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Enhancement of Nutritional and Biological Values of Labneh by Adding Dry Leaves of *Moringa oleifera* as Innovative Dairy Products

[1]Aida S. Salem, [2]Wafaa M. Salama, [2]A.M. Hassanein and [3]Hanan M.A. El Ghandour

[1]Dairy Tech Department Animal Production Research Institute,
Agricultural Research Center, Dokki, Giza, Egypt
[2]Dairy Research Department, Food Technology Research Institute,
Agricultural Research Center, Giza, Egypt
[3]Regional Center for Food and Feed (RCFF), Agricultural Research Center, Giza, Egypt

Abstract: Dry leaves of *Moringa oleifera* (DLMO) were added to Labneh cheese at concentration 1, 2, or 3%. Subsequently, the chemical, microbiological and organoleptic properties of Labneh cheese during storage 3 weeks at 5±1°C were determined. Nutritional and biological values of Labneh were evaluated when fresh. Addition of DLMO had considerable effect on Total Solid (TS), protein, acidity, carbohydrate and ash. The highest values were recorded with Labneh fortified with DLMO (innovative Labneh). The addition of DLMO had a significant effect on carbohydrate. Acidity increased gradually for all treatments during storage. The highest values were obtained with Labneh fortified with DLMO. Labneh fortified with DLMO can be considered as a good source of minerals (Ca, Fe, Zn and Si) and vitamins (A, B1, B2 and E). The results indicated that total counts were higher in Labneh fortified with DLMO. Yeast & mould and coliform bacteria were not detected in Labneh fortified with DLMO when fresh and till the end of storage. Labneh fortified with DLMO characterized with high biological value (BV), true protein digestibility (TD) and net protein utilization (NPU). Organoleptic scores revealed that the Labneh fortified with DLMO was acceptable during storage period.

Key words: Innovative Labneh · Chemical composition · Microbiological quality · Minerals and vitamins · Biological values (BV, TD, NPU)

INTRODUCTION

The dairy industry offer a wide range of products from raw milk to the specialist products aimed at special market or consumers and offers ingredients to other industries. Consumers are rather conservative and cautions in accepting entirely new ingredients and new food products and prefer to look for new benefits in more or less familiar products [1]. Consumers demand keep changing over time. These changes range from basic considerations such as improving food safety, shelf life and reducing wastage to demands for increasingly sophisticated foods having special characteristics in terms of nutritional value, palatability and convenience. The actual product development process is determined by the interaction between consumer expectations and demand, the technical capacity of the food producer and emerging knowledge from Food Science research [2, 3]. Enhancing human health and wellness through food, nutrition and innovative products is one of the priorities identified in the world. Innovation is defined as an implementation of a new or significantly improved product (good or service) or process, new marketing method or new organizational method in business practices, workplace organization or external relations. Innovation spectrum is defined as new to the world, product improvement and cost reduction [2, 4]. A range of innovative applications are known to enhance nutritional quality of food including novel materials and nutrient delivery mechanisms.

Corresponding Author: Wafaa M. Salama, Dairy Research Department, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt.

Today, innovation food technology plays crucial role in translating nutrition information into consumer products to produce new health food ingredients and added specific nutrient or functional ingredients [5]. In modern society, people desire both good health and longevity and hence demand nutritious and functional food that promotes their wellbeing, enjoyment and active life style [5, 6]. Convenient health food or foods that impart extra value in the form of health benefits are now the highest priority for product development in the food industry. A variety of foods are now manufactured that provide essential or specific nutrients or functional ingredients to improve nutrition and to prevent both malnutrition and disease. In the area of food processing, food manufactures are adding value to their products to meet the current consumer demand for healthier food products [5]. The innovation success of new products is improved when there is true added value to consumer. Increased consumption of minerals (Ca, Zn, Fe, Se) and multivitamins are clearly recognized goods of many public health programmes and in food industry [5, 7, 8]. Moringa has gained much importance in the recent days to its multiple use and benefits to agriculture and industry. Regards as a miracle plant, all the parts of Moringa plant are used for medicinal, enhancing plant growth, livestock feed and fodder, water purification agent, biogas and bio pesticide. Moringa belongs to Moringaceae a single-genus family with 13 known species. *Moringa oleifera* lam is the most common known and utilized species. It is indigenous to many countries in south East Asia, Africa, Arabia and South America [9].

