Survival of *Listeria Monocytogenes* in Tomato Juice at 5 and 30°C Storage

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ABSTRACT

Listeria monocytogenes is a food-borne pathogen and has been associated with a variety of food products including fruits and vegetables, which are important for a healthy human diet. *L. monocytogenes* survives and grows at low temperatures and thus it can be multiplied to dangerous levels in a product which is kept at refrigeration temperatures. This work examines the ability of *L. monocytogenes* to survive, in a product of high consumption frequency, such as tomato juice, in correlation to storage temperature. The results indicate that a significant number of cells survived in tomato juice whether the storage temperature was 30°C or 5°C, although the refrigerator temperature slightly reduces the survival of *L. monocytogenes* cells. An understanding of the cold stress response of the pathogen will be helpful in the design of effective methods to control *L. monocytogenes* in freezing foods in order to provide consumers with a safe product.

Keywords: *Listeria monocytogenes*; tomato juice; refrigeration; vegetables; food safety

INTRODUCTION

Fresh or minimally processed vegetables and fruits are an important part of a healthy human diet rich in vitamins, fibers and nutrients. However, it should be considered that they are also very important as causative agents for several food-borne illnesses. The fresh vegetables and fruit juices can be contaminated with pathogenic microorganisms during growing, harvesting, postharvest handling, processing or post-pasteurization contamination of the product. Outbreaks of human infections, correlated with the consumption of raw fruits and vegetables, have been increased during the past decades[1-3]. The pathogen *Listeria monocytogenes* is a Gram-positive acapsular, asporogenous, facultatively anaerobic bacterium and is regarded as one of the leading causes of food-borne outbreaks with high fatality rates (listeriosis)[1,4]. The pathogen has been associated with a variety of food products, including dairy foods, meat, poultry, seafood, as well as fruits and vegetables[1,5-7].

L. monocytogenes can survive in multiple environmental conditions used in food industry such as low temperature, acidic pH, presence of disinfectants, osmotic pressure[8-11]. In a food processing plant, this bacterial pathogen can experience refrigeration temperatures, leading to cold adaptation, which may enhance its survival in vegetable products, and thus the organism can be multiplied to dangerous levels when the product is kept at refrigeration temperatures[11-13]. Changing food habits of the consumer, with a trend towards

consumption of minimally processed ready-to-eat convenience foods and refrigerated or frozen food products have affected the incidence of listeriosis over the past years[5]. Since refrigeration is one of the most common ways to increase the shelf life of foods, understanding the survival and growth of *L. monocytogenes* at low temperature could provide information to help develop more effective control methods. This study was therefore undertaken to examine the ability of *L. monocytogenes* to survive, in a product of high consumption frequency, such as tomato *(Solanum lycopersicum* L.) juice, in correlation to storage temperature.

MATERIALS AND METHODS

Preparation of tomato juice

Fresh tomatoes (*Solanum lycopersicum* L.) were purchased from a local market (Athens, Greece). Prior to experimental studies, the tomatoes were washed with tap water for 2 min, follows with distilled water, and dried with absorbent paper at room temperature. The tomatoes were cut into pieces and the stems were removed using a common kitchen knife, before they were transferred into a household blender (Izzy E450 Multi Plus) and converted to juice. The juice was then batch-pasteurized at 65°C for 30 min in a water bath (Memmert WNB7-45, Schwawbach, Germany) and was cooled rapidly at room temperature.

Culture preparation and inoculation processing of tomato juice

An avirulent strain *Listeria monocytogenes*, DP-L1044 (D. Portnoy, University of Pennsylvania) prepared by a transposon insertion[14] in the parent strain (Lm10403S), was grown in Brain Heart Infusion broth (BHI, BD, Franklin Lakes, NJ, USA) at 30°C for 24 h. A 10mL aliquot of the above inoculum was transferred into 1 L of BHI broth, which was then incubated at 30°C; another 10 mL aliquot was used to inoculate 1 L of BHI broth, which was then incubated at 5°C. The growth of *L. monocytogenes* for each incubation temperature was determined by measuring absorbance (OD) at 600 nm over time.

Aliquots (1 mL) of *L. monocytogenes* culture grown at 30°C until early stationary phase (OD_{600nm} = 0.850), were serially decimally diluted to attain the desired inoculum level for tomato juice samples. Each inoculum was added to each sample to yield a final concentration of 5.0×10^5 CFU g^{-1} tomato juice (5.70 log CFU g^{-1}).

Storage experiments

Two sets of tomato juice samples were utilized, the first for storage at 30°C and the second for storage at 5°C. The first set of 8 samples (storage at 30°C) was enumerated at 1, 3, 5 and 7 days and this procedure was repeated (n=2, 2×4 samples). The second set of 8 samples (storage at 5°C) was enumerated at 2, 4, 8 and 12 days and also was repeated (n=2, 2×4 samples).

Uninoculated blank samples were also stored under the same conditions and analyzed with the same method for the presence of the pathogen and no cells were found.

