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Investigation of the Antimicrobial Preservatives in the Dairy Product (Labneh)

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Abstract
The aim of this study was to investigate the presence and levels of antimicrobial preservatives (natamycin, benzoic acid and sorbic acid) in the dairy product, Labneh. One hundred and fifty Labneh samples of 10 different trademarks were collected from the Jordanian markets in January, April and July 2013 to be analyzed. The obtained results indicated that 30% of the samples contained natamycin, 20% contained sorbic acid, 10% contained benzoic acid, and none of the detected preservatives were found in 40% of the samples. Results revealed that the highest level of antimicrobial preservatives detected in the Labneh samples was benzoic acid, which ranged from 5.70 to 365.4 mg/kg, while natamycin ranged from 11.74 to 76.98 mg/kg and sorbic acid ranged from 77.08 to 321.6 mg/kg. None of the samples contained more than one preservative. This study demonstrated preservative addition and amount was made with no consideration of seasonal variation. Results also showed that Labneh samples produced in facilities that apply HACCP systems met the required conditions without the addition of any preservatives.
Keywords: antimicrobial preservatives, natamycin, benzoic acid, sorbic acid, dairy product, Labneh

1. Introduction

The dairy product, Labneh, is defined as a milk product obtained by fermentation of milk or its products by a suitable and pure culture of lactic acid bacteria with the resulting product then being concentrated (JISM, 2003). It is also known as a semi-solid product obtained from yogurt which is concentrated by the removal of whey (Mihyar et al., 1999). Production of Labneh in Jordan is still carried out by the traditional method, which includes setting of yoghurt in permeable cheese cloth bags which are hung in refrigerators until the desired concentration is obtained. Addition of preservatives is not permitted (JISM, 2003). On the contrary, some dairies in Jordan tend to add natamycin to the Labneh product to protect it from spoilage caused by yeasts and molds. Nantamycin has recently been added as an alternative to benzoic and sorbic acids which were used previously. This can mislead the food control authorities in Jordan, since inspection program is designed to detect the addition of sorbic and benzoic acids and their salts to Labneh for the purpose of restricting the dairies to abide by the Jordanian standard of Labneh that do not allow the addition of any preservative agents including natamycin to Labneh (JFDA, 2010). Natamycin (E235), is also called pimaricin, is a naturally occurring antimicrobial agent produced by the bacterium Streptomyces natalensis and related species. It is commonly added to some dairy-based products in order to control spoilage by fungi(Eps et al., 2002; FSANZ, 2004; EAEMP, 1998). Natamycin, which is specified as a food additive for surface treatment of cheese, is an antibiotic that specifically inhibits the growth of yeast and mold, and is also used in a variety of foods and beverages (FAAM, 2005), and is permitted as a food additive for surface treatment of certain cheeses (EAEMP, 1998). Natamycin exhibits a broad spectrum of activity against spoilage due to fungi and yeast-like organisms including strains pathogenic to man; It is a very stable product which is effective against Aspergillus flavus and aflatoxin production (Rusul and Marth 1988; Raab 1972). It has no effect on the bacteria used in the fermentation and maturation processes of such dairy products (Adams, 2008). Regarding absorption, distribution, and excretion or metabolism of natamycin in the body, less than 1 µg/ml could be detected in the blood following ingestion of 500 mg by human subjects (ANONYMOUS, 1968). This helps to substantiate the statement that natamycin is not absorbed from the gut in animals or man. Joint FAO/WHO Expert Committee on Food Additives (JECFA) reconfirmed the existing acceptable daily intake (ADI) of 0-0.3 mg/kg/body weight (Raab, 1972). On the other hand, Jordanian standards do not allow the addition of any preservative agent to any dairy products (JISM, 2003).

