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Original Research Article

The survival of listeria monocytogenes in yoghurt and ice cream

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ABSTRACT

The ability of *L. monocytogenes* as an opportunistic pathogen of humans and animals, to survive and grow under various adverse environmental conditions, makes it a potential health hazard after the consumption of contaminated dairy products, it often implicated in several outbreaks of listeriosis. This study was conducted to investigate the survival of *L. monocytogenes* strain *(NCTC13372)* when inoculated with a population level of 6.95 log cfu/g and 7.64 log cfu/g and stored at 4ºC for 15 days and 3 months at -18ºC for yoghurt and ice cream respectively. The obtained results indicated that complete inactivation of the tested organism wasn't achieved till the end of storage periods and the inoculated *L. monocytogenes* was survived in both yoghurt and ice cream throughout the trial. It is concluded that in the dairy industry, we cannot rely upon either fermentation process and storage at refrigerating temperature or upon storage at freezing temperature during yoghurt and ice cream manufacturing to control *L. monocytogenes* pathogen in order to provide safe products for consumption.

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1. Introduction

Milk and dairy products, because of their high nutritional value, are very suitable for development of microorganisms, including pathogenic bacteria **(Farber and Peterkin, 1991; Kasalica et al, 2011)**. To what extent it will develop depends primarily on the type of product, its composition, manufacturing and storing.

Yoghurt is one of the most popular fermented dairy products which have a wide acceptance worldwide. It considered a nutrient dense food, whereas it highly nutritious, easily digestible and it is a source of more than ten essential nutrients, certain minerals and vitamins **(Weerathilake et al., 2014)**

Ice cream is one of the widely accepted dairy products that dominates interest of large segments of populations **(Anonymons, 2012),** it consider as very suitable environment for microbial growth due to its high nutritional value, almost neutral pH value (pH ~6-7) and long storage periods **(Windrantz and Aries 2000; Molla et al., 2004;Gougouli et al., 2008).**

*L. monocytogenes*is a foodborne pathogen that is widely distributed in the nature and mainly associated with a serious and often life-threatening disease owing to its elaborate physiological adaption mechanism, *L. monocytogenes* can survive and even proliferate in a variety of foods under adverse environmental conditions such as low pH, high salinity and low temperature **(Angelidis et al.,2010).**

Several outbreaks of *L. monocytogenes* infection were mainly associated with the consumption of milk and dairy products **(CDC, 2011; Gaulin et al., 2012)** which subjected to minimal further processing and post processing contamination **(McDonald et al., 2005; Swaminathan and Gerner-Smidt, 2007).**

The survival of *L. monocytogenes* mainly depends upon many factors including the type of culture of microorganisms **(Schaack and Marth, 1988)**, size of inoculums **(Zuniga-Estrada et al., 1995**), the condition during manufacturing and fermentation **(Gameiro et al, 2007)**, different physical and chemical stresses and storage condition of the dairy products **(Faleiro et al., 2003; Arques et al., 2005).**

Therefore, the objective of the present study was to monitor the behavior and survival of *L. monocytogenes* during refrigerating storage of yoghurt and during freezing process of ice cream.

2. Material and Methods:

2.1. Bacterial strain and Media:

L. monocytogenes strain **(NCTC 13372)** was kindly provided by Animal Health Research Institute (AHRI), Dokki, Egypt.

The strain of *L. monocytogenes* was maintained on tryptic soya agar with 0.6% yeast extract (oxoid) slants at 4°C.The strain was activated by inoculation in tryptic soya broth with 0.6% yeast extract (Oxoid) and incubated at 30°C for 24 h`, then ten-fold serial dilution was made. Inoculation level of listeria strain was determined by direct plating on oxford agar medium.

2.2. Preparation of yoghurt:

Yoghurt was prepared in the laboratory according to **(Gulmez and Guven, 2003).**

One liter UHT milk was warmed to the 40-43°C, whereas starter culture (2-3%) was

added to milk with thoroughly mixed then cultured milk was divided into 2 parts.1st part of cultured milk was inoculated with *L. monocytogenes* strain **(NCTC 13372)** with a population level of 6.95 log cfu/g, while other part left to serve as a control. Inoculated milk was distributed into sterile small cups and incubated at 45°C for 5-6h to be coagulated and then stored at refrigerator temperature (4°C).

