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Fortification of Labneh with the Extract of Guava Leaves Powder as a Functional Products

Hayam A. El-Gazzar; M. A. M. Moussa and Salaala Aln Aby E. Abo El- khair
Dairy Chem.,Res.,Depar - Animal production Research Institute,A.R.C., Dokki, Giza, Egypt.
Mohammedmoussa997@gmail.com

ABSTRACT

Making functional labneh from buffalos milk with added guava (*Psidium guajava*) leaves extract was examined. Methanol was used for extraction of the phenolic compounds. The total phenols of 890, 879 and 883 ug/g powder were, respectively, used at an extraction ratio of 1:12. Different concentrations of water extract of guava leaves were added to milk before adding the starter resulted in significant changes in pH, titratable acidity during cold storage. Reducing activity of all samples significantly (P>0.05) decreased up to the end of storage period. Concentration of phenolic compounds till 300ug/100ml milk used in making labneh increased, followed by a slight decrease during storage. Viable cell counts increased in labneh with added guava leaves extract, phenolic component 75ug /100 ml from960 CFU\ml after one day to 9 .77 on the fifth day. A decline in CFU\ml throughout storage was detected. Sensory evaluation data indicate no significant differences (p >005) between the control and treated samples. Labneh with added water extract of guava leaves powder as natural antioxidants source could be proposed for making it as natural antioxidants source.

INTRODUCTION

An accumulation of oxidative radicals such as hydrogen peroxide and other hydroxyl radicals could be detected in human beings and certain microorganisms, which contribute to damage of cells and tissues (Sikora *et al.,* 2008).

The presence of phenolic compounds in the diet could retard or prevent the oxidation of lipids, with consequent delay of occurrence many chronic diseases. Phenolic compounds of plant origin were extensively used in the present time for safety and to prolong the food shelf life, and for their benefits in treating many diseases (Shety 1997, Adam *et al*., 1998, Akyon 2002, Botsoglou *et al*.,2002, Chen and Yen 2007, and Tachakittirumgrod *et al*., 2007)

Lebneh is widely consumed in the Mediterranean and the Middle East countries, due to its nutritional and health benefits. Developed and recent methods such as of making Labneh from ultrafiltration milk could later be applied. (Tamime and Robinson 1978, Tamime *et al* 1989 a, b 1991 a ,b), Nsabimana *et al*., 2005 and Shamsia and El ghanam 2012).

Fermented milk foods are of great important for their nutritional and safety values. Numerous types of traditional fermented foods exist worldwide (Khurana and Kanawjia 2007).

Fermented milks as an important component of nutrition and diet were greatly developed as a mean of preserving (Khurana and Kanawjia 2007. and Beena 2000).

The aim of this research, therefore, was to prepare functional labneh, with different levels of natural antioxidant from water extract of guava leaves to study the effect of storage period on the changes of some antioxidant activities, including inhibition of ascorbate autoxidation and reducing activity in functional labneh.

MATERIALS AND METHODS

Buffalo's milk was obtained from the herds of Mehalet Moussa Experimental Station. Agricultural Research Center, Ministry of Agriculture. Fresh guava leaves were obtained from Kafer Elsheikh Egypt.

The leaves were washed well with tap water ,and dried at 40°C under vacuum. Leaves powder was obtained by drying and milting of fresh leaves, followed by filtration until the whole sample passed through a 0.125 mm sieve.

The dried guava powder was extracted with various solvents (water, methanol and ethanol) at different ratios of 1 : 6 ,1 : 8, 1 : 10 , 1 : 12 and 1 : 14 g/ml for 48 hours at room temperature.

The highest phenolic containing extracts were chosen for further analysis. The antimicrobial activity was detected after filtration through 0.45 um (Nacalai tesque japan) filters, and kept in a freezer at – 20°C until use.

Milk used in making Lebneh was heat treated (90°C/10 min, cooled to 45°C), distributed into 1000 ml flasks. 75, 150, 225, 300 and 375 ug extracted phenolic components were added to 100 ml milk portions.

