

**PRODUCTION OF LABNEH FORTIFIED WITH ORGANIC SELENIUM USING
 BUFFALO'S MILK RETENTATE
 BY**

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ABSTRACT

The effect of organic selenium on the growth and activity of some lactic acid bacterial strains which can be used as a starter culture in dairy industry was tested. Then organic selenium incorporated into Labneh using different concentrations of 25, 50 and 100 ppm. The various attributes investigated were chemical composition, microbiological quality and sensory evaluation over a storage period of 21 days. No significant differences ($P < 0.05$) in growth and activity were found between the control and the presence of organic selenium up to 100 ppm. No significant differences ($P < 0.05$) were observed for the chemical characteristics of the fortified Labneh when compared to the control. Over storage, total lactic acid bacterial count did not differ significantly ($P < 0.05$) neither among treatments nor between the control and the treatments. Similarly, flavour, body and texture and appearance scores of the fortified Labneh showed no significant differences ($P < 0.05$) when compared to the control. Fortification of Labneh or other fermented dairy products with organic selenium can be accomplished without adversely affecting product characteristics to produce a new nutraceutical dairy product.

Key words: organic selenium, Labneh, concentrated yoghurt, retentate, buffalo's milk, nutraceutical.

INTRODUCTION

Although minerals comprise only 4-6 % of human body weight, they are of critical importance in the diet. Selenium (Se) is one of these minerals, which is essential for maintaining good health in many life forms, including human and other mammals (Freeland Graves and Trotter, 2003; Berlau *et al.*, 2004). Se is incorporated into proteins to form selenoproteins, which are important antioxidant enzymes (e.g., Glutathione peroxidase). Selenoproteins help preventing cellular damage from free radicals which are natural by-products of oxygen metabolism (Beck, 2007; Tinggi, 2008). Se was also identified in type I iodothyronine 5'-deiodinase, an enzyme involved in thyroid gland functions (Rayman, 2000). Moreover, Se supplements improve the immune system ability to respond to viral infections, decrease the rate of tumors growth

and induce P450 enzymes in the liver leading to detoxification of some carcinogenic molecules in lung, prostate, colon and breast cancer (Donaldson, 2004).

The recommended daily allowance of Se for adults is 50 and 70 µg for men and women, respectively with 40µg /day as a minimum requirement and 200 µg as the upper limit (El- Bayoumy *et al.*, 2002). In addition, several forms of Se enter the body as part of amino acids within proteins (e.g., selenocysteine) but the two most common forms of Se are organic and inorganic ones. The organic Se is easier to be absorbed in the digestive system and its absorption rate is higher than the inorganic one (Rayman, 2004). However, several studies have shown that, bioconversion of the inorganic Se (sodium

selenite) to an intercellular organic one (selenomethionine) is achievable by some species of lactic acid bacteria used in yoghurt culture (Zommara, 2007).

In human diet, plant foods are the major dietary source of Se. The amount of Se in soil, which varies by region, determines the amount of Se in the plant foods that are grown in that soil. Se also can be found in some meats. Animals that eat grains or plants that were grown in Se-rich soil have higher levels of Se in their muscles (Yanardag and Orak,

1999). In the dairy field, studies have shown that, Se concentration in milk is strongly and positively correlated to Se concentration in the dairy cow feed (Givens *et al.*, 2004). Therefore, there is a raising interest in improving Se status in milk and dairy foods, which would have widespread public health benefits.

In the present study, Labneh (concentrated yoghurt) made from buffalo's milk retentate was fortified with three different concentrations of organic Se to produce a novel dairy product.

