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Impact of lemon peel extracts utilization on the biological values of the Labneh during storage

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ABSTRACT

Lemon peel extract is a natural source of improving biologically and physiological functions. The aim of this study is to evaluate antioxidant, phenolic compounds, cytotoxicity and antimicrobial potential of lemon peel water extracts. Fresh and dried lemon peel extracts at concentrations of 1,2 and 4% were used to enhance Labneh quality. The hemical, physical, rheological, microbiological and sensory properties of the tested samples were evaluated (at 5±1°C) after 30 days of storage. Dried lemon peel extract (DLPE) recorded high radical scavenging activity, total phenolic and flavonoids contents compared to fresh lemon peel extract (FLPE). Dried lemon peel extract has potent, also, antiproliferative effects with CC50 = 61.2 µg/ml which provided a promising approach to its safety. DLPE possessed a great antimicrobial activity against all tested microorganisms. Meanwhile, total solids, ash, fat and protein values were slightly affected by extracts addition in Labneh samples. Antioxidant activity was increased with increasing the concentration of lemon peel extracts. Control sample recorded the highest values of hardness, while cohesiveness, gumminess and chewiness values were gradually decreased with higher concentrations of lemon peel extracts till the end of storage period. Total bacterial counts of Labneh samples were increased, reached the maximum after 7 days of storage. Psychrophilic bacteria, Yeasts & moulds and coliform bacteria were not detected in processed Labneh up to 3 weeks of storage period. The addition of lemon peel water extracts (fresh or dried up to 4%) improved Labneh flavor, body & texture and appearance at 5±1°C for 30 days of storage.

Keywords*:* Lemon peel extracts, Antimicrobial, Phenolic compounds, Cytotoxcicity, Sensory evaluation.

INTRODUCTION

Citrus lemons contain large amounts of vitamin C, fibre, phenolic, and flavonoid compounds. It is affordable, has antioxidant effects, and is widely accessible in a variety of countries around the world (Dosoky and Setzer, 2018; Mahmoud, *et al.,* 2019). Peels are the main citrus byproducts produced during processing, accounting for between 50 and 65 % of the fruit weight. They are discarded and are thought to represent a significant environmental burden (Nayak, *et al.,* 2015)**.** Peels from citrus fruits are a rich source of flavonoids and many flavones, which are relatively rare in other plants. Polyphenols from citrus peels have beneficial benefits on people that protect their health. It is a revolutionary technique for boosting pharmacological kinetic potential and bioavailability in an effort to minimize disease burden and prolong life. Citrus peels are a less expensive source of antioxidants and potentially bioactive substances because flavonoids like naringenin and hesperidin have few side effects and are generally considered safe for intake (Imran, *et al*., 2020).

The lemon peel extract has a high potential antimicrobial role against the activities of *Escherchia coli* (Henderson, *et al.,* 2018). Citrus flavonoids have a wide spectrum of biological activity included antibacterial, antifungal, antidiabetic, anticancer and antiviral activities. Peel extracts' antimicrobial effectiveness is directly related to their constituents (Hindi and Chabuck, 2013). Aqueous extracts of citrus peel and juice from fresh, dried, and sweet lemon demonstrated a range of inhibitory effects when used as antimicrobial agents against 6 Gram-positive, 8 Gram-negative, and 1 yeast isolate (Hassan, 2017). Some of the compounds that have been found to inhibit some hazardous bacteria and fungus (*Staphylococcus aureus, Salmonella enteritidis,* and *Listeria monocytogenes*) include catechins, oleuropein, ferulic acid, ellagic acid, and coumaric acid (Shan, et al., 2011). According to Pandey, *et al.* (2011), the peel of C. limon contains tannins, reducing sugars, and flavonoids but lacks saponins and phlobatannins. Concentrated yoghurt (Labneh), which plays a vital part in family nutrition, is a widely accepted fermented milk product, especially in the Middle East (Abd El-Salam, *et al.,* 2011). The characteristics of labneh include a cream or white hue, a soft and silky texture, good flavour and spreadability, and a mild acidity. Additionally, because it

ferments into lactic acid rather than lactose, Labneh has a lower lactose concentration, making it more suitable for use by those who are lactose intolerant. These factors, along with its perceived nutritional benefits and long shelf life, have contributed to the product's growing economic importance (Nsabimana, *et al.,* 2005). Numerous herbs and spices in various forms (such as powder, fresh, extract, essential oils, etc.) have been added to some dairy products to broaden the product variety of currently available dairy-based foods. (El-Sayed and Youssef, 2019). To increase Labneh's functionality and shelf life, papaya seeds extract was used (Mohamed, *et al.,* 2016).

In labneh, aqueous extracts of licorice, oregano, marjoram, and sage were added as flavour enhancers and antimicrobials (Al-Turki, *et al.,* 2008). In order to improve its antioxidant qualities, functional labneh was prepared with varying concentrations of natural antioxidant from guava leaf water extract (El-Gazzar, *et al.,* 2018).Sensory and textural properties of food products have axial roles and encourage companies to manufacture and modify new types of dairy products to stratify customer demand (Atamian, *et al.,* 2014). The flavoured milk samples that contained 15 percent Gaz-angubin and 0.075 percent bitter orange peel extract claimed to have the highest overall acceptability score, the highest percentage of inhibition of free radicals, the lowest total microbial count, and the lowest total polyphenol content (TPC). These samples also had the lowest total microbial count (TMC) (Jalilzadeh-Afshari and Fadaei, 2021).

The objective of this work is to evaluate the antioxidant, phenolic compounds, cytotoxicity and antimicrobial activities of fresh and dried of water lemon peel extracts and the effect of this extracts on the physicochemical, microbiological, texture and sensory properties of the stored Labneh at 5±1°C for 30 days.

MATERIALS AND METHODS

MATERIALS:

A mixture of fresh cow and buffalo milk at a ratio of 1:1 (Total solids: 15.3% and fat: 3.5%) was obtained from the processing unit belonging to Dairy Dept. Fac. Agric., Cairo Univ., Giza, while the traditional yoghurt starter culture (YSC) containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii sub sp. bulgaricus* (DVS) from Misr Food Additives Company (MIFAD) as a product of Ch. Hansen Lab, Denmark, Egypt.

Fresh Baladi lemon fruits *(Citrus lemon*) were obtained from Horticulture Research Institute, Agriculture Research Center, Giza.

Folin-Ciocalteu reagent, methanol, ethanol purchased from E. Merck. Quercetin, gallic acid, 2,2-bipyridyl, and 2,2-diphenyl-1-picrylhydrazyl were purchased from Sigma Chemical Co. (St. Louis, Mo) were purchased from El-Nassr Pharmaceutical Chemical Co., Egypt).

Gram positive bacteria (*Bacillus cerues, Staphylococcus aureus, Listeria monocytogen, Bacillus subtilis* RCMB015 (1) NRRL B-543), Gram negative bacteria (*Escherichia coli* (RCMB010052), *Salmonella typhimurium, Pseudomonas aeruginosa* and yeast & fungi (*Candida albicans* RCMB 005003 (1), *Aspergilus fumigatus* (RCMB 002008) were obtained from The Regional Center for Mycology and Biotechnology, Cairo, Egypt.

