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Survival of local *Listeria monocytogenes* after treatment with sodium benzoate in trypticase soy broth

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Abstract

Survival of *Listeria monocytogenes* (*L. monocytogenes*) by various concentrations (0.1, 0.2 and 0.3%) of sodium benzoate was studied in trypticase soy broth. The medium was adjusted to pH 5.0 or 5.5 and incubated at 4 or 37°C for 5weeks. After incubation, number of the bacteria was estimated in the treated and untreated samples. Average counts of *L. monocytogenes* of untreated (control) samples were higher than those of all sodium benzoate treated samples in both pH values and after incubation at both temperatures. None of the three benzoate concentrations tested was able to completely inhibit growth of *L. monocytogenes* at both temperatures and pH values used. It was concluded that survival of pathogenic of *L. monocytogenes* was highly influenced by sodium benzoate as well as pH of the medium and incubation temperature.

Keywords: *Listeria monocytogenes*, Survival, Sodium benzoate, Trypticase soy broth.

Introduction

*Listeria monocytogenes*is an intracellular pathogen which induces what is known as listeriosis, a severe disease with high hospitalization and mortality rates. It estimated to cause nearly 1600 illnesses each year in the United States with more than 1400 hospitalizations and 250 deaths. Based on EFSA report, the incidence in Europe was 3 cases/millions of inhibitions. According to FDA studies, 1 of 10 of humans may be intestinal carriers of Listeria (Vilar *et al.,* 2007; Scallan *et al.,* 2011; CDC. 2011; EFSA, 2012). *L. monocytogenes*is widespread in the environment, it is found in soil, decayed vegetation, water and sewage, and it can be isolated from humans and domestic animals. Infection by *L. monocytogenes*is caused almost exclusively by the consumption of a wide variety of contaminated foods, including dairy, meat, fishery, and sea food products .It has been associated with some of the outbreaks in the United States and Europe (Lovett *et al.,* 1987; Said *et al.,* 1994; Colette *et al.,* 2008; Jackson *et al.,* 2011; Konosonaka *et al.,* 2012; Gambarin *et al.,* 2012; Zhang *et al.,* 2012).

*L. monocytogenes*is considered an ubiquitous microorganism capable of adapting to a wide range of environmental conditions such as refrigeration temperature, low pH and high salt concentrations. These abilities make it well adapted to food environments, which normally restrict bacterial growth (Baqir *et al.,* 1998; Gandhi and Chikindas, 2007; Degenhardt and Sant' Anna, 2007; Chan and Wiedmann, 2009; Walker *et al.,* 2008).

Sodium benzoate is used as a chemical preservative in a wide variety of food for its effect against microorganisms particularly the bacteria (Jay, 2000). It is often used to conserve margarine, fresh juices, and sweets, European Commission limits for benzoic acid and sodium benzoate are 0015-0.5 (EC, 1995). The FAO/WHO Joint Expert Committee on Food Additives (JECFA) assigned an acceptable daily intake (ADI) of 0.5 mg/kg of body weight to benzoic acid and sodium benzoate (FDA, 2012). The aim of this work was to investigate the efficiency of different concentrations of sodium benzoate against growth of *L. monocytogenes* in trypticase soy broth medium with two pH values and after incubation at two different temperatures.

Materials and Methods

Bacterial culture: *Listeria monocytogenes* used in this study was isolated from local soft white cheese sold at Baghdad markets by the method of the United States Food and Drug Administration (FDA) as described by (Lovett, 1988). Bacterial cultures were maintained on trypticase soy agar slants (Alpha Bioscience, USA).

Sodium benzoate treatment: Three concentrations (0.1, 0.2, and 0.3%) of sodium benzoate were used; such concentrations are in accordance with the

levels permitted to be used as food preservative. They were prepared and added to trypticase soy broth medium as mentioned by (El-Shenawy and Marth, 1988).

Growth condition: Two different pH values (5 and 5.5) have been used in this study and numbers of *L. monocytogenes* were estimated in the samples before and weekly after incubation at both 4 and 37°C for 5 weeks.

Counting techniques: After cultures were vigorously shaken, serial dilutions (10^{-1} to 10^{-7}) was prepared from each culture by using 0.85% of normal saline physiological solution. Surface plating of 0.1 ml (in duplicate) of specific dilutions on trypticase soy agar was also made. After plates were incubated at 37°C for 48h under normal atmospheric conditions colonies were counted with the aid of a Darkfield Quebec colony counter apparatus. Total counts were calculated by multiplying the number of colonies by the dilution factor (Atlas *et al.,* 1995).

Results and Discussion

Average counts of *L. monocytogenes* in untreated (control) samples were higher than those of all sodium benzoate treated samples in the two pH values (5 and 5.5) used after both incubation temperatures (4 and 37°C).