Benzoic and sorbic acids and their salts are considered as a permitted food preservatives in many types of foods, and according to the U.S. food and drug administration (FDA) these acids and their potassium and sodium salts are generally recognized as safe (GRAS), therefore, determination of the levels of benzoic and sorbic acids in dairy products including Labneh is necessary in order to ensure that they are within the permitted limits (Mihyar et al., 1999). The addition of benzoic and sorbic acids and their salts are effective in inhibiting yeast and mould

growth. They also exhibit inhibitory effects against a wide range of bacteria. Benzoic acid occurs naturally in yoghurt, but there is no reference to its deliberate use in the preservation of yoghurt(CAC, 2001).

The main objectives of this study were to investigate whether the Jordanian dairies comply with the Jordanian standard of Labneh, regarding the addition of natamycin, benzoic acid and sorbic acid, to ensure Labneh products available in the markets are free from preservatives. An additional objective was to evaluate the influence of seasonal variation and the application of HACCP system on Labneh production.post until a replacement is found. If such disturbance has caused a large number of tasks become unattended and overdue, the company is then vulnerable to overtime cost, shrunk capacity and productivity, extra queuing time, lost business income, etc. In order to prevent these deteriorative effects, optimising the number of workers can be helpful. As a fundamental branch of knowledge in manufacturing business, workforce management will never fall behind the times. Therefore, it is worth an attempt to incorporate a novel methodology, such as HMS, into the state of the art of workforce sizing.

2. Material and Methods

2.1 Samples Collection

One hundred and fifty samples of Labneh from ten dairy plants were collected from the Jordanian markets during at 3 times during the year (January, April and July, 2013). Fifty samples were collected during each month; five sub-samples from the same trademark of each month were analyzed. Only sixty of the Labneh samples were produced by four local dairy plants that apply HACCP system. The collected samples were sent directly to the laboratory under refrigeration (2-4 ˚C) conditions and kept refrigerated until analysis. Analysis was carried out on the last day of the shelf life period (15 days) recorded by the producer.

2. 2 Determination of Natamycin

High-performance liquid chromatography (HPLC) was used to determine the concentrations of natamycin according to the procedure described by the ISO (2007). Briefly, 5.00 g of test sample was weighed into 100 ml volumetric flasks. Then, a 50 ml of methanol:water (2:1) was added to the mark and sample was shaken for 30 min by vortex mixer. The extract was filtered (pore size 0.45 µm) by syringe into an HPLC vial. The first drops of filtrate were discarded. The clear supernatant was injected into a HPLC system (PerkinElmer Series 200, USA) at an injection volume of 10µl. Chromatographic separation was performed using a Ksomosil 100 C8 (5 µm, 250mm × 4.6mm) (Teknokroma, Spain) column and DAD detector (Series 200 EP) with an isocratic mobile phase of methanol: water (70:30). Glacial acetic acid (1 ml) was added to the mobile phase. The mobile phase was filtered through a 0.45 µm nylon membrane (Whatman, UK) and degassed. The used mobile phase was eluted at flow rate of 1 ml/min and detected at a UV wavelength of 303 nm.

2. 3 Determination of Benzoic and Sorbic Acids

The concentrations of benzoic and sorbic acids in Labneh were determined by the HPLC technique according to the method described by Saad et al. (2004). Briefly, 5.00 gm of test sample were weighed into 50 ml volumetric flasks. Then, 50 ml of ethanol:water (70:30) sufficient quantity was added and sample was shaken for 30 min by vortex mixer. The extract was filtered through a nylon membrane microfilter (pore size 0.45 µm, Whatman, UK) by syringe, into an HPLC vial. The first drops of filtrate were discarded. The clear supernatant was injected into an HPLC system (PerkinElmer Series 200) at injection volume of 10 µm. Chromatographic separation was performed using a Brownlee Analytical C18 (5µm, 250mm × 4.6mm) (PerkinElmer, USA) column and DAD detector (Series 200 EP) with a mobile phase of methanol: acetate buffer (60:40). The pH adjusted to 4.0 by using glacial acetic acid. The mobile phase was filtered through a 0.45 µm nylon membrane (Whatman, UK) and degassed. The mobile phase used was eluted at a flow rate of 1 ml/min and detected at an Ultraviolet (UV) wavelength of 230 nm. Figure 2 shows LC chromatogram of standard solution of benzoic and sorbic acid (25 µg/ml of each).

