J. Food and Dairy Sci., Mansoura Univ., Vol. 7(10): 415 - 419 , 2016

Labneh Fortified with Olive Leaves as Innovative Dairy Products

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ABSTRACT

The aim of this study was to investigate the compositional, microbiological and organoleptical characteristics of labneh as affected by adding sterilized dried olive leaves. Dried olive leaves powder were added to Labneh cheese at concentration 1, 2, 3, 4, 5%. Subsequently, the chemical, microbiological and organoleptic properties of Labneh cheese were determined during storage 3 weeks at 5±1°C. The results indicated that the protein and lactose content as well as titratable acidity of labneh decreased with increasing the ratio of dried olive leaves. However, fat content , ash, pH values increased with increasing the added amount of dried olive leaves. Acidity and diacetyle contents of labneh increased up to 21 days of storage, while acetaldehyde content increased up to 14days. Organoleptic scores revealed that the Labneh fortified with dried olive leaves till 3% was acceptable during the storage period.

Keywords: olive leaves, Innovative Labneh , Chemical composition, Sensory properties, Microbiological quality.

INTRODUCTION

Some early studies claimed that maximum antioxidant capacity and better health benefit could be gained by ingesting milk proteins-phenols complex, however, later studies reported reduced bioavailability of phenolics after ingestion with milk (Hoffman *et al*. 2001), Serafini *et al*. 2009).

Nutritional and therapeutic properties of labneh are considered similar to or even better than those of yogurt. Labneh has 2.5 times higher protein content, 50% more minerals, and a considerably larger number of viable microorganisms than common yoghurt. The traditional manufacture of labneh is labour intensive and unhygienic. Besides the losses of product due to its adherence to the cloth bags are quite higher, therefore, during over the past three decades, several techniques of labneh manufacture have been developed. In one of them, Labneh is produce from yoghurt by centrifugation (El-Kenany, 1995, Nsabimana *et al*., 2005).

Recently, the physiological effects of polyphenol-rich foods ,such as olive leaves received much attention as dietary sources of antioxidants that are valuable for human health. In addition,the antioxidant compounds can increase shelf-life by delaying oxidative deterioration of substrates such as unsaturated lipids (lipid oxidation), which can lead to the development of off-flavours and is considered one of main causes of deterioration of food products during processing and storage (Rhee, *et al*., 1996 Yokozawa, *et al*., 2007)..

Olive tree (Olea europaea L.) is of great economic and social importance and of its possible benefits being derived from utilization of any of its byproducts. The leaves of oil tree are important for their secondary metabolites such as the secoiridoid compounds oleacein and oleuropein, the former responsible for hypotensive activity (Hansen *et al*.,1996)..

Olive leaves contain high quantities of phenol substances very similar to those present in olives and their derived products. Furthermore, olive leaf extract and its individual constituents are considered safe and non-toxic for human and animal consumption. (Ritchason,1999, Abaza *et al*.,2008; De Leonardis *et al*., 2008,

The leaves of this tree have been used for medical purposes and were introduced recently into the pharmacopoeia, especially, in remedy of diabetes, (Sehefflera,*et al*. 2008)

Olive leaves contain different groups of constituents, such ,as iridoid glycoside , polyphenols , flavones and carbohydrates , polyphenols are the most abundant antioxidant in our diets , clinical studies have suggested association between the consumption of polyphenol-rich foods or beverages and the prevention of many diseases . Polyphenols are considered a reducing agents and together with other dietary reducing agents to protect the body tissues against oxidative stress , various diseases, inflammation and cardiovascular disease. Oleuropein is the major component of olive polyphenols and i extensively studied for health benefits concerning variety of ailments such as high blood pressure , cancer , heart problems and an array of viral and bacterial diseases(Scalbert and williamsont,2000, Malik and Bradford, 2008).