Survival of *L. monocytogenes* was detected in milk at time of inoculation (zero time), in curd (5-6 h after inoculation) and the finished product was examined daily up to 15 days.

2.3. PH measurement: The pH was measured with pH meter (Adwa, AD 111).

2.4.Preparation of ice cream:

Ice cream was prepared in the laboratory according to **(Mastronicolis et al, 2011).**

Full dissolution of the dry ingredients of ice cream into the liquid materials; the mix was then batch pasteurized at 76°C for25 min in a water bath and the ice cream mix was divided into 2 parts. The 1st part was inoculated with *L. monocytogenes strain **(NCTC13372)*** with a population level of 7.64 log cfu/g. and the other part left to serve as a control. The inoculated ice cream mix was distributed into small cups then the mixes were cooled rapidly at 4°C and aged at 4°C for 18 h. Finally, the aged mixes were stored at freezing temperature of -18°C.

The survival of *L. monocytogenes* was detected in ice cream at time of inoculation (zero time), daily for the first week , then each ten days till the end of the storage period.

2.5.Microbiological analysis:

All procedures were applied according to the FDA-Bacteriological Analytical Manual **(Hitchins, 2011).**

10 g of each sample was transferred to sterile flasks containing 90 ml of 0.85% sterilized physiological saline solution and homogenized. The homogenate was prepared for (10-Fold) diluted serial dilutions up to 10^6 and plated by the spread plate technique onto the selective medium (oxford listeria selective agar (CM0856, Oxoid) supplemented with listeria selective supplement (SR0140, Oxoid) and incubated in 37°C for 24-48 h.

3. Results and Discussion

Food safety is a complex issue that has an impact on all segments of the society, from the public to government, industry and academia.

3.1. The Survival of Listeria monocytogenes in yoghurt during refrigeration storage at 4°C for 15 days :

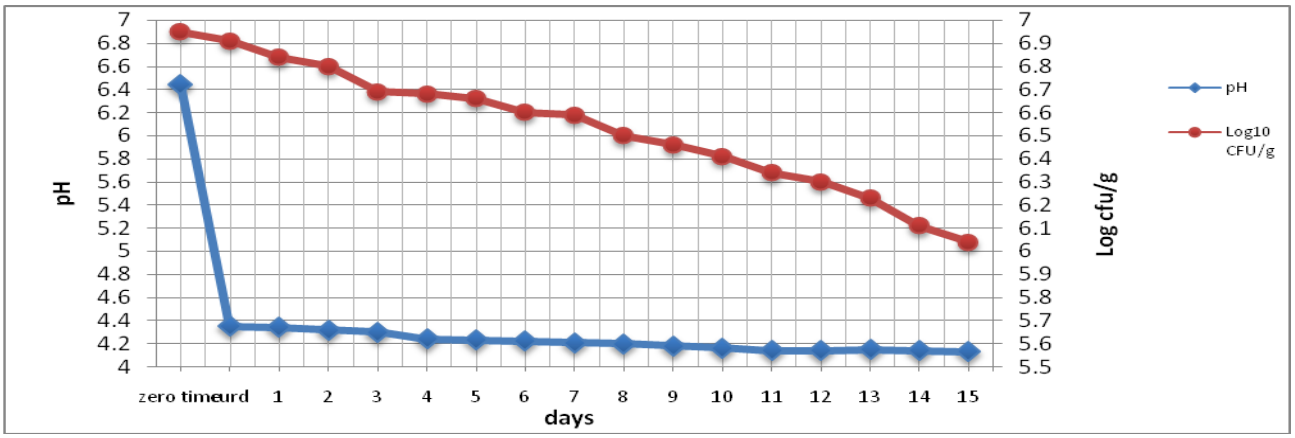
The interest in fermented dairy products is continuously increasing owing to their nutritional and microbiological quality and thepotential health promote. Yoghurt received the least attention due to the fact that, its high acidity and milk pasteurization before addition of starter culture are effective barriers to pathogens including *L. monocytogenes* **(Benkerroum et al., 2003; Liu and Puri, 2008).**

