnaciea extract) labneh was 2.42 log cfu/g and 5.60 log cfu/g of labneh after 7 days and after 21 days of storage respectively. The labneh prepared with echnaciae extract lead to the reduction in the maximal growth levels in the labneh and inhibition and delay of the growth of mold and yeasts. Data in Table (5) showed that there was enhancement in the growth of lactic acid bacteria (*Str. thermophilus* and *L. bulgaricus*) in treatment fortified with 1and 1.5 % echnaciae extract compared with control sample, whereas there was decrease in the growth of lactic acid bacteria (*Str. thermophilus* and *L. bulgaricus*) at ratio 2% echnaciae extract. These results were in agreement with [31].

Table 5 .Effect of echnaciae extract concentrations (%) on microbiological quality of Labneh (log cfu/g).

Composition (%)	Treatments				SE
	C	L_1	L_2	L_3	
0 time					
Aerobic mesophilic	9.48^{a}±0.09	9.44^{a}±0.18	9.39^{b}±0.08	9.22^{c}±0.32	0.005
Str. thermophilus	8.57^{c}±0.05	8.71^{b} ±0.03	8.90^{a} ±0.07	8.21^{d}±0.06	0.002
L. bulgaricus	8.19^{c}±0.05	8.57^{b}±0.07	8.82^{a}±0.03	8.15^{d}±0.07	0.003
Mold & Yeast	ND	ND	ND	ND	
7 Days					
Aerobic mesophilic	9.45^{a}±0.08	9.43^{a} ±0.04	9.31^{b}±0.05	9.12^{c}±0.04	0.004
Str. thermophilus	8.15^{c}±0.03	8.60^{b} ±0.05	8.85^{a} ±0.07	8.10^{d}±0.05	0.002
L. bulgaricus	7.80^{c}±0.05	7.89^{b}±0.08	7.98^{a}±0.04	7.35^{d}±0.06	0.003
Mold & Yeast	2.42^{a}±0.02	2.40^{a}±0.03	ND	ND	
14 Days					
Aerobic mesophilic	9.71^{a}±0.08	9.58^{b}±0.05	9.52^{c}±0.04	9.35^{d}±0.07	0.004
Str. thermophilus	7.45^{c}±0.05	7.56^{b}±0.06	7.75^{a} ±0.04	7.40^{d} ±0.05	0.003
L. bulgaricus	7.43^{c}±0.07	7.50^{b}±0.05	7.60^{a}±0.04	7.12^{d}±0.07	0.002
Mold & Yeast	4.25^{a}±0.04	4.02^{b}±0.05	2.90^{c}±0.08	2.43^{a}±0.07	0.004
21 Days					
Aerobic mesophilic	9.90^{a}±0.06	9.75^{b}±0.05	9.55^{c}±0.07	9.41^{d}±0.08	0.004
Str. thermophilus	7.15^{c}±0.07	7.32^{c}±0.08	7.50^{a} ±0.09	7.02^{c}±0.06	0.003
L. bulgaricus	7.00^{c}±0.06	7.24^{b}±0.09	7.43^{a}±0.08	6.95^{d}±0.07	0.002
Mold & Yeast	5.60^{a}±0.05	5.55^{b}±0.08	3.40^{c}±0.07	2.90^{d}±0.08	0.004

C= Control sample. **L_1**= Labneh with 1% echnaciae extract. **L_2**= Labneh with 1.5% echnaciae extract. **L_3**= Labneh with 2% echnaciae extract. $^{a\text{-}d}$ The different superscript letter have a significant difference in the same row. (Duncan's test $P<0.05$)

