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INCIDENCE AND SURVIVAL OF LISTERIA MONOCYTOGENES IN YOGHURT IN ASSIUT CITY

(With 1 Table)

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مدى تواجد وحيوية ميكروب اللستيريا مونوسيتوجينس في الزبادي في مدينة أسيوط

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جمعت ١٥٠ عينة عشوائية من الزبادي العادي (٧٥ عينة) والزبادي بالفواكه (٧٥ عينة) "بالموز والمشمش والمانجو والفراولة والخوخ" من أماكن مختلفة بمدينة أسيوط ، وذلك لمعرفة مدي تلوثها بميكروبات اللستيريا المختلفة. وقد أظهرت النتائج أن جميع العينات المفحوصة لم تسفر عن عزل ميكروب اللستيريا مونوسيتوجينس أو أي من أنواع اللستيريا الأخرى. وكذلك تم دراسة مدي حيوية وبقاء ميكروب اللستيريا مونوسيتوجينس في الزبادي ، ولذلك تم تصنيع الزبادي معمليا من اللبن بعد تعقيمه ثم حقنه ب ٧١٠×٥,٧ خلية/مللي من هذا الميكروب، وتم تقسيم اللبن المحقون إلى أربعة أجزاء بعد إضافة بادئ الزبادي ، منها ثلاثة أجزاء تم إضافة الفاكهة لكل منها (موز ومشمش ومانجو) وتم تصنيعها زبادي وذلك لعمل أربع محاولات لدراسة مدى نمو وبقاء ميكروب اللستيريا مونوسيتوجينس ، ثم حفظت عينات الزبادي بالثلاجة (٥ ± ١° م) لمدة سبعة أيام. وقد تم أخذ عينات من اللبن بعد الحقن، ومن الزبادي بعد التصنيع مباشرة ،وكذلك عينات يومية من الزبادي المحقون والمحفوظة بالثلاجة لتقدير عدد هذا الميكروب والرقم الهيدروجيني بهذه العينات، وتم تسجيل متوسط الأربع محاولات. وقد بينت النتائج أن ميكروب اللستيريا مونوسيتوجينس يتناقص في العدد تدريجيا من ٥,٧ × ١٠^٧ إلى ٥,٦ × ١٠^٧ خلية /جرام بعد التصنيع مباشرة حتى وصل إلى٣,٣ × ١٠^٣ خلية /جرام في نهاية اليوم السابع. أما بالنسبة للرقم الهيدروجيني فقد لوحظ انخفاض شديد من ٦,٤٦ إلى ٤,٧٩ بنهاية التصنيع ثم إنخفض تدريجيا حتى وصل إلى ٣,٩٨ بنهاية فترة التخزين . ولقد نوقشت الأهمية الصحية والطرق والإجراءات الواجب اتباعها لمنع تلوث الزبادي بهذه الميكروبات.

SUMMARY

A total of 150 random samples of plain (75 samples) and fruit yoghurt (75 samples) with banana, apricot, mango, strawberry and peach were collected from different localities in Assiut City and examined for the presence of Listeria spp. The obtained results pointed out that L. monocytogenes and other Listeria spp. could not be isolated from all the samples examined. Furthermore, four trials of yoghurt were manufactured in the laboratory and artificially inoculated with L.monocytogenes at an initial inoculum of $5.7X10^7$ cells/ml, 3 trials were containing fruits (banana, apricot and mango). Then they were refrigerated at $5\pm1^{\circ}C$ for seven days. Numbers of L.monocytogenes, as well as, pH values of yoghurts were determined at zero time and daily thereafter. The number of organisms decreased and reached to $9.9X10^4$ cells/g by the end of the fourth day, and gradually declined till the end of storage to achieve a minimum count of $3.3X10^3$ cells/g. The pH value decreased sharply from 6.46 to 4.79 by the end of yoghurt preparation and decreased gradually to reach 4.34 on the fourth day, and its minimum value (3.98) was recorded by the end of storage period (seven days). The public health importance and the sanitary measures for control of Listeria spp. were mentioned.

Key words: *Isolation, Survival, L.monocytogenes, Plain & Fruit Yoghurt.*

INTRODUCTION

Yoghurt is considered as one of the fermented milk products consumed by different ages of population throughout the world. The great popularity of yoghurt is due to its refreshing and thirst-quenching in hot weather, and it is also considered more digestible than ordinary milk and particularly recommended for sick and convalescent people. Economically, the development of yoghurt manufacture is very necessary because the product represents a major source of income to the dairy industry. The development in the 1950s of fruit and flavoured yoghurt, however, resulted in this product becoming of major importance in the dairy industries of western Europe, the US and other non-traditional markets.