Moringa oleifera is the most nutrient rich plant yet discovered. It provides a rich and rare combination of nutrients, amino acids, antioxidantes, antiaging and anti-inflammatory properties used for nutrition and healing. The World Health Organization has promoted Moringa as an alternative to imported food supplies to treat malnutrition [9-11]. *Moringa oleifera* is a miracle tree with a great indigenous source of highly digestible proteins, Ca, Fe and vitamin C. it contains all the essential nutritional elements that are essential for livestock and human beings as well [9, 12, 13]. Moringa contains a range of fairly unique phytochemicals containing the simple sugar, hamnose and it is rich in a fairly unique group of compounds called glucosinolates and isothiocyanates [14, 15]. Moringa has been used in the traditional medicine for centuries in many cultures a round the world, for skin infections, anemia, anxiety, asthma, chest congestion, blackheads, blood impurities, catarrh, cholera, conjunctivitis, cough, diarrhea, eye and ear infections, abnormal blood pressure, respiratory disorders, for intestinal worms, lactation, diabetes and pregnancy [9, 16].

Therefore, the present study was planned firstly, to produce novel functional Labneh using dry leaves of *Moringa oleifera* as innovative labenh. Secondly, to evaluate nutritional and biological values in albino rats.

MATERIALS AND METHODS

Materials: Fresh buffalo's milk was obtained from the Faculty of Agriculture, Cairo University. *Moringa oleiferia* (dry leaves) was obtained from Breadbasket of Egypt Association. Pure cultures of *Str. salavarius sub sp. thermophilus* and *Lactobacillus delbruckii sub sp. bulgaricus* were obtained from Hansen Laboratories (Denmark).The composition of buffalo's milk and dry leaves of *Moringa oleiferia* is shown in Table 1.

Preparation of Labneh: Labneh was made from buffalo's milk standardized to 3% fat by the traditional method as described by El-Samergy *et al*. [17]. The Labneh was divided into four batches. The first was served as a control and the others three batches mixed individually with 1, 2 and 3% of *Moringa oleifera* for T1, T2 and T3, respectively. Each batch redivided into equal portions 100 gm packed in a plastic container and stored at 5±1°C for three weeks. The experiment was carried out in triplicate. Data were reported as the average of three independent trials.

Methods of Analysis

Chemical Analysis: Moisture, fat, ash and total protein contents of Labneh were determined according to AOAC [18]. Total carbohydrates in innovative Labneh were calculated by difference as described by Ceirwyn [19]. Titratable acidity was determined according to Richardson [20]. Antioxidant activity as indicator of antioxidant

Table 1: Chemical composition (%) of *Moringa oleifera* and buffalo's milk.

Item	TS	Fat	Protein	Ash	Carbohydrate	Antioxidant activity	pH
Moringa leaves powder	96.75	2.30	44.20	10.81	38.20	97.02	6.08
Standardized Buffalo's milk	13.00	3.00	3.96	0.80	5.24	--	6.75

contents was determined according to Olivera *et al*. [21]. The pH values were measured using a digital laboratory pH meter (HI 93 1400, Hanna instruments) with glass electrode. Minerals Zn, Fe, Si, Ca contents were analyzed by using Atomic absorption 3300 Perkin Elmer U.S.A. AOAC [18]. Vitamin A (retinol) and Vitamin E (α – tocopherol) were determined by using HPLC method described by Leth and Sonderyaro [22].

