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EFFECT OF VARIATION IN REGION AND SEASONS ON SENSORY, CHEMICAL AND MICROBIA CHARACTERISTICS OF LABNEH MANUFACTURED BY TRADITIONAL METHODS

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ABSTRACT

This work investigated the chemical, physical, microbiological, and sensory characteristics of labneh, made from fresh cow's milk in the northern, middle and southern regions of Jordan, in the spring and summer seasons. The chemical composition (%) fell within the limits specified for labneh by the Jordanian standards. . where the data shows that the mean moisture levels ranged from 72.40 - 73.22 % , fat between 8.73 - 9.38 and protein between 12.66 – 13.30,for the samples from the northern, middle and southern regions, respectively. also the results for all chemical composition values, showed no significant ($p>0.05$) differences between both the seasons and the regions .

Palmitic acid (33.32–36.22%) was the predominant fatty acid, followed by oleic acid (19.68–23.34%). fifty-six samples contained coliforms, *Escherichia coli* and yeast, due to the production method. However, all samples were free of *Salmonella and Staphylococcus aureus.* The sensory evaluation results showed that all labneh samples met the panellists' satisfaction, Where the data showed that the scores for all the samples ranged between 3.0 - 4.03 of 5 scores. for flavour , texture , appearance and colour.

1.Introduction

Labneh (concentrated yogurt) is a traditional fermented milk product manufactured by draining away a proportion of the yogurt water, using a cheese cloth bag at 6 °C, until reaching the desired total solids (23–25%). Under these conditions, the titratable acidity of labneh reaches 1.8 – 2.0% (as lactic acid), while the fat content is around 9 – 11% (KÖse and Ocak, 2011). Labneh has a cream or milky white appearance, a soft and smooth body, and good spread ability, with minor syneresis and a slightly acidic flavour (Nsabimana *et al*., 2005).

Labneh is regarded as one of the most popular foods, in various parts of the world. It plays a significant role in the family diet, particularly in the Middle Eastern countries and the Balkans (Haddad *et al*., 2007; El-Salam, 2011; KÖse and Ocak, 2011). Moreover, it is considered an intermediate product between sour cream and unripe cheeses, with a characteristic sharp acidic flavour that is modulated by diacetyl, produced during fermentation (Haddad *et al*., 2007).

The popularity of labneh has increased around the world due to the rise in consumer awareness. Its nutritional benefits and storage characteristics have increased its economic importance and nutritional value. Labneh is valued more than yogurt because it has 2.5-fold higher protein content and 50% more minerals (Nsabimana *et al*., 2005). In addition, the lactose content in labneh is low (approximately 6%) due to its fermentation into lactic acid by lactic acid bacteria. Thus, it can be consumed by lactose intolerant patients (Özer and Robinson, 1999; Nsabimana *et al*., 2005). Furthermore; there is a global interest in probiotic microorganisms, which can enhance the health benefits of dairy products (e.g. labneh). Consequently, these products can be considered functional foods (Rocha, et.al. 2014; Songtummin and Leenanon, 2016).

Labneh that is prepared by the traditional method, with total solids around 22%, has a short shelf life of about 2 weeks, even if refrigerated (Tamime and Crawford, 1984; Shamsia and El-Ghannam, 2012). This may be due to the sanitary problems that are usually associated with the cloth bags used in its production and the unhygienic handling of the product, which increases the microbial contamination (El-Samragy, 1997). The high microbial load of labneh coupled with the packaging and storage conditions result in the formation of off-flavours and undesirable physicochemical changes that eventually lead to rejection of the product (Otaibi and Demerdash, 2008). Burt, 2004 mentioned that there is a possibility to extend the shelf life of perishable food products, such as labneh, through the use of bio-preservatives such as essential oils. In this regard, the most common way to extend labneh shelf life is to increase its solid concentration up to 40%, then, store it in vegetable oil at room temperature. This would extend the expiry date up to 2 years (Kecel*i et. al*., 1999; Otaibi and Demerdash, 2008).

This study focuses on the chemical, microbiological and sensory characteristics of Jordanian labneh, made from cow's milk from three different Jordanian regions (northern, middle and southern) in spring and summer seasons.

2. Materials and methods

2.1.Materials

2.2.1.Samples collection

One-hundred-and-eighty samples of labneh, made from fresh cow's milk, were collected from northern, middle and southern regions of Jordan (60 samples from each region). Labneh samples were collected in two seasons (30 samples in spring and 30 samples in summer). The collection process always took place on the same day of production. Chemical and microbiological analysis, in addition to sensory evaluation, were conducted on the collection day.