Listeria monocytogenes has become of significant concern in recent years as a serious foodborne pathogen (Gellin et al., 1991). In human the primary manifestations of listeriosis included septicemia, endocarditis, pneumonia, conjunctivitis, pharyngitis, cutaneous papules and pustules, urethritis, meningitis or abortions (Banwart, 1989; Miller et al., 1997 and Ryser, 1998). L.monocytogenes and other spp. of *Listeria* may gain entrance to plain and fruit yoghurts through use of inferior quality raw materials, insufficient heat treatment of milk or contaminated equipment used for its preparation and distribution. On the other hand, yoghurt as a product is characterized by its high acidity, low moisture content and its fat content which may affect *Listeria spp.* and other foodborne pathogens if present due to exposure to lactic acid and other inhibitory compounds produced by lactic acid bacteria. The free fatty acids released during storage period also aid in destruction of foodborne pathogens (Wang and Johnson, 1992).

L.monocytogenes and other spp. of *Listeria* could not be isolated from yoghurt examined by different investigators (Kerr et al., 1992; Rola et al., 1994; Abou-Eleinin, 1999 and El-Prince, 1999). The failure of *Listeria* to grow in yoghurt may be attributed to the high content lactic acid and the resultant lowering of its pH value. While, the lower incidence (2%) of L.monocytogenes obtained by Greenwood et al. (1991) may be due to the post-processing contamination from the plant environment. Likewise, the growth and survival of L.monocytogenes in yoghurt have been noted elsewhere (Siragusa and Johnson, 1988; Ahmed, 1989; Singh and Chander, 1990 and Zuniga Estrada et al., 1995). However, Abdel-Hady (1998) and El-Sherbini and El-Dweny (1999) investigated the behaviour of some pathogenic bacteria in fruit yoghurt. Recently, in Egypt, the incidence and behaviour of L.monocytogenes in milk and its products were studied by several workers (Abdel-Khalek and El-Gamal, 1998; Abou-Eleinin, 1999; El-Prince, 1999; Deeb, 2000 and Abou-Zeid et al., 2001). Moreover, reports on food borne illness are progressively increased and the need of detection, identification as well as studying the factors that enhance and retard growth and survival of microorganisms become of a public health concern.

Therefore, the goal of the present study is to examine the plain and fruit (banana, apricot, mango, strawberry and peach) yoghurts sold

in Assiut City for detection and isolation of L.monocytogenes and other spp. of Listeria. Also, to study the growth and survival of L..monocytogenes artificially injected in yoghurt stored at refrigeration temperature (5±1°C).

MATERIAL and METHODS

I- Incidence of Listeria species in different types of yoghurt sold in Assiut City:

1 – Collection of samples:

150 random samples of plain (75 samples) and fruit yoghurt (75 samples) with banana, apricot, mango, strawberry and peach were collected from different localities in Assiut City. The collected samples were transferred to the laboratory with a minimum of delay where they were examined for presence of *Listeria spp.*

2- Preparation of samples:

Samples were prepared following the technique described by APHA (1992).

3– Isolation and identification of Listeria spp.:

The technique recommended by FDA (Lovett et al., 1987) was adopted by selective enrichment in *Listeria* enrichment broth (LEB) followed by selective plating onto Oxford agar plates (Curtis et al., 1989). Suspected colonies of *Listeria* were picked up and purified before being identified according to Hitchins (1995).

II- Growth and survival of *Listeria* monocytogenes in yoghurt:

- Culture:

L.monocytogenes strain was obtained from Institute of milk hygiene and technology, Vet. Med. Univ., Vienna, Austria. The strain was inoculated into LEB followed by plating on Oxford agar for typical colonial morphology and purity. The amount of inocula was determined by plating 0.1 ml from decimal dilution onto Oxford agar. The incubation was done at 37°C for 24-48h.

- Experimental procedure:

Yoghurt samples were manufactured in the laboratory from sterile milk. Milk was inoculated with L.monocytogenes at 45°C immediately after the starter to provide $5.7X10^7$ cells/ml. Addition of starter cultures was done according to Lampert (1975). Four trials were

made to study the survival of the organism in yoghurt. The first trial was done in plain yoghurt, and the other three trials were performed in yoghurt containing fruits (banana, apricot and mango). This was done by dividing the inoculated sterile milk after addition of starter cultures into four portions. The first portion was made without fruits, while the other 3 portions were made to contain fruits and 1% added sugar. The prepared yoghurt with their control were kept at 5±1°C in a refrigerator. Samples were taken from, the inoculated sterile milk, and as well from yoghurts just after preparation and then daily up to 7 days to determine numbers of L.monocytogenes and the pH value. The average counts of L.monocytogenes and pH value of the four trials were recorded.