Inoculation of milk with added 2% (v/v) yoghurt starter in the presence of phenolic copounds 2% (v/v), mixed well and poured into 100 ml cups, followed by incubation at 42°C until coagulation, cooled to 10°C overnight, mixed and transferred into sterilized cheese cloth bags hanged in refrigerated room at 6 – 8°C to allow whey drainage for 12 hr. Sodium chloride was added at 0.5 %), and the resultant Labneh was filled into plastic containers and stored at 6–8°C for 21 days. Three replicates of each treatment were conducted. Samples of labneh were chemically, microbiologically and organo-leptically analyzed. Each treatment was analyzed when fresh and after 7, 14 and 21 days.

Protein, fat, ash contents and total solids were determined as described in AOAC,(2007).

Titratable acidity was estimated according to Richardson (1986), The pH value was measured using pH- meter (HANNA 8417). crude fiber according to A O A C (1995), and acetaldehyde was estimated as mentioned by Lees and Jago (1969).

Total phenolic contents and antioxidant of the examined treatments were carried out as described by

Mathaus (2002). 5g of labneh was mixed thoroughly in mortar with 22ml of 75% methanol.

The suspension of Labneh was shacked, and centrifuged at 10000g for 15 min at 10° C, followed by filtration, and stored at 20 °C until analyzed for total phenolic and ferric reducing power as described by Arcan and Yemenicioglu (2009). Ferric reducing antioxidant powder (FRAP) was determined according to Benzie and Strain (1999).

The folin- ciocalteu reagent was used to detect the soluble phenolic compounds (PC) in the different treatments as mentioned by Nassar *et al*, (2014) with slight modifications.

Suitable volumes of sample extract of standard concentrations were taken, 1.0ml of 10% folin-ciocaltey reagent and 0.8ml of 7.5% Na_2Co_3 were added, mixed thoroughly and incubated at room temperature for 90min.

The absorbance was read at 725 nm. The values were expressed as mg equivalents of Gallic acid.

For measuring of inhibition of ascorbate autoxidation, the method mentioned by Mishra and Kovachich (1984) was applied. The absorbance of mixture of A 0.1 ml of sample and ascorbate solution (0.1ml, 5.0 mM, and phosphate buffer (9.8 ml, 0.2 M, pH 7.0) was measured at 265 nm. The ascorbate autoxidation inhibition rate of the sample was then calculated according to the following equation:

Inhibition effect (%) = [absorbance's ample / absorbance control - 1] X100%

The reducing activity of sample was determined according to Oyaizu (1986). sodium phosphate buffer (pH 7.0), incubated at 50°C., treated with10% trichloroacetic, centrifuged, and the absorbance was measured at700 nm.

For the microbiological evaluation of supplemented labneh, the resultant of fresh labneh control and supplemented with Guava leaves extract were microbiologically examined for the total bacterial count (TBC), yeast & mold and coliform group as described in Difco (1985).

Labneh samples were examined for Sensory test by 10 panelists from the staff of Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture. A hedonic scale of 1 (dislike extremely) to 10 (like extremely) was used.

The panelists were asked to evaluate 2 weeks old samples. They were also given a further option of writing comments, if any was observed. As evaluation scheme proposed by Saldamli *et al*. (1991).

Statistical analysis:

Results were conducted as completely randomized design (CRD) .Statistical analysis was performed using the statistical analysis systems (SAS) software (Version 9.3, Cary, NC USA, 2013). using the Duncan's multiple – range test to compare treatments. Significance was set at $P \leq 0.05$.

RESULTS AND DISCUSSION

Results in Table (1) show the influence of adding different levels of Guava extract to fresh labneh on the chemical composition of the resultant labneh, compared to the control. An increase in acidity and decrease in pH values were detected by using different levels of supplementation. Considerable increase could also be observed in the total solids (TS) by using the same concentration of supplementation. This decrease ranged from 23.96 (T5) to 22.60 (T1) .

