

THERMAL RESISTANCE OF TWO STRAINS OF *LISTERIA MONOCYTOGENES*

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SUMMARY: The thermal resistance of two strains of *Listeria monocytogenes*, strain NCTC 7973 and NCTC 5105, was demonstrated at five different temperatures (52, 57, 62, 67, 72°C) in pasteurized and whole boiled milk. 3 ml portions of milk suspended at concentrations of ca. 2.23×10^5 cells ml⁻¹ was heated at temperatures ranging from 52 to 72°C. The D-value of NCTC 7973 for fresh boiled milk ranged from 1620 to 60 sec. In pasteurized milk the D-value was computed ranging from 1860 to 75 sec. Moreover the Z-value for the same strain was 14.2°C and 11.6°C for fresh boiled milk and pasteurized milk respectively. The D-value of strain NCTC 5105 ranged from 1050 to 120 sec. for fresh boiled milk whereas for pasteurized milk it ranged from 2100 to 69 sec. The Z-value of NCTC 5105 was 21.5°C and 13.8°C for fresh boiled milk and pasteurized milk respectively.

Key Words: *Listeria monocytogenes*, D-value, Z-value, strain.

INTRODUCTION

Listeria monocytogenes has recently been reported as food borne pathogen in four food borne outbreaks in United States and Canada. Food associated with these outbreaks were vegetables, pasteurized milk and soft cheese (8,14,19). Besides such outbreaks *L. monocytogenes* has frequently been isolated from uncooked meat (7,10,16) milk (5,9,12,17) and soft cheese (5,15). The ability to survive and grow at refrigeration temperatures (11) and tolerance to the preserving agents, sodium chloride and sodium nitrite (20), makes *Listeria* of particular concern as a post contamination agent for refrigerated food. The ability of organism to grow at refrigeration temperature is not well documented. Consequently present study was under taken to determine the thermal resistance of *L. monocytogenes* in whole fresh boiled milk and pasteurized milk between 52 and 72°C. It is expected that studies like this would provide some knowledge about the role of listeric infections.

MATERIALS AND METHODS

Bacterial strains

Two strains of *L. monocytogenes* NCTC 7973 serovar 1/2a and NCTC 5150 serovar 3a were used in the present study.

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Milk samples

Different brands of pasteurized milk samples were obtained from the local market, whereas fresh boiled milk samples were obtained from home delivery service and boiled before use.

Stock cultures

Cultures of *L. monocytogenes* were maintained on TPA (Tryptose phosphate agar) slopes with monthly transfers and stored at 4°C in screw capped bottles.

Thermal resistance of *L. monocytogenes* in fresh boiled milk and pasteurized milk samples

To determine the thermal resistance of two strains of *L. monocytogenes* 100 ml of milk sample was withdrawn aseptically in a sterile conical flask. The sample was then inoculated with 1 ml inoculum ca. 2.23×10^7 cells ml⁻¹ and flask was shaken well for even distribution of organisms. Portions of 3 ml of inoculated milk sample was dispensed in sterile test tube. Samples were heated in a water bath (Kottermann). After intervals tubes were taken out from water bath and ten fold dilution of the sample was made. Number of survivors was determined by SPC. D-value was calculated from the data plotted on semilog paper, whereas Z-value was calculated from the curve D-value versus heating temperatures.

RESULTS

Results of thermal resistance of two strains of *L. monocytogenes* NCTC 7973 and NCTC 5105 in fresh boiled milk and commercially available pasteurized milk at temperatures between 52-72°C are shown as D-value in Table 1, moreover Z-value of each strain for both types of milk is also shown in the same table.

The D-value estimated for NCTC 5105 in case of fresh boiled milk ranged from 1050-120 seconds. The observed Z-value was 21.5°C. Initially ca. 2.23×10^5 cells were suspended in fresh boiled milk and heated at different temperatures. Results obtained indicated a linear decrease in population density. D-value obtained for NCTC 7973 ranged from 1620-66 seconds. The observed Z-value was 14.2°C. The D-value at 72°C was 66 seconds for NCTC 7973 whereas it was D-value at 72°C was 66 seconds for NCTC 7973 whereas it was 120°C for NCTC 5105 at the same temperature. The comparative study of thermal resistance of these two strains revealed that NCTC 5105 was more heat resistant than NCTC 7973 in fresh boiled milk.