Microbiological Estimation: Total bacterial counts were determined according to the method described by Houghtby *et al*. [23].Whereas, molds & yeast and coliforms were determined according to Marshail [24].

Sensory Evaluation: All innovative Labneh treatments were graded when fresh and after 1, 2 and 3 weeks of refrigerator storage 5±1°C by staff members of Dairy department, Food Technology Institute, Agricultural Research Center according to Pappas *et al*. [25].

Statistical Analysis: All data were statistically analyzed using the General Linear Models procedure of the Statistical Analysis System SAS [26]. Significance of difference was defined at $p \leq 0.05$. All experiments as well as the related analyses results were repeated three times and all obtained data are expressed as averages.

Determination of Protein Quality by Biological Evaluation: The experimental procedure has been described by Eggum [27]. A total 15 albino rats, 6 weeks old, were used in the experiment. The animals were divided into control and two treated groups, each with five male rats, within average weight 70g. The experiment started by preliminary period of 4 days and balance period of 5 days. The rats were housed individually in plexiglas cages with stainless steel mesh bottoms in an environmentally controlled room. Temperature and relative humidity were maintained at 24°C and 60%, respectively, lighting 12hr day light/dark. The diets were composed of a N-free mixture (starch, 80.7%, sucrose, 8.9%, cellulose, 5.2% and fresh corn oil, 5.2%), vitamins (1%), minerals (4%) and dried Labneh cheese (control), dried Labneh cheese fortified with DLMO and casein as control were added to provide 10% protein in dry weight. Each animal around 10g dry matter (150 mg N) of diet daily. Body weight and diet intake were daily recorded up to the end period. During the balance period, urine and faces were collected separately. Total nitrogen was determined using the micro-Kjeldahl method and protein was calculated as nitrogen × 6.38 in the obtained diet, feces and urine. True protein digestibility (TD), Biological value (BV), net protein utilization (NPU) were used as biological indices and determined as already described, including appropriate correction factors.

The calculation was carried out according to Eggum [27] as follows:

$$TD = [N_i - (NF_1 - NF_2)] \div [N_i] \times 100$$

$$BV = [N_i - (NF_1 - NF_2)] - [(NU_1 - NU_2)] \div [N_i - (NF_1 - NF_2)] \times 100$$

Where: N_i, Nitrogen intake of animal fed test diet, NF_1, Nitrogen excreted in feces of animals fed test diet, NF_2, Nitrogen excreted in feces of animal fed protein-free diet, NU, Nitrogen excreted in urine of animals fed test diet, NU_2, Nitrogen excreted in urine of animals fed protein-free diet.

Net protein utilization (NPU) was calculated according to Eggum [27] as follows:

$$NPU = [BV \times TD] \div 100$$

RESULTS AND DISCUSSION

Data presented in Table 2 summarize the chemical composition of the Labneh fortified with DLMO (innovative Labenh). Addition of DLMO had considerable effect on TS, protein, carbohydrates and ash. The highest values were recorded with treatments fortified with 3% DLMO. This variation could be attributed to the high TS, protein, carbohydrates and ash in DLMO (Table 1). The addition of DLMO had significant effect on carbohydrates contents of Labneh compared to control. In contrast, addition of DLMO had no significant effect on fat contents of Labneh. TS ranged from 25.26–27.98% among different treatments and control. In addition, this consistent with the values reported by Tamime and Robinson [28] and Mehaia and El-Khadragy [29] who reported that TS of Labneh ranged between 22-26%. Moreover, the final total solids content of Labneh is 23-29 g/100g [30]. It could be noticed that protein was affected by level of addition. The increase in protein content was due to the presence of higher concentration of protein in Moringa leaves. The quality of protein in dry leaves of *Moringa oleifera* is better than all vegetable proteins and similar to egg and milk proteins as it contains all essential amino acids in appreciable amount manner [9, 11]. These leaves could be a great boon to people who

Table 2: Chemical composition and antioxidant activity (%) of Labneh cheese fortified with different levels of dry leaves of *Moringa oleifera*.