METHODS:

Preparation of Lemon Peels (LP):

Lemon fruits were washed by distilled water and squeezing the juice to obtain the residue of lemon peels (flavedo and albedo) and cutting it by a knife to get small parts. The peels were divided in two parts: the first was used as fresh, while the second was air dried in a ventilated oven at 40°C for 48 h and was grinding in a kitchen grinder to obtained a fine powder and passed through a 24-mesh sieve according to Van Acker, *et al*., (2011).

Preparation of lemon peel extracts (LPEs):

The fresh (76.74% moisture) or dried lemon (5.7% moisture) peels (50 g) were put (to be extracted) in 250 mL of distilled water in a shaker (Lab-Line L.E.D. Orbit Shaker Model 3518) at room temp. for 48 h. These extracts were filtered through a perlon filter cloth. Then, the extracts were concentrated in a rotary evaporator (Stuart Rotary Evaporator Model RE300) at 40±1ºC and were transferred to a sterile tube in the refrigerator for later usage as concentrated extracts (1, 2 and 4%).

Manufacture of Labneh:

Mixed milk was heat treated at 85ºC for15 min., then cooled in a water bath at 45ºC, inoculated with activated yoghurt starter culture *(Streptococcus thermophilus* and *Lactobacillus delbrueckii sub sp. bulgaricus)* at 2% and incubated at 42ºC for 4h or until the pH reached 4.6. The resultant coagulant was mixed and put into cheese cloth bags, which were hung in the refrigerator at 5±1ºC and overnight drained. Then 1.0% of NaCl was added and mixed well and equally divided into seven batches. The first batch is with no fresh or dried of lemon peel extracts served addition as a control. Fresh lemon peel extract (FLPE) were added to three portions at a ratio of 1, 2, and 4% (F1, F2, and F4), while the last three portions were prepered with dried lemon peel extract (DLPE) at ratios at 1, 2, and 4% (P1, P2 and P4), respectively. Each treatment was well mixed and filled into 50 gm plastic containers and stored at 5º±1C for 30 days. The Labneh treatments were taken at day 1 and after storage at 30 days for analysis (Tamime and Robinson, 1999).

Chemical analysis of lemon peel extracts and Labneh:

Moisture contents in fresh and dried lemon peels were determined, Ash contents, minerals, pH values and the titratable acidity (as citric acid %) were determined in fresh and dried lemon peel extracts. Fresh and 30 days stored Labneh samples were chemically analyzed for total solids%, ash, fat, total protein, titratable acidity (as lactic acid %) and pH (Hanna 8417). Minerals (K, Ca, Na, and Fe) were determined by Atomic Absorption spectrophotometry (Varian 100 & 200) in lemon peel extracts and Labneh. All of these tests were determined according to AOAC (2012).

Total phenols of the lemon peel extracts (FLPE and DLPE)) were measured following Folin-Ciocalteu (FC) reagent method (Yoo, *et al.,* 2008). The results were recorded as mg gallic acid equivalent (GAE)/ g.

The extracts' total flavonoid concentration was measured using a colorimetric technique, as done by (Hogan, et al., 2009). In terms of mg catechin (CA)/g, the results for total flavonoids were presented.

HPLC analysis of polyphenols and flavonoids fractions:

The polyphenols fractions were estimated using HPLC Agilent 1200 series equipped with a C18 column reverse phase (Zorbax ODS 5 pm, 4.6 x 250 mm) maintained at 35º C, auto sampler, solvent degasser, ultraviolet (UV) detector and a quarter HP pump (series 1050). All chromatograms were recorded at 330 nm for flavonoids and 280 nm for estimated phenolic acids. By comparing peak areas with outside standards, all components were discovered and measured (Schieber, et al., 2001).

Antioxidant assay:

An aliquot of the lemon peel extracts (FLPE and DLPE) and Labneh (at fresh and after 30 day of cold storage, 5± 1° C) has been used in the DPPH assay according to Li, *et al.*, (2009).

Antimicrobial activity of lemon peel extracts:

Agar well diffusion method was used to test antimicrobial action of lemon peel extracts (FLPE and DLPE) versus Gram Positive (*Bacillus cerues, Staphylococcus aureus, Listeria monocytogen, Bacillus subtilis* RCMB015 (1) NRRL B-543), Gram negative (*E. coli* (RCMB010052), *Salmonella typhimurium, Pseudomonas aeruginosa*, yeast and fungi (*Candida albicans* RCMB 005003 (1), *Aspergilus fumigatus* (RCMB 002008) test organisms. The sets were compared with standard antibiotics **(**Gentamycin and ketoconoazole). About 20 mL of media was placed into sterile plates (9 cm) and allowed to harden before cutting 5mm diameter holes in the agar with a sterile cork borer (plates were in triplicate sets for each plant extract). The plates were dried for 30 minutes. 50, 75, and 100 µl of each concentrated solution were poured into the holes. Plates were placed in a cooled refrigerator at 4°C for one hour to allow for diffusion; the inhibition zones were measured in millimetres at the end of the incubation period (Yosri, et al., 2020).

Cytotoxic activity of Lemon peel extracts:

We gratefully acknowledge The Regional Center for Mycology and Biotechnology at Al Azhar University for their assistance in obtaining normal Vero cells. Cells were cultured under standard culture conditions in Dulbecco's modified Eagle's medium, which was augmented with heat-inactivated foetal bovine serum (10 percent), L-glutamine (1 percent), HEPES buffer, and gentamycin at a concentration of 50 micrograms per millilitre (g/ml). In preparation for further research, the cells were kept alive at 37 degrees Celsius in a humidified environment containing 5 percent carbon dioxide. After being pretreated with fresh and dried extracts of lemon peel, the cells were then allowed to incubate in DMSO. Crystal violet was used to colour the cells after the media were aspirated. After the stain was eliminated and glacial acetic acid at a concentration of thirty percent was applied to each well, the absorbance of the plates was determined by measuring it at 490 nm (Gomha, *et al.,* 2015).

When compared to untreated controls, the concentration of extract that caused a fifty percent decrease in cell viability was determined to be the fifty percent cytotoxic concentration (also abbreviated as CC50).

Microbiological examination of Labneh:

Fresh and stored Labneh samples (1,2,3 and 4 weeks), containing of lemon peel extracts (FLPE and DLPE) were tested for enumeration for total bacterial and psychrotrophic bacterial counts, however, *Salmonella typhimurium, Staphylococcus aureus* and *Listeria monocytogen* were enumerated by spread plate method. Yeasts and moulds were counted on potato dextrose agar (Şahan, *et al.,* 2004).

Textural properties:

Texture profile analysis (TPA) such as hardness, springiness, cohesiveness, gumminess, adhesiveness and chewiness of Labneh was measured with an Instron Universal Testing Machine (Model 4302, Instron Corporation, Canton M.A, England) according to Bourne, (1978).

Sensory evaluation:

The labneh's organoleptic qualities were assessed by a trained panel of 10 Food Technology Research Institute, Agric, employees. Res. Giza Cent. Keating and Rand-white estimated the organoleptic qualities, which

included flavour (50 points), body and texture (40 points), and appearance (10 points) when the food was fresh and after 30 days in the refrigerator (5±1°C) (1990).

Statistical Analysis:

Statistical analysis of the obtained data was performed according to SAS (1990) Institute using liner Model (GLM). All data from the current study is expressed as mean values ±SD. Duncan's multiple rang was used to separate among means of three replicates of the data, unless otherwise stated.