Highest log. number ($10^{11.3}$/1g) of *L. monocytogenes* was recorded in the control sample after incubation at 4°C for 4 weeks, while the lowest number ($10^{4.7}$/1g) was in the sample of pH 5.0 and 0.3% Na-benzoate concentration after incubation at same temperature for 5 weeks. However, numbers of the bacteria in the control sample of pH 5.5 increased from$10^{9.53}$/1g in the first week to $10^{11.2}$/1g after the last week of incubation. When such results are compared with those of control sample but with pH 5.0, the number was increased to just $10^{8.9}$/1g after the last week (Figures 1, 2).

Generally, inconsiderable differences were recorded in the numbers of *L. monocytogenes* incubated at 37°C for both pH (5 and 5.5) values during the same periods of incubation. All numbers of sodium benzoate treated and untreated samples were higher than 10^{8}/1g. Moreover, slight decreases in numbers of the bacteria of treated samples were noticed upon prolonging the period of incubation at this temperature (Figures 3 and 4).

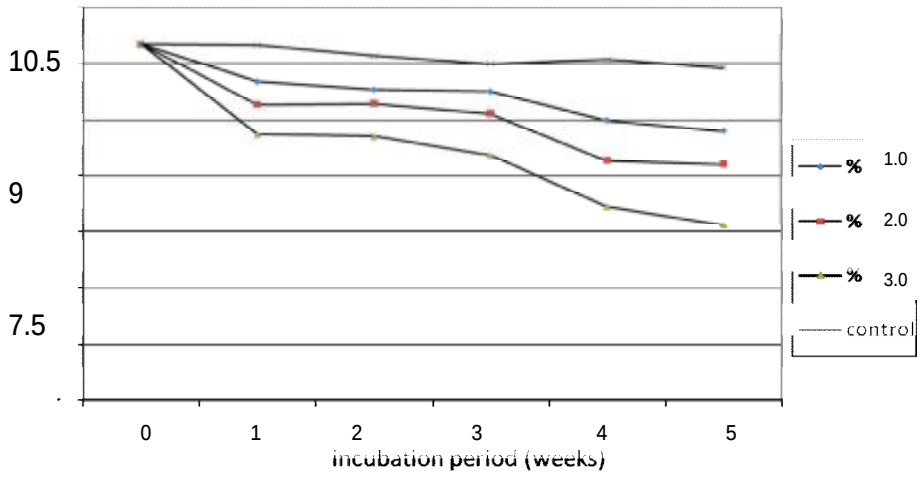

Figure (1): Behavior of *Listeria monocytogenes* in the presence of various concentrations of sodium benzoate at pH 5.0 and 4°C

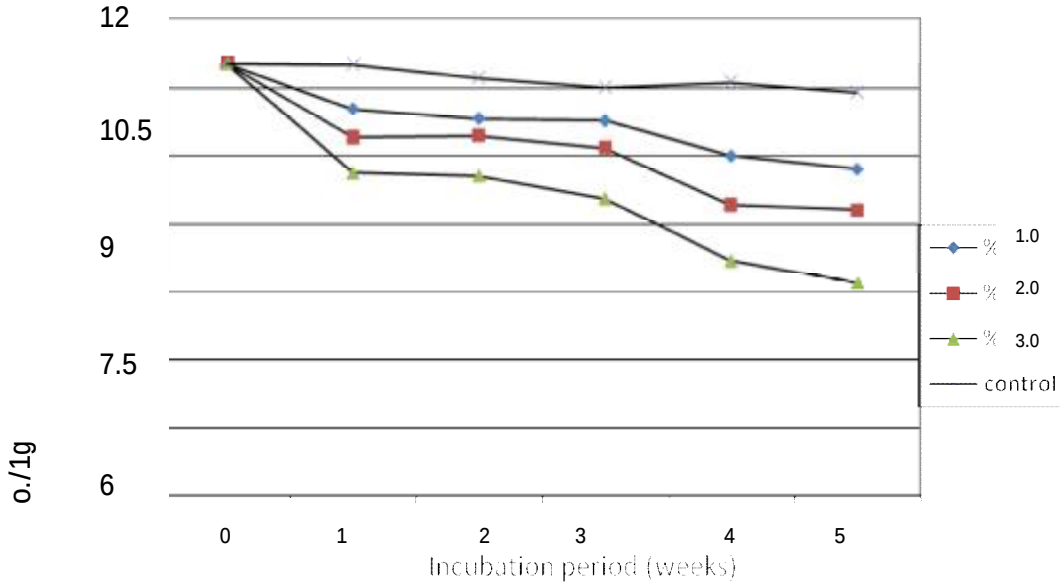

Figure (2): Behavior of *Listeria monocytogenes* in the presence of various concentrations of sodium benzoate at pH 5.5 and 4°C