2. 4 Statistical Analysis

Statistical analysis was conducted according to SAS statistical program version 8 , where data presented are the mean of two replicates and standard deviations. Differences between the means were tested using the Least Significant Difference (LSD) test at $p \leq 0.05$.

3. Results and Discussion

3.1 Addition of Natamycin

The analysis shown in Table 1 indicated that all Labneh samples from three dairies A, C and G during January, April and July contained natamycin at varying concentrations, with the levels ranging from (54.0-59.4), (10.1-13.5), and (76.2-78.4) mg/kg for those months, respectively. Those levels differed significantly ($P < 0.05$) from one dairy to another. It was noted three dairies do not apply HACCP systems to their products. Labneh samples

from dairies B, D, E, F, H, I, and J did not contain natamycins. From the above results it is obvious that the decision to add natamycin to Labneh at what level was made by the individual the Jordanian dairies in spite the fact that they are not legally authorized to practice such a process. Two out of the ten dairies added higher levels of natamycin to Labneh (54.0-78.4) mg/kg, which is above the presumptive quantity needed to prevent the growth of yeast and mould in the product, since lower concentrations of natamycin has strong cidal activity towards susceptible microorganisms and is particularly effective against fungi (FSANZ, 2004). Though the results in table 1 show that the content of natamycin in Labneh did not differ significantly (P>0.05) due to the month of production , the content of natamycin in the Labneh samples are almost equal for the three different months January, April and July for dairies A (56.52 ± 5.4, 56.2 ± 5.0, and 56.14± 4.9 mg\kg respectively), C (11.74 ± 2.4, 12 ± 2.2, and 12 ± 2.4 mg/kg respectively), and G (77.26 ± 5.7, 76.84 ± 5.5, and 76.98 ± 6.1 mg/kg respectively). It did differ between dairy G and other dairies with dairy G having the highest concentrations and Dairy C having the lowest concentration. Although addition of natamycin to Labneh is not permited according to Jordanian standards (JISM, 2003), it may be used for the surface treatment of semi-hard and semi-soft cheese (EFSA, 2009).

Clark et al. (1964) concluded that natamycin concentrations as high as 100 ppm are safe in food, and all samples that contain natamycin not exceeding 100 ppm are safe. The world health organization (WHO) and food and agriculture organization (FAO) established the acceptable daily intake (ADI) at 0.30 mg/kg (JECFA, 1976). Labenh consumed in Jordan was estimated as annual per capita about 1.6 kg / year (DOS, 2011). It seems that the daily intake of natamycin in the Labaneh product by Jordanian people would be within the WHO\FAO acceptable range (JECFA, 1976).

Table 1. Natamycin levels in labneh samples collected from jordanian markets at different months of 2013.

Symbol of trademark Samples	Levels of Natamycin (mg/kg)		
	January	April	July
A	$56.52 \pm 5.4^{*}{}_{b\,1}$	$56.2 \pm 5.0_{b\,1}$	$56.14 \pm 4.9_{ab\,1}$
B	$0.0 \pm 0.0_{d\,1}$	$0.0\,0.0_{d\,1}$	$0.0\,0.0_{d\,1}$
C	$11.74 \pm 2.4_{c\,1}$	$12 \pm 2.2_{c\,1}$	$12 \pm 2.4_{c\,1}$
D	$0.0 \pm 0.0_{d\,1}$	$0.0 \pm 0.0_{d\,1}$	$0.0 \pm 0.0_{d\,1}$
E	$0.0 \pm 0.0_{d\,1}$	$0.0 \pm 0.0_{d\,1}$	$0.0 \pm 0.0_{d\,1}$
F	$0.0 \pm 0.0_{d\,1}$	$0.0 \pm 0.0_{d\,1}$	$0.0 \pm 0.0_{d\,1}$
G	$77.26 \pm 5.7_{a\,1}$	$76.84 \pm 5.5_{a\,1}$	$76.98 \pm 6.1_{a\,1}$
H	$0.0 \pm 0.0_{d\,1}$	$0.0 \pm 0.0_{d\,1}$	$0.0 \pm 0.0_{d\,1}$
I	$0.0 \pm 0.0_{d\,1}$	$0.0 \pm 0.0_{d\,1}$	$0.0 \pm 0.0_{d\,1}$
J	$0.0 \pm 0.0_{d\,1}$	$0.0 \pm 0.0_{d\,1}$	$0.0 \pm 0.0_{d\,1}$

