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# Effect of Heat and Food Preservatives on Survival of Thermophilic *Campylobacter* Isolates in Food Products*

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**Abstract:** Major purpose of this study was to investigate survival of thermophilic *Campylobacter* spp. isolates from environmental samples in sterile chicken extract and milk and to determine sensitivity of them to food preservatives. Six thermophilic *Campylobacter* isolates were subjected to determine their survival and the decimal reduction time in sterile chicken extract and milk at -15, 4, 32°C and 55, 60, 65°C, respectively. Minimal Inhibitory Concentrations (MICs) of food preservatives against the *Campylobacter* isolates were determined by E-test. The results obtained indicated that *Campylobacter* isolates survived relatively longer in the milk. The decimal reduction time for the isolates was approximately 1 min at 55°C and less than 1 min at 60°C. Most of the isolates were rapidly inactivated at 65°C. Although, most of the *Campylobacter* isolates were sensitive to food preservatives, MIC values of lactic and acetic acid against the isolates were relatively low. Overall, the thermal sensitivity of Campylobacters would not allow them to survive in the food even with moderate cooking. Hence, heat, lactic and acetic acids could be considered as functional physical agent and food preservatives against *Campylobacter*.

**Key words:** Survival, heat, food preservatives

## Introduction

Thermophilic campylobacters are widespread in the environment, where they are a sign of recent contamination of the environment with animal and avian feces (Jones, 2001). Gastroenteritis caused by *Campylobacter* spp. has been recognized as one of the important food borne disease in the developed countries (Chaveerach *et al*., 2002). The most important pathogenic species belong to the group of so-called thermophilic campylobacters. *Camp. jejuni*, *Camp. coli* and *Camp. lari* are most important pathogenic *Camprlobacter* (Skirrow, 1994). Transmission of thermophilic *Campylobacter* spp. from environment to human could be often through contaminated food as well as contaminated water. Several report have revealed that 30-100% of poultry, 40% of cattle and 60-80% of swine carry *Campylobacters* in their intestinal tract. For this reason, the organism is principally associated with foods of animal origin (Doyle, 1984). Household pets with diarrhea have been shown to be the source of infection for man (Frost, 2001). The infectious dose of *Campylobacter* required to cause foodborne disease is very low. It means only a few hundred cells can produce illness in babies; young children and debilitated people. Symptoms of the infection vary from mild to severe with bloody diarrhoea as the most characteristic symptom (Ketlety, 1997). However, treatment of infection using antibiotics is always final remedy. But prevention of infection must be considered most important than that of treatment. Prevention of campylobacter enteritis depends largely on interrupting the transmission of the organism to humans from farm and domestic animals, food of animal origin, or contaminated water. Individuals can reduce the risk of campylobacter enteritis by using properly

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cooked and stored meat and dairy products, (Tosh *et al*., 1981; Dawkins *et al*., 1984; Diker *et al*., 1987; Spacciapoli *et al*., 2001). In view of above background and in order to reduce campylobacter enteritis, the present study was undertaken to determine survival of thermophilic *Campylobacter* isolates in the sterile foods, the decimal reduction time (D-values) for the isolates at different temperatures and to evaluate the effect of food preservatives on the thermophilic *Campylobacter* isolates from environment.

## Materials and Methods

*Islation and Identification of Thermophilic Campylobacter*

Twenty-five strains of *Campylobacter* spp. were isolated from different sources viz., animal feces, poultry and meat using KB device and preT- KB methods (Baserisalehi *et al*., 2004a, b). All suspected colonies have grown on the KB medium were confirmed by typical morphology, darting motility, Gram staining, oxidase and catalase tests. The isolates exhibiting characteristics typical of *Campylobacter* were characterized using standard *Campylobacter* phenotypic identification tests recommended by Atabay and Corry (1997). These tests included, $H_2S$ lead acetate strip, nitrate reduction, growth in 1% glycine and 3.5% NaCl, growth at different temperatures, viz., 25, 37 and 42°C and resistance to nalidixic acid (30 μg disc) and cephalothin (30 μg disc). All thermophilic campylobacters isolated from each sample were confirmed using hippurate hydrolysis, Indoxyl acetate and Urease tests.