On the other hand, total protein (TP), total volatile fatty acids (TVFA), and ash revealed considerable increase in all treatments. Guava leaves extract treatments showed the highest values for fibers and ash. While the treatment (T5) characterized with the highest value of total protein (TP).

Table 1. Chemical property of fresh Labneh with different levels of Guava leaves extract

Items	Treatments					
	Control	T1	T2	T3	T4	T5
Acidity%	1.20^{a}±0.05	1.26^{b}±0.06	1.87^{b}±0.05	1.88^{b}±0.05	1.89^{b}±0.05	1.89^{b}±0.05
pH	4.49^{c}±0.07	4.42^{bc}±0.07	4.41^{bc}±0.07	4.43^{ab}±0.07	4.43^{ab}±0.07	4.44^{a}±0.07
TS%	22.60^{cd}±0.15	23.05^{bc}±0.11	23.50^{b}±0.11	23.82^{ab}±0.11	23.85^{a}±0.11	23.96^{a}±0.10
TP%	9.52^{b}±0.11	9.65^{ab}±0.09	9.76^{ab}±0.09	9.77^{a}±0.09	9.89^{a}±0.10	9.98^{a}±0.11
Fat%	6.5^{a}±0.06	6.5^{a}±0.06	6.5^{a}±0.06	6.5^{a}±0.06	6.5^{a}±0.06	6.5^{a}±0.06
TVFA*	0.37^{b}±0.06	0.38^{b}±0.06	0.40^{ab}±0.07	0.41^{a}±0.07	0.43^{b}±0.07	0.42^{ab}±0.07
Ash%	0.83^{c}±0.02	0.85^{bc}±0.02	0.86^{b}±0.03	0.88^{ab}±0.03	0.89^{a}±0.03	0.90^{a}±0.03
Fibers%	----------	0.51^{c}±0.04	0.57^{b}±0.04	0.45^{ab}±0.04	0.61^{a}±0.04	0.62^{a}±0.04
Acetaldehyde**	162.4^{d}±13.2	160.11^{ab}±11.85	161.33^{a}±11.51	161.75^{a}±11.33	165.55^{cd}±11.91	166.33^{bc}±12.31

T1: Labneh mixture and 1:6 Guava (leaves extract T2: Labneh mixture and 1:8 Guava leaves extract
T3: Labneh mixture and 1:10 Guava leaves extract. T4: Labneh mixture and 1:12 Guava) leaves extract
T5: Labneh mixture and 1:14 Guava leaves extract *0.1N- NaoH/10g labneh.
****u mole/100g labneh. Averages with different superscripts differed significantly (P≤0.05).**

Results in Table (2) demonstrate the chemical composition and certain properties of Labneh fortified with Guava leaves extract during storage. A relatively high increase of acidity and decrease in pH values were found at the end of storage periods (5 days for the control and 10 days for treatments). Total solids (TS) considerably increased in T1, T2, while slightly increased in T4 and T5, which ranged from 4.5% to 6.5%. Also, acetaldehyde, TP, TVFA, fibers and ash increased during storage period, however, fat slightly decreased in the stored samples.

Table 2. Chemical property of stored Labneh enriched with different levels of extract of Guava leaves .

