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COMPARATIVE STUDY BETWEEN THE MICROBIOLOGICAL QUALITY OF COMMERCIAL AND HOMEMADE LABENAH

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ABSTRACT

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A total of 50 commercial and homemade Labenah samples were collected randomly from supermarkets and houses (n=25 for each product) in Asiut Governorate. It was concluded that the average of total counts of yeasts and molds and total coliforms were $8.8x10^2$ and $9.6x10^2$cfu /g in commercial labneh and $6x10^2$ and $7.6x10^4$cfu/g in homemade labenah, respectively. *Pathogenic E.coli* could be isolated in percentage of 28% from homemade one. The isolated *pathogenic E.coli* could be serologically identified to *O103 : H2(EHEC), O26 : H11(EHEC), O125 : H21(ETEC), O26 : H11(EHEC), O55: H7(EPEC), O91(EPEC) and O125: H21(EHEC). Serratialiquefaciens, Klebsiella pneumonia*, *Providenciarettgeri*, *Proteus mirabilis, Enterobacteraerogenes* and *Serratiamarcescens* could be isolated and identified in percentages of 4%, 12%, 12%, 12%, 12% and 4% in the examined samples of homemade labenah. *Coliforms* which could be detected in examined samples of commercial labenah were *Citrobacterdiversus, Proteus mirabilis* and *Enterobacteraerogenes* in percentages of 4%, 4% and 4%, respectively. The results obtained show that labenah samples collected from Supermarkets were safer than that made at home.

Key words: *Microbiological quality, Commercial, Homemade labenah*

INTRODUCTION

Modern socio-economic changes mean that some traditional technologies for the production of fermented foods might eventually be lost together with the associated microorganisms (Akabanda *et al.,* 2013). This underscores the importance of studying indigenous fermented products for their microbiota which might yield technologically important species and strains. Microorganisms present in traditionally fermented milk products have been documented in various studies (Gonfa *et al., 1999; Beukes et al., 2001;* Lore *et al., 2005;* El-Baradei *et al.,* 2008; Mathara *et al.,* 2008; Njage *et al.,* 2011; Akabanda *et al.,* 2013).

Nutritional and therapeutic properties of labenah are considered similar to or even better than those of yogurt. Labenah has 2.5 time's higher protein content, 50% more minerals, and a considerably larger number of viable microorganisms than common yoghurt *(Nsabimana et al.,* 2005). In addition, the lactose concentration of labneh is low (approximately 6%) due to its fermentation into lactic acid, which makes it more suitable for use by lactose intolerant individuals (Nsabimana *et al.,* 2005 and Özer and Robinson, 1999). Due to its high total solids content, labenah may be considered a suitable matrix for probiotics since it offers protection when added to them (Abd El-Salam *et al.,* 2011). The high microbial load of labenah ،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 (Muir and Banks, 2000).

Labenah is a white to creamy paste product that has a smooth texture, with a taste crossing between sour cream and cottage cheese and a characteristic sharp flavor that is largely modulated by diacetyl produced during fermentation (Varnam and Sutherland, 1994; Tamime and Robinson, 1999). Concentrated yogurt, known as labneh in the Middle East, is widely consumed, chiefly as a sandwich spread, in the Middle East and Balkan regions (Tamime *et al.,* 1989 and Özerand Robinson, 1999). Labneh is produced by removing a proportion of the whey from cow's milk yogurt until fat and total solids contents of 9 to 11 and 23 to 25% are attained, respectively (Tamime and Robinson, 1999).

In Lebanon, and other Middle Eastern countries, labenah is produced by straining yogurt in cloth bags to the desired total solids level. The product is packaged in plastic containers that prevent access of light and air, and displayed under refrigeration (5 to 7°C) in retail outlets. The presence of live starter bacteria and yeast and mold contaminants (Salji *et al.,* 1987) coupled with packaging/storage conditions lead to the formation of off-flavors and other undesirable physicochemical changes that eventually lead to product failure (Muir and Banks, 2000). The stated shelf life of cloth-bag labenah, produced by major dairy processors, is between 14 and 21 d and is largely based on commercial experience. The variation in quality of Labneh (yoghurt cheese) in different countries is due to the variation of starter cultures used (Sharaf *et al.,* 1996).

The product may be considered as intermediate between conventional fermented milks and high moisture, unripened soft cheese such as quarg (Varnam and Sutherland, 1994).