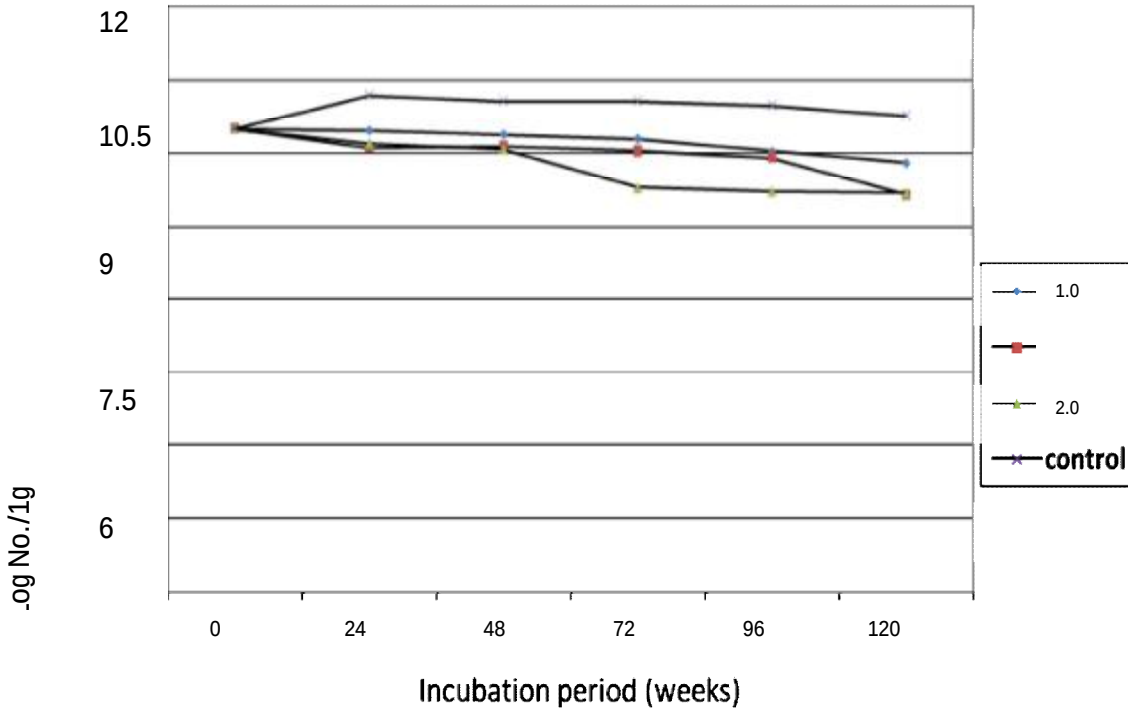

Figure (3): Behavior of *Listeria monocytogenes* in the presence of various concentrations of sodium benzoate at pH 5.0 and 37°C

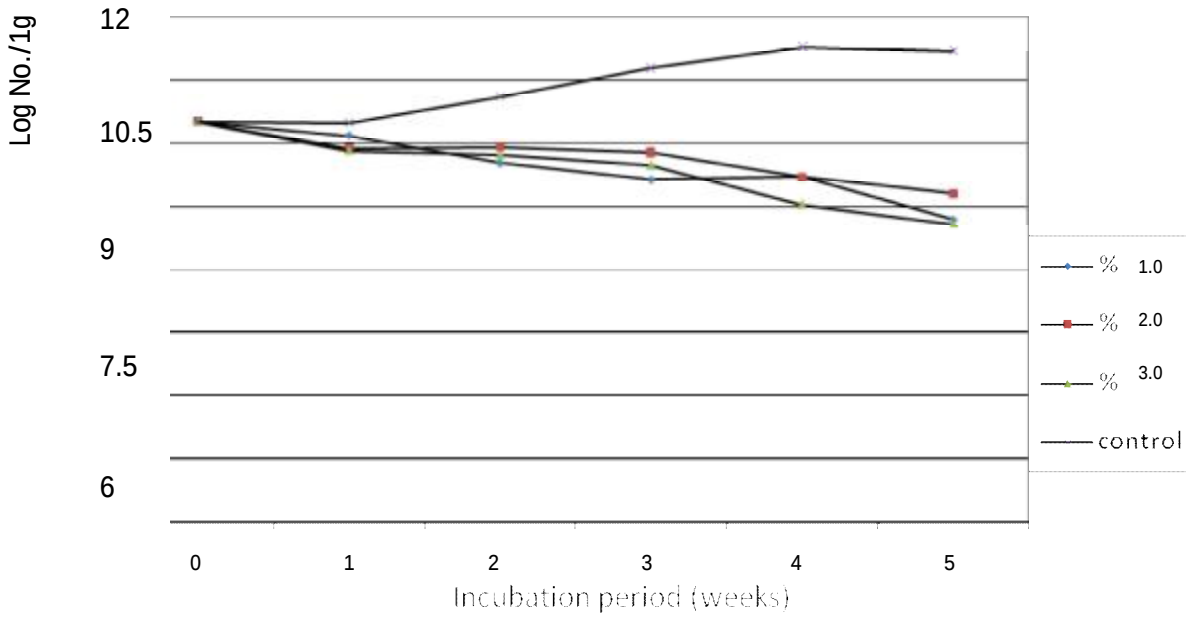

Figure (4): Behavior of *Listeria monocytogenes* in the presence of various concentrations of sodium benzoate at pH 5.5 and 37°C