*Means the average of five sub samples followed by standard deviation
Column with the same letters is not significantly different (P > 0.05)
Row with same numbers is not significantly different (P > 0.05).

3.2 Addition of Benzoic and Sorbic acid

The analysis shown in Table 2 indicated that all Labneh samples from dairy D contained benzoic acid in quantities ranging from 323.7 to 365.4 mg/kg, while all Labneh samples from dairies I and J contained sorbic acid in quantities ranged ranging from 77.0 to 321.6 mg/kg. The remaining Labneh samples from different dairies contained neither sorbic acid nor benzoic acid, which indicates that the addition of benzoic (and\ or) sorbic acid to Labneh as well as the quantity to be added depend on the dairy producer decision.

Most of the Labneh samples contained small quantities of benzoic acid ranging from 5.7 to 9.8 mg/kg. This can be explained by the natural formation of benzoic acid in yoghurt (Mroueh et al., 2008). These low quantities detected are due to the fact that the tested samples were subjected to analysis while they were still in fresh state, as samples were analyzed at the last day of its shelf life (14 days) and were not subjected to long fermentation process (JISM, 2004). A similar study carried out on Lebanon samples; found that the indigenous benzoic acid levels in laboratory-produced yoghurt, under aseptic conditions, ranged between 5.5 and 14.7 mg/kg after storage for a period of four weeks, whereas all samples contained benzoic acid with levels ranging between 12 and 479.1 mg/kg. 14 out of 30 samples of commercial Labneh produced in Lebanon and collected from Lebanon markets contained sorbic acid ranging from 83.9 to 3719.1 mg/kg (Mroueh et al., 2008).

According to another study carried out in Jordan in 2009, some commercial Labneh samples obtained from Jordanian markets contained benzoic acid in the range of 0 – 199 mg/kg, other samples contained benzoic acid at levels between 200 and 2000 mg/kg, while the control samples (preservative free) Labneh samples contained 25 mg/kg benzoic acid and some samples contained sorbic acids in the range of 87.2 – 988.8mg/kg.

None of the samples contained more than one added preservative, while 3 out of 14 samples of Labneh contained high levels of both benzoic and sorbic acids (table 2 and 3), and just 1 out of 30 sample contained both benzoic and sorbic acids in high quantities 231.2 and 232.6mg\kg respectively (Mihyar et al., 1999).

Table 2. Benzoic Acid levels in Labneh samples collected from Jordanian markets at different months of 2013

Symbol of trademark samples	Levels of benzoic acid (mg/kg)		
	January	April	July
A	7.70 ±1.0 * b 1	7.72 ±0.9 cde 1	7.60 ±0.7 bc 1
B	5.70 ±0.5 b 1	6.42 ±0.7 f 1	6.1 ±0.6 c 1
C	7.58 ±0.8 b 1	7.52 ±0.9 def 1	7.14 ±0.9 bc 1
D	365.4 ±4.2 a 1	323.76 ±3.8 a 1	323.42 ±3.9 a1
E	9.8 ±1.2 b 1	9.32 ±1.0 b 1	7.76±1.3 bc 1
F	7.96 ±0.9 b 1	8.08 ±0.9 cd 1	8.06 ±1.2 bc 1
G	7.76 ±1.0 b 1	7.48 ±0.8 de 1	7.56 ±0.8bc 1
H	6.40 ±0.6 b 1	6.36 ±0.7 ed 1	6.08 ±0.6 c 1
I	6.70 ±0.8 b 1	6.64 ±0.7 def 1	6.76 ±0.6 c 1
J	8.82 ±0.9 b 1	9.02 ±1.0 bc 1	9.12 ±1.1 b 1