RESULTS

Bioactive compounds, chemical and physical properties of lemon peel extracts (LPEs):

Lemon peels contain high content of phytochemicals and have the greater antimicrobial and antioxidant activities. **Table (1)** summarizes the proximate bioactive compounds, chemical and physical properties of fresh and dried lemon peel extracts. From the results, it could be noticed that the dried lemon peel extract (DLPE) showed higher values of all the tested parameters except pH value and titratable acidity content than fresh lemon peel extract (FLPE). The dried lemon peel extract recorded the highest content of total phenolic and flavonoid (7.49 and 4.55 mg/g, respectively) compared to fresh lemon peel extract which were 4.52 and 2.83 mg/g, respectively. The radical-scavenging activities recorded higher values for DLPE (86.95%) compared to the fresh lemon peels extract (69.58%). The high phenolic and flavonoid contents of peels cause a high antioxidant activity.

Potassium (K) recorded the highest mineral content in DLPE (18200 ppm), followed by calcium (8200 ppm) and the lowest mineral values in DLPE were iron (110.58 ppm). Sodium (Na) plays an important role in the transport of metabolites.

The fresh lemon peel extract recorded a slightly higher content of titratable acidity (0.75%) than dried lemon peel extract (0.61 %). The acidity (as citric acid) of FLPE (0.75%) is slightly higher than DLPE (0.61%).

Table 1. Bioactive compounds, chemical and physical properties of lemon peel extracts (on dry weight)

Ingredients	Fresh lemon extract	Dried lemon extract
Total Phenolic (mg/g)	$4.52^{b} \pm 0.03$	$7.49^{a} \pm 0.04$
Total flavonoids (mg/g)	$2.83^{b} \pm 0.03$	$4.55^{a} \pm 0.03$
Antioxidants activity (%)	$69.58^{b} \pm 0.04$	$86.95^{a} \pm 0.04$
Ash (%)	$1.49^{b} \pm 0.03$	$1.57^{a} \pm 0.02$
Minerals (ppm)		
K	$15600^{b} \pm 10.00$	$18200^{a} \pm 13.00$
Ca	$6250^{b} \pm 5.00$	$8200^{a} \pm 7.00$
Na	$5630^{b} \pm 3.00$	$7240^{a} \pm 4.00$
Fe	$25.075^{b} \pm 0.02$	$110.58^{a} \pm 0.04$
pH value	$6.21^{a} \pm 0.02$	$6.11^{b} \pm 0.01$
Titritable acidity% (as citric acid)	$0.75^{a} \pm 0.02$	$0.61^{b} \pm 0.03$

Data are means ±SD (n=3).

Different superscript letters are significantly different at 5% level.

Phenolic and flavonoid compounds fraction content in lemon peel extracts(LPEs):

The fraction contents of phenolic and flavonoid compounds for fresh and dried lemon peel extracts are presented in **Table (2)**. Pyro catechol was the major phenolic compound of both fresh and dried lemon peels extract and the dried lemon peels extract recorded the highest content (195.64 µg/ml) compared to the fresh lemon peels extract which was 177.04 µg/ml. As well as, DLPE had a higher content of Gallic, Coumaric and Chlorogenic acids which recorded 125.73, 99.44 and 94.21 µg/ml, respectively compared to FLPE (60.03, 15.17 and 65.77 µg/ml, respectively). On the other hand, the lowest phenolic concentration was 0.60 and 1.94 µg/ml for Cinnamic acid content in fresh and dried lemon peels extract.

Consistent with the data on flavonoid components, Hesperidin was the highest flavonoid component in the dried and fresh lemon peel extracts (1563.45 and 865.12 µg/ml, respectively). Meanwhile, the lowest one was Taxifolin which recorded 2.77 and 19.49 µg/ml, for fresh and dried lemon extract, respectively. These results may be due to the lower moisture content of dried lemon peel (5.7%) which concentrated the ingredients. The DLPE recorded higher values of phenolic and flavonoid compounds compared to FLPE.

Table 2. Phenolic and flavonoid compounds fraction of fresh and dried lemon peel extracts

Phenolic components Conc. (µg/ml)		
Component	Fresh extract	Dried extract
Gallic acid	60.03	125.73
Chlorogenic acid	65.77	94.21
Methyl gallate	1.33	2.08
Caffeic acid	13.96	2.57
Syringic acid	7.26	22.93
Pyro catechol	177.04	195.64
Coumaric acid	15.17	99.44
Catechein	19.20	55.10
Ferulic acid	16.27	35.03
Cinnamic acid	0.60	1.94
Pyrogallol	18.05	47.79
Flavonoid components Conc. (µg/ml)		
Component	Fresh extract	Dried extract
Hesperidin	865.12	1563.45
Rutin	10.02	24.48
Quercetin	297.3	657.4
Naringenin	38.69	99.11
Taxifolin	2.77	19.49
Kaempferol	15.92	46.14
Quercetrin	92.78	188.86

***In vitro* cytotoxic activity of lemon peel extracts (LPEs)**

In the present results **Table (3)**. the cytotoxic activity against mammalian cells from African Green Monkey Kidney (Vero) cells was detected for fresh and dried lemon peel extracts. The DLPE had more potent antiproliferative effects and higher cytotoxicity than FLPE with CC_{50} (cell cytotoxic concentration) 29.3 ± 0.7 and 61.2± 2.9 µg/ml, respectively, which provided a promising approach to its safety as depicted. The current results reveled that citrus peel extracts increased the cell viability and antimutagenic activity (simultaneous treatment).

Table 3. Cytotoxicity of lemon peel extracts on Vero cells (normal cell)

	Fresh extract			Dried extract		
Conc. (µg/ml)	Viability %	Inhibitory %	(±) SD	Viability %	Inhibitory %	(±) SD
0	100	0.0	0.0	100	0.0	0.50
3.9	100	0.0	0.0	90. 2	9.38	0.64
7.8	100	0.0	0.0	82.39	17.61	0.87
15.6	98.03	1.97	0.53	69.44	30.56	1.73
31.25	89.41	10.59	0.97	47.36	52.64	2.98
62.5	67.28	32.72	2.84	32.89	67.11	2.72
125	41.37	58.63	2.95	15.68	84.32	1.49
250	26.19	73.81	1.73	7.92	92.08	0.67
500	11.28	88.72	0.64	3.43	96.57	0.00
*CC_{50}	61.2 ± 2.9 µg/ml.			29.3 ± 0.7 µg/ml		

*CC_{50}: The extract concentration that reduced the cell viability by 50% when compared to untreated controls.

Antimicrobial activity of lemon peel extracts (LPEs)

Plant extracts are rich in antimicrobial compounds. Previous studies have shown that lemon peel extracts (LPEs) possessed a high antimicrobial activity against several foodborne pathogens. So, the present study was done against a variety of Gram-positive bacteria including *S. aureus*, *L. monocytogen*, *B. cerues, and B. subtilis* as well as *E. coli*, *P. aeruginosa*, and *S. typhimurium* as Gram-negative bacteria. Meanwhile, *C. albicans* and *A. fumigatus* were used as yeast and fungal strains compared to gentamycin and Ketoconoazole as commercial antibiotics. The inhibition zones (mm) of the lemon peel fresh and dried lemon peel extracts on selected microorganisms are given in **Table (4).**

The data of fresh and dried lemon peels extract indicated that lemon peels exhibited antibacterial activity against all tested microorganisms. The inhibition zones were gradually increased with raising the concentration of the extracts from 50, 75 to 100 µg/ml. The highest zones of inhibition (55.5, 51.0, and 49.5 mm, respectively) were observed in the dried lemon peels extract against *L. monocytogen, S. aureus, and B. cerues* as Gram-positive bacteria at 100 µg/ml compared to fresh lemon peels extract. While the highest antibacterial activity for DLPE against Gram-negative bacteria was observed in *E. coli* and *P. aeruginosa,* at 100 µg/ml. In this study, DLE exhibited the highest antimicrobial activity against *C. albicans* and *A. fumigatus* at 100 µg/ml (33.0 and 30.0 mm, respectively) compared to FLPE (18.5 and 19 mm, respectively). DLPE possessed a greater antimicrobial activity against all tested bacterial strains than control (gentamycin, and ketoconoazole) due to the presence of components such as ascorbic acid, flavonoids and phenolic compounds.