2.2. Chemical analysis

Water content, fat and crude protein (N × 6.38) were determined according to AOAC, 2000 methods. Titratable acidity (expressed as a percentage of lactic acid), was determined as described by Marshall (1992).

All determinations were carried out on duplicate samples.

Fat from Labneh was extracted using chloroform-methanol (2: 1; v/v) according to the Modified Folch's technique (Prandini et al., 2007, and then converted into fatty acids methyl esters (FAME) according to IUPAC (International Union of Applied and Pure Chemistry) method (Commission Regulation (EC) No 796/2002 of 6 May 2002.

Fatty acids profiles were determined using a gas chromatography (variancp -3800, Japan) equipped with a flame ionization detector and restek capillary column(50m×0.25mm×0.25µm thickness). The column oven temperature was programmed: the initial temperature was165 °C and held at this temperature for 10 min, then temperature was increased to 180 ° C at a rate of 10 ° C /minute and remained at this degree for 30 minutes and then rose to 240 ° C, then sample injection volume 1 µl (injector temperature was 240 °C) and the flame ionisation detector temperature rises to 260 °C. High purity nitrogen gas was used as the

mobile phase and flow rate was 1.5 mL min–1. The FAMEs were identified using a chromatogram of fatty acid standards (Sigma Chemical Co.). Peak identification was achieved by retention time and comparing with reference standards. Peak areas were measured with an HP computing integrator. Data were expressed as percentages of total FAME content.

2.3. Microbial analysis

Total Coliform numbers were enumerated by the pour plate technique from the diluted samples (dilution of 1 g of sample in 9 mL of sterile distilled water with 0.8% NaCl), then, cultured on violet red yellow agar (Scharlau Chemie, Spain) and incubated at 37 °C for 24 h.

Salmonella spp. were isolated, after enrichment in selenite cystine broth (SC), at 37 °C for 24 h, then plated on xylose lysine deoxycholate agar and incubated at 37 °C for 24 – 48 h.

Staphylococcus aureus was isolated from the diluted samples on Baird-Parker agar, with egg yolk tellurite emulsion (Scharlau Chemie, Spain) and then incubated at 37 °C for 48 h.

Yeasts and moulds were also isolated by the spread plate technique, using potato dextrose agar at 25°C for 5 days. Furthermore, *Escherichia coli* bacteria were isolated by the most probable number method, using three tubes per dilution (10^{-1}, 10^{-2}, and 10^{-3}), containing 9 mL of lauryl tryptose broth (Scharlau Chemie, Spain). All tests were duplicated for each isolated bacterial species (Yousef and Carlstrom, 2003).

2.4. Sensory evaluation

Sensory evaluation was performed by 12 specialists in the field of food, using a 5-point hedonic scale (1 = dislike too much, 2 = dislike moderately, 3 = neither like nor dislike, 4 = like and 5 = like very much) (Meilgaard et al., 2006). The sensory characteristics of the samples that were evaluated included the appearance, flavour, colour and texture.

3. Results and discussion

3.1. Chemical composition of labneh

Table 1 shows the chemical composition of the labneh samples. The data shows that the mean moisture levels were 72.40 - 73.22, 73.00, and 72.90 – 73.00 % for the samples from the northern, middle and southern regions, respectively. In the same order of the regions, the samples contained between 9.25 - 9.38, 8.73 - 8.83, and 8.98 -9.05% fat, and correspondingly, 12.74 – 12.68, 12.66 – 13.30, and 12.90 – 13.03% protein. Also, the acidity value of the samples from the northern, middle and southern regions ranged from 1.51 - 1.58, 1.48 - 1.58, and 1.54 - 1.87%, respectively, and the corresponding total solids, as dry matter, ranged from 26.48 -27.59, 26.93 - 27.00, and 27.10 - 27.85%.

The aforementioned results, for all chemical composition values, showed no significant ($p>0.05$) differences between both the seasons and the regions. Furthermore, the chemical values were within the acceptable limits of the Jordanian legislation standard 108/2003 (JS, 2003), which states that labneh content should not contain less than 9%, 12%, 23% and 2.5% fat, protein, total solids and titratable acidity, respectively.