	Treatments*			
Tests	Control	T1	T2	T3
T.S	25.26^{B}	26.17^{B}	27.19^{A}	27.98^{A}
Fat	9.00^{A}	9.10^{A}	9.20^{A}	9.30^{A}
Protein	11.00^{A}	11.35^{A}	11.58^{A}	11.89^{AB}
Ash	0.85^{A}	0.97^{A}	1.09^{A}	1.14^{AB}
Carbohydrate	4.41^{C}	4.75^{BC}	5.32^{AB}	5.65^{A}
Antioxidant activity	17.14	29.82	40.54	54.21

*Control: Labneh cheese without dry leaves of *Moringa oleifera*.

T1: Labneh cheese with 1 % dry leaves of *Moringa oleifera*

T2: Labneh cheese with 2 % dry leaves of *Moringa oleifera*

T3: Labneh cheese with 3 % dry leaves of *Moringa oleifera*

A, B, C: Means with the same letter among treatments are not significantly different

do not get protein from meat. *Moringa oleifera* leaves are especially important for infant who are unable to make enough protein for their growth requirements and people who suffer from protein deficient [9, 11]. Fortification of diets with *Moringa oleifera* significantly improved the health of young children and pregnant women. Also, it is helpful in increasing breast milk in nursing mothers during breast feeding [31]. It was recommended that fortification of food from various parts of this miraculous tree should be carried out to rectify the malnutrition problems of the poor nations. *Moringa oleifera* leaves as rich protein sources which can be used by doctors, nutritionists and community health cautious persons to solve worldwide malnutrition or under nutrition problems [9, 32]. With regard to carbohydrate, it could be noticed that carbohydrate content of Labneh was significantly increased by approximately 20.63% and 28.11% in Labneh cheese supplemented with 2% and 3% DLMO respectively. Also, ash content increased by 28.23% and 34.11 % at 2% and 3% addition level in order. This variation could be attributed to the high carbohydrate and ash contents in dry leaves of *Moringa oleifera*.

Data presented in Table 2 show that Labneh fortified with DLMO had the highest values of antioxidant activity compared to control. In addition, antioxidant activity increased with increasing *Moringa oleifera* level of addition. This variation could be attributed to the high antioxidant activity in dry leaves of *Moringa oleifera* (Table 1). These results are in line with those reported by Sreealathe and Padma [10] and Ashfaq *et al*. [9], they reported that the extract of *Moringa oleifera* both nature and tender leaves have potent antioxidant activity against free radicals, prevent oxidative damage to major bimolecules and afford significant protection against oxidative damage. In addition, Moringa contains a range of fairly unique phytochemicals containing the simple sugar, hamnose and its rich in a fairly unique group of compounds called glucosinolates and isothiocyanates [9]. It was reported that the hydroalcoholic extract of the leave of *Moringa oleifera* contains 90 mg/g of β- sitosterol, 8 µg /ml and 27 µg /ml of total phenolic and flavonoid compounds, respectively [15]. Thus, the therapeutic potential of *Moringa oleifera* may be due to the presence of these major phytoconstituents.

The changes in total acidity are very important factor, since it affects the shelf life and the acceptability of Labneh. Based on the results presented in Fig. 1, it is evident that acidity values of Labneh increased during storage. The highest values were obtained with fortified Labneh when fresh and at the end of storage period. It could be concluded that dry leaves of *Moringa oleifera* had stimulatory effect on the starter culture and total count. Furthermore, Abbas and Osman [33] reported that the TA increased gradually during storage period. Moreover, Mahdian and Tehrani [34] discovered that high total solids content improved the growth and activity of starter cultures and increased acidity. In addition, increasing milk total solids had significant effect on decreasing rate of pH during fermentation [35]. The trend of the changes in pH values of all treatments was opposite to that of acidity.