Six thermophilic *Campylobacter* belonging to *Camp. jejuni, Camp. coli* and *Camp. lari* randomly were selected for future study. *Camp. jejuni* P1, *Camp. lari* P2 and *Camp. lari* P3, isolated from poultry, *Camp. jejuni* M1 and *Camp. coli* M2 isolated from beef and *Camp. coli* F5 isolated from animal feces. All the isolates were maintained at -15°C in the Brucella broth with 15% glycerol.

*Food Samples and Their Processing*

Fresh raw milk (buffalo's milk) obtained from animal husbandry in Pune city was immediately sterilized upon arrival in the laboratory, at 121°C for 15 min. Chicken meat was obtained from retail market in Pune city. The sterile chicken extract was prepared by adding 50 mL distillated water to 250 g ground meat and sterilized at 121°C for 15 min.

*Effect of Temperature on the Survival of Thermophilic Campylobacter Isolates in Sterile Chicken Extract and Milk*

Six sets of chicken extract and milk tubes, each with three tubes of chicken extract and three tubes of milk were made inoculated with six different isolates of campylobacter separately. Three subsets of each with one tube of chicken extract and one tube of milk, of each set were made and incubated at three different temperatures (-15, 4 and 32°C). At different time intervals (6, 12, 18, 24, …h) viable count of each isolate was determined by standard plate count technique using Luria Bertani agar.

*Sensitivity of the Thermophilic Campylobacter Isolates to Heat as a Function of Time*

The decimal reduction time (D-value) is a measure of sensitivity of microorganisms to heat, was determined for six isolates belonging to *Camp. jejuni*, *Camp. coli* and *Camp. lari* at varied temperatures (55, 60 and 65°C). To determine the D-value at each temperature, test tubes (5×1/2 inch), containing 1 mL of food products (sterile chicken extract or milk) were placed in the water bath to required temperature. After 10 min, 0.1 mL culture of one of the *Campylobacter* isolates ($1.5\times10^8$ cells $mL^{-1}$) was inoculated into each tube in the water bath. After every 20 sec 2 tubes one each of milk and chicken extract were placed in an ice bath for 10 min. Then the suspension was suitably diluted in

sterile distillated water and plated onto the Luria Bertani agar (LB agar) for viable count (Gill and Harris, 1982). This procedure was repeated for remaining isolates of *Campylobacter* at the different temperatures. The D-value was calculated as the time in minutes required for reducing 90% of microbial population.

$$D_t = \frac{t_2 - t_1}{LogN_0 - LogN}$$

$LogN_0$ = Log. *Campylobacter* population at time $t_1$
LogN = Log. *Campylobacter* population at time $t_2$
$t_1$ = Initial incubation time.
$t_2$ = Final incubation time.

*Minimal inhibitory concentrations of food preservatives for thermophilic Campylobacter isolates*

Six isolates of *Campylobacter* belonging to *Camp. jejuni*, *Camp. coli* and *Camp. lari* were grown in 5 mL Nutrient Broth (NB) in 15×2 cm tubes at 37°C for overnight. The number of cells per mL of culture was determined in terms of turbidity (Mcfarland scale No. 0.5 $1.5\times10^8$ cfu $mL^{-1}$). The food preservatives were diluted (0.05, 0.1, 0.25, 0.5, 1, 2 and 3%) in the sterile distilled water and sterilized using membrane filter (0.45 μm pore size).

Minimal inhibitory concentration of food preservatives for theremophilic *Campylobacter* isolates was determined by E-test (Baker, 1992). E-test strip for each food preservative was made using a piece of filter paper (Whatman No. 10, size 1×7 cm). Each strip was divided into seven squares (1×1 cm). The squares were labeled by respective concentration in increasing order. The diluted food preservatives were dropped (10 μL) at the respective square of the strip and allowed to dry.