The results of this study were in agreement with results reported by previous researchers. For instance, the values of fat, protein, total solids and acidity found by Thabet et. al. (2014), were 8%, 11%, 23% and 1.4%, respectively, while Atamian et. al. (2014) reported corresponding values of 9%, 8%, 26% and 1%, respectively. For the same parameters, Tamime et al.(1991) reported 11.0%, 8.23%, 24.23% and 1.46%, respectively. The differences in the mentioned values might be due to many factors, such as the animal breed and its feeding and processing conditions.

There were no significant differences among the samples collected from the middle region, except for the fat percentage, which was less than required by the Jordanian standards (JS, 2003).

Also, previous studies have found

similar fat contents, reporting values ranging from 5.51% 11.61% (KÖse and Ocak, 2011; SÖMER and KiliÇ, 2012).

3.2. Fatty Acids content of labneh

The fatty acids in milk are derived from two sources, the feed and the microbial activity in the rumen of the cow (Parodi, 2004).

There are many factors that are related to variations in the fatty acid profile of cow milk lipids, such as animal origin, genetics, stage of lactation, mastitis and ruminal fermentation, or they may be feed-related factors including fibre and energy intake, dietary fats, and seasonal and regional effects (Jensen, 2002).

Table 1. Chemical composition of labneh produced in various regions of Jordan, in two seasons.

Region	Season	Moisture (%)	Fat (%)	Protein (%)	Acidity (%)	Total solids (%)
North	Spring	72.40±4.31^a	9.25±1.32^a	12.74±2.34^a	1.51±0.31^b	27.59±3.11^a
	Summer	73.22±3.11^a	9.38±1.67^a	12.68±3.10^a	1.58±0.21^b	26.48±2.98^a
Middle	Spring	73.00±4.92^a	8.73±1.85^a	13.03±2.98^a	1.47±0.32^b	27.00±3.24^a
	Summer	73.00±3.33^a	8.83±1.93^a	12.66±2. 65^a	1.58±0.22^b	26.93±3.33^a
South	Spring	73.00±4.21^a	9.05±1.56^a	13.03±2.87^a	1.87±0.42^a	26.85±2.67^a
	Summer	72.90±3.98^a	8.98±1.94^a	12.90±2.32^a	1.94±0.41^a	27.10±2.45^a

Values are averages of two repetitions ± standard deviation values in the same columns in the same season followed by different letters are significantly different ($p<0.05$).

Table 2 displays the fatty acid profile of labneh. The fatty acid content of labneh varied according to the region and season. The results revealed that the most abundant fatty acid in labneh was palmitic acid (C16:0), which ranged from 33.32 – 36.22 g/100 g lipid, followed by oleic, myristic, stearic, linoleic, capric,palmitoleic, caprylic, caproic, and, finally, linolenic. Generally, the results of this study were in agreement with Mirelle and Mattar (2015). Also, the fatty acid profiles of all the labneh samples were within the acceptable limits of the Jordanian food legislation for labneh (JS, 108/2003) which indicated that the fat in the labneh is of animal origin.

The minor differences between the fatty acids content of labneh samples were due to the region from which the samples were collected and the season in which the sample were collected. Published research has shown that milk obtained from cows fed on fresh green forage, particularly those that graze grass, had a significantly higher level of unsaturated fatty acids(Alqaisi*et al*. 2013)

In Jordan, cows rely on the use of concentrated feed. Therefore, the absence of grassland has led to the dominance of concentrated feed in cow's diet (Jordanian Ministry of Agriculture, 2016).

3.2. Microbial analysis

As stated in the Jordanian standard 108/2003, labneh products should be free of pathogenic microorganisms, and the total coliforms count should not be more than 10 CFU/g. The existence of coliforms in labneh, indicates the substandard hygiene conditions during conventional processing, which lead to recontamination at one or more stages of processing, together with the possibility of contaminates in the water used during labneh processing, by the same microorganisms. Moreover, these contaminations have a negative effect on the consumer's health, as well as the general public health (SÖmer and KiliÇ, 2012).

The mean values of the general microbial counts of labneh, collected from the various regions and in different seasons, are presented in Table 3. The data show that *Salmonella* spp. and *S. Aureus* ,were not detected in all the labneh samples collected from the three

regions. However, the results show that some samples (in all regions and for both seasons) contained total coliforms, and *E. coli*, in addition to yeasts.