The behavior of *L. monocytogenes* during yoghurt manufacturing and storage at 4°C is summarized in **(Table 1 and Figure1).**

Table 1: The survival of *L. monocytogenes* in yoghurt during the storage periods at 4°c for 15 days

Incubation period/ day	pH	Log_{10} CFU/ml
Zero time	6.44	6.95
curd	4.35	6.91
1st day	4.34	6.84
2nd day	4.32	6.80
3rd day	4.30	6.69
4th day	4.24	6.68
5th day	4.23	6.66
6th day	4.22	6.60
7th day	4.21	6.59
8th day	4.20	6.50
9th day	4.18	6.46
10th day	4.16	6.41
11th day	4.14	6.34
12th day	4.14	6.30
13th day	4.15	6.23
14th day	4.14	6.11
15th day	4.13	6.04

Figure 1: The survival of *L. monocytogenes* in yoghurt during the storage periods at 4°C for 15 days



In the current study, the initial population of *L. monocytogenes* inoculated in yoghurt at zero time was 6.95 log cfu/g with pH value 6.44. Slow and slight reduction in the counts of *L. monocytogenes* was detected from 6.95 log cfu/g to 6.04 log cfu/g (with 0.91 log cfu/g decline) at the end of trial, while pH value decreased sharply from 6.44 to 4.35 at end of yoghurt preparation, then gradually decreased till reaching to its minimal value 4.13 at the end of storage period (15 days).

Complete inactivation of *L. monocytogenes* was not achieved and the counts of *L.monocytogenes* were never less than 6 log cfu/g at the 15^{th} days of trial.

In a study conducted by **Ashenafi (1994)** demonstrated substantial numbers of *L. monocytogenes* strains still survived even with markedly decrease of pH to as low as 3.9, on the other hand **Sabreen and Korashy, (2001)** investigated the survival of *L. monocytogenes* during short storage period at 4ºC and they found that *L. monocytogenes* was decreased from its initial numbers of 5.7×10^7 cells /ml to 3.3×10^3 cells /ml at the end of storage (at7^{th}day) with decline in pH value from 6.46 to 3.9.

Another study performed by **Schaack and Marth (1988)** reported that *L. monocytogenes* was survived during yoghurt manufacture and fermentation from 1 day (final **pH 4.13**) to 12 day (with final **pH 3.93**) during refrigerated storage of the products at 4°C.

The study of **Massa et al. (1991)** stated that inoculated *L. monocytogenes* either at level of 10^3 and 10^7 were only survived for 48h and 7 days respectively. Whereas initial pH (at time of preparation) decreased from 4.9 to 4.2 (in low inoculate), and from 5 to 4.2 (in high inoculate).

Other studies were investigated that exposure to pH 4.0 can totally inactivate *L. monocytogenes* **(Samelis et al., 2003; Aygun and Pehlivanlar, 2006; Tiganitis et al., 2009).**

Another study of **Ahmed et al., (2014)** reported that inoculated *L. monocytogenes* showed dramatically dropped from initial population of 3×10^7 to 22×10^3cfu/g on the ninth day of storage period (12 day), when the pH value radically decreased to 3.98.

On the other hand, in the study performed by **Zuniga-Estrada et al., (1995**) recorded that *L. monocytogenes* was survived for 8h , 10 days and 32 days in fermented milks when inoculated with 10^3,10^5 and 10^7cfu/ml respectively during refrigerating storage at 4ºC .

In the study conducted by **Tirloni et al., (2015)** the survival of inoculated *L. monocytogenes* with a conc. of 2 log cfu/g and 5 log cfu/g into plain and flavored yoghurt was investigated. They found that inoculated *L. monocytogenes* was survived till the end of trial (68 days) in the two type of yoghurt with conc. 2 log cfu/g and only in flavored yoghurt in conc. 5 log cfu/g while it survived till the day 61 of the trial in the plain yoghurt with conc. 5 log cfu/g.