The objective of this study was to study the produce novel functional Labneh using dry leaves of olive powder as innovative labenh and their effect on chemical, microbiological and sensory properties of labneh

MATERIALS AND METHODS

The fresh olive leaves samples were collected from the planted trees in the farm of Horticulture Research Institute, Agriculture Research Center, Giza ,Egypt. After cleaning ,the leafs were dried in an oven air drier at 65 C , then be ground to a fine powder and kept on20 C. the leaves have been sterilized . Olive oil was obtained from food technology Institute, Ministry of Agriculture. Fresh buffalo's milk was obtained from the Faculty of Agriculture, Cairo University. Pure cultures of Str. salavarius subsp. *thermophilus* and *Lactobacillus delbruckii subsp. bulgaricus* were obtained from Hansen Laboratories (Denmark).

Labneh was made from buffalo's milk standardized to 3% fat by the traditional method as

described by El-Samergy *et al*.1988. The Labneh was divided into six batches. The first was as a control and the others five batches mixed individually with 1, 2, 3, 4 and 5% of dry olive leaves for T1, T2, T3,T4 and T5 respectively. Each batch redivided into equal portions. The labneh stored in a jar, covered with olive oil after being rolled into balls and stored at 5±1°C for three weeks. The experiment was carried out in triplicate. Data were reported as the average of three trials.

Moisture, fat, ash and total protein contents of olive leaves and Labneh were determined according to AOAC,2007. Total carbohydrates in Labneh were calculated by difference . Titratable acidity was determined according to Richardson, 1986. The pH values were measured using a digital laboratory pH meter with glass electrode model SA 720 (Orion, U.S.A). Acetaldehyde and diacetyl content were determined according to Lees and jago (1969). Refractive index, acid value and peroxide value of different oils used were measured according to methods described by A.O.A.C (2000). The total phenolic content of olive oil and leaves extract was estimated by Folin-Ciocalteu colorimetric method, based on the procedure mentioned by Singleton *et al* .(1998). Procedure: Briefly, the crude extract (50 mg) was mixed with Folin-Ciocalteu reagent (0.5 mL) and deionized water (7.5 mL). The mixture was kept at room temperature for 5 min, then, 10 mL of 7% sodium carbonate was added to the mixture, and then incubated for 90 min at room temperature. After incubation the absorbance against the reagent blank was determined at 760 nm. The total phenolic content of the plant was expressed as Gallic acid equivalent (mg/100 g dry weight).

Gas chromatography analysis of fatty acids was determined as described by Cossignani *et al*., (2005). *Methylation of fatty acids* was done by dissolving an aliquot of oils, about 10mg, in 2ml hexane and then 0.4ml 2N KOH in anhydrous methanol was added after 3 min, 3ml water was added. The organic layer, separated, dried over anhydrous sodium sulfate, and then concentrated with a N_2 stream to around 0.5 ml for GC analysis of fatty acids methyl esters (FAME) as described below.

Agilent 6890 series GC apparatus provided with a DB-23 column (60m x 0.32mm x 0.25µm) was used for Identification of fatty acids methyl esters by GLC . Fatty acids methyl esters directly injected into the GC. Carrier gas was N_2 with a flow rate of 2ml/min, splitting ratio of 1:100. The injector temperature was 250^{o}C and that of Flame ionization detector (FID) was 270^{o}C. The temperature settings were as follows: 150^{o}C to 225°C at 5^{o}C/min, and then held at 225^{o}C for 20 min. Peak identification was performed by comparison of the retention time (RT) for each peak with those of standard fatty acids. The peaks areas were measured using Chemstation Program, and relative areas of the identified fatty acids were recorded.

Total bacterial counts were determined according to the method described Houghtby *et al*.(1992) .Lactic acid bacteria was enumerated according to Elliker *et al*. (1956) Coliforms were enumerated according to Harrigan and McCance (1996) using Violt Red Bile agar media. lipolytic bacteria, caseolytic bacteria and yeast & mould were determined according to American public health Association methods (APHA, 1994)

Sensory Evaluation:

All Labneh treatments were graded when fresh and after 1, 2 and 3 weeks by staff members of Dairy department, Food Technology Institute, Agricultural Research Center according to Pappas *et al*. (1996).

RESULTS AND DISCUSSION

The gross chemical components; crude protein, crude fiber, ash and carbohydrate contents for olive leaves powder was determined, and the obtained results are tabulated as in Table (1).