MATERIALS AND METHODS

Materials

1. Buffalo's milk retentate (approximately 28 % total solids (TS)) was obtained from the Dairy Production Unit at the Animal Production Research Institute. Retentate fat content was standardized to 10 %.
2. Bacterial strains: *Bifidobacterium longum* ATCC 1570, *Streptococcus thermophilus* DSMZ 20470, *Lactococcus lactis* subsp. *lactis* ATCC 12315, *Lactococcus lactis* subsp. *cremoris* DSMZ 2006, *Lactobacillus acidophilus* ATCC 4321, *Lb. delbrueckii* subsp. *bulgaricus* DSMZ 20080 and *Lb. rhamnosus* DSMZ 20245 were obtained from Food Science Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. A yoghurt starter containing *Streptococcus thermophilus* and *lactobacillus delbrueckii* subsp. *bulgaricus*, was obtained from Chr. Hansen, Copenhagen, Denmark. The growth and activity of the different strains were investigated by growing the different strains in sterilized skim milk containing 25, 50 and 100 µg of organic Se. Viable cell count as well as titratable acidity were determined every 4 h up to 24 h. Sterilized skim milk without adding organic Se was used as a control.
3. Organic Se: a food grade organic selenium (Sel-Plex), approved by FDA, was purchased from Alltech, Kentucky, USA.

Methods

1. Preparation of Labneh

Labneh was manufactured following the method of Ozer *et al.* (1999). Yoghurt starter culture was added at a rate of 2 % to ferment the retentate at 40 ± 1 °C. After

incubation (~ 3 h and pH, 4.1), the curd was divided into four parts. One part served as the control and the other three parts were mixed with 25, 50 and 100 ppm Se, respectively. The resultant Labnehs were packed into equal portions of approximately 100 g in plastic cups and stored at 5 ± 1 °C for 21 days. Chemical, microbiological, and organoleptic attributes were conducted on the fresh samples and every seven days up to 21 days storage period.

2. Chemical characteristics:

TS and titratable acidity (expressed as % of lactic acid) were determined according to AOAC (2005). Lactose content was determined according to Nickerson *et al.* (1976). Total nitrogen (TN) and soluble nitrogen (SN) were determined by Kjeldahl method according to Ling (1963).

3. Microbiological analysis:

Lactic acid bacteria (LAB), proteolytic bacteria, lipolytic bacteria, and yeast and mould counts were determined according to Standard Methods for the Examination of Dairy Products (2004).

4. Organoleptic properties:

Fresh and stored Labneh samples were subjected to sensory evaluation by experienced panelists of Dairy Department at the Animal Production Research Institute. A total score of 100 reflected overall Labneh quality with appearance contributing 10 points, body and texture 40 points and flavor 50 points according to a score card suggested by Keating and White (1990).

5. Replication and statistical analysis:

All treatments were triplicated and duplicate analyses were performed on each replicate. Analysis of variance (ANOVA) was

performed by the SAS General Linear Methods (SAS, 1994) and differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Effect of organic Se concentration on growth and activity of some lactic acid bacterial Strains:

The growth and activity (as indicated by viable cell count and titratable acidity) of the different lactic acid bacterial strains namely, *Bifidobacterium longum*, *Streptococcus thermophilus*, *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactobacillus acidophilus*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. rhamnosus* and the yoghurt culture did not differ significantly ($P < 0.05$) by using different concentrations of organic Se.

Chemical analyses:

Table 1 shows the changes during storage in TS and fat-to-dry matter (F/DM) of labneh fortified with several concentrations of organic Se. The TS increased slightly in both the control and all treatments as the storage period proceeded. However, no significant differences ($P < 0.05$) were observed in the TS or F/DM of the labnehs either when fresh or during storage. Al-Otaibi and El-Demerdash (2008) ascribed the increase in TS to moisture loss during storage (21 days). The data is in agreement with those of Tamime (1978).

Titratable acidity (TA) is a very important factor, since it affects acceptability of the final product. Results presented in Table 1 reveal a gradual increase in the TA over the storage period. As TA increased, a corresponding decrease in lactose content was observed which is readily explained based on fermentation of lactose by starter culture added. These findings are in agreement with that obtained by Abbas and Osman (1998) and Al-Otaibi and El-Demerdash (2008) who reported a gradual increase in the TA of Labneh during storage.