- Enumeration of L.monocytogenes:

Counting of L.monocytogenes was achieved by direct plating of decimal dilutions of prepared samples (APHA, 1992) onto plates of Oxford agar. The plates were incubated at 37°C for 24-48h, and typical colonies presumed to be L.monocytogenes were counted.

- pH determination:

The pH values of yoghurt were determined by using an Orion pH meter model 701, equipped with standard combination electrode.

RESULTS

The obtained results were recorded in Table 1.

DISCUSSION

The obtained results proved that L.monocytogenes and other spp. of *Listeria* could not be detected in any of the 150 examined samples of yoghurts (plain and fruit "banana, apricot, mango, strawberry and peach"). Similar results were obtained by Kerr et al. (1992); Rola et al. (1994); Abou-Eleinin (1999) and El-Prince (1999). Likewise, Siragusa and Johnson (1988) who investigated the fate of L.monocytogenes added to commercial yoghurt, and by using low inoculum level (10^2 cells/g), no L.monocytogenes cells were detected after 3 days. The failure of these organisms to grow in yoghurt may be due to lactic acid production and the resultant lowering pH value of such product (Irvin, 1968; Ahmed, 1989; Huang et al., 1993 and Marth, 1993). Also, Seeliger and Jones (1986) recorded that L.monocytogenes could only grow at pH

values from 5.6 to 9.6, with optimal growth occurring at neutral to slightly alkaline pH values. On the other hand, Greenwood et al. (1991) isolated L.monocytogenes from one sample (2.13%) out of 47 yoghurt samples and the authors attributed the presence of *Listeria spp.* in yoghurt to the post-processing contamination from the plant environment. Although, milk used to produce the industrially fermented milk is pasteurized, contamination of the product with L.monocytogenes may occur after pasteurization if complex and less easily cleaned equipment is used in the packaging/filling rooms or if bulk starter cultures are contaminated with the organism (Charlton et al., 1990). Generally, lowering the pH value of food is widely used as a measure for food safety. Yoghurt is a very popular product in Egypt and other countries, and it is used all the year round, specially in summer. Moreover, during the last decade the addition of fruits (banana, apricot, mango, strawberry, peach and others) to yoghurt is a new innovation. The fruit yoghurt has increased remarkably and occupied the largest sales (Marshall, 1982).

Results from the second part of the study, as indicated in Table 1 showed that the effect of pH value on growth and survival of L.monocytogenes during preparation and storage of yoghurt at refrigeration temperature (5± 1°C). The average viable cell count of L.monocytogenes slightly decreased during preparation of yoghurt from $5.7X10^7$ to $5.6X10^7$ CFU/g. The microorganism significantly decreased during the first day of storage ($7.1X10^5$ cells/g). Then, the bacterium gradually decrease in count from $4.9X10^5$ to $9.9X10^4$ organism/g on the fourth day till reach its minimum numbers ($3.3X10^3$ cells/g) by the end of storage. A sharp drop in the average pH value of yoghurt from 6.46 to 4.79 occurred by the end of its prepartion. A low value of 4.34 was achieved on the fourth day and reached to its minimum value by the end of storage (3.98). These findings go parallel with the results achieved by Irvin (1968); Ryser et al. (1985); Ahmed (1989) and Marth (1993). Also, the obtained results are in good agreement with those reported by Siragusa and Johnson (1988) who studied the fate of L.monocytogenes added to commercial yoghurt. Detectable numbers of *Listeria* cells, inoculated at high levels (10^7 cells/g), survived in yoghurt at pH 4.1 for 6 days by direct plating, while enrichment procedure showed some cells persisted for up to 9 days. The authors concluded that there is a

possibility for persistence of L.monocytogenes in yoghurt if introduced as a contaminant in sufficiently high numbers at a post-fermentation step such as the carton filler station.

Moreover, L.monocytogenes survived in yoghurt for 1 to 12 days during refrigerated storage of the product (Singh and Chander, 1990). Likewise, Zuniga Estrada et al. (1995) illustrated that L.monocytogenes survived 32 days in the cultured milk with a yoghurt starter culture, when the inoculum was 10^7 CFU/ml, and inhibition of the pathogen was associated with a decrease of pH to <4.0 and increase in acidity. The authors also added that this pathogen was able to survive several weeks in milk cultured with a starter culture, despite the general assumption that this would be very difficult due to the low pH. It is concluded that cultured milks may play an important role in the transmission of this bacterium.