Labenah is produced by strains of thermophilic lactic acid bacteria (LAB), which ferment the lactose present to produce organic acids, mainly lactic acid (El-Samragy, 1997). Industrially, excess liquid is removed from the yoghurt by mechanical separators (Tamime and Robinson, 1999). The shelf life of traditional labenah is short, even if stored at low temperatures. This may be due to the sanitary problems usually associated with the cloth bags used in its production and due to unhygienic handling of the product, which increases microbial contamination (El-Samragy, 1997).

Coliforms and *E. coli* are often used as marker organisms. Recovery and counting of *E. coli* is used as reliable indicator of fecal contamination and indicates a possible presence of enteropathogenic and/or toxigenic microorganisms which constitute a public health hazard. *E. coli* is one of the main inhabitants of the intestinal tract of most mammalian species, including humans and birds. Most *E. coli* are harmless, but some are known to be pathogenic bacteria, causing severe intestinal and extra intestinal diseases in man (Kaper *et al.,* 2004).

Strains of *E. coli* are traditionally characterized by serological identification of somatic O, flagellar H, capsular K, and fimbrial F antigens (Quinn *et al.,* 2002 and Gyles *et al.,* 1993). Differentiation of pathogenic strains from normal flora strains depends on the identification of virulence characteristics. *E.coli* strains can further be classified according to the presence of virulence factors such as enterotoxigenic *E. coli* (ETEC), attaching and effacing *E.coli* (AEEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), and Shiga toxin-producing *E. coli* (STEC or VTEC) *(Franck et al.,* 1998 and Nagy and Fekete 1999). Virulence factors associated with strains of *E. coli* include adhesions, toxins, cell wall, capsule production, and serum resistance (Gyles *et al.,* 1993).

The labenah samples were microbiologically examined in order to determine whether they were hygienically safe to be consumed by customers or not.

MATERIALS and METHODS

A) Collection, preparation and serial dilutions of samples:

A total of fifty random samples of commercial and homemade labneh were collected from different shops and supermarkets and houses (25 samples of each), respectively. The commercial samples were still valid for consumption for 6 months from production time and they were transferred to the laboratory in their packages to be analyzed microbiologically to evaluate their quality. Eleven grams of the prepared samples were mixed with 99 ml of sterile 0.1 % peptone water and thoroughly mixed to give a dilution of 1/10, and then tenfold serial dilutions were carried out according to (A.P.H.A., 1992).

B) Experimental techniques:

1) Enumeration of total yeasts and molds count according to Harrigan and MacCance (1976) by using malt extract agar (containing 500 mg each of chlortetracycline and HCL chloramphenicol).

2) Enumeration of total coliform count according to Ray and Speck (1978) by using violet red bile glucose agar.

3) Isolation and identification of E. coliaccording to Dilielo (1982).

A portion (10 g or 10 ml) from the centre of each sample was extracted aseptically and homogenized with 90 ml sterile enrichment MacConkey broth. The enriched sample was cultured on selective medium Levine Eosin Methylene Blue (EMB) agar and incubated at 37 °C for 24 hours. Morphologically typical colonies (at least 4 / plate) producing metallic sheen were taken into nutrient broth for further identification.

Biochemical tests were performed to confirm *E.coli* using Gram staining, Catalase test, Indole, Methyl red, Voges- Proskauer test, Nitrate reduction, Urease production, Simon citrate agar, and various sugar fermentation tests.

Serodiagnosis of *E.coli*:
The isolates were serologically identified according to Kok *et al.* (1996) by using rapid diagnostic *E.coli* antisera sets (*DENKA SEIKEN Co., Japan*) for diagnosis of the Enteropathogenic types.

Technique:
- Two separate drops of saline were put on a glass slide and a portion of the colony from the suspected culture was emulsified with the saline solution to give a smooth fairly dense suspension.

- To one suspension, control, one loopful of saline was added and mixed. To the other suspension one loopful of undiluted antiserum was added and tilted back and forward for one minute.

- Agglutination was observed using indirect lighting over a dark background. When a colony gave a strongly positive agglutination with one of the pools of polyvalent serum, a further portion of it was inoculated onto a nutrient agar slant and incubated at 37°C for 24 hours to grow as a culture for testing with mono-valent sera.

- A heavy suspension of bacteria from each slope culture was prepared in saline, and slide agglutination tests were performed with the diagnostic sera to identify the O-antigen.