A very slight increase in all treatments as well as control during storage was observed for percentage of soluble nitrogen

per total nitrogen (SN/TN %) of labneh fortified with different concentrations of organic Se (Table 1). Ismail *et al.* (2006) found the same trend in flavored labneh and they attributed it to an increase in LAB activity. SN/ TN % trend was also similar to that of Al-Otaibi and El-Demerdash (2008).

Microbiological quality:

Several studies have proposed using ultrafiltration retentate as a better alternative to the traditional Labneh making process, which is uneconomical and unhygienic (Tamime *et al.*, 1989). Data presented in Table 2 indicate a clean production of the Labneh from buffalo's milk retentate. since no proteolytic, lipolytic bacteria, and yeasts and moulds were detected. Al-Kadamany *et al.* (2002) reported the presence of yeasts and molds as well coliforms in traditional Labneh prepared by cloth-bag method which was attributed to a post pasteurization contamination at one or more stage during processing. Moreover, fortifying Labneh with Se had no effect ($P < 0.05$), in terms of inhibition, on LAB count. LAB count followed a pattern of an increase up to 14 days then started to decrease thereafter. Similar results were obtained by Al-Otaibi and El-Demerdash (2008).

Sensory evaluation:

Sensory evaluation scores are shown in Table 3. Although a slight decrease, probably owing to acidity development was observed for the over all Labneh acceptability during storage, no significant differences ($P < 0.05$) were detected in the sensory attributes. Among these attributes, flavor is considered to be the most important factor for determining consumer's response. Results reveal no significant differences ($P < 0.05$) in the flavor scores among Labnehs fortified with deferent concentrations of Se. Similarly, appearance and body and texture values were not affected ($P < 0.05$) by adding Se to the Labneh. The results obtained are probably because Se was used in such small amounts that they had no influence on the product organoleptic

properties. Hekmat and McMahon (1997) reported that, consumer panels did not find significant differences in the over all quality among yogurts fortified with iron chloride, casein chelated iron, and whey protein

chelated iron. Similar results were also reported for yogurts fortified with different minerals like magnesium, manganese, zinc, molybdenum, and chromium (Achanta *et al.*, 2007).

Table (1): Chemical properties of Labnah manufactured from buffalo's milk retentate and fortified with different concentrations of organic selenium.

| Storage period (days) | TS (%) | | | | TA (%) | | | |
|-----------------------|--------------------------------|---------------------|---------------------|---------------------|-------------------|--------------------|--------------------|--------------------|
| | Organic Se concentration (ppm) | | | | | | | |
| | Control | 25 | 50 | 100 | Control | 25 | 50 | 100 |
| 0 | 28.38 ^a | 28.37 ^a | 28.38 ^a | 28.38 ^a | 0.75 ^a | 0.74 ^{ab} | 0.75 ^a | 0.76 ^a |
| 7 | 28.43 ^{ab} | 28.45 ^{ab} | 28.42 ^{ab} | 28.47 ^a | 0.84 ^a | 0.82 ^{ab} | 0.83 ^a | 0.83 ^a |
| 14 | 28.54 ^{ab} | 28.60 ^a | 28.59 ^a | 28.57 ^{ab} | 0.90 ^a | 0.88 ^{ab} | 0.90 ^a | 0.90 ^a |
| 21 | 28.72 ^{ab} | 28.79 ^a | 28.80 ^a | 28.78 ^a | 0.98 ^a | 0.98 ^a | 0.96 ^{ab} | 0.97 ^{ab} |

TA, total solids; TA, titraTable acidity (expressed as % of lactic acid)

Different letters within the same row are significantly different ($P < 0.05$).