The numbers of samples containing total coliform from the northern, middle and southern regions were 6, 5, and 9, respectively, of which 4, 4, and 7 contained *E. coli*. The coliform load ranged from $1.0 – 1.5 \times 10^3$ CFU/g in the northern region in spring, up to $7.0 – 7.3 \times 10^3$CFU/ g in the southern region in the summer. AL-kadamany *et al.* (2002) reported that the coliforms were detected at a level of 1.5×10^2 CFU/g These results showed that the coliform contaminations were high exceeded the limit reported in the Jordanian standard specification108/2003(JS,2003), particularly in summer .

Table 3. Microbiological characteristics of labneh produced in different region of Jordan, in two different seasons.

Types of microbes	**North region *No. of total sample 58/n**		**Middle region *No. of total sample 58/n**		**South region *No. of total sample 58/n**	
	1	2	1	2	1	2
Salmonella	ND	ND	ND	ND	ND	ND
E.colicfug^{-1}	2-3 x 10^2 1n	3 -4 x 10^2 3 n	1-1.2 x 10^2 2 n	1.5-2 x10^2 2 n	3-4 x 10^2 3n	4-5 x 10^2 4n
Total coliform cfug^{-1}	1-1.5x10^3 2 n	2-3 x10^3 4n	2.2-x10^3 2n	2.8-.5x10^3 3n	5.6-6 x10^3 4n	7-7.3 x10^3 5n
Staphylococcus *aureus*	ND	ND	ND	ND	ND	ND
Yeast cfug^{-1}	3-4 x10^3 5n	5-6 x10^3 6n	4-5 x10^3 5n	4.5-5.2x10^3 7 n	5.2-5.6x10^3 6n	6-6.5 x10^3 8n

*No. of samples from north, n: no. of the samples detected *Season 1=spring (29 samples), 2= summer(29 samples)

Table 4. Sensory attribute scores of labneh produced in various regions of Jordan, in two different seasons

Region	**Season [a]**	**Attributes**			
		Appearance	**Color**	**Flavor**	**Texture**
North	1	3.90±0.32^a	3.98±0.41^a	3.72±0.31^{ab}	3.65±0.41^a
	2	3.82±0.25^a	3.87±0.37^a	3.71±0.33^a	3.58±0.26^a
Middle	1	3.71±0.37^a	3.60±0.33^a	4.03±0.25^a	3.25±0.12^a
	2	3.87±0.42^a	3.78±0.40^a	3.75±0.43^a	3.37±0.33^{ab}
South	1	3.00±0.30b	3.16±0.26^b	3.50±0.21^b	3.45±0.22^a
	2	3.90±0.32^a	3.00±0.21^b	3.00±0.23^b	3.00±0.14^b

Values are averages of two repetitions of 12 panelists ±standard deviation.

Values in the same columns in the same season followed by different letters are significantly different ($p<0.05$), according to Duncan's multiple range test.

[a]1 = spring, 2 = summer.

Table 2. Fatty acid profile (g/100 g lipid) of labneh produced in various regions of Jordan, in two seasons.

Region	Season[a]	Caproic C6:0	Caprylic C8:0	Capric C10:0	Lauric C12:0	Myristic C14:0	Palmitic C16:0	Palmitoleic C16:1	Stearic C18:0	Oleic C18:1	Linoleic C18:2	Linolenic C18:3
North	1	1.91±0.21^{ab}	1.21±0.03^{b}	3.05±0.65^{a}	3.72±0.76^{a}	11.61±2.31^{ab}	34.77±4.24^{ab}	1.69±0.12^{ab}	9.64±1.23^{b}	20.29±3.53^{a}	3.70±1.03^{a}	0.36±0.01^{b}
	2	1.98±0.13^{a}	1.27±0.06^{a}	3.08±0.72^{a}	3.74±0.64^{a}	11.84±1.87^{a}	34.95±3.45^{a}	1.93±0.08^{a}	8.73±1.11^{b}	19.77±2.33^{ab}	3.05±0.98^{b}	0.33±0.01^{b}
Middle	1	1.93±0.15^{a}	1.27±0.11^{a}	2.64±0.57^{b}	3.57±0.85^{ab}	11.18±2.32^{b}	33.32±4.26^{b}	2.06±0.42^{a}	8.23±1.31^{b}	21.34±2.89^{a}	3.39±1.05^{ab}	0.35±0.01^{b}
	2	1.98±0.33^{a}	1.14±0.05^{b}	2.95±0.98^{ab}	3.75±0.74^{a}	11.63±2.12^{ab}	35.08±3.66^{a}	1.62±0.26^{ab}	7.51±1.02^{bc}	18.91±2.64^{b}	3.28±0.89^{ab}	0.48±0.01^{ab}
South	1	1.89±0.26^{ab}	1.20±0.03^{b}	2.58±0.99^{b}	3.37±0.59^{b}	11.55±1.87^{ab}	36.22±4.21^{a}	1.67±0.22^{ab}	9.85±1.37^{b}	19.68±3.22^{ab}	3.59±0.96^{a}	0.44±0.01^{ab}
	2	1.86±0.32^{b}	1.17±0.04^{b}	2.73±0.89^{b}	3.23±0.73^{b}	11.17±1.95^{b}	34.19±2.78^{a}	1.41±0.12^{b}	11.57±2.34^{a}	20.60±2.45^{a}	3.56±1.11^{a}	0.56±0.01^{a}