Therefore In the current study, the survival of *L. monocytogenes* during preparation of yoghurt and at refrigerating storage, Contrary to the general belief which considered it is very difficult due to its low pH, make fermentation process and

refrigerating storage did not seem to be effective in controlling of *L. monocytogenes* growth that made the fermented milks could be potentially hazardous to the public health.

3.2. The survival of *Listeria monocytogenes* in ice cream during storage at -18°C for 3 months:

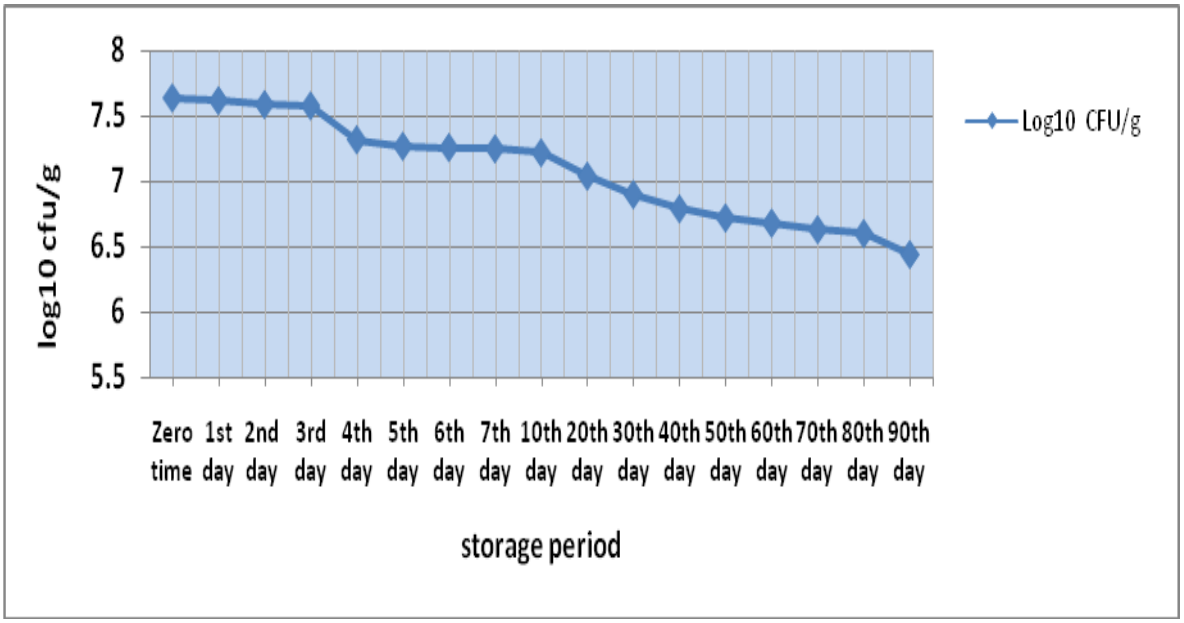
L. monocytogenes as a phychrotrophic microorganism has the ability to grow at refrigerator temperature which allow for the possibility of proliferation and cause a disease through frozen foods **(Schillinger et al., 1991).** In a dairy processing plant**,** *L. monocytogenes* can experience coldconditions leading to cold adaption of this organism which enhance its survival in frozen dairy products.

Data concerned about the behavior of *L. monocytogenes* in ice cream during frozen storage are very limited by (**Palumbo and Williams, 1991; Dean and Zottola, 1996; Gougouli et al., 2008; Mastronicolis et al., 2011)).**

Table 2: The survival of *L. monocytogenes* in ice cream during the storage at –18°C for 3 months

Incubation period	Log_{10} CFU/g
Zero time	7.64
1st day	7.62
2nd day	7.59
3rd day	7.58
4th day	7.32
5th day	7.27
6th day	7.26
7th day	7.25
10th day	7.22
20th day	7.04
30th day	6.9
40th day	6.79
50th day	6.72
60th day	6.68
70th day	6.63
80th day	6.6
90th day	6.44

Figure 2: The survival of *L. monocytogenes* in ice cream during the storage at –18°C for 3 months