The obtained results show that , crude protein, fat, carbohydrate, fiber, and ash contents for olive leaves were 28.50, 2.29, 3,40,32.83and 32.62 on dry basis. These results indicate that olive leaves have high level of protein and have high level of ash lead to increasing in minerals content ,the main element is calcium followed by potassium ,iron and magnesium which were 13.5,4.68,2.91,2.79g/100g respectively.

Physicochemical characteristics and oxidative stability of olive oil was determined and results were tabulated in Table 1. Results revealed that the refractive index (RI) was 1.4698,. Meanwhile, free fatty acids levels are the first measure of olive oil quality. Table(2) shows the changes in the % free fatty acids (FFA) as oleic acid of the olive oil , free fatty acids % (FFA %) of olive oil was 0.40 also, peroxide value (meq O 2 Kg^{-1} oil) was 2.28. Moreover, stability at 100^{o}C was found to be 28 hr for olive oils (Codex Alimentarius, 2005)

Table 1. Chemical composition and minerals content of olive leaves on dry basis:

Chemical composition g/100g		Mineral content mg/100g	
Fat	2.29	Mg	2.79
Protein	28.50	Na	0.35
Ash	32.62	Zn	2.23
Carbohydrates	3.40	Mn	0.83
Fiber	32.83	Fe	2.91
		K	4.68
		Ca	13.5

Table 2. Physicochemical characteristics of olive oil

Characteristics	olive oil
% FFA (as Oleic acid)	0.40
Peroxide value (meq O 2 / kg oil)	2.28
Refractive index at 25°C	1.4693

Data presented in Table (٣) showed that total phenolic contents ١٦٦.69 mg /g in Sterilized olive leaves. The results of current analysis revealed that Sterilized olive leaves contained a considerably high amount of phenolic ingredients. Results were in agreement with Djeridane *et al*., (2006) and Mylonaki *et al*., (2008)

Data presented in Tables (4, 5) summarize the chemical composition of buffalo's milk and the Labneh fortified with sterilized dread olive leaves. Addition of sterilized dried olive leaves had considerable affect on T.S and ash. Values of T.S. ranged from 25.4–26.50%

among different treatments and control. Results were in agreement with Mehaia and El-Khadragy 1999, who reported that T.S. of Labneh ranged between 22-26%.

Table 3. Polyphenols content of olive leaves mg/١٠٠g

olive oil	619.63
Sterilized olive leaves	166.69

Table 4. Chemical composition % of buffalo's milk on dry basis :

Item	TS	Fat	Protein	Ash	Carbohydrates	pH	Acidity as lactic acid
buffalo's milk	16	6.5%	4.16%	1.05%	4.29	6.7	1.0

Table 5. Gross composition % of Lebneh made by adding sterilized olive leaves

Properties	Level of sterilized dread olive leaves					
	0%	1%	2%	3%	4%	5%
T.S.%	25.4	25.7	25.88	25.94	25.98	26.5
Protein%	9.7	9.6	9.55	9.5	9.45	9.4
Fat%	10	10.4	10.5	10.6	10.65	10.7
Lactose%	4.2	4.1	4	3.95	3.9	3.85
Ash%	1.8	1.9	2.0	2.1	2.3	2.5

The changes in total acidity are very important factor.scince it affects the shelf life and the acceptability of Labneh . Titratable acidity and pH values were determined in all labneh treatments when fresh and during cold storage. The results in Table (6) show that the labneh acidity decreased as the level of olive leaves increased. Also, the acidity was further increased by cold storge up to 21days. The trend of the changes in pH values of all treatments was opposite to that of acidity which may led to lactic acid production as a results of microorganisms metabolism (Abd-Allah *et al*., 1993).

Table (7) demonstrates the acetaldehyde (AC) and diacetyl (DA) contents in labenh treatments when fresh and during cold storage. The results reveal that both AC and DA content gradually increased until 14th day for storage period. These results are in agreement with the results obtained by Hassanein *et al*. (2008).