- The diagnostic *E.coli* antisera sets used for identification include the following sets:

Set 1 : O- antisera:
Polyvalent antisera 1: O1, O26, O86a, O111, O119, O127a and O128.

Polyvalent antisera 2: O44, O55, O125, O126, O146 and O166.

Polyvalent antisera 3: O18, O114, O142, O151, O157 and O158.

Polyvalent antisera 4: O2,O6, O27, O78, O148, O159 and O168.

Polyvalent antisera 5: O20, O25, O63, O153 and O167.

Polyvalent antisera 6: O8, O15, O115 and O169.

Polyvalent antisera 7: O28ac, O112ac, O124, O136 and O144.

Polyvalent antisera 8: O29, O143, O152 and O164.

Set 2: H- sera.
H2, H4, H6, H7, H11, H18 and H21.

RESULTS

Table 1: Statistical analytical results of total yeast and molds count of the examined samples.

Sample	Positive samples		Count/g		
	No.	%	Min.	Max.	Average
Commercial Labenah (No. :25)	**5**	**20%**	**<100**	**$5x10^3$**	**$8.8x10^2$**
Homemade Labenah (No. :25)	**7**	**28%**	**<100**	**$^4X10^3$**	**$^6X10^2$**

No. : Number of examined samples

Table 2: Statistical analytical results of total coliforms count of the examined samples.

Sample	Positive samples		Count/g		
	No.	%	Min.	Max.	Average
Commercial Labenah (No. :25)	**7**	**28%**	**<100**	**$^9X10^3$**	**$^{9.6}X10^2$**
Homemade Labenah (No. :25)	**18**	**72%**	**<100**	**$^{3.15}X10^5$**	**$^{7.6}X\ 10^4$**

No. : Number of examined samples

Table 3: Incidence of some microorganisms could be isolated from the examined.

Microorganisms Products	Commercial Labenah		Homemade Labenah	
	No./25	%	No./25	%
Pathogenic E. coli	**0**	**0**	**7**	**28%**
Serratialiquefaciens	**0**	**0**	**1**	**4%**
Klebsiella pneumonia	**0**	**0**	**3**	**12%**
Providenciarettgeri	**0**	**0**	**3**	**12%**
Proteus mirabilis	**0**	**0**	**3**	**12%**
Enterobacteraerogenes	**0**	**0**	**3**	**12%**
Serratiamarcescens	**0**	**0**	**1**	**4%**
Citrobacterdiversus	**1**	**4%**	**0**	**0**
Proteus mirabilis	**1**	**4%**	**0**	**0**
Enterobacteraerogenes	**1**	**4%**	**0**	**0**

Table 4: Frequency % of *pathogenic E. coli* could be isolated from the examined samples of labenah.

Identified bacterium	Products				Strain characteristic
	Commercial Labenah		Homemade Labenah		
	No./ 0	%	No./7	%	
O103 : H2	**0**	**0%**	**1**	**14.3%**	**EHEC**
O26 : H11	**0**	**0%**	**1**	**14.3%**	**EHEC**
O125 : H21	**0**	**0%**	**1**	**14.3%**	**ETEC**
O26 : H11	**0**	**0%**	**1**	**14.3%**	**EHEC**
O55 : H7	**0**	**0%**	**1**	**14.3%**	**EPEC**
O91	**0**	**0%**	**1**	**14.3%**	**EPEC**
O125 : H21	**0**	**0%**	**1**	**14.3%**	**ETEC**
TOTAL	**0**	**0%**	**7**	**100%**	

DISCUSSION

Examination of commercial Labenah samples for detection of total yeast and molds count, revealed that 5 sample were positive in percentage of 20% with average count of $8.8x10^2$cfu/g, while the samples of homemade Labenah were counted in 28% (7 samples) with average count $6X10^2$ and minimum of <100 and maximum of $4X10^3$cfu/g (table1). The results are online with those reported by Al-Kadamany *et al.* (2002) who stated that psychrotrophic yeasts increased in stored Labenah at 5 and 15°C. Yeasts and molds may grow over a wide range of temperature and gain entrance to milk either from the milk used, air contamination or utensils. So, their presence is indicative of unsatisfactory sanitation during processing and handling of the product. The high level of these microorganisms may be due to post pasteurization contamination (Mihyar *et al.*, 1997).