Items	Treatments*					
	Control	T1	T2	T3	T4	T5
Acidity%	1.58^{a}±0.06	1.61^{ab}±0.06	1.75^{ab}±0.05	1.88^{ab}±0.07	1.91^{ab}±0.06	1.99^{ab}±0.05
PH	4.0^{a}±0.06	4.10^{a}±0.06	4.05^{a}±0.07	4.15^{a}±0.07	4.10^{a}±0.07	4.05^{a}±0.07
TS%	24.93^{bc}±0.10	25.51^{a}±0.10	25.47^{ab}±0.11	25.59^{ab}±0.11	25.67^{b}±0.11	25.71^{a}±0.12
TP%	4.40^{ab}±0.10	4.50^{a}±0.08	4.48^{a}±0.07	4.33^{b}±0.07	4.39a^{b}±0.08	4.42^{ab}±0.07
Fat%	6.50^{a}±0.05	6.40^{ab}±0.05	6.40^{ab}±0.05	6.30^{b}±0.05	6.20b^{c}±0.05	6.20^{bc}±0.05
TVFA	0.45^{b}±0.06	0.44^{ab}±0.05	0.46^{ab}±0.06	0.48^{a}±0.06	0.49^{ab}±0.07	0.51^{ab}±0.06
Ash%	1.05^{c}±0.04	1.15^{bc}±0.04	1.20^{ab}±0.04	1.18^{b}±0.04	1.18^{ab}±0.04	1.22^{a}±0.04
Fibers%	----------	0.65^{bc}±0.05	0.69^{b}±0.05	0.73^{ab}±0.05	0.77^{a}±0.05	0.79^{a}±0.05
Acetaldehyde	211.5^{de}±13.11	215.5^{bc}±13.51	217.5^{ab}±13.77	205.3^{a}±12.86	208.3^{e}±12.70	210.3^{de}±12.97

***See legend to Table (1) for details**

Regarding the total phenolic compounds (Table 3), the Labneh made with Guava leaves extract was of higher content of phenolic compounds, compared with the control. Furthermpore, the concentration of phenolic compounds increased with the increase of the concentration of Guava, which could be due to the high content of phenolic compounds in Guava, which agrees with Sreelatha and Pedma (2009) and Ashfagqu *et al*, (2012). It could also be observed that the addition of Guava leaves the added Guava extract resulted in changes in texture parameters including hardness, springiness, adhesiveness, cohesivness, resilience, gumminess, and chewiness of labneh (Table 4). Hardness and adhesiveness increased, while springiness, cohesiveness, resilience and chewiness decreased in the samples treated with the examined Guava extract. While slight change was observed in the gumminess. Increase (4%) of hardness was detected, which reflects the unfavorable character of the rubbery property formed in labneh. Cohesiveness decreased with 6, 11, 14, 18 and 21% in all of the examined treatments. Resilience decreased in all of the above mentioned treated with Guava leaves extract treatments. The decrease of resilience in control and the treatments were 5, 7, 11, 12, and 14, in the same order. Values of resilience, chewiness and gumminess were in the same trend with those detected of cohesiveness.

Table 3. Antioxidant scavenging activity (FRAP) and total phenolis of Labneh made with different concentrations of aqueous extract of Guava leaves (Average ±SE of 3 replicates).

Treatments*	FRAP (mg Fe2So4 Eq/100g)	Total phenos (mg GAE** /100g FW)
Control	70.55^{f}± 0.04	4.98^{f} ± 0.03
T1	72.35^{e} ± 0.03	6.90^{e} ± 0.04
T2	72.60^{d} ± 0.03	8.50^{d} ± 0.04
T3	75.12^{c} ± 0.05	11.03^{c} ± 0.04
T4	76.87^{b} ± 0.05	13.55^{b} ± 0.04
T5	77.01^{a} ± 0.05	15.05^{a} ± 0.04

***See legend to Table (1) for details. **GAE Gallic acid equivalent**

Table 4. Textural properties of Labneh made with different levels of Guava leaves extract (Average±SE of 3 replicates).