Acetaldehyde can be converted into the ethanol by alcohol dehydrogenase (Tamime and Robinsonm 1983). This may explain the lower amount of acetaldehyde observed during the cold storage period.

Table 6. Titratable acidity (TA%) and pH values of labneh treatments during cold storage

Cold storge period	Level of sterilized dread olive leaves											
	0%		1%		2%		3%		4%		5%	
(days)	TA	pH	TA	Ph	TA	pH	TA	pH	TA	pH	TA	pH
Fresh	2	3.95	1.95	3.99	1.9	4.0	1.85	4.1	1.8	4.2	1.75	4.3
7	2.1	3.85	2	3.95	1.95	3.95	1.9	4.0	1.8	4.1	1.74	4.25
14	2.3	3.8	2.1	3.85	2	3.9	1.95	3.97	1.75	4.0	1.7	4.2
21	2.5	3.7	2.2	3.8	2.1	3.85	2	3.95	1.7	3.95	1.68	4.15

Table 7. Acetaldehyde (AC) and diacetyl (DA) (µmol/100g) of labneh treatments during cold storage

Cold storge period	Level of sterilized dread olive leaves											
	0%		1%		2%		3%		4%		5%	
(days)	AC	DA	AC	DA	AC	DA	AC	DA	AC	DA	AC	DA
Fresh	185	100	190	105	190	106	195	107	196	107	197	110
7	200	120	220	125	220	125	225	130	225	130	225	130
14	250	130	280	132	285	135	285	140	290	141	290	142
21	300	150	310	155	310	158	315	160	320	160	320	160

Form results presented in Table (8), it confirmed that 1%,2%, 3% olive leaves concentrations and control of fortified Labneh possessed the best appearance, with no significant difference in between, but significantly differed in comparison with the 4 % and 5% evaluation. consistency 1%,2%,3% and control were the most preferable by the panelists with non significant differences. With respect to the flavor of the tested labneh, 4% and 5% recorded the lowest value of flavor compared to the other labneh. Also 1%, 2% ,3% and control of labneh were the most preferable by the consumers with non significant differences. As the concentration of olive leaves was increased in fortified Labneh the score of flavor, consistency, appearance and total score was decreased. In general, all Labneh made with different levels of olive leaves had acceptable flavor, consistency and appearance during storage period. Our results are in agreement with Peker and Arslan, (2016)

Table 8 . Organoleptic scoring of labneh during cold storage

Sensory attributes	Control	1 %	2%	3%	4%	5%
			Fresh			
Appearance (5 points)	4.83^{a}	4.866^{a}	4.9^{a}	4.86^{a}	3.50^{b}	3.40^{b}
Consistency (5 points)	4.90^{a}	4.90^{a}	4.96^{a}	4.80^{a}	3.4^{b}	3.33^{b}
Flavour (5 points)	4.93^{a}	4.96^{a}	4.93^{a}	4.83^{a}	2.96^{b}	2.93^{b}
Total (15 points)	14.7^{a}	14.73^{a}	14.8^{a}	14.5^{a}	9.86^{b}	9.33^{c}
			One week			
Appearance (5 points)	4.93^{a}	4.93^{a}	4.93^{a}	4.50^{b}	3.50^{c}	3.37^{a}
Consistency (5 points)	4.86^{a}	4.90^{a}	4.86^{a}	4.86^{a}	3.40^{b}	3.36^{b}
Flavour (5 points)	4.90^{a}	4.93^{a}	4.93^{a}	4.83^{a}	2.93^{b}	2.46^{c}
Total (15 points)	14.70^{a}	14.76^{a}	14.73^{a}	14.20^{b}	9.83^{c}	9.20^{a}
			2 weeks			
Appearance (5 points)	4.90^{a}	4.93^{a}	4.8 0^{a}	4.76^{a}	3.33^{b}	3.07^{c}
Consistency (5 points)	4.83^{a}	4.80^{a}	4.76^{b}	4.60^{b}	3.30^{c}	3.26^{c}
Flavour (5 points)	4.90^{a}	4.86^{a}	4.8 0^{a}	4.66^{a}	2.96^{b}	2.96^{b}
Total (15 points)	14.63^{a}	14.60^{a}	14.36^{ab}	14.03^{b}	9.60^{c}	8.43^{a}
			3weeks			
Appearance (5 points)	4.5^{a}	4.5^{a}	4.30^{ab}	4.10^{b}	3.33^{c}	2.50^{a}
Consistency (5 points)	4.53^{a}	4.43^{ab}	4.30^{ab}	4.13^{b}	2.5^{c}	2.16^{a}
Flavour (5 points)	4.40^{a}	4.36^{a}	4.07^{ab}	4.13^{b}	2.50 ^{c}	2.17^{a}
Total (15 points)	13.43^{a}	13.30^{ab}	12.66^{bc}	12.23^{c}	8.46^{a}	6.96^{e}

