

## Research Article

### **Determination of Thermal Sensitivity of *Staphylococcus aureus* isolate CHK3**

**Shah Chirag, Yagnik B N\***

Dept. of Biotechnology, Government Science College, K. K. Shastri Educational Campus,  
Maninagar (E), Ahmedabad-380 008, India

#### **\*Corresponding author**

Yagnik B N

Email: [yagnikbn@gmail.com](mailto:yagnikbn@gmail.com)

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**Abstract:** Food-borne diseases, around 250 different types been described so far, have gained the worldwide concern and among two thirds of such outbreaks are linked to bacteria as causative agents. The pathogenic capability of bacteria causing food-borne infections is largely attributed to their ability to penetrate, survive and multiply inside the host cells, whereas that of bacteria causing food-poisoning depends on their capacity to do so also within the food, along with production of potent enterotoxins. The Gram positive spherical bacteria *Staphylococcus aureus* remains the predominant causative agent involved under such conditions. Formation and denaturation of bonds is accompanied by release and utilization of energy, respectively. Such an energy change attribute to a molecular activity and even vice versa. Hence, heat treatment, as the source of energy, is the choicest and commonly employed mean at home and industries to control its propagation within food for human consumption. Present study describes effectiveness of thermal denaturation over the bacteria along the structural and physiological aspects.

**Keywords:** Food-borne disease, Enterotoxin, Decimal Reduction Time, Deactivation Rate Constant, Activation Energy, *S. aureus*

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#### **INTRODUCTION**

*Staphylococcus aureus* being a leading cause of gastroenteritis, is a major concern in human health and food processing sector [1, 2]. *S. aureus* are Gram-positive sphere, occurring singly or in pair or clusters, facultative anaerobic, non-motile bacteria producing catalase and coagulase. The staphylococcal cell wall is resistant to lysozyme, but sensitive to lysostaphin, which specifically cleaves the pentaglycine bridges within the cell wall scaffold [3].

Despite *S. aureus* being non-sporing unlike the other causative agents *Clostridium perfringens*, *C. botulinum*, and *Bacillus cereus*; can be readily deactivated by heat treatment of food, its control remains a major concern during preparation and processing of food products.

*S. aureus* is able to grow at a wide range of temperatures (7-48.5°C with an optimum of 30-37 °C), pH (4.2 to 9.3, optimum 7-7.5) and sodium chloride concentrations (up to 15%). These characteristics enable *S. aureus* to grow within a wide variety of foods explaining their widespread incidence in foodstuffs that warrants control manipulation during processing [4, 5]. The assessment of risk in the foodstuffs relies upon classical microbial detection and quantification of coagulase positive staphylococci, in many countries,

low level of contaminations by *S. aureus* in food stuff is not regarded a risk for public health [6]. *S. aureus* is a potential threat to human health due to a repertoire of toxigenicity, virulence, invasiveness, and marked antibiotic resistance [7].

Some strains of *S. aureus* are able to produce potent staphylococcal enterotoxins (SEs) and are the causative agents of staphylococcal food poisonings [8]. Staphylococcal food poisoning (SFP) is an intoxication by pre-formed enterotoxin, that normally exert its effects on the gastrointestinal tract that render individuals incapacitate for up to two weeks [9]. Staphylococcal enterotoxins are a potential biological threat because of their stability at high temperatures (100°C for 1 h) [10, 11]. It is observed that growth of *Staphylococcus* in perishable foods can be prevented at the internal temperature at or below ~5°C, and staphylococci do not multiply in foods heated to temperatures of ~47°C and above [12]. The purpose of this present study is to understand thermal deactivation of the pathogen with a view to develop heat treatment regime for food that can assure safeguard against food-poisoning by staphylococci.

#### **Death kinetics**

Control of microbial presence and growth is essential in home and industry to prevent food spoilage

and diseases. Heat which causes detrimental effects to most cellular structures and molecules, have remained very effective mean for control. Exposure to heat brings about injury to peripheral walls, alter colloidal state of cytoplasm, irreversibly inactivate enzymes and interfere with structure and function of nucleic acids. The destruction of microbial cells using heat implies loss of viability. The rate equation for destruction of microorganism by heat at specific temperature follows first order reaction kinetics explained by the following reaction [13, 14].

$$dN/dt = -k \cdot N \text{ or } \ln N/N_0 = -k \cdot t$$

Where,  $k$  = cell deactivation rate constant  $\text{minute}^{-1}$ .