To perform the E-test, each culture was spread inoculated onto Mueller-Hinton agar plate separately. Then two strips of two food preservatives were applied on each plate. The plates were incubated at 37°C for 48 h under microaerophilic conditions and inhibitory concentration of each preservative was read at the point where the elliptical zone of inhibition intersected the E-test strip.

## Results

*Effect of Temperature on Survival of Thermophilic Campylobacter Isolates in Sterile Chicken Extract and Milk*

The population of *Campylobacter* increased initially upto 2-4 days at 32°C. The rate of decline on subsequent incubation was significantly high indicating relatively less survival of all *Campylobacters* at 32°C. The results from survival of thermophilic *Campylobacter* isolates in the foods indicated that the population of *Campylobacter* isolates during storage at -15 and 4°C declined. However, the rate of decline was relatively less than that at 32°C. The rate of decline of *Campylobacter* at -15°C was relatively more than that 4°C. In general, survival of the *Campylobacter* isolates was relatively greater in the milk however, survival of *Camp. coli* M2 and *Camp. jejuni* M1 was relatively greater in the chicken extract. However, the population of *Camp. jejuni* P1, *Camp. coli* F5, *Camp. lari* P2 and P3 inoculated in milk decreased. But rate of decline was relatively less in milk. The population of *Camp. jejuni* M1, *Camp. coli* M2 inoculated in milk decreased more than that in chicken extract (Fig. 1).

*Sensitivity of the Thermophilic Campylobacter to Heat as a Function of Time*

D-values of thermophilic *Campylobacter* isolates in sterile chicken extract and milk are given in the Table 1. As seen in this table, thermophilic *Campylobacter* isolates were inactivated when incubated at 55, 60 and 65°C. Virtually the D-value for all isolates was 1 min at 55°C and less than 1 min at 60°C. D-value for thermophilic *Campylobacter* isolates at 65°C was not determined because,