Low count of total yeasts and molds count in commercial labenah may be due to Lactic acid bacteria (LAB) occur naturally in labenah or are added as pure cultures to products. They are considered to be harmless or even to have an advantage for human health (probiotics) (Stiles *et al.*, 2002). LAB are well known for their use as starter cultures in the manufacture of dairy products such as acidophilus milk, yoghurt, buttermilk, cottage cheeses, hard cheeses (Cheddar and Edam) and soft cheeses (Brie and Camembert) (Carr *et al.*, 2002). Lactic and acetic acids are produced as end products during lactic acid bacterial fermentation causing a reduction in pH, but other substances such as hydrogen peroxide, formic acid, propionic acid, acetoin and diacetyl, are also produced (Lindgren and Dobrogosz, 1990). Studies on the effect of LAB on fungi are complicated by the fact that some fungi are sensitive to the normal by-products of LAB-metabolism, most notably lactic and acetic acids (Piard and Desmazeaud, 1992 and Bonestroo *et al.*, 1993).

The results recorded in Table 2 revealed that, coliforms was detected in 28% of commercial Labenah samples in counts ranging from <100 to 9 X 10^3 with an average count of $9.6X10^2$cfu/g. while,

from estimated results in the same Table, it is obvious that the coliforms were counted in 72% (18 samples) of homemade Labenah and counts ranging from <100 to $3.15X10^5$ with an average count of $7.6X10^4$cfu/g. Coliforms being non-spore formers should be susceptible to pasteurization. Their post pasteurization presence in the examined samples may be due to either faulty heat process or to post pasteurization contamination by handlers with poor sanitary practices. The presence of these organisms in food had been described as an index of food hygiene (Frazier and Westhoff, 1978; Jay 1978).

The results determined in this study revealed that, out of the 25 samples of homemade Labenah examined, 28% (7 samples) were positive for *pathogenic E. coli* (Table 3). While, the samples of commercial labenah found to be free from *pathogenic E. coli*. A decrease in pH occasioned by the production of organic acids in fermented milk products leads to inhibition of *E. coli* and other coliforms (Gran *et al.,* 2003). Also, Table (3) gives information about the other microorganisms could be isolated from examined samples were *Serratialiquefaciens, Klebsiella pneumonia, Providenciarettgeri, Proteus mirabilis, Enterobacteraerogenes* and *Serratiamarcescens* could be isolated and identified in percentage of 4%, 12%, 12%, 12%, 12% and 4% in the examined samples of homemade labenah. In addition, *Coliforms* which could be detected in examined samples of commercial labenah were *Citrobacterdiversus, Proteus mirabilis* and *Enterobacteraerogenes* in percentage of 4%, 4% and 4% respectively. *Enterobacter spp.*, particularly *E. aerogenes,* has been associated with nosocomial outbreaks, and is considered opportunistic pathogens. The detection of *Enterobacteraerogenes, Klebsiella, and Serratia species* in commercial and homemade Labenah as the case may be, indicates possible faecal contamination. Being enteric bacteria, their presence indicates poor hygienic practices among handlers of commercial and homemade Labenah. Due to the significance of the faecal-oral route transmission for many bacterial food-borne diseases, basic hygiene measures assume a decisive importance in food safety management (Utermann, 1998).

Enterobacteriaceae are normally associated with poor hygiene and their presence may be a pointer toward a potential health risk. Dirar (1993) observes that lack of pasteurization in traditionally fermented milk products is a major risk-enhancing factor. Even though the milk is boiled for prolonged periods of time, this is insufficient to minimize the risk of contamination, *coliforms* were still detected, an indication of post heat treatment contamination. *Enterobacter spp*. can cause numerous infections, including cerebral abscess, pneumonia, meningitis, septicemia, and wound, urinary tract (particularly catheter-related UTI), and abdominal cavity/ intestinal infections. In addition, *Enterobacter spp*. has been noted in intravascular device-related infections, and surgical site infections (primarily postoperative or related to devices such as biliary stents). Many species can cause extra-intestinal infections (Pagotto *et al.,* 2003 and Farmer *et al.,* 2007).