Treatments*	Hardness (g)	Springiness (mm)	Adhesiveness (g sec)	Cohesiveness (g/cm)	Resilience	Gumminess (g/cm)	Chewiness (g/cm)
Control	887^{c}±4.75	0.79^{a}±0.003	-565^{f}±1.12	0.39^{a}±0.002	0.12^{a}±0.002	451^{a}±29	486.5^{a}±4.92
T1	897^{bc}±4.75	0.76^{a}±0.003	-501^{e}±1.12	0.31^{b}±0.002	0.10^{ab}±0.002	475^{a}±28	466.1^{ab}±4.92
T2	915^{b}±4.75	0.72^{b}±0.003	-471^{d}±1.12	0.24^{c}±0.002	0.09^{abc}±0.002	488^{a}±28	432^{bc}±4.92
T3	932^{ab}±4.75	0.69^{bc}±0.003	-442^{c}±1.12	0.20^{d}±0.002	0.08bc±0.002	498^{a}±28	411^{c}±4.92
T4	945^{ab}±4.75	0.65^{bc}±0.003	-412^{b}±1.12	0.17^{e}±0.002	0.08^{bc}±0.002	521^{a}±28	398.5^{c}±4.92
T5	959^{a}±4.75	0.62^{c}±0.003	-389^{a}±1.12	0.15^{f}±0.002	0.08^{c}±0.002	542^{a}±28	377.4^{c}±4.92

The inhibitory effect of ascorbate greatly increased with adding 300ug phenolic compounds per 100 ml labneh, with consequent slight decrease. The extent of the inhibition was 22.4, 30.8, 39.0, 48.1 and 46.7% with added 75,150,225,300 and 375 µg phenolic compound/100ml milk used in making labneh, in the same order, which might be due to the formation of phenoxy radical formed from phenolic compounds. The effect of guava extracts at high concentrations might be correlated with phenoxy radical formed by the changes of phenolic compounds. Which came in harmony with those results obtained by Bowry, *et al*., 1992 & Chen and Yen, 2007).

Results presented in Table (6) indicate slight and insignificant differences in the total counts of bacteria could be detected in the examined treatments with the different concentrations of the Guava leaves extract. However, all of the examined treatments were found completely free from both of moulds & yeasts and coliform bacteria.

Organoleptic evaluation of labneh made with different levels of Guava leaves extract is shown in Table (7). Supplementation with different concentrations of Guava leaves extract resulted in an effect on the labneh texture compared with the control treatment. Results in Table (8) reveal that the organoleptic scores quality increased for all samples throughout the cold storage, however, at the end of storage, slight decrease was observed in all treatments, with the exception with the flavored with Guava leaves extract. No changes in sensory properties were observed throughout storage.

Table 5. Ascorbate autoxidation of Labneh made with different concentrations of Guava leaves extract.

Treatments*	Ascorbate autoxidation (%)
Control	18.2
T1	22.4
T2	30.8
T3	39.0
T4	48.1
T5	46.7

***See legend to Table (1) for details.**

Table 6. Microbiological content of Labneh made with different concentrations of Guava leaves extract.

Treatments*	Total bacterial count(cfu/g)	Mould and yeast (cfu/g)	Coliform group (cfu/g)
Control	8830^{ab}	Nil	Nil
T1	8910^{b}	Nil	Nil
T2	8795^{a}	Nil	Nil
T3	8800^{bc}	Nil	Nil
T4	9100^{c}	Nil	Nil
T5	9250^{cd}	Nil	Nil

Table 7. Organoleptic properties of fresh Labneh made with different levels of Guava leaves extract.

Treatments*	Color (10)	Taste (10)	Odour (10)	Texture (10)	Appearance (10)
Control	$9.15^{a} \pm 0.06$	$8.75^{a} \pm 0.06$	$8.85^{a} \pm 0.06$	$8.55^{a} \pm 0.07$	$8.35^{a} \pm 0.07$
1	$9.05^{a} \pm 0.06$	$8.55^{a} \pm 0.06$	$8.75^{a} \pm 0.06$	$8.35^{a} \pm 0.07$	$8.25^{a} \pm 0.07$
2	$9.25^{a} \pm 0.06$	$8.45^{ab} \pm 0.06$	$8.65^{a} \pm 0.06$	$8.35^{a} \pm 0.06$	$8.35^{a} \pm 0.07$
3	$8.55^{ab} \pm 0.05$	$8.15^{ab} \pm 0.06$	$8.35^{ab} \pm 0.06$	$8.15^{b} \pm 0.05$	$8.00^{b} \pm 0.06$
4	$8.75^{ab} \pm 0.05$	$7.95^{b} \pm 0.06$	$8.05^{b} \pm 0.06$	$8.00^{b} \pm 0.05$	$7.75^{bc} \pm 0.06$
5	$8.45^{b} \pm 0.05$	$7.65^{c} \pm 0.06$	$7.75^{bc} \pm 0.05$	$7.80^{c} \pm 0.05$	$7.45^{b} \pm 0.05$