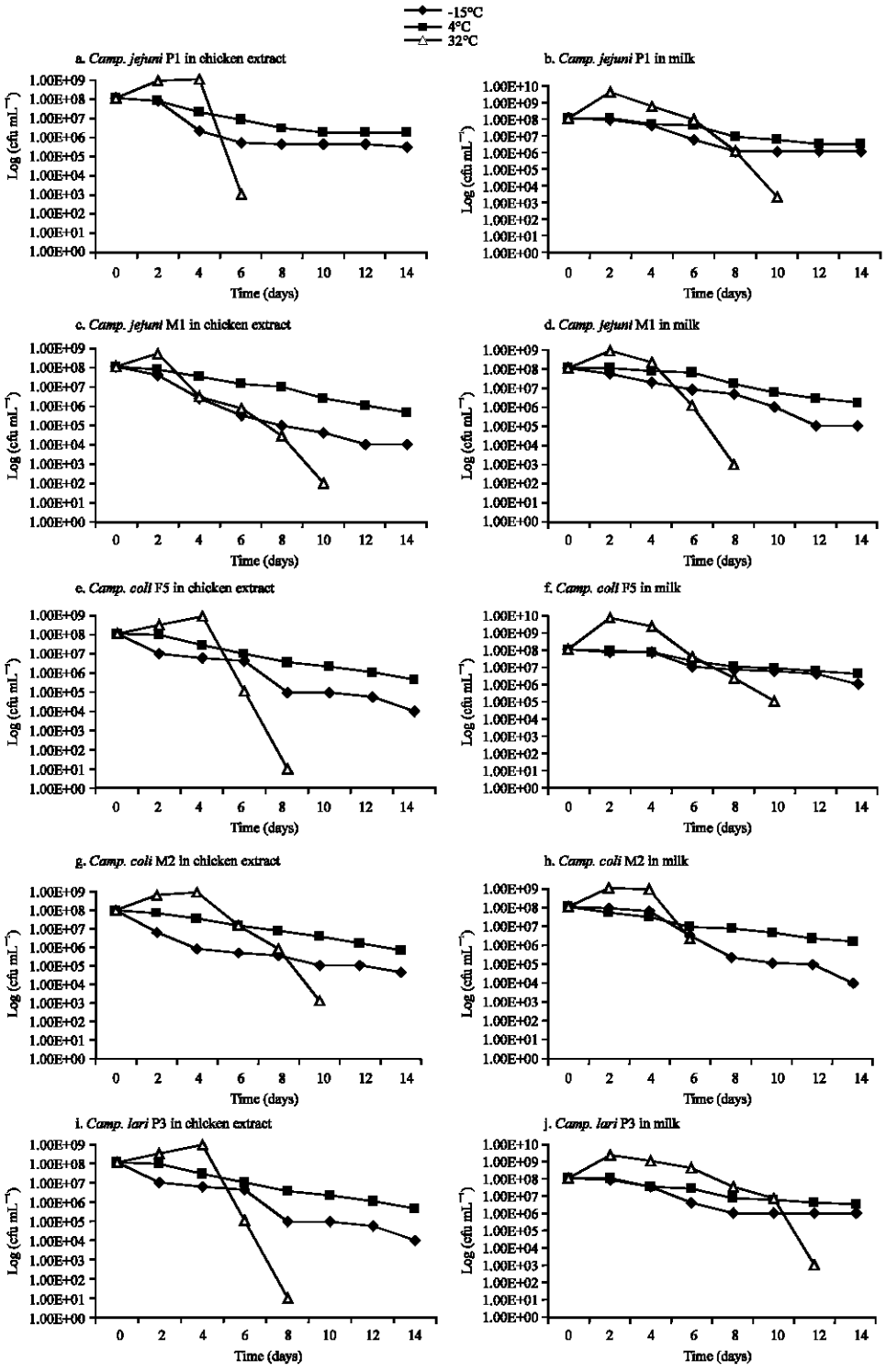


Fig. 1: Continued

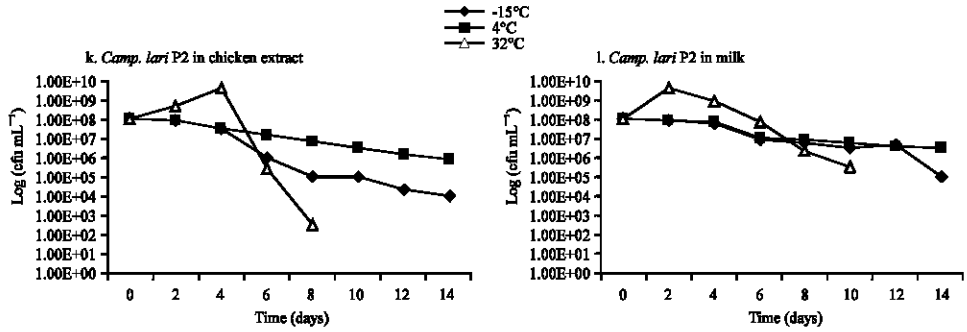


Fig. 1: Effect of temperature on survival of *Campylobacter* isolation in food products

Table 1: D-values of *Campylobacter* isolates in food products at different temperatures

| | D- value (sec) in | | | | | |
|---|---|---|---|---|---|---|
| | Chicken extract at | | | Milk at | | |
| Isolate | 55°C | 60°C | 65°C | 55°C | 60°C | 65°C |
| *Camp. jejuni* P1 | 51 | 28 | - | 48 | 24 | - |
| *Camp. jejuni* M1 | 62 | 35 | - | 62 | 35 | - |
| *Camp. lari* P3 | 58 | 28 | - | 65 | 41 | - |
| *Camp. lari* P2 | 61 | 30 | - | 64 | 38 | - |
| *Camp. coli* F5 | 48 | 23 | - | 51 | 25 | - |
| *Camp. coli* M2 | 56 | 31 | - | 53 | 33 | - |