It is noteworthy from these trials that the drastic reduction in viable cell number of L.monocytogenes in yoghurt during its refrigerated storage, and the loss in its viability may be due to the decrease of pH value of yoghurt from 4.79 to 3.98 and acidity increased as lactic acid percent. Also, it is worthy to state that the combination of Lactobacillus bulgaricus and Strept. thermophillus in yoghurt having strong effect on the growth and survival of such bacteria and L.bulgaricus was the most detrimental to L.monocytogenes. These findings were also supported by Kerr et al. (1992) who failed to isolate any of the *Listeria spp.* from 100 yoghurt samples examined, and the low pH value of the samples was between 3.6 and 4.6 with an average of 4.24. On the other hand, the contamination of yoghurt by L.monocytogenes from the view point of a potential health hazard should not be ignored.

In general, L.monocytogenes is widely distributed in nature and has been existed in soil, foods, water and faeces, besides containers where yoghurt is filled in, temperature where it is kept at and the process of filling in final containers which delivered to the consumers considered as sources for contamination (Weis and Seeliger, 1975; Laciar et al., 1999 and Baek et al., 2000). Furthermore, L.monocytogenes has become a pathogen of concern for food industry, the organism is one of the most studied causes of food poisoning in the last few years. In addition to acute gastroenteritis, listeriosis can cause miscarriages or result in meningitis in-patients with chronic diseases (Nelson, 1990; Pritchard et

al., 1994; Miller et al., 1997 and Ryser, 1998). On the other hand, fruit yoghurts are very popular types of milk products, and pasteurization in flavoured yoghurt represents an extremely important stage in the pre-treatment of fruit additives to inactivate all vegetative microorganisms, but without impairing the taste and structure of the fruits (Alfa-Laval-AB, 1983).

In conclusion, from this work it is clear that contamination of plain and fruit yoghurts by *Listeria spp.*, specially L.monocytogenes from the view point of a potential health hazard should not be ignored. Listeria could contaminate the yoghurt through raw milk used without sufficient heat treatment or through contaminated equipment used for preparation or distribution of the plain and fruit yoghurts. Therefore, application of good hygienic measures during production, storage and distribution of such products are essential to safe yoghurt quality, consequently prevent the risk of human hazard. Likewise, rapid development of lactic acid by good starter culture and use of clean milk are essential for making the product unfavourable for growth and survival of L.monocytogenes. In addition, it is important for food hygienists and employes working in the field of production of yoghurt to understand the pattern of microbial growth specially those of a public health concern such as L.monocytogenes in order to safeguard consumers and human health.

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Table 1: Growth and survival of L.monocytogenes during preparation and storage of yoghurt at 5±1°C.

Time (days)	Trial 1		Trial 2		Trial 3		Trial 4		Average	
	Count/g	pH	Count/g	pH	Count/g	pH	Count/g	pH	Count/g	pH
I	5.7×10^7	6.46	5.7×10^7	6.46	5.7×10^7	6.46	5.7×10^7	6.46	5.7×10^7	6.46
P	5.9×10^7	4.81	5.7×10^7	4.87	5.5×10^7	4.72	5.3×10^7	4.75	5.6×10^7	4.79
1	3.8×10^5	4.77	8.3×10^5	4.62	2.1×10^5	4.63	1.4×10^6	4.71	7.1×10^5	4.68
2	2.4×10^5	4.52	9.7×10^5	4.59	9.8×10^4	4.61	6.6×10^5	4.74	4.9×10^5	4.62
3	1.9×10^5	4.58	4.6×10^5	4.41	3.6×10^4	4.33	8.6×10^4	4.55	1.9×10^5	4.47
4	8.3×10^4	4.44	2.5×10^5	4.37	1.5×10^4	4.17	4.7×10^4	4.36	9.9×10^4	4.34
5	6.5×10^4	4.32	1.4×10^5	4.42	4.3×10^4	4.08	9.2×10^4	4.27	8.5×10^4	4.27
6	9.2×10^3	4.19	3.8×10^4	4.23	2.8×10^3	3.97	7.0×10^3	4.05	1.4×10^4	4.11
7	4.3×10^3	4.02	7.2×10^3	4.16	6.0×10^2	3.82	9.0×10^2	3.94	3.3×10^3	3.98

I= Inoculated sterile milk. P= Prepared yoghurt.