From the data in Table 3, it could be noticed that adding DLMO to Labneh was accompanied by high levels of Ca, Fe, Zn and Si due to the high content of these elements in DLMO. Therefore, Labneh fortified with DLMO (T3) can be considered a good source of minerals Ca, Fe, Zn and Si. Iron content of T3 increased from 1.27 to 6.40 mg/100g while Ca, Zn and Si increased from (570 to 690), (1.52 to 3.54) and (0.170 to 0.220) mg/100g, respectively. *Moringa oleifera* is a miracle tree with a great indigenous source of highly digestible proteins, Ca, Fe and carotenoids [11, 12]. In addition, Owusu-Ansah *et al*. [13] reported that dried leaf powder of Moringa can serve

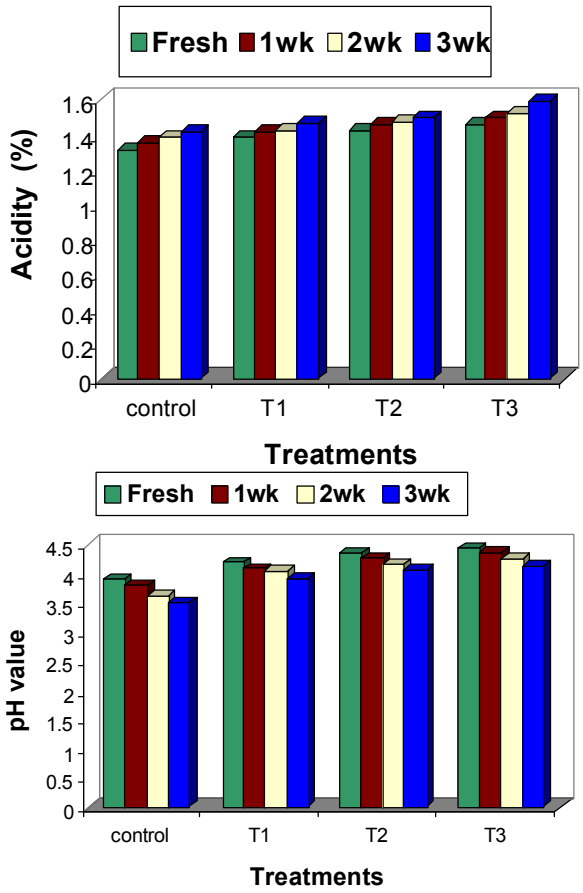

Fig. 1: Acidity (%) and pH values of Labneh cheese as affected by using different levels of dry leaves of *Moringa oleifera* during storage in a refrigerator 5±1°C.

as an excellent source of minerals. Some studies reported that the dry leaves of *Moringa oleifera* contain 17times calcium than milk and 25 times iron than spinach [31], 100 g of *Moringa oleifera* powder contains 2003 mg of Ca, 368mg of Mg 204 mg of P, 1324 mg of K, 28.2 mg of iron and 270 mg of selenium [36]. Vitamins content in fortified Labneh increased by increasing the addition level of DLMO Table 3. Vitamins A, B1, B2 and E increased by 85.7, 100, 63.15 and 1413.9% respectively. The increase in vitamins content was due to the presence of higher concentrations of these vitamins in the dry leaves of *Moringa oleifera*. According to some researchers, Moringa has the potential to combat Vit. A and other micronutrient deficiencies [37]. According to an analysis of 100 gm of fresh (raw) leaves and dried leaf powder of *Moringa oleifera* have been shown to contain as much of the following water soluble vitamins 2.6 mg of Vit. B1 (thiamin), 20.5 mg of Vit.B2 (riboflavin), 8.2 mg of Vit.B3 (nicotinic acid) and 220mg of Vit. C. In addition this same portion contains as much of the following fat- soluble vitamins 16.3 mg of Vit. A and 113mg of Vit. E (alpha tocopherol acetate [36].