$N$  = number of viable cells at time  $t$  minutes.

$N_0$  = number of viable cells at time  $t = 0$ .

Here  $N/N_0$  represents the fraction of viable cells which survive after heat treatment for time  $t$ . Since, the time needed to reduce the population to one-tenth of its original number is the time interval during which the straight line traverses one complete logarithmic cycle representing Decimal reduction time (D value). From the rate equation, when,

$$N/N_0 = 1/10, t = D,$$

$$\ln 1/10 = -k \cdot D,$$

$$D = 2.303/k,$$

$$k = 2.303/D.$$

However, the thermal death time of bacteria is dependent upon the temperature employed, the initial microbial load and physiological age of the culture, of these factors, especially the initial concentration of bacteria have been ignored by a number of workers [15]. When this factor is considered, the thermal death time follows a regular order which is adequately described by applying the equation for the "mono-molecular reaction rate".

## EXPERIMENTAL SECTION

### Culture media

All the culture media and ingredients used were obtained from HiMedia Laboratories, Mumbai 400 086, India. The culture used for the study was isolated, maintained and grown over Nutrient agar (M001).

### Bacterial test culture

A bacterial strain *S. aureus* isolated in our laboratory as CHK3 was identified based upon the cultural, morphological and biochemical characteristics employing standard methods [16]. The test culture was maintained over nutrient agar slope with periodic transfer. Prior to use, a loop-full of test culture was activated in nutrient broth (5ml) at 37 °C for 18 hours.

### Heat exposure

Test culture activated over nutrient agar medium was suspended in sterile distilled water and was diluted to cell density of  $10^8$  cells  $\text{mL}^{-1}$  judged by

microscopic count. For each temperature value, 5 tubes for varying time exposure time along with a control were made. All the tubes except control were exposed to varying time intervals by placing in a water-bath maintained at constant temperature. Periodically, 0.1 mL culture was aseptically sampled out and plated for determining survivor number.

### Viable bacterial count

Bacteria surviving after heat treatment were counted using Standard Plate Count (SPC) method [17, 18]. Test cultures (0.1 mL) were diluted and spread over Nutrient agar surface. After incubation at 37 °C for 24 hours, plates having colonies within 30-300 numbers were considered to find viable count as Colony-forming Units (CFU) using formulae:

$$\text{CFU mL}^{-1} = \text{Average number of colonies} / \text{Dilution} \times \text{Volume plated in mL}.$$

### Measurement of microbial death by heat treatment

A graph of  $N/N_0$  against time was plotted and from it decimal reduction time and thus the deactivation rate constant and the activation energy of the test organism was calculated.

## RESULTS AND DISCUSSION

The negative co-relation between exposure time and log survival number at varying temperatures is evident from Fig. 1. Higher temperature with increased exposure time exerts the most detrimental effect results but for practical purposes, both temperature and time is restricted. Hence, the maximum temperature employed was 60°C and maximum exposure time was 50 minutes, which gave satisfactory results.

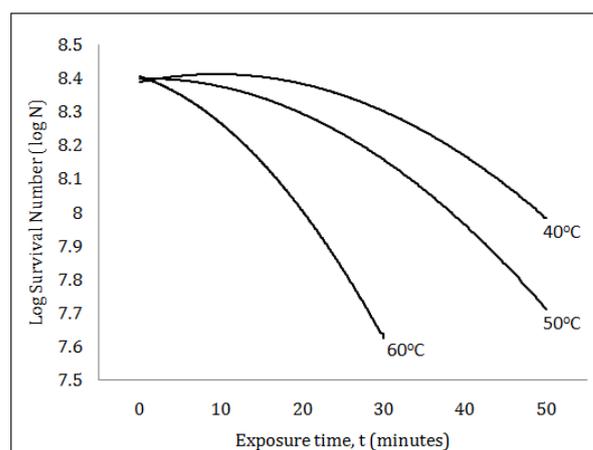
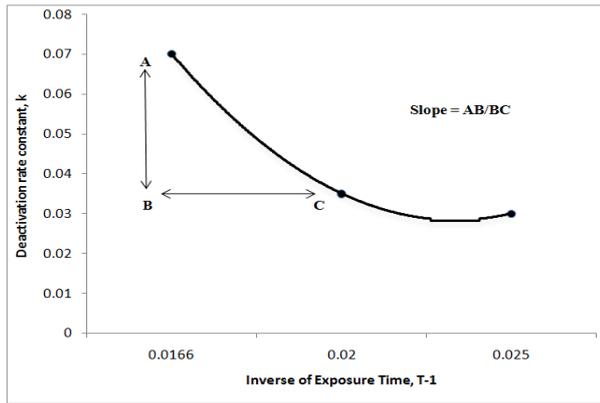