Table 4. Antimicrobial activity of lemon peel extracts

Concentration (µl/ml)	Fresh extract			Dried extract			Control (100µg/ml)
	50	75	100	50	75	100	
Diameter of zone of inhibition (mm)							
Pathogenic	Gram-positive						Gentamycin
Bacillus cerues	30	33.2	39.8	18	36	49.5	24
Staphylococcus aureus	28	35	37	-	30	51	24
Listeria monocytogen	31.5	38	42	25	43	55.5	25
Bacillus subtilis RCMB015 (1) NRRL B-543	-	10	20	-	12	22	21
Gram-negative							Ketoconoazole
E. coli (RCMB010052)	11	16	29.2	20	38	46	28
Salmonella typhimurium	14	15.8	19	-	12	28	35
Pseudomonas aeruginosa	31.5	38	42	25	32	40	
Yeast & fungi							Ketoconoazole
Candida albicans RCMB 005003 (1)	10	13	18.5	9	19	33	20
Aspergilus fumigatus (RCMB 002008)	9	16	19	8	12	30	12

Chemical composition of Labneh:

The chemical composition of control and Labneh samples with fresh and dried lemon peel extracts addition at different concentrations (1, 2 and 4%) and the effect of storage period for 30 days at 5±1ºC show in **Table (5)**. Data showed that the total solid contents slightly increased in control and all treatments at the end of storage period due to slight loss in moisture during storage. F4 and P4 samples recorded slightly increase in ash at fresh and at the end of storage compared to other treatments. No significant differences (p≤0.05) observed in Fat content of the different Labneh, either when fresh or at the end of storage. There were significant differences (p≥0.05) in total protein contents in Labneh samples compared to control at fresh and after storage period. This data may due to increase ratio of lemon peel extract added to Labneh samples. In the same Table, it could be noticed that a significant effect (p≥0.05) on the level of antioxidant activity of all samples with the raising of concentrations of lemon peel extracts either fresh or dried in comparison with the control sample.

Table 5. Chemical composition of Labneh as affected by lemon peel extracts addition (on dry weight)

Parameters	Storage period	Treatments						
		Control	F1	F2	F4	P1	P2	P4
TS	Fresh	24.43^{aB}±0.03	24.45^{aB}±0.03	24.44^{aB}±0.03	24.37^{bB}±0.02	24.45^{aB}±0.04	24.46^{aB}±0.02	24.35^{bB}±0.04
	30 days	24.56^{aA}±0.02	24.58^{aA}±0.03	24.58^{aA}±0.04	24.43^{bA}±0.04	24.58^{aA}±0.04	24.35^{aA}±0.02	24.46^{bA}±0.04
Ash	Fresh	1.08^{fB}±0.02	1.16^{eB}±0.04	1.21^{cdB}±0.03	1.35^{bB}±0.03	1.17^{deB}±0.01	1.25^{cB}±0.03	1.46^{aB}±0.02
	30 days	1.14^{eA}±0.02	1.25^{dA}±0.03	1.28^{dA}±0.02	1.44^{bA}±0.04	1.26^{dA}±0.02	1.36^{cA}±0.02	1.58^{aA}±0.04
Fat	Fresh	9.12^{aA}±0.01	9.11^{aA}±0.01	9.11^{aA}±0.01	9.12^{aA}±0.01	9.12^{aA}±0.01	9.12^{aA}±0.01	9.12^{aA}±0.01
	30 days	9.12^{abA}±0.01	9.12^{abA}±0.01	9.12^{abA}±0.01	9.12^{abA}±0.01	9.12^{abA}±0.01	9.13^{aA}±0.01	9.13^{aA}±0.01
Protein	Fresh	10.23^{cdB}±0.05	10.24^{cB}±0.03	10.39^{aB}±0.03	10.38^{aB}±0.04	10.17^{dB}±0.02	10.2^{bcB}±0.15	10.32^{bB}±0.03
	30 days	10.34^{cA}±0.03	10.39^{bcA}±0.02	10.47^{aA}±0.04	10.50^{aA}±0.03	10.26^{dA}±0.03	10.34^{cA}±0.03	10.41^{bA}±0.02
Antioxidant activity	Fresh	75.68^{dB}±0.09	75.78^{dB}±0.01	76.48^{cB}±0.07	77.28^{bB}±0.04	76.88^{cB}±0.06	77.58^{bB}±0.02	78.68^{aB}±0.04
	30 days	92.95^{fA}±0.06	93.15^{eA}±0.06	96.85^{dA}±0.06	98.35^{bA}±0.06	96.15^{dA}±0.06	97.25^{cA}±0.08	99.95^{aA}±0.03

Means (±SD) with the same small letter among the samples at the same time are not significantly different (P ≤ 0.05), however, means with the same capital letter between fresh and stored samples for each treatment are not significantly different (P ≤0.05).

F1: labneh + 1% fresh lemon peel extract P1: labneh + 1% lemon peel dried extract

F2: labneh + 2% fresh lemon peel extract P2: labneh + 2% lemon peel dried extract

F4: labneh + 4% fresh lemon peel extract P4: labneh + 4% lemon peel dried extract

pH values and titratable acidity (%):

The changes in the pH values and titratable acidity (%) in Labneh after 30 days of storage at 5±1ºC affected by the addition of peel extracts (fresh or dried) were shown in **Fig (1)**. The pH values for all samples decreased at the end of storage period. F1 recorded the highest pH value (4.44) and P4 sample was the lowest value (4.37) among all fresh samples. In addition, after 30 days of storage periods, the control sample showed the highest pH value (4.28), while F4 sample recorded the lowest value (4.19). Whereas, the titratable acidity (%) for all tested samples increased with increasing storage period. The differences in pH values among different Labneh samples were probably due to the lemon extracts used.