*Means the average of five sub samples followed by standard deviation
Column with the same letters is not significantly different (P > 0.05)
Row with same numbers is not significantly different (P > 0.05).

Table 3. Sorbic Acid levels in Labneh samples collected from Jordanian markets at different months of 2013

Symbol of trademark samples	Levels of sorbic acid (mg/kg)		
	January	April	July
A	0.0 ±0.0 * c 1	0.0 ±0.0 c 1	0.0 ±0.0 c 1
B	0.0 ±0.0 c 1	0.0 ±0.0 c 1	0.0 ±0.0 c 1
C	0.0 ±0.0 c 1	0.0 ±0.0 c 1	0.0 ±0.0 c 1
D	0.0 ±0.0 c 1	0.0 ±0.0 c 1	0.0 ±0.0 c 1
E	0.0 ±0.0 c 1	0.0 ±0.0 c 1	0.0 ±0.0 c 1
F	0.0 ±0.0 c 1	0.0 ±0.0 c 1	0.0 ±0. 0 c 1
G	0.0 ±0.0 c 1	0.0 ±0.0 c 1	0.0 ±0.0 c 1
H	0.0 ±0.0 c 1	0.0 ±0.0 c 1	0.0 ±0.0 c 1
I	320.32 ±4.7 a 1	321.6±5.0 a 1	321.5±4.9 a1
J	77.08 ±7.4 b 1	78.24±7.2 b 1	77.76 ±7.0 b 1

*Means the average of five sub samples followed by standard deviation.
Column with the same letters is not significantly different (P > 0.05)
Row with same numbers is not significantly different (P > 0.05).

The results in table 2 and 3 indicate that the contents of benzoic and sorbic acids in Labneh did not differ significantly (P>0.05) in due to the month of production January, April and July, but there were significant differences (P<0.05) in accordance to trademarks. The presence of antimicrobial preservatives such as natamycin, benzoic and sorbic acids in Labneh products did not affect its sensory acceptability by consumers and no complaints were recorded. Some dairies tend to add preservatives to Labneh to mask the non hygienic condition during production as a sort of fraud (JFDA, 2010). This assumption agrees with results reported was obtained by Rajarajan et al., (2010).

According to Tables 1 - 3, all Labneh samples dairies B, E, F, and H did not contain any preservatives. These Labneh samples were produced in dairy plants that apply the HACCP system to production of its products. Accordingly, dairy premises applying the HACCP system are significantly (P<0.05) satisfied by the quality of its Labneh products without the addition of any preservatives.

4. Conclusion

This study revealed the following conclusions:

1- 30% of Labneh samples contained natamycin, 20% contained sorbic acid, and 10% contained benzoic acids.

2-The addition of natamycin, sorbic acid, and benzoic acid and the amount to be added to Labneh is an individual decision made by some dairies on their own 3 as this practice is not legally permitted, according to several standards and regulations such as the Jordanian standard of Labneh and the Food Control Act.

3-The application of the HACCP system in dairy plants proved to insure that dairy products such as Labneh meet

the required standards without any antimicrobial preservative addition.

5. Recommendation

In order to overcome this illegal practice by some dairies and to prevent fraud, the authorized agencies must enforce the farm-to-fork, preventive approach by restricting all parties involved in the chain of Labneh production to the application and implementation of HACCP system, in a sense of tackling the responsibility for educating them, on the importance of the application of such system and to provide them with the proper training on technical practices.

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