Other strains of *E. coli* could be isolated and identified with serodiagnosis were documented in Table 4 where *O103: H2(EHEC), O26: H11(EHEC), O125: H21(ETEC), O26: H11(EHEC), O55: H7(EPEC), O91(EPEC) and O125 : H21(EHEC)* in frequency percentage of 14.3%; 14.3%; 14.3%; 14.3%; 14.3%; 14.3% and 14.3% respectively, in the examined samples of homemade labenah. Riley *et al.* (1983) stated that enterohaemorrhagic *E. coli* is a new emerging pathogen causing two principle types of illness in human, Hemorrhagic Colitis (HC) and Hemolytic Uremic Syn-drome (HUS). It was firstly identified as a cause of human illness in 1982 when it was associated with two food related outbreaks of HC in the states of Oregon (26 cases) and Michigan (21 cases). Varnam and Evans (1991) subdivided the pathogenic strains of *E. coli* on the basic of clinical symptoms, mechanisms of pathogenesis, biochemical and serological markers into five groups: enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteroaggregative, and enterohaemorrhagic (EHEC). While, Piercefield *et al.* (2010) stated that one of the common non-*O157* VTEC in the USA is O111:H8 and one of the largest outbreaks was caused by an EHEC O111 in the USA in 2008 causing 341 illnesses.

The higher microbial load may be due to contamination during post-preparation handling, transportation and storage of the finished product. The method of production, handling, transportation and marketing of the homemade products are entirely depend upon traditional method. Such method could pose favorable environment for bacterial contamination. The unclean hands of workers, poor quality of milk, unhygienic conditions of manufacturing unit, inferior quality of material used and water supplied for washing the utensils could be the source of accelerating the bacterial contamination of milk products and post manufacturing contamination (Kulshrestha, 1990).

In the present study, the bacteriological evaluation of homemade Labenah found to be contaminated with different bacterial pathogens *like pathogenic E. coli, coliforms and mold and yeast*. All these bacterial pathogens are responsible for the food borne and diarrheal diseases. The Local Government and the ministry should consider establishment of adequate facilities and utility services as well as provision of necessary information, education and training programmes for consumers.

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دراسة مقارنة بين الجودة الميكروبيولوجية للبنة التجارية والمنزلية

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تم تجميع ٥٠ عينة من اللبنة التجارية والمصنعة منزليا بواقع ٢٥ عينة لكل منهما من محلات السوبر ماركت والمنازل في مدينة اسيوط لفحصها ميكروبيولوجيا. وقد وجد ان متوسطات العدد الكلي للخمائر والفطريات والعدد الكلي للكوليفورم $8.8x10^2$ و $9.6x10^2$ لكل جرام علي التوالي في عينات اللبنة التجارية بينما كانت المتوسطات في عينات اللبنة المصنعة منزليا علي التوالي كالاتي $6x10^2$ و$7.6x10^4$ لكل جرام. أمكن عزل ميكروب الايشيريشيا كولاي الممرض من عينات اللبنة المنزلية بنسبة ٢٨ % (٧عينات) بينما كانت عينات اللبنة التجارية خالية من ميكروب الايشيريشيا كولاي الممرض. وتم تعريف الايشيريشيا كولاي المعزولة من اللبنة المصنعة منزليا باستتخدام الاختبارات السيرولوجية الي : *O103 : H2(EHEC);O26 : H11(EHEC);O125 : H21(ETEC);O55 : H7(EPEC);O91(EPEC) و O125 : H21(EHEC) من ناحية اخري تم تصنيف Serratialiquefaciens; Klebsiella pneumonia; Providenciarettgeri; Proteus mirabilis; Enterobacteraerogenes و Serratiamarcescens* وعزلها والتعرف عليها في عينات اللبنة المصنعة منزليا بنسب ٤% ; ١٢% ;١٢% ; ١٢% ; ٤% علي التوالي. اما اللبنة التجارية فقد وجدت ملوثة بميكروب *Proteus mirabilis* و *Citrobacterdiversus*; بنسب ٤% ; ٤% و ٤% علي التوالي. وبناءا علي النتائج السالف ذكرها، فان هذه الدراسة تظهر ان اللبنة المصنعة *Enterobacteraerogenes* منزليا تحوي انواع كثيرة من الميكروبات الضارة بالجسم. وعلية يحذر من عدم استخدام الطرق الصحية في اعداد اللبنة في المنزل مما يعرضها للتلوث بكميات كبيرة من البكتريا وخصوصا الايشيريشيا كولاي التي تسبب االتسمم الغذائي واستخدام مصادر موثوقة من اللبن والزبادي. كما يجب ايضا الحرص علي مراقبة اعدادها وحفظها بطريقة صحية تمنع تلوثها حتي تصل للمستهلك بطريقة امنة.