Values are averages of two repetitions ± standard deviation.

Values in the same columns in the same season followed by different letters are significantly different (p 0.05) Duncan's multiple range test.

[a] 1 = spring, 2 = summer.

There were 11, 12, and 14 samples containing yeast, in the northern, middle and southern region, respectively, and the yeast load ranged from 3.0 - 4.0 × 10^3CFU in the northern region in spring to 6.0 – 6.5 × 10^3CFU in the southern region in summer. The values in this study revealed were lower than the values detected by Muir and Banks (2000), which were ≥ 10^6CFU/ml, as well as Yamani and Abu-Jaber (1994) of >10^6CFU. Yamani and Abu-Jaber (1994) documented 2.6 × 106 and 4.4 × 10^6CFU, in the northern and southern regions, respectively, whereas, in the current study, the yeast loads were comparatively lower, ranging from 1.0 - 1.5 × 10^3CFU in the northern region and up to 7.0 - 7.3 × 10^3CFU in the southern region. Furthermore, Yamani and Abu-Jaber (1994) claimed that yeasts were the main cause of labneh spoilage since they provided ideal conditions for the growth of yeast, such as high concentration of lactic acid and little of air during refrigerated storage.

It is known that Middle Eastern consumers prefer labneh that is prepared by traditional methods, even though the yeast flavour may be present. Somer and KiliÇ (2012) mentioned that the transfer of yoghurt from cloth bags, which are used for straining, to the packaging materials, may contribute to contamination of labneh with fungi and coliforms. In addition, the hygienic conditions of the packaging material, storage, manufacturing place, practices of the workers and market places would increase microbial contamination which lead to rejection of the product(Otaibi and El.demerdash,2008).

3.4. Sensory evaluation

The sensory attributes studied were appearance, flavor , colour and texture.

A 5-point hedonic scale was adopted, to obtain the scores. The mean sensory attribute scores for the samples obtained from the various regions and both seasons are shown in Table 4. The data showed that the appearance and colour of the samples collected from northern and middle regions in both seasons, scored higher, and were significantly different ($p \leq 0.05$) than the samples obtained from the southern region.

The scores for all the samples ranged between 3.0 - 3.9. The flavour scores of samples for all the regions and both seasons ranged between from 3.00 to 4.03. However, the labneh samples collected from the northern and middle regions of the two seasons were significantly different ($p \leq 0.05$) than the southern region. This may be due to the higher acidity value of the labneh from the southern region. The texture of all labneh samples scored 3.00 - 3.65.

The scores also showed that there was no significant difference detected in the texture, among the samples from all regions in spring. However, the northern region scored a higher value in the summer, matching the results in Table 1, which showed these samples had the highest total soluble solids. This result concurred with Mahdian and Tehrani (2007), who reported that the texture acceptability increased significantly with increasing total solids. 4.

4.Conclusions

The labneh that was produced in various regions was within the acceptable limits of the Jordanian standards. The predominant fatty acid in labneh was palmitic acid (C16:0), ranging from 33.32 – 36.22 g/100 g lipid, followed by oleic acid. Also, no significant variations ($p > 0.05$) in the average values of caproic acid (C6:0) and palmitic acid (C16:0) were observed, whereas the remaining fatty acids in labneh showed only minor variations. Moreover, the microbiological analysis revealed that some samples of labneh contained coliforms, E. coli and yeast, indicating poor hygienic conditions. However, all the samples were free of pathogenic bacteria (*Salmonella* spp. and *S. aureus*). Regarding the sensory evaluation, the labneh was considered acceptable by all the panellists.

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