Table 2: MIC-values of food preservatives* by E-test** against *Campylobacter* isolates

| | MIC (%) of | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Isolate | LA | CA | AA | PA | SA | BA | $NaNO_2$ | NaCl |
| *Camp. jejuni* P1 | 0.10 | 0.1 | 0.1 | 0.25 | 0.50 | 0.50 | 0.1 | 2 |
| *Camp. jejuni* M1 | 0.10 | 2 | 0.25 | 0.25 | 0.25 | 0.10 | 0.5 | 2 |
| *Camp. lari* P3 | 0.20 | 2 | 0.5 | 0.50 | 0.50 | 0.50 | 0.1 | 2 |
| *Camp. lari* P2 | 0.25 | 2 | 0.25 | 0.50 | 0.25 | 0.25 | 1.0 | 3 |
| *Camp. coli* F5 | 0.25 | 2 | 0.5 | 0.50 | 0.25 | 0.25 | 0.1 | 3 |
| *Camp. coli* M2 | 0.25 | 2 | 0.1 | 0.25 | 0.25 | 0.25 | 0.5 | 2 |

* LA lactic acid, CA citric acid, AA acetic acid, PA propionic acid, SA sodium sorbate, BA sodium benzoate, $NaNO_3$ sodium nitrite, NaCl sodium chloride. ** On Mueller- Hinton agar at 37°C for 48 h under microaerophilic conditions

most of them were rapidly inactivated at 65°C. These results indicated that D-values of the isolates in milk and chicken extract were similar. Therefore, there is no significant correlation between resistance of the *Campylobacter* isolates to heat and type of the foods. Although, D-value for *Camp. jejuni* M1was relatively high and for *Camp. coli* F5 it was relatively low, rest of the isolates had similar D-value.

*Minimal Inhibitory Concentrations of Food Preservatives for Thermophilic Campylobacter Isolates*

MIC values of six weak acid preservatives and two salt preservatives were determined against thermophilic *Campylobacter* isolates. As seen in the Table 2, MIC values of weak acid preservatives ranged from 0.1% for lactic acid to 2% for citric acid.

Although, *Camp. coli* and *Camp. jejuni* isolates showed similar behavior against organic acids tested, *Camp. lari* isolates were relatively resistant. Lowest MIC values of lactic acid was against *Camp. jejuni* isolates, citric acid against *Camp. jejuni* strain P1, acetic acid against *Camp. jejuni* P1 and *Camp. coli* M2, propionic acid against *Camp. jejuni* isolates and *Camp. coli* M2, sorbic acid was against *Camp. jejuni* M1, *Camp. lari* P2 and *Camp. coli* isolates and benzoic acid was against

*Camp. jejuni* M1. The results of MIC of salts against *Campylobacter* isolates indicated varied MIC values of sodium nitrate and sodium chloride. However, the MIC values of sodium nitrite against the isolates were significantly less than that of sodium chloride.