Microbiological Examination: The results in Table 4 showed the changes in total count of different treatment of Labneh products during storage period at refrigerator temperature 5±1°C. The results indicated that there is a gradual increase was observed throughout the storage period in all Labneh cheeses. The results indicate that the total bacterial count were higher in Labneh fortified with dry leaves of *Moringa oleifera* compared to control. The obtained results suggest that bacterial population was stimulated by adding dry leaves of *Moringa oleifera*. This may be due to high nutritional composition of dry leaves of *Moringa oleifera* such as carbohydrates, proteins, minerals and vitamins. Moreover, increases in the level adding (DLMO) led to enhance in bacterial count. On the other hand, the high bacterial count of such treatments may be due to contamination from the DLMO added.

Table 3: Minerals (mg/100g) and vitamins (µg/100g) contents of Labneh cheese fortified with 3% of dry leaves of *Moringa oleifera* (T3).

	Treatments	
Character assessed	Control	T_3
	Minerals (mg/100g)	
Ca	570	690
Fe	1.27	6.40
Zn	1.52	3.54
Si	0.17	0.22
	Vitamins (µg/100g)	
Vit.(A)	77	143
Vit.(B1)	120	240
Vit.(B2)	380	620
Vit.(E)	43	651

Table 4: Microbiological analysis (log cfu/g) of Labneh cheese fortified with different levels of dry leaves of *Moringa oleifera*.

Properties	Storage period	Treatments*			
Total Count (TC	Fresh	Control	T1	T2	T3
	1wk	7.27	7.41	7.47	8.15
	2wk	7.34	7.45	7.56	8.29
	3wk	7.41	7.53	7.68	8.38
Moulds &Yeast	Fresh	7.53	7.75	7.76	8.42
	1wk	ND**	ND	ND	ND
	2wk	ND`	ND	ND	ND
	3wk	ND	ND	ND	ND
Coliform	Fresh	2.58	ND	ND	ND
	1wk	ND	ND	ND	ND
	2wk	ND	ND	ND	ND
	3wk	ND	ND	ND	ND

* see Table 2.

**Not detected

Table 5: True digestibility (TD), biological values (BV) and net protein utilization (NPU) parameters of Labneh cheese fortified with 3% of dry leaves of *Moringa oleifera*

	Experimental diets		
Properties	Casein diet	Labneh control diet	Labneh + Moringa diet
TD	97.68	86.48	90.07
BV	85.76	80.20	83.35
NPU	83.81	69.40	75.00

Mould and yeast counts are considered indicative of the quality and shelf life of Labneh. In this regard, moulds and yeasts were not detected in fresh Labneh of all treatments and throughout the storage period. This may be due to *Moringa oleifera* leaves had antifungal and antimicrobial activities. *Moringa oleifera* has been reported to contain phenolic and flavonoid compounds that are primarily responsible for their antimicrobial properties [15]. However, moulds and yeasts were detected in control at the end of storage period. Notably, coliforms were not detected in fresh Labneh and during storage period in all resultant Labneh, which indicated the good hygienic condition followed in its production.

Biological Evaluation: The parameter of true digestibility (TD), biological values (BV) and net protein utilization (NPU) are investigated in Table 5 against the reference of casein diet. The TD values of Labneh fortified with DLMO (90.07) and without DLMO (86.48) were lower than that for casein diet (97.68). The biological value (BV) 85.76 was obtained for rats on the reference protein. A noticeable decrease was observed in BV in Labneh fortified with DLMO (83.35) and followed by Labneh control (80.20). Our results indicated that adding DLMO increased BV. The values of BV of the Labneh fortified with DLMO might be related to the quality of added ingredient.