Table 8. Organoleptic properties of stored Labneh made with different levels of Guava leaves extract .

Treatments*	Color (10)	Taste (10)	Odour (10)	Texture (10)	Appearance (10)
Control	$9.45^{a} \pm 0.05$	$9.25^{a} \pm 0.05$	$9.25^{a} \pm 0.05$	$8.60^{a} \pm 0.06$	$8.75^{a} \pm 0.07$
1	$9.75^{a} \pm 0.05$	$9.15^{a} \pm 0.05$	$9.25^{a} \pm 0.05$	$8.65^{a} \pm 0.06$	$8.65^{a} \pm 0.07$
2	$9.65^{a} \pm 0.05$	$9.05^{a} \pm 0.05$	$9.15^{a} \pm 0.05$	$8.75^{a} \pm 0.06$	$8.85^{a} \pm 0.07$
3	$8.75^{b} \pm 0.05$	$8.25^{b} \pm 0.05$	$8.35^{b} \pm 0.05$	$8.45^{ab} \pm 0.05$	$8.45^{ab} \pm 0.06$
4	$8.45^{bc} \pm 0.05$	$7.35^{bc} \pm 0.05$	$8.25^{b} \pm 0.05$	$8.25^{b} \pm 0.05$	$8.25^{b} \pm 0.06$
5	$8.15^{c} \pm 0.05$	$7.25^{c} \pm 0.05$	$8.15^{bc} \pm 0.05$	$8.15^{c} \pm 0.05$	$7.95^{bc} \pm 0.05$

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اللبنه المدعمه بمستخلص مسحوق اوراق الجوافه كمنتج وظيفى

هيام عبد الرحمن الجزار ، محمد عرفة محمد موسى و صلا على النبى ابراهيم ابو الخير

قسم بحوث كيمياء الالبان- معهد بحوث الانتاج الحيوانى- مركز البحوث الزراعية- وزارة الزراعة

تتناول الدراسة إمكانية تصنيع لبنة ذات خواص وظيفية من اللبن الجاموسى وبإضافة مستخلص ورق الجوافة. وتم إستخلاص الموادالفينولية من ورق الجوافة بإستخدام كل من الماء والإيثانول والميثانول، وإختيار الآخير نظرا لإعطائه أفضل النتائج. وق تم إضافة مستخلص الجوافة إلى اللبن المستخدم فى التصنيع بتركيزات مختلفة والمقارنة بالكونترول. وقد أدت الإضافة إلى تغيرات جوهرية فى كل من الحموضة والpH خلال التخزين، وإنخفاض فى النشاط الإختزالى بالنسبة لكل المعاملات، فى حين ملاحظة إرتفاع تدريجى لتثبيط الأكسدة الذاتية بإستخدام حامض الأسكوربيك، وتبع ذلك إنخفاض طفيف فى التأثير على الأكسدة. كما لوحظ زيادة محدودة بالنسبة لأعداد خلايا البكتريا الحية، فى حين خلت جميع المعاملات من الخمائر والفطريات وكذا من بكتريا القولون. وإتضح عدم وجود فروق جوهرية فى التقييم الحسى لجميع المعاملات. ويترتب على ذلك إمكانية إستخدام مستخلص أوراق الجوافة كمصدر لمضادات الأكسدة الطبيعية فى اللبنة.