Table (1) (continue): Chemical properties of Labnah manufactured from buffalo's milk retentate and fortified with different concentrations of organic selenium.

| Storage period (days) | Lactose (%) | | | | F/DM | | | |
|-----------------------|--------------------------------|--------------------|--------------------|--------------------|--------------------|---------------------|---------------------|---------------------|
| | Organic Se concentration (ppm) | | | | | | | |
| | Control | 25 | 50 | 100 | Control | 25 | 50 | 100 |
| 0 | 5.62 ^a | 5.62 ^a | 5.63 ^a | 5.63 ^a | 35.24 ^a | 35.25 ^a | 35.24 ^a | 35.24 ^a |
| 7 | 5.34 ^{ab} | 5.37 ^a | 5.33 ^{ab} | 5.34 ^{ab} | 35.17 ^a | 35.15 ^{ab} | 35.19 ^a | 35.13 ^{ab} |
| 14 | 5.16 ^a | 5.11 ^{ab} | 5.10 ^{ab} | 5.14 ^a | 35.04 ^a | 34.97 ^{ab} | 34.98 ^{ab} | 35.00 ^a |
| 21 | 4.68 ^a | 4.64 ^{ab} | 4.67 ^{ab} | 4.70 ^a | 34.82 ^a | 34.73 ^{ab} | 34.72 ^{ab} | 34.75 ^{ab} |

F/DM, fat/dry matter

Different letters within the same row are significantly different ($P < 0.05$).

Table (1) (continue). Chemical properties of Labnah manufactured from buffalo's milk retentate and fortified with different concentrations of organic selenium.

| Storage period (days) | SN/TN (%) | | | |
|-----------------------|--------------------------------|---------------------|---------------------|--------------------|
| | Organic Se concentration (ppm) | | | |
| | Control | 25 | 50 | 100 |
| 0 | 15.40 ^a | 15.39 ^a | 15.40 ^a | 15.40 ^a |
| 7 | 15.56 ^a | 15.50 ^{ab} | 15.52 ^{ab} | 15.55 ^a |
| 14 | 15.69 ^a | 15.61 ^{ab} | 15.60 ^{ab} | 15.64 ^a |
| 21 | 15.83 ^a | 15.80 ^{ab} | 15.79 ^{ab} | 15.82 ^a |

SN/ TN, soluble nitrogen / total nitrogen

Different letters within the same row are significantly different ($P < 0.05$).

Table (2): Microbiological characteristics (log cfu/ g) of Labnah manufactured from buffalo's milk retentate and fortified with different concentrations of organic selenium.

| Storage time (days) | Organic Se concentration (ppm) | | | |
|---------------------|--------------------------------|--------------------|-------------------|--------------------|
| | LAB | | | |
| | Control | 25 | 50 | 100 |
| 0 | 8.12 ^{ab} | 8.15 ^a | 8.16 ^a | 8.14 ^{ab} |
| 7 | 8.20 ^{ab} | 8.22 ^a | 8.24 ^a | 8.18 ^{ab} |
| 14 | 8.22 ^{ab} | 8.22 ^{ab} | 8.28 ^a | 8.20 ^{ab} |
| 21 | 8.13 ^{ab} | 8.14 ^a | 8.18 ^a | 8.12 ^{ab} |
| | Proteolytic bacteria | | | |
| 0 | <1 | <1 | <1 | <1 |
| 7 | <1 | <1 | <1 | <1 |
| 14 | <1 | <1 | <1 | <1 |
| 21 | <1 | <1 | <1 | <1 |
| | Lipolytic bacteria | | | |
| 0 | <1 | <1 | <1 | <1 |
| 7 | <1 | <1 | <1 | <1 |
| 14 | <1 | <1 | <1 | <1 |
| 21 | <1 | <1 | <1 | <1 |
| | Moulds and yeasts | | | |
| 0 | <1 | <1 | <1 | <1 |
| 7 | <1 | <1 | <1 | <1 |
| 14 | <1 | <1 | <1 | <1 |
| 21 | <1 | <1 | <1 | <1 |

LAB, lactic acid bacteria.

Different letters within the same row are significantly different ($P < 0.05$).