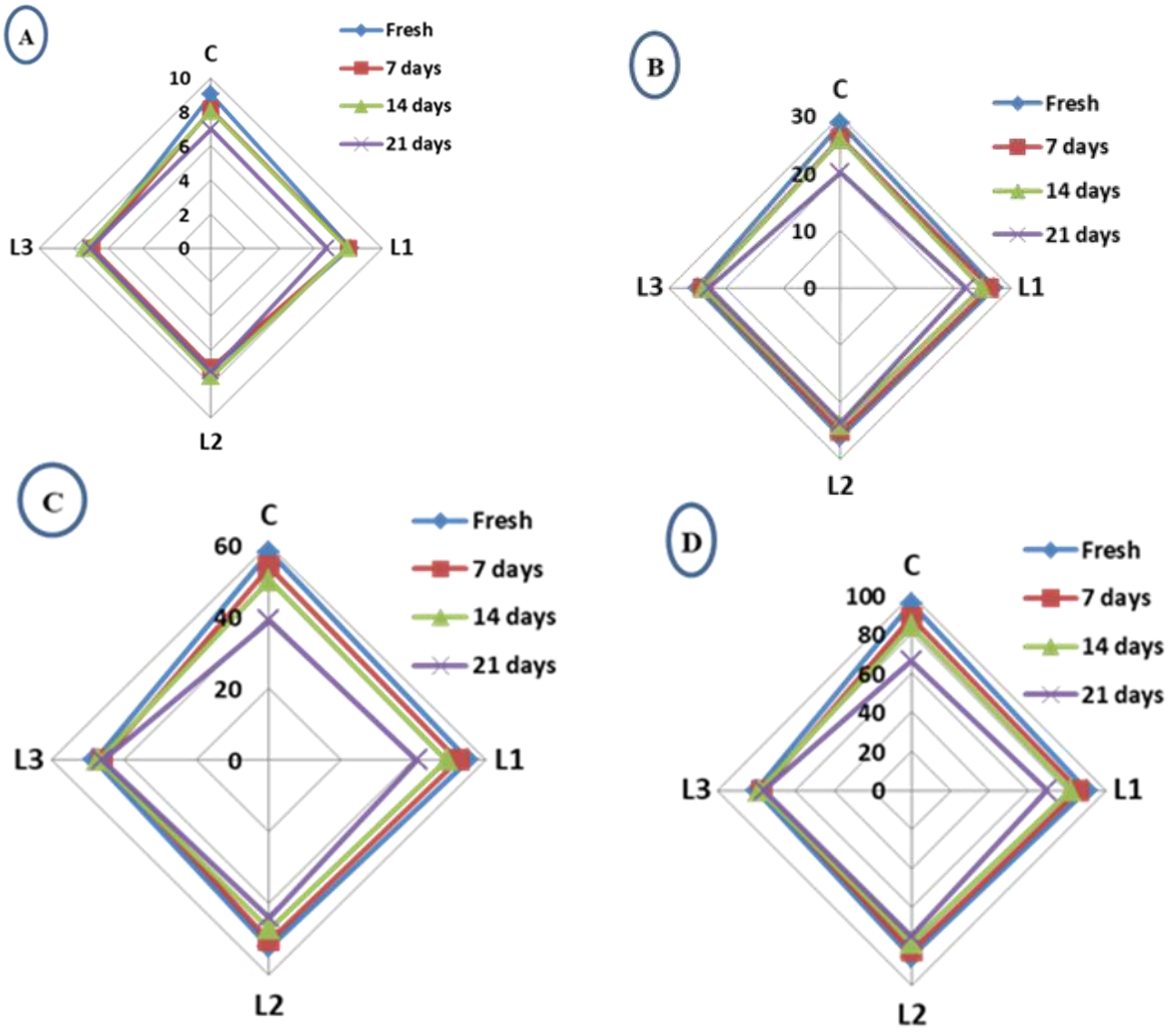
3.7. Sensory evaluation of labneh with echnaciea extract during storage

The sensory characteristics of labneh are the most important criteria that determine the acceptability and increase consumer demand on this commodity. These attributes include appearance, texture & texture, flavor and the overall impression of labneh produced characteristics. The current phase of the study focuses on the evaluation of these features using panelists recruited to represent the Product Judging Panel. Appearance the first parameter that the consumer experience when tasted the products and the final score is 10 points. Figure (5a) indicates that during the whole storage period (21 days) the four treatments of labneh samples got the acceptable score significantly ($p\leq0.05$). The corresponding scores ranged between 7.02 for labneh with the highest concentration from echnaciae extract (0.2 %) to (9.02) with control treatment at fresh time samples. At the end of the storage period, little change in the color from bright while to just white was observed, indicating little change in the color, which was still in the acceptable score range. The change in color may be due to a loss a little moisture. However, after 14 till 21 days, L3 sample with got the high score significantly ($p\leq0.05$) comparison to other samples.

In this parameter the whole score is 30 points. The Body &Texture of labneh is very important parameter that makes the consumer prefer it. Fig (5b) indicated that these parameters that the texture of the all samples was acceptable significantly ($p\leq0.05$) at the fresh and 7 days. The reason may be due to the concentration of echnaciea extract in the samples with a loss of a little moisture, which leads to an increase in the hardness of the texture.

Regarding flavor acceptability, in this parameter the whole score is 60 points. Fig. (5c) indicates that samples with all echnaciae extract concentrations at the zero were acceptable, and in the end of storage period with 0.2% echnaciae extract was the significantly highest acceptable sample ($p\leq 0.05$). On the other hand, the flavor preference decreased along the storage period for all samples.

Over all, putting the four evaluations (appearance, body & texture flavor) all together Fig. (5d) we end up with a final conclusion which indicates that all samples at zero time got preferred score significantly ($p\leq0.05$). On the other hand after storage for 14 days, L3 sample maintained the preferred status and the lowest value was for control sample. There were significant differences ($p\leq0.05$) between all samples during the storage period. These results were in agreement with [32].



Conclusion

The present study explained the effect of using Echinacea extract on shelf life of labneh. Echinacea is a good source of antioxidant and antimicrobial properties were determined, Treatments with Echinacea purpurea L. extract gave the best results for improve the shelf life of labneh, and increased acceptability of flavor, body and texture of resultant labneh compared with control without *Echinacea purpurea* L extract.

Conflicts of interest

The authors have no conflicts of interest to declare.

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