The D-value in pasteurized milk ranged from 2100-60 seconds and 1860-75 seconds for NCTC 7973 and NCTC 5105 respectively. Whereas Z-value in pasteurized milk was 13.8°C for NCTC 5105 and 11.6°C for NCTC 7973. The D-value obtained at 72°C was 60 seconds which is less than D-value obtained for NCTC 5105 and NCTC 7973 in fresh boiled milk at the same temperature. Data indicate that both tested strains of *L. monocytogenes* appeared to be more heat resistant in fresh boiled milk as compared to pasteurized milk.

DISCUSSION

The role and importance of *L. monocytogenes* as an agent of food borne disease is becoming increasingly apparent. Recent outbreaks of listeriosis have led to the implication of a variety of foods, particularly dairy products as source of the pathogen (8,13,19). It was sug-

gested that pasteurized milk used to make cheese was a possible source of an outbreak of listeriosis in 1979 (18). Reports on thermal resistance of *L. monocytogenes* have produce conflicting results (1,2). The most comprehensive study on heat resistance of *L. monocytogenes* was reported by Bradshaw *et al.* in 1985. Their data revealed that *L. monocytogenes* does not tolerate the lowest legal pasteurization temperature. Whereas Garayzabal *et al.* in 1986 isolated *L. monocytogenes* from ca. 21% of milk samples pasteurized at 78°C for 15 seconds in processing plant in Spain. This finding was supported by Doyle *et al.* (1987) who interpreted their results that *L. monocytogenes* is more thermotolerant than most non spore forming bacterial pathogenes and may in some instance survive minimum pasteurization temperature treatment.

There is no information available about the presence of *Listeria* in fresh raw milk sued in our country. It is possible that *Listeria* may be present in the milk because of shedding from a symptomatic animals. Such occurrence in raw milk has previously been noted in several countries (18). Hence it seems reasonable to believe that fresh raw milk of different animals used here may contain considerable amount of *Listeria* which may cause a hazard at any moment. The same fresh milk contaminated with *Listeria* when undergoes pasteurization process and if the organism survive the minimum pasteurization temperature as reported by Doyle *et al.* (6) then people using this type of milk are really at risk. Data of present study on thermal resistance of *L. monocytogenes* demonstrated that the thermal resistance of NCTC 7973 appeared to be higher in fresh boiled milk as compared to pasteurized milk. Similar results were obtained for NCTC 5105. Thermal resistance of *L. monocytogenes* has also been observed by Bradshaw *et al.* in 1986, who used *L. monocytogenes* strain Scott A, using different raw and sterile milk products found that *L. monocytogenes* showed resistance in sterile milk products.

Table 1: D-Value estimates for two strains of *L. monocytogenes*.

Temperature	D-Value (Seconds)			
	Fresh Boiled Milk		Pasteurized Milk	
	NCTC 7973	NCTC 5105	NCTC 7973	NCTC 5105
1 52°C	1620	1050	1860	2100
2 57°C	900	630	1410	750
3 62°C	150	150	168	360
4 67°C	90	138	111	102
5 72°C	66	120	75	60
Z-Value °C	14.2°C	21.5°C	11.5°C	13.8°C

We report that the D-value for NCTC 7973 ranged from 1620 - 66 seconds in case of fresh boiled milk, whereas it was 1860 - 75 seconds in pasteurized milk. NCTC 5105 showed a D-value range from 1050 to 120 seconds and from 2100 to 60 seconds for fresh boiled and pasteurized milk respectively. The Z-value for NCTC 7973 in pasteurized milk was 11.6°C, whilst it was 14.2°C in fresh boiled milk. The Z-value for NCTC 5105 was 13.8°C and 21.5°C, for pasteurized and fresh boiled milk respectively. These findings supported the idea that higher thermal resistance of both the strains in fresh boiled milk as compared to pasteurized milk may be due to some unknown growth promoting factors present in fresh boiled milk. These factors presumably inactivated in commercially available pasteurized milk because of prolonged storage before use.

Comparative account of thermal resistance of both the strains of *L. monocytogenes* in pasteurized milk and fresh boiled milk showed that heat resistance of NCTC 5105 was more than NCTC 7973 in both types of milk products. Our data did not support the idea of Bradshaw *et al.* (1985) that the current pasteurization process guide lines of the U.S. Food and Drug Administration (FDA) 1978 are adequate to destroy *L. monocytogenes* in milk. Present study indicates that *L. monocytogenes* can survive a temperature of 72°C for four minutes, hence the minimum high temperature, short time treatment (71.7°C for 15 sec) is inadequate to destroy *L. monocytogenes* however, there is a need for further study.

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