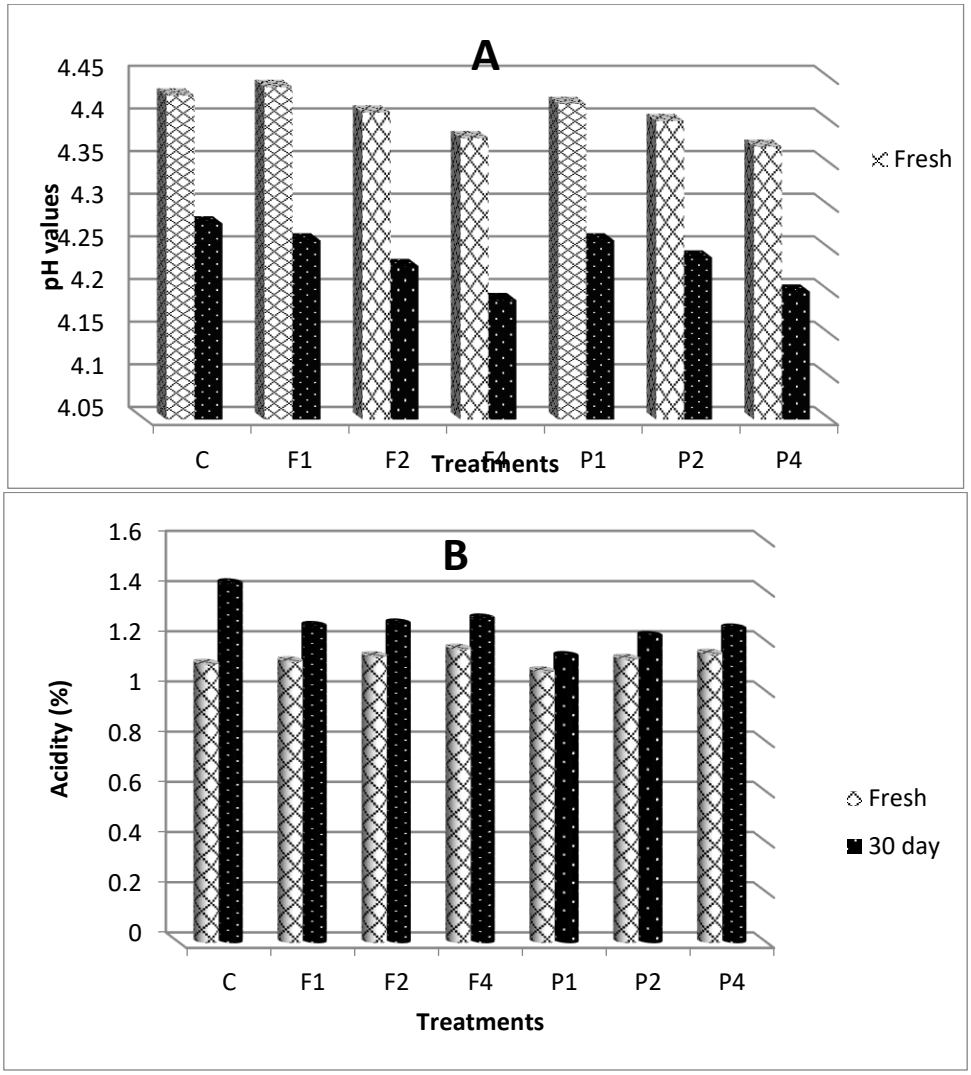

Fig 1. Changes in the pH values and acidity % (as dry weight basis) in Labneh of fresh and after 30 days of storage

Rheological properties of Labneh:

Rheological properties of fresh and stored Labneh samples as affected by lemon peel extracts (LPEs) are shown in **Table (6)**. The control sample recorded the highest significant hardness values followed by F1 and P1 (2.60, 2.57 and 2.53 N, respectively). Hardness value was low in all tested samples by addition of fresh or dried lemon peel extracts compared to control, either fresh or after 30 days of storage period at 5±1ºC.

Cohesiveness, gumminess and chewiness values followed the same trend of hardness were gradually decreased with higher concentrations of water lemon peel extracts, either fresh or dried at the end of storage period.

In all treatments, adhesiveness and springiness compared to control in fresh Labenh were gradually significantly increased with the addition of higher concentrations of LPEs (P≥0.05) and till the end of storage period at 5±1ºC.

Data in **Table (6)** show that P4 and P2 samples containing 4 and 2% of DLPE had the highest adhesiveness values, while the control sample was relatively low at fresh and at 30 days of storage (0.35 and 0.43mJ). The panellists are told that springiness is the sample's ability to bounce back after numerous bites. The values of this characteristic that were acquired at Labneh were impacted by LPEs as a result of various additions, ranging from 3.21 (P1 at the lowest value in fresh) to 5.16 mm (F4 at the maximum value after 30 days), which suggests that the springiness demonstrated the opposite tendency of hardness.

Table 6. Rheological properties of Labneh as affected by lemon peel extracts addition

Parameters	Storage period	Control	F1	F2	F4	P1	P2	P4
Hardness (N)	Fresh	2.60^{aA}±0.17	2.57^{aA}±0.21	2.37^{bcA}±0.21	1.93^{dA}±0.25	2.53^{abA}±0.25	2.43^{bA}±0.21	2.17^{cA}±0.11
	30 days	1.97^{aB}±0.21	1.70^{bB}±0.26	1.60^{bcB}±0.17	1.23^{cB}±0.25	1.78^{bB}±0.44	1.57^{bcB}±0.15	1.30^{cB}±0.2
Adhesiveness (mJ)	Fresh	0.35^{cB}±0.10	0.41^{bcB}±0.11	0.43^{bcB}±0.03	0.45^{bcB}±0.13	0.52^{abB}±0.09	0.56^{abB}±0.07	0.65^{aB}±0.08
	30 days	0.43^{cA}±0.10	0.45^{cA}±0.09	0.48^{bcA}±0.10	0.53^{bcA}±0.07	0.63^{abA}±0.08	0.65^{abA}±0.12	0.78^{aA}±0.11
Cohesivenees (Ratio)	Fresh	0.96^{aA}±0.03	0.82^{abA}±0.04	0.80^{abA}±0.15	0.72^{bcA}±0.16	0.94^{aA}±0.11	0.81^{abA}±0.07	0.79^{abA}±0.08
	30 days	0.91^{aB}±0.06	0.70^{bB}±0.14	0.73^{bB}±0.05	0.67^{bcB}±0.09	0.87^{abB}±0.08	0.62^{bcB}±0.08	0.51^{cB}±0.06
Springness (mm)	Fresh	3.58^{bcB}±0.02	3.77^{abB}±0.11	3.87^{abB}±0.20	3.95^{aB}±0.20	3.21^{eB}±0.06	3.31^{deB}±0.04	3.49^{cdB}±0.13
	30 days	4.60^{cA}±0.04	5.01^{abA}±0.48	5.08^{abA}±0.18	5.16^{aA}±0.05	4.55^{cA}±0.06	4.71^{bcA}±0.07	4.84^{bA}±0.24
Gummness (N)	Fresh	2.53^{bcA}±0.35	1.97^{cdA}±0.45	1.83^{cdA}±0.35	1.77^{dA}±0.15	3.17^{abA}±0.25	2.87^{abA}±0.50	1.93^{cdA}±0.35
	30 days	1.43^{bcB}±0.35	1.23^{cB}±0.15	0.97^{cB}±0.42	1.03^{cB}±0.21	2.00^{abB}±0.53	1.90^{abB}±0.3	0.97^{cB}±0.42
Chewieness (mJ)	Fresh	7.38^{cA}±0.68	7.18^{cdA}±0.65	6.65^{dA}±1.22	6.13^{dA}±0.77	9.88^{aA}±0.29	9.50^{abA}±1.87	8.87^{bA}±0.47
	30 days	6.93^{cB}±0.36	6.86^{cB}±0.16	6.01^{cdB}±0.43	5.42^{dB}±0.81	9.11^{aB}±0.69	8.41^{bB}±0.69	8.28^{bcB}±0.70

Means (±SD) with the same small letter among the samples at the same time are not significantly different (P ≤ 0.05), however, means with the same capital letter between fresh and stored samples for each treatment are not significantly different (P ≤0.05).