Despite that none of the sodium benzoate concentrations was able to completely retard growth of *L. monocytogenes*at at both pH values used, generally, numbers in samples incubated at lower temperature (4°C) were less than those at higher temperature (37°C) as show in (Figures 1 and 4). However, no such considerable findings were recorded for the control samples at both incubation temperatures used.

Regarding pH of media used in treated and untreated samples, results show that adjusting pH to 5.5 led to higher populations of *L. monocytogenes* than those of pH 5.0 after incubation at 4°C during periods used.

George *et al*. (1988) found that *L. monocytogenes* was able to grow in nutrient broth medium acidified with HCl to pH values of (4.2 to 7) and incubated for (4, 7, 10, 20 and 30°C) with the exception of 0.25 and 0.3% all lower concentrations of potassium sorbate permitted slight growth of *L. monocytogenes* in tryptose soy broth followed by population decreases after 60 and 66 days of incubation.

Similar effects have been found by El-Shenawy and Marth (1988) they applied several concentrations of sodium benzoate in trypticase soy broth and found that *L. monocytogenes* was able to grow at low temperature in presence of low concentrations of the benzoate. Buazzi and Marth (1992) found out that over 99% of viable cells of *L. monocytogene* were injured after treated with a solution of 8.5% sodium benzoate at pH 7.0 for 1h. In their study, Elci and Akopolat (2003) investigated the effect of different sodium benzoate concentrations, incubation temperatures and pH values of trypticase soy broth with an inoculums size of *L. monocytogene* of 10^3 CFU/*ml*. Growth of *L. monocytogene* in the study was recorded by all sodium benzoate concentrations used. Islam *et al.* (2002) found that *L. monocytogene*s was effectually controlled on chicken luncheon meats after treatment with 25% wt/vol sodium benzoate sprayed on surface of this food. Log number of *L. monocytogenes* was decreased from 3.2 (in the untreated control) to 1 log after storage at 13°C for14 days. Glass *et al*., (2007) found that the addition of combination of 0.05% sodium propionate and 0.05% sodium benzoate prevented growth of *L. monocytogenes* in cured pork-beef bologna stored at 4°C for 13 weeks. In another studies, Glass *et al*. (2007a,b) tested cured ham with 0.1% sodium benzoate or 0.2% sodium propionate and found that growth of *L. monocytogenes* was inhibited after storage at 4°C for 12 weeks, in the uncured turkey 0.2% sodium propionate inhibited *L. monocytogenes* under same conditions. Seman *et al*. (2008) studied the effect of sodium benzoate (0.08 to 0.25%) in combination with different concentrations of sodium diacetate (0.05 to 0.015%) and sodium chloride (0.8 to 2%)

and different finished product moisture contents (55 to 75%) on growth of *L. monocytogenes* in ready-to-eat meat products. It was found that high-moisture ready-to-eat products presented by 0.1% sodium benzoate may need additional ingredients to effectively inhibit growth of *L. monocytogenes*. Stanojevic *et al.* (2009) investigate the antimicrobial effects of sodium benzoate, sodium nitrate and potassium sorbate and their synergistic action on food-spoilage bacteria and fungi, it was found that the sodium nitrate potassium sorbate and sodium nitrate sodium benzoate combinations of preservatives exhibited synergestic and additive effects. Safari and Saeidi (2011) studied the effect of sodium benzoate on *L. monocytogenes* in silver carp (*Hypophtalmichtys moltrix*) fillet during storage period (0, 3, 6, 9 and 12) days at 4°C, it was concluded that application of sodium benzoate showed inhibitory effects on *L. monocytogenes* in culture media and silver carp.

In these concerns, previously mentioned reports which suggested that *L. monocytogenes* can survive in a wide range of environmental conditions, allows the pathogen to overcome food preservative.

Conclusions

Based on the result of this study, it can be concluded that inhibitory effect of sodium benzoate, as a preservative, can be depends on the interaction between pH, temperature and sodium benzoate and their levels. So further studies are needed to provide a sufficient prediction for concentration of sodium benzoate required commercially to preserve foods because each food product have its own environmental conditions such as water activity, temperature pH, microbial flora, food component that should be considered.

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