## Discussion

In general, survival of the most thermophilic *Campylobacter* isolates was greater in milk compared to chicken extract. The population of *Campylobacter* increased initially up to 2-4 days at 32°C then declined on subsequent incubation. Although, the population of the isolates declined at 4 and -15°C, the rate of decline was relatively high at -15°C. Parallel to our finding, Stern and Kotula (1982) reported that the levels of *Camp. jejuni* inoculated in ground meat decreased during storage at -15°C, while it remained constant during storage at 4°C. Solow *et al*. (2003) reported that the population of thermophilic *Campylobacter* isolates remained constant in the foods during storage at 4°C. On the other hands, based on our observations most of the isolates were survived relatively longer in the milk however, in contrary of our data Doyle and Roman (1982) reported that the combination of lactoperoxidase with $H_2O_2$ and $SCN^-$ in raw milk elicited to produce metabolites with bactericidal property and the survival of Gram-negative bacteria was less in raw milk. As an interpretation, it should be noted that the sterilization of the milk-culminated in the inactivation of its antimicrobial compounds, therefore, antimicrobial property of sterile milk is different with raw milk. Hence *Campylobacter* could survive greater in the sterile milk compared to chicken extract. The results indicated that the survival of some *Campylobacter* isolates viz., *Camp. coli* M2 and *Camp. jejuni* M1 was greater in the chicken extract than in milk. Probably the source of isolates must be considered as reason for the results obtained. It is because *Camp. coli* M2 and *Camp. jejuni* M1 were isolated from meat therefore their survival in the meat is relatively more due to their previous adaptation. In this case, Lior *et al*. (1981) reported that *Campylobacter jejuni* strains of the same serogroup could be isolated from a variety of different animals. Hence, similar strains of the same serogroup may be isolated from both poultry and cattle. Therefore, it could be concluded that probably due to previous adaptation of these isolates to meat, the survival of *Camp. coli* M2 and *Camp. jejuni* M1 was greater in chicken extract compared to milk.

The results obtained from decimal reduction time (D-values) for thermophilic *Campylobacter* isolates indicated that $D_{55}$ and $D_{60}$ values for thermophilic *Campylobacter* isolates were 1 min and less than 1 min, respectively. While all the isolates were rapidly inactive at 65°C. A number of publications indicated that campylobacters are more sensitive to heat than other Gram negative pathogens (ICMSF, 1996). Doyle and Roman (1981) found D-values for five strains of *Camp. jejuni* in milk ranging from 1.56 to 1.95 min at 53°C and 0.74 to 1 min at 55°C. They opined that " the times and temperatures used to pasteurize milk should be sufficient to free milk of even unusually large numbers of viable cells of *Camp. fetus* subsp. *jejuni*. Then it has been interpreted that nonhomogenous fat may serve to create microenvironments to protect microorganisms against heat therefore protection of the microorganisms by nonhomogenous fat must be considered for adjusting temperature to make milk free from the microorganisms. Blankenship and Craven (1982) found D-value for *Camp. jejuni* ranging from 8.8 min at 51°C to 0.8 min at 57°C in ground chicken meat. Gill *et al*. (1981) reported that the high temperature, short time process (71.7, 15 sec) is sufficient for inactivating campylobacters. Therefore, it can be concluded that thermal sensitivity of these bacteria would not allow the organism to survive in the food even with moderate cooking.

The results obtained from minimal inhibitory concentration of food preservatives for thermophilic *Campylobacter* isolates indicated that all of the isolates tested were sensitive to organic acids with varied MIC values. Probably these results are due to varied responses of species as well as different strains belonging to a species. Several studies reported that organic acids, such as formic, acetic,

ascorbic and lactic acids, rapidly inhibited the growth of *Campylobacter* species (Chaveerach *et al*., 2002; Cudjoe and Kapperud, 1991; Waterman and Small, 1998; Cuk *et al*., 1987; Fletcher *et al*., 1983), while, on the basis of our observations lactic and acetic acids inhibited growth of the *Campylobacter* isolates relatively with high efficiency.

However, most of the *Campylobacter* isolates were sensitive to 2% NaCl. But *Camp. lari* P2 and *Camp. coli* F5 were resistant to 2% NaCl. Therefore, it can be concluded that most of the isolates were sensitive to NaCl however; MIC values of sodium chloride against the isolates were relatively high. Although, sodium nitrite eliminated growth of all the *Campylobacter* isolates, MIC values of this salt against them were relatively low.

In conclusion, the people working in kitchen and those who are in contact with cooking should take necessary precautions regarding personal hygiene and cleanliness. In fact the present study illustrated that thermal sensitivity of *Campylobacters* would not allow the organism to survive in the food even with moderate cooking. Therefore, heat and some food preservatives such as lactic and acetic acids can be considered as functional physical and chemical agents against *Campylobacter*.

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