F1: labneh + 1% fresh lemon peel extract P1: labneh + 1% lemon peel dried extract

F2: labneh + 2% fresh lemon peel extract P2: labneh + 2% lemon peel dried extract

F4: labneh + 4% fresh lemon peel extract P4: labneh + 4% lemon peel dried extract

Microbiological properties of Labneh:

The total bacterial count and psychrotrophic bacteria, as well as yeasts & moulds were examined for fresh and stored Labneh samples after 30 days at 5±1°C. The results in **Fig. (2)** showed that initial total bacterial count (TBC) in fresh Labneh samples was about 6.88-6.82 log CFU/g and then continuously reduced to reach 6.77, 6.74, 6.66, 6.76, 6.72 and 6.61 log CFU/g using amount 1, 2 and 4% of fresh and dried lemon peel extracts, respectively at the end of storage in compared with control (6.81 log CFU/g). These findings may be due to the antibacterial effect of lemon peel extracts on Labneh during storage. It could be noticed, in the current study, that the lowest microbial growth where upon using fresh lemon peel extract (F4) as well as dried lemon peel extract (P4). Data represented the promising role of lemon peel extracts in regulating total bacterial count.

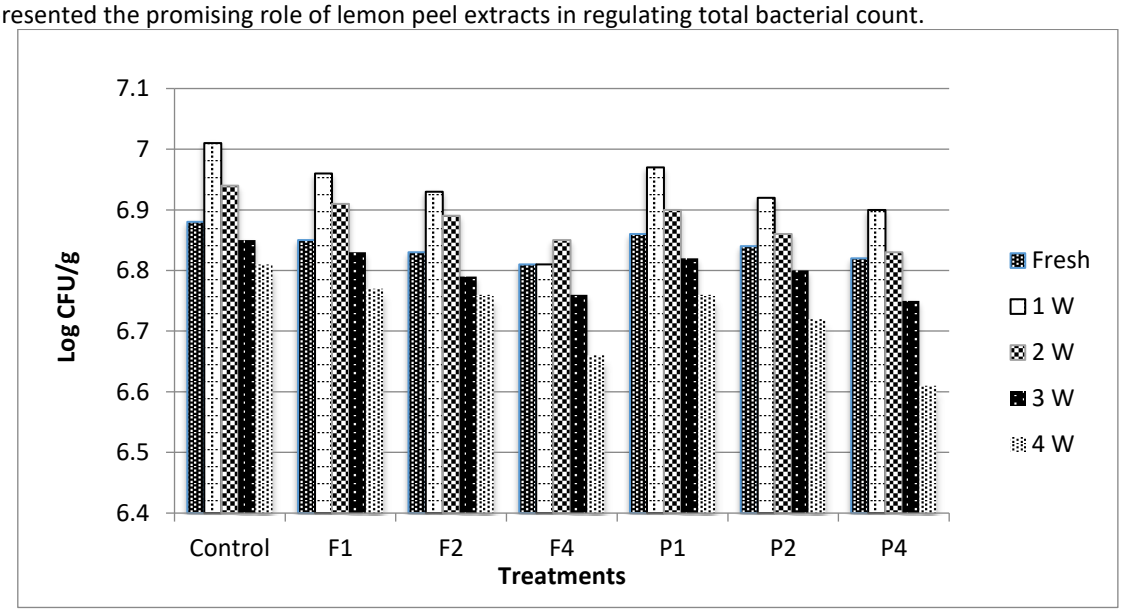

Fig 2. Total bacterial count (Log CFU/g) of Labneh as affected by lemon peel extracts addition

The current results revealed that the Labneh samples showed that no coliform bacteria, *S. typhimurium*, *P. aeruginosa*, *S. aureus* and *L. monocytogen* growth were detected, due to the good hygiene and effectiveness of the LPE. Colonies of psychrophilic bacteria were not be detected either in fresh or after storage of Labneh for 3 weeks in **Fig. 3A** and a minimal total count of psychrophilic bacteria could be detected at the 4th week of storage period. The viable psychrophilic bacterial counts were reached 1.96, 142, 1.20, 1.10 and 0.80 log CFU/g for control, F1, F2, P1and P2, respectively at the end of the storage period. Yeast and mould counts are considered indicative of the quality and the shelf life of Labneh. Data in **Fig.3B** showed that the yeasts and moulds were not detected in Labneh containing aqueous extracts of lemon peel until the 15 days but only after 21 and 30 days of storage (1.6, 3.21; 1.48, 3.04; 1.13, 278 and1.16, 1.32 CFU/g) were recorded in control, F1, F2 and P1, respectively.

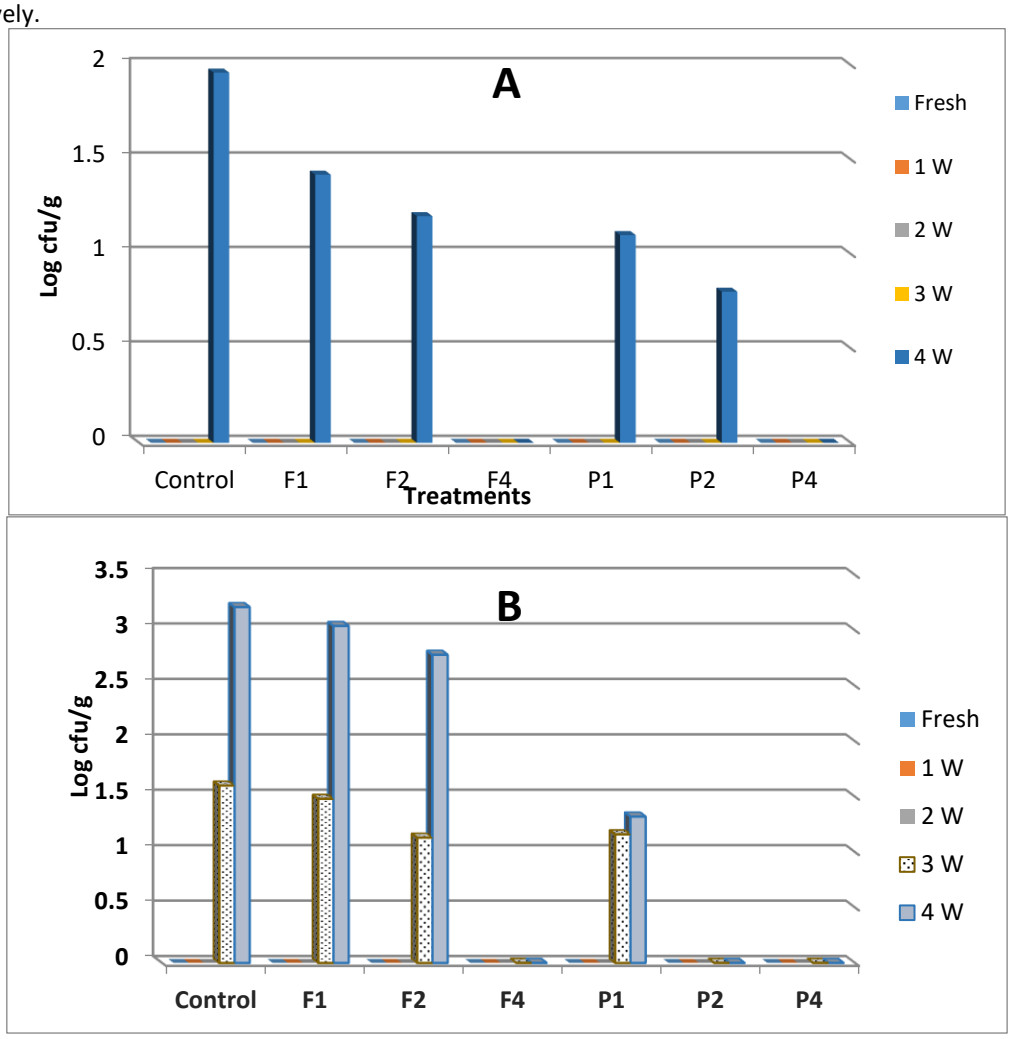

Fig 3. Psychrotrophic bacteria (A) and Yeast & Mould (B) counts (Log CFU/g) of Labneh as affected by lemon peel extracts addition