Regarding to NPU, the results in Table 5 show that the NPU value of Labneh under study were lower than that of casein diet. However, casein diet recorded highest value (83.81) followed by Labneh fortified with DLMO (75.00) then control (Labneh without DLMO) (69.40). The high NPU value of Labneh fortified with DLMO may be attributed to the well pattern of amino acids which make by their utilization more efficient and highly digestible. It is clear from Table 5, that TD, BV and NPU were higher in Labneh fortified with DLMO than control Labneh and near to the result obtained for casein diet. This could be due to excess of digestibility protein and essential amino acids. Some studies reported that the dry leaves of *Moringa oleifera* contain 9 times protein than yoghurt, which can be used by doctors nutritionists and community health cautious persons to solve worldwide malnutrition or under nutrition problems [9, 11, 31, 32]. Moreover, Moringa leaves contain all essential amino acids in a good proportion. These leaves could be a great boon to people who do not get protein from meat [9, 38]. *Moringa oleifera* leaves meal possess good dietary protein quality for optimal growth of rabbits and be incorporated in the rabbits diets up to 15% inclusion levels without any detrimental effects on the performance, hematology, serum biochemistry and carcass and organ weights of growing rabbits [39].

Table 6: Sensory evaluation of Labneh cheese fortified with different levels of dry leaves of *Moringa oleifera*

	Treatments*			
Sensory attributes	Control	T1	T2	T3
		Fresh		
Flavor (50)	47^{Aa}	46^{ABa}	45^{BCa}	44^{Ca}
Body&Text. (40)	38^{Aa}	37^{Aa}	37^{Aa}	36^{Bab}
Appearance (10)	10^{Aa}	10^{Aa}	10^{Aa}	9^{Aa}
Total score (100)	95^{Aa}	93^{Aa}	92^{ABa}	89^{Ba}
		One week		
Flavor (50)	46^{ABa}	45^{ABab}	44^{BCab}	43^{Cab}
Body&Tex. (40)	37^{Aa}	37^{Aa}	36^{Aab}	35^{Bab}
Appearance (10)	9^{Aab}	9^{Aab}	9^{Aab}	8^{Aa}
Total score (100)	92^{Aab}	91^{Ab}	89^{ABb}	86^{Bb}
		Tow weeks		
Flavor (50)	45^{Aab}	45^{Aab}	43^{ABbc}	42^{Bbc}
Body&Tex. (40)	36^{Aab}	36^{Aab}	36^{Aab}	34^{Bb}
Appearance (10)	9^{Aab}	8^{Aab}	8^{Aab}	8Aa
Total score (100)	90^{Ab}	89^{ABc}	87^{Bc}	84^{Cc}
		Three weeks		
Flavor (50)	42^{ABb}	43^{Abc}	42^{ABc}	41^{Bc}
Body&Tex. (40)	35^{Aab}	35^{Aab}	34^{ABb}	33^{Bc}
Appearance (10)	8^{Ab}	8^{Ab}	8^{Aab}	7^{Ab}
Total score (100)	85^{ABc}	86^{Ad}	84^{Bd}	81^{Cd}

*see Table 2.

A, B, C: Means with the same letter among treatments are not significantly different (P=0.05)

a, b, c: Means with the same letter during storage period are not significantly different (P=0.05)

Sensory Evolution: As can be seen from Table 6 results of organoleptic properties revealed that addition of DLMO had significant effect on flavour, appearance, body & texture and total score. As the concentration of DLMO was increased in fortified Labneh the score of flavour, body & texture, appearance and total score was decreased. In general, all Labneh made with different level of DLMO had acceptable flavour, body & texture and appearance during storage period. The addition of DLMO tended to make the color (appearance) greener. However the people are already use green leaves of coriander& parsley and other herbs in Labneh.

CONCLUSION

Therefore it can be conclude that DLMO can be used in fortification of Labneh to increase its nutritional and biological values without marked effect on its acceptability up to 2%. In addition, it can be serves as innovative Labneh.

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