Table (3): Sensory evaluation of Labnah manufactured from buffalo's milk retentate and fortified with different concentrations of organic selenium.

| Storage time (days) | Flavor (50) | | | | Body and texture (40) | | | | Appearance (10) | | | |
|---------------------|--------------------------------|------------------|-----------------|------------------|-----------------------|------------------|------------------|------------------|-----------------|-----------------|-----------------|-----------------|
| | Organic Se concentration (ppm) | | | | | | | | | | | |
| | Control | 25 | 50 | 100 | Control | 25 | 50 | 100 | Control | 25 | 50 | 100 |
| 0 | 45 ^a | 45 ^a | 45 ^a | 45 ^a | 39 ^a | 38 ^{ab} | 38 ^{ab} | 38 ^{ab} | 10 ^a | 9 ^{ab} | 10 ^a | 10 ^a |
| 7 | 44 ^{ab} | 44 ^{ab} | 45 ^a | 44 ^{ab} | 38 ^a | 38 ^a | 38 ^a | 38 ^a | 10 ^a | 9 ^{ab} | 9 ^{ab} | 10 ^a |
| 14 | 44 ^a | 43 ^{ab} | 44 ^a | 44 ^a | 38 ^a | 38 ^a | 38 ^a | 37 ^{ab} | 10 ^a | 9 ^{ab} | 9 ^{ab} | 9 ^{ab} |
| 21 | 43 ^a | 43 ^a | 43 ^a | 43 ^a | 37 ^{ab} | 37 ^{ab} | 38 ^a | 37 ^{ab} | 9 ^a | 9 ^a | 9 ^a | 9 ^a |

Different letters within the same row are significantly different ($P < 0.05$).

CONCLUSION

Adding of organic selenium up to 100 ppm did not affect the growth and the activity of some lactic acid bacteria. Using different concentrations of organic Se (25, 50 and 100 ppm) to fortify Labneh did not affect the chemical, microbiological properties of the

final product which showed acceptable organoleptic characteristics during storage. Thus it can be recommended to use organic selenium in fermented dairy products up to 100 ppm to produce nutraceutical dairy products for special use.

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إنتاج لبننة من مركز اللبن الجاموسي المدعم بالسليينيوم العضوي

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تم إجراء هذا البحث بهدف إختبار قدرة نمو ونشاط بعض سلالات بكتريا حمض اللاكتيك المستخدمة في منتجات الألبان المتخمرة في وجود تركيزات مختلفه من السليينيوم العضوي حتى ١٠٠ جزء في المليون وكذلك تأثير استخدام السليينيوم العضوي على خواص وجودة احد المنتجات اللبنيه المتخمرة وتم اختيار اللبنه كنموذج ، لذلك تم إضافة السليينيوم العضوي إلى اللبنه المصنعة من مركز اللبن الجاموسي بتركيزات ٢٥، ٥٠، ١٠٠ جزء في المليون وقد تم دراسة الخواص الكيماوية، الميكروبيولوجية والحسية للمنتج طوال مدة التخزين ومقدارها ٢١ يوم .

أظهرت نتائج البحث عدم وجود أي فروق معنوية بين نمو ونشاط سلالات بكتريا حمض اللاكتيك المختبره حتى تركيز ١٠٠ جزء في المليون . كذلك لم توجد فروق معنويه مابين لبننة المقارنة والمعاملات في الخواص الكيماوية والتي تضمنت النسبة المئوية للجوامد الكلية، الحموضة، اللاكتوز، نسبة الدهن / المادة الجافة وكذلك نسبة النتروجين الذائب / النتروجين الكلي . بالمثل لم يلاحظ وجود فروق معنوية مابين العينات فيما يتعلق بالمحتوى الميكروبي من بكتريا حمض اللاكتيك مع عدم إحتواء العينات على اي من البكتريا المحللة للبروتين أو المحلله للدهون وكذلك الخمائر والفطريات .

وجدت العينات قبولاً عند إجراء التحكيم الحسي خلال مدة التخزين والتي إستمرت ٢١ يوماً. بوجه عام يشير البحث إلى إمكانية إنتاج لبننة ذات خواص صحية من مركز اللبن الجاموسي المدعم بالسليينيوم العضوي بدون أي تغيير في خواص المنتج النهائي أو أية منتجات لبننيه متخمرة أخرى.