Sensory evaluation of Labneh:

The effect of LPEs on the sensorial properties of Labneh is shown in **Table (7).** The addition of lemon peel extracts (fresh and dried) to Labneh had a significant (P≥ 0.05) impact on flavor, body & texture and appearance characteristics at fresh and the end of storage period at 5±1ºC. Labneh flavor with added 4% of FLE (F4) and DLE (P4) showed the highest score compared to the control trials at fresh (47.80 and 47.91), and after 30 days of storage (45.27 and 45.63).

Data showed that control, F1 and P1 treatments recorded the lowest values in body& texture at fresh and after 30 days, while F4 and P4 recorded a high score (34.67 and 35.60)at the end of storage. No significant effect ($p \leq 0.05$) on appearance at fresh manufacturing between control, and samples treated with either FLE or DLE and at the end of storage.

Table (7): Sensory evaluation of Labneh as affected by lemon peel extracts addition

Parameters	Storage period	Control	F1	F2	F4	P1	P2	P4
Flavor (50)	Fresh	46.40^{cA} ±0.10	46.53^{cA}±0.18	47.31^{bA}±0.15	47.80^{aA}±1.00	46.32^{cA}±0.08	47.67^{abA}±0.53	47.91^{aA}±0.15
	30 days	43.67^{cB}±0.18	44.33^{bcB}±0.18	44.67^{bB}±0.21	45.2^{aB}±0.26	43.8^{dB}±0.14	44.77^{bB}±0.13	45.63^{aB}±0.23
Body& texture(40)	Fresh	35.50^{cA}±0.11	35.83^{bcA}±0.15	36.40^{bA}±1.00	36.6^{bA}±0.18	35.4^{cA}±0.23	36.33^{bA}±0.24	37.47^{aA}±0.17
	30 days	32.00^{dB}±0.20	33.22^{dB}±0.30	34.33^{cB}±0.23	34.6^{bB}±0.23	33.5^{dB}±0.10	34.13^{cB}±0.23	35.60^{aB}±0.10
Appearance (10)	Fresh	8.50^{aA}±0.20	8.50^{aA}±0.19	8.50^{aA}±0.08	8.5^{aA}±0.05	8.5^{aA}±0.16	8.5^{aA}±0.19	8.50^{aA}±0.05
	30 days	8.00^{bB}±0.13	8.50^{aA}±0.15	8.50^{aA}±0.03	8.50^{aA}±0.05	8.5^{aA}±0.12	8.5^{aA}±0.05	8.50^{aA}±0.10

Means (±SD) with the same small letter among the samples at the same time are not significantly different (P ≤ 0.05), however, means with the same capital letter between fresh and stored samples for each treatment are not significantly different (P ≤0.05).

F1: labneh + 1% fresh lemon peel extract P1: labneh + 1% lemon peel dried extract

F2: labneh + 2% fresh lemon peel extract P2: labneh + 2% lemon peel dried extract

F4: labneh + 4% fresh lemon peel extract P4: labneh + 4% lemon peel dried extract

DISCUSSION

To assess the capacity of diverse samples to scavenge free radicals, the DPPH radical scavenging model is frequently utilised (Lee, et al., 2003). Food products made from plant sources frequently contain phenols and polyphenolic chemicals, such as flavonoids, which have been demonstrated to have strong antioxidant properties (Van Acker, et al., 1996). In addition, Diab, (2016) recorded that lemon peels had the highest total phenolic compounds (TPC) and possessed the strongest antioxidant activity as indicated by the highest DPPH radical scavenging.

The ratio of Na /K in any food is an important factor in hypertension arteriosclerosis prevention, with K depressing and Na enhancing blood pressure as was recorded by Abdelwahab and Abouelyazeed, (2018). Data show pH values of FLPE and DLPE with the same line reported by Irkin, *et al.* (2015). Food products made from plant sources commonly contain phenols and polyphenolic substances like flavonoids, which are greatly variable in different citrus fruit segments (Van Acker, et al., 1996, Mehmood, *et al.,* 2018). Data in the same line with Manthey and Grohman, (1996) who mentioned that Hesperidin accumulates in citrus peel in considerably high amounts.

The majority of chemotherapy drugs are not only cytotoxic to the cancer cells but also toxic to healthy cells and have some immune-suppressive side effects. As a result, the discovery of novel compounds that possess not only cytotoxic activity against cancer cells but are also non-toxic to healthy cells and modulating the immune response has become an important goal of researches in the biomedical sciences (Sak, 2012). Charoensinphon, *et al.,* (2013) who tested citrus peel effects on various cell lines to assure its safety on normal cells and found that bioactive compounds of lemon peel mediated G2/M cell cycle. As a result of the citrus pomace water (CPW) extract's protective actions against induced apoptosis, the number of apoptotic bodies was noticeably decreased in the cells treated with CPW. Additionally, the percentages of cells that underwent apoptosis matched those reported by Selim *et al.* (2019), who found that citrus peel extracts had antitumor efficacy against various cancers such colon, liver, and breast cancers. These characteristics might be brought about by the presence of phenolic chemicals, such as flavonoids and coumarone compounds, which are crucial in causing their anticancer effects to be exerted through the antimutagenic pathway. According to earlier studies, citrus peels are potent antioxidants that defend against a variety of mutagens by removing reactive oxygen species (ROS, pro-oxidants) produced by mutagens and shield humans from diseases caused by mutation (Diab, *et al.,* 2015). The immune stimulation by plant extracts is believed to be a promising way to prevent and cure disease (Kumar *et al.,* 2012). Data agreement with Diab (2016) who recorded that lemon peel extracts possessed a lower effective concentration (EC_{50}= 42.97 g extract µg/ ml).

These results are in agreement with John *et al*., (2017). Henderson, *et al*., (2018) mentioned that antimicrobial activities of lemon peel extract indicated its efficiency after drying process due to inhibiting the bacterial specific enzymatic activity by disrupting the membrane, damage the lipids and proteins layers of microbes. Additionally, against Gram-positive bacteria (Bacillus spp.), Citrus reticulata peel extracts were discovered to be more effective than juice extracts (20.33±1.527). However, it did not exhibit a zone of inhibition when exposed to E. coli, *E. coli S.*ATCC 25922 and E. typhi and P. aeruginosa that Shakya, et al., recorded (2019). According to Mutlag and Hassan (2008), adding three different essential oils to labneh did not result in any noticeable differences in the TS or fat content. Additionally, they found that the total solid contents in Labneh ranged from 23 to 25 g/100 g in accordance with Tamime and Robinson's (2007) and Alolu and Ner's (2013)

findings. Results that are in line with those reported by Janati et al. (2012) who said that 9.42 percent of lemon peels contain protein.