The changes of viable count of lactic acid bacteria during storage period are presented in Table (9). Data indicated that the viability remained higher for 7 days of cold storage and then started to decline. The results indicated also that, the count of lactic acid bacteria was higher in labneh with lower concentration of olive leaves. As a result of high hygienic conditions during manufacturing and storage, molds and yeasts and pathogenic bacteria were not detectable in all treatments when fresh and throughout storage period. These results are in agreement with those reported by Hareedy *et al.*, (2008).

Table 9. Lactic acid bacteria, mold and yeast, lipolytic and proteolytic counts (log cfu/ml) of labneh affected by the levels of olive leaves added.

Treatments	Cold storage periods (days)	Microbiological quality			
		Lactic acid bacteria	mold and yeast	Lipolytic	Proteolytic
0%	Fresh	8.6	ND	ND	ND
	7	8.9	ND	ND	ND
	14	8.8	ND	ND	ND
	21	8.6	ND	ND	ND
1%	Fresh	8.5	ND	ND	ND
	7	8.6	ND	ND	ND
	14	8.5	ND	ND	ND
	21	8.4	ND	ND	ND
2%	Fresh	8.4	ND	ND	ND
	7	8.4	ND	ND	ND
	14	8.5	ND	ND	ND
	21	8.4	ND	ND	ND
3%	Fresh	8.5	ND	ND	ND
	7	8.4	ND	ND	ND
	14	8.4	ND	ND	ND
	21	8.3	ND	ND	ND
4%	Fresh	8.5	ND	ND	ND
	7	8.4	ND	ND	ND
	14	8.4	ND	ND	ND
	21	8.3	ND	ND	ND
5%	Fresh	8.5	ND	ND	ND
	7	8.4	ND	ND	ND
	14	8.3	ND	ND	ND
	21	8.2	ND	ND	ND

N.D.=Not detected.

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تدعيم اللبنة بأوراق الزيتون كمنتجات لبنية مبتكرة

عواطف ابراهيم أساعيل , ناهد زكى لطفى و هويدا عبد اللة الشاذلى

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تهدف هذة الدراسة إلى التعرف على التركيب الكيميائى والميكروبيولوجى والخصائص الحسية للبنة المضاف لها اوراق الزيتون المجففة والمعقمة تم اضافة مطحون أوراق الزيتون المجففة إلى اللبنة بتركيز ١، ٢،٣، ٤،٥ % ، تم تقدير التركيب الكيميائى والميكروبيولوجي والخواص الحسية للبنة أثناء التخزين ٣ أسابيع في ٥ ± ١ درجة مئوية.أ شارت النتائج إلى أن البروتين واللاكتوز وكذلك الحموضة للبنة انخفضت مع زيادة نسبة أوراق الزيتون ومع ذلك، فإن محتوى الدهون والرمادpH زاد مع زيادة كمية إضافة أوراق الزيتون المجففة. أثناء التخزين البارد للبنة نجد محتوى الحموضة وداى اسيتيل زاد حتى ٢١ يوم فى حين محتوى الاسيتالدهيد زاد حتى ١٤ يوم ، اظهرت الصفات الحسية للبنة المدعمة باوراق الزيتون المجفف مقبولة حسي حتى تركيز٣% خلال فترة التخزين.