In the present study, The viable cell counts of the initial population of *L. monocytogenes* (7.64 log cfu/g) inoculated into ice cream samples and stored at freezing conditions were remained almost constant with no apparent reduction (from 7.64 log cfu/g to 7.58 log cfu/g) at the 3rd day of storage, then very slow reduction in the counts (from 7.32 log cfu/g to 7.22 log cfu/g) with 0.1 log decline was observed from the fourth day till the tenth day of storage. Over the tenth day of storage the viable cell counts were obviously decreased and reached to 6.44log cfu/g (1.20cfu/g lower than the initial population) at the end of storage period. **(Table2 and Figure2).**

Gougouli et al., (2008) reported that the inoculated *L. monocytogenes* with ($10^3$and 10^6cfu/g) into ice cream samples stored at constant freezing conditions showed no changes in the population during storage period of 90 days.

Another study conducted by **Palumbo and Williams, (1991)** reported the survival of *L. monocytogenes* for 14 weeks in ice cream stored at -18°C with no apparent cell death or injury, whereas **Dean and Zottola (1996)** detected the survival of this organism throughout a 3 month of frozen storage.

In a study of **Mastronicolis et al. (2011)** reported that non adapted *L. monocytogenes* was survived for 182 days storage and the initial populations were reduced from 6.30 to 4.09 log cfu/g (with 2.21 log cfu/g decline).

Similar behavior of *L. monocytogenes* was observed by **El-Kest and Marth (1990 and 1991)** in frozen milk and frozen milk components, in which the intial population of *L. monocytogenes*

was decreased over 7 weeks. Also **Papageorgiou et al. (1997)** showed that 98% of *L. monocytogenes* population survived for7.5 months in frozen ewe`s milk and feta cheese curd at -38°C or -18°C. from the previous studies, the survival of *L. monocytogenes* differ not only in food substrates and the strain of *L. monocytogenes*, but also in the time period of storage, freezing rate and other conditions (**Mastronicolis et al., 2011).**

Therefore, freezing storage cannot be used by the industry as a means of reducing the level of pathogen in a contaminated batch. Ideally, ice cream should be kept under freezing temperature conditions during its transportation and storage in which there is a potential for sever temperature abuse.

4. Conclusion:

The results obtained in this study revealed that the ability of *L. monocytogenes* to survive and resist the acidic condition during the storage period of yoghurt may represent a serious risk for the public health. Also the data obtained about the behavior of *L. monocytogenes* in ice cream stored under freezing conditions may be used as an effective tool in the dairy industry to predict of this organism during manufacturing, distribution and storing. HACCP systems must be focused on the selection of good quality raw milk and strict hygienic measures should be applied during processing, packing, distribution and storing conditions to ensure the safety of the products for human consumption.

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بقاء ميكروب الليستيريا مونوسيتوجينس فى الزبادى والآيس كريم

نظرا لقدرة ميكروب الليستيريا مونوسيتوجينس كميكروب ممرض لكلا من الانسان والحيوان على البقاء والنمو تحت الظروف البيئية المعاكسة والمختلفة ،مما يجعله مسببا للعديد من المخاطرعند استهلاك الالبان ومنتجاتها والذى غالبا ينتج عنه العديد من الاوبئة الخطيرة لمرض الليستيريوزس .وقد اجريت هذه الدراسة لاكتشاف مدى قدرة ميكروب الليستيريا عترة (NCTC 13372)على التواجد عندما يوجد بتركيز٦.٩٥ log cfu/g و٧.٦٤ log cfu/g ويتم تخزينه عند ٤ درجة مئوية لمدة ١٥ يوم و٣ شهور عند درجة -١٨ درجة مئوية فى الزبادى والآيس كريم على التوالى . وقد اظهرت النتائج الآتى انه لم يتم حدوث تثبيط كامل لميكروب الليستيريا وانما استطاع الميكروب البقاء حيا حتى نهاية التجربة .وقد استخلص من ذلك انه فى مجال صناعة الالبان لا يمكننا الاعتماد على عملية التخمر والتخزين فى درجة حرارة الثلاجة او التخزين عند درجة التجمد للسيطرة والقضاء على ميكروب الليستيريا اثناء تصنيع الزبادى والايس كريم للحصول على منتج آمن صالح للاستهلاك.