The titratable acidity of Labneh samples, according to Tarakci et al. (2011), ranged from 1.26 to 1.32 percent. In a related experiment, Ztürk and Ner (1999) found that the biggest pH drop occurred after 20 days of refrigerator storage. This could be because of the increased bacterial metabolic activity brought on by lactose consumption, which led to an increase in lactic acid and galactose levels. The consumption of sugar and organic acids by bacteria may be to blame for the pH values dropping (Vahedi, et al., 2008). According to certain studies (Tarakçi and Kucukoner, 2003; Celik, et al., 2006), the titratable acidity of yoghurt rose with storage times. Regardless of how the samples changed during storage, all samples, after 20 days of cold storage, reach similar pH values and lactic acid concentrations of 0.80-1.50 percent (Celik and Bakirci, 2003; Kucukoner and Tarakci, 2004).

Nasser, *et al.,* (2017) found that Labenh treatments with the addition of peppermint aqueous extracts cause a decrease in hardness, cohesiveness, springiness, chewiness and gumminess compared to control in fresh samples. The main differences are concerned with the rheological properties of Labneh, which are the consequence of its higher total solids level (Özer and Robinson, 1999).

These results are in agreement with Sahan, *et al.,* (2004) who reported that the total aerobic bacterial counts decreased during the storage. Zaky, *et al.,*(2013) and Al- Mutlag and Hassan, (2008) reported that total viable bacterial counts (TVCs) increased in the treated samples with the addition of some essential oils and reached a maximum population after 7 days of storage then, they decreased till the end of storage. Dostálová, *et al.,* (2013) reported that the best inhibitory effects were noticed with the addition of thyme, peppermint and fennel, due to the highest concentration of active compounds in aqueous extract which inhibited the growth of microorganisms.

According to research by Min et al. (2014), low-fat, whole, and skimmed milk may benefit from using the acid hydrolysis extract from Citrus unshiu peel as a natural antibacterial. Irkin, et al. (2015) reported that citrus fruit juices and their peels can be used in the production of functional foods and probiotics, for sustaining and developing vitality in probiotic microorganisms, and for enriching products in terms of phenolic constituents. This finding is in line with the data. El-Sayed and El-Sayed both reported results with the same trend (2021). According to Hashemi, et al. (2016), citrus aurantium L. In traditional yoghurt, flowers (Bahanarang Extract (BE) as a source of antioxidant and antibacterial activity had significant influence on the development of harmful bacteria such as *Pseudomonas aeruginosa, Escherichia coli* O157:H7, *Bacillus cereus* and *Staphylococcus aureus.* In accordance with Mutlag and Hassan, (2008) who reported that natural plant extract had antifungal and antimicrobial activities, these results might be due to the antimicrobial effect of lemon peel extracts in treated Labneh. Burt, (2004) demonstrated that the presence of phenolic compounds, which have antibacterial capabilities, caused the use of particular essential oils in the manufacturing process to suppress microbial development. On the other hand, Hervert-Hernandez and Goni (2011) found that the phenolic compounds present can boost nutrient consumption and, as a result, stimulate bacterial growth.

All labans (Labneh) samples from mixed cultures, according to Béal and Chammas (2012), exhibit a complex scent composition that includes all of the aroma chemicals made by the two species *S. thyrophilus* and *L. Delbrueckii* species *bulgaricus*. According to Hashemi et al. (2016), employing *Citrus aurantium* L. Traditional yoghurt's quality and shelf life were improved by using flowers (Bahanarang Extract (BE)) as a source of antioxidant and antibacterial agents at concentrations ranging from 500 to 2000 ppm.

Each component of the food's visually perceptible structure—its appearance—possesses colour, translucency, gloss, and surface texture (roughness) characteristics. With time and processing, each of these qualities exhibits a characteristic behaviour that influences how we perceive the food in front of us overall (Caivano and del Pilar Buera, 2012). Nasser, *et al.* (2017) found that the highest score points were in Labneh treated with 1.5% aqueous extracts till the end of storage compared with control and the total scores of the sensory evaluation decreased gradually during storage. It can be concluded that the addition of water lemon peel extracts, either fresh or dried (up to 4%) improved the flavor, body & texture and appearance of produced Labneh and keep at 5±1°C for 30 days.

CONCLUSION

The current study demonstrated that the consumption of water lemon peel extracts, whether fresh or dried at concentrations of 2 and 4 percent with Labneh contains high antioxidant activities and takes out a wide range of activity against pathogenic bacteria, yeasts, and moulds while also exhibiting no cytotoxicity. Therefore, the addition of fresh and dried LPEs (*Citrus limon*) as a low-cost agricultural waste material can be employed to improve the rheological qualities and sensory attributes of Labneh, which can increase its shelf life to up to 30 days.

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تأثير استخدام مستخلصات قشر الليمون على القيمة البيولوجية للبنة أثناء التخزين

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يعتبر مستخلص قشر الليمون مصدر طبيعى لتحسين الوظائف البيولوجية والفسيولوجية. حيث تم دراسة كلا من مستخلص قشر الليمون المجفف والطازج وتقييم مضادات الأكسدة والمركبات الفينولية والسمية الخلوية والتاثير المضاد للميكروبات للمستخلصات المائية من قشر الليمون. وتم استخدام مستخلصات قشر الليمون الطازج والمجفف بتركيزات 1،2 و 4٪ لتحسين جودة اللبنة. وتم تقييم الخصائص الكيميائية والفيزيائية والريولوجية والميكروبيولوجية والحسية للعينات المختبرة (عند 5 ± 1 درجة مئوية) بعد 30 يومًا من التخزين. لوحظ ان مستخلص قشر الليمون المجفف (DLPE) سجل نشاطًا عاليًا كمضاد للاكسدة ، والمحتوى الكلى للفينول والفلافونويد مقارنة بمستخلص قشر الليمون الطازج (FLPE). كما ان السمية الخلوية لمستخلص قشر الليمون المجفف كانت مل / ميكروغرام 61.2= CC_{50} مما يدل علي انه آمن. كما يمتلك DLPE نشاطًا هائلا كمضاد للميكروبات ضد بعض السلالات المختبرة. بينما تأثرت قيم المواد الصلبة الكلية والرماد والدهون والبروتين بشكل طفيف عند إضافة المستخلصات الى عينات اللبنة. و زادت الفعالية المضادة للأكسدة مع زيادة تركيز مستخلصات قشر الليمون. وسجلت عينة الكنترول أعلى قيم للصلابة ، بينما انخفضت قيم التماسك والصموغية والمضغ تدريجيًا مع ارتفاع تركيزات مستخلص قشر الليمون وذلك حتى نهاية فترة التخزين. وحدث زيادة فى العد البكتيري الكلى لعينات اللبنة إلى الحد الأقصى بعد 7 أيام من التخزين. ولم يظهر نمو البكتيريا المحبة للبرودة والخمائر والاعفان والبكتيريا القولونية في اللبنة المعالجة لمدة وصلت إلى 3 أسابيع من فترة التخزين. وأدى إضافة المستخلصات المائية من قشر الليمون (طازجة أو مجففة حتى 4٪) إلى تحسين نكهة اللبنة وقوامها ومظهرها على درجة حرارة 5 ± 1 درجة مئوية لمدة 30 يومًا من التخزين.

الكلمات المفتاحية: مستخلصات قشر الليمون ، النشاط المضاد للميكروبات ، المركبات الفينولية ، السمية الخلوية ، التقييم الحسي.