Enumeration of Listeria monocytogenes

Samples of tomato juice (25 g) were aseptically transferred and homogenized with 225 mL sterile Buffered Peptone Water (code CM0509, Oxoid LTD, Basingstoke, Hampshire, United Kingdom) in stomacher bags (stomacher 400, Light Interscience, Rockland, MA). Appropriate serial decimal dilutions were made in Buffered Peptone Water. *L. monocytogenes* was counted by surface-plating 0.1 mL of appropriate serial dilutions of the homogenate on PALCAM Agar Base plates (code CM0877, Oxoid LTD, Basingstoke, Hampshire, United Kingdom). The Petri dishes were incubated at 30°C for 48

Statistical analysis

All the above experiments were carried out in duplicated. Student's t-test was used to determine statistical significance at a confidence level of 95%.

The results on the enumeration of *L. monocytogenes* in tomato juice inoculated at 5×10^5 CFU g^{-1} (5.7 log g^{-1}) are shown in Fig. 1. In tomato juice stored at 30°C, *L. monocytogenes* viable cells counts reduced slightly but with no significant difference during the first day (0.55 log CFU g^{-1} lower than the initial inoculated sample). The population of the bacteria stabilized over the next 6 days and remained at 5.14 ± 0.11 log CFU g^{-1} during the seventh day of storage.

The cells of *L. monocytogenes* were reduced significantly (1.31 log decline, P<0.05) compared to the initial population after 2 days storage in tomato juice stored at refrigeration temperature (5°C). In the next 10 days storage period, the number of the cells remained constant and was 4.05 ± 0.05 log CFU g^{-1} after 12 days of storage. The refrigeration temperature reduces the survival of *L. monocytogenes* cells (~1 log CFU g^{-1}) compared to 30°C storage temperature, however, in both cases the population remained constant at each storage temperature.

A significant number of *L. monocytogenes* cells had the ability to overcome the acidic nature of the tomato juice and survived in the product whether the storage temperature was 30°C or 5°C. *L. monocytogenes* has the ability to survive and grow at low temperatures and thus the organism can multiply to dangerous levels when the product is kept at refrigeration temperatures. Modification of membrane lipid composition is clearly an important adaptation mechanism in *L. monocytogenes*, which allows it to grow in a stressful environment such as low temperature[15,16]. Changes in lipid composition can lead to changes in cytoplasmic membrane permeability and fluidity, which may in turn contribute to tolerance[11,17].

Previous studies observed that *L. monocytogenes* is able to survive in tomatoes[18-20] and in vegetables and fruits such as lettuce[21], cabbage[22], vegetable broth[23], bell pepper[24], leafy endives[25], apple[26], pear and melon[27] at different temperatures.

Data regarding the behavior of *L. monocytogenes* in tomato juice during freezing storage are limited. Our findings support that *L. monocytogenes* has the ability to survive in tomato juice regardless of freezing storage or not, although when the storage temperature is 5°C the survival potential of the bacteria is lower than the storage temperature of 30°C.

Understanding response mechanisms of cold adaptation will improve the strategies, which will help to manipulate the survival and growth of *L. monocytogenes* in freezing foods, and exert microbial control at these points. Although, pasteurization of the product is adequate to control small amounts of the bacterium, *L. monocytogenes* is able to attach to and survive on surfaces found in food processing plants, forming biofilms and post-pasteurization contamination of the product is possible[13]. However due to the specific abilities of this pathogen to overcome the processing hurdles, its control remains a challenge. Compliance with

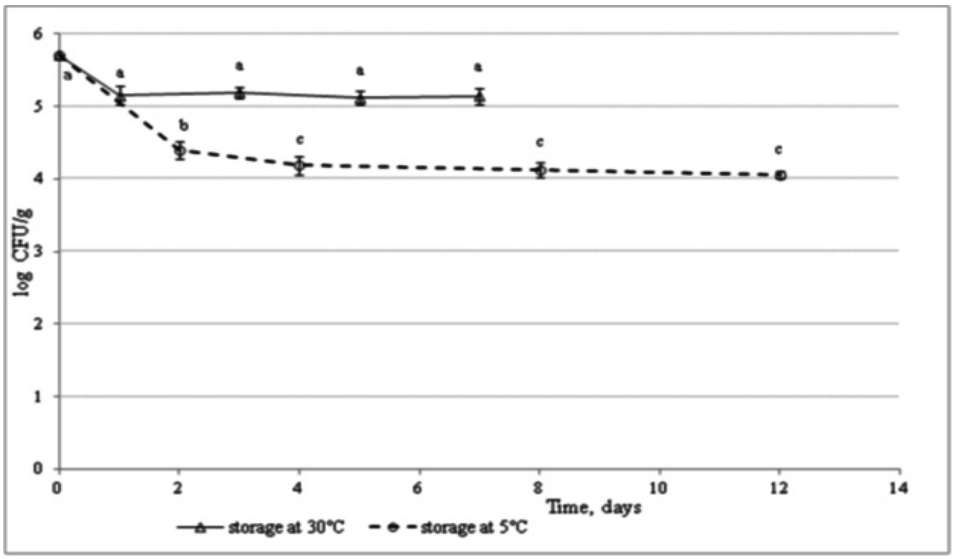

Fig. 1: Listeria monocytogenes population in tomato juice stored at 30°C (triangles) or at 5°C (circles). Values with different superscript letters indicate statistically significant differences (P<0.05).

the Good Manufacturing Practices, implementation of Hazard Analysis Critical Control Points (HACCP) and the surveillance of the pathogen in the processing environment are crucial in order to provide consumers with a safe product [28].

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