Fig. 1: Survival rate of *S. aureus* at varied time temperatures



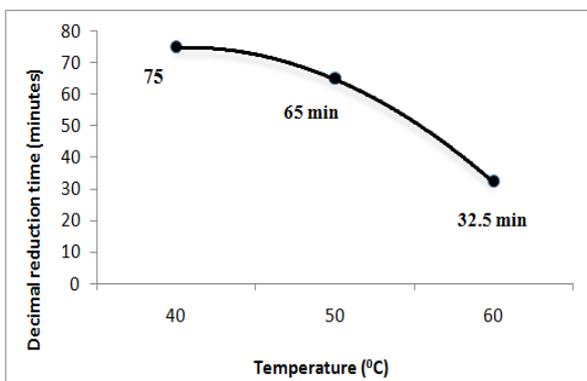
**Fig. 2: Calculation of activation energy from the graph**

The slope of the line relating deactivation rate constants at given temperature vs. inverse of exposure time is a measure of activation energy. At varied temperature treatments, the activation energy marked in Figure 2 remains constant showing a characteristic specific to the test strain. The activation energy calculated as 8.75 from the graph is shown in Table 1.

**Table 1: Summary of result processing**

Temperature	D Value (Fig. 1)	Deactivation Rate Constant $k$ $\text{min}^{-1}$ (Fig. 1)	Activation Energy (Fig. 2)
40	75.0	0.031	8.75
50	65.0	0.035	
60	32.5	0.070	

Fig. 3 shows the relation between Decimal Reduction Time and Temperature. This relationship shows inverse proportionality. As temperature increases, the Decimal Reduction Time decreases which denotes the measure of the tolerance of the organism for temperature.



**Fig. 3: Relation between Decimal reduction time and Temperature**

Moist heat owing to its greater permeability and hydrolyzing effects, kills microbial cells more rapidly than dry heat. Since microbes show inherent and remarkable diversity with regards to vulnerability

towards heat, surrounding factors like water content of medium and cell,  $\text{H}^{+}$  ion concentration, composition of medium, age of the cell, sporulation stage and incubation temperature of the recovery cells vastly affects killing rate [19]. Interestingly, high but sub-lethal temperature promotes higher metabolic activities [20]. This is because rise of every 10  $^{\circ}\text{C}$  in temperature in the multiples of Temperature Quotient (Q10) value although need for increased repair work can slower down growth rate. These considerations necessitate careful evaluation of target cells ability to sustain heat dosage as a critical aspect to microbial control by heat.

The approach for quantitative bacteriological analysis used herein was viable count that has inherent limitations, despite that an estimate of heat survivor is accurate enough to understand heat tolerance limits of the bacterial culture.

Judging from the values determined for  $D_{40}$ ,  $D_{50}$ , and  $D_{60}$ , 90 % destruction of the population of *S. aureus* occurred in approximately 75 minutes, 65 minutes, and 32.5 minutes, giving Deactivation Rate Constants,  $k$  ( $\text{min}^{-1}$ ) of 0.030, 0.035, and 0.070, respectively (Fig. 1). When the number of staphylococci remaining in food after a 90 % reduction is not equivalent to an infective dosage, subsequent contamination or growth of the remaining organisms is prevented, hence little danger is associated with the ingestion of such a food. The adult oral infective dose for several strains of *S. aureus* ranges between 587 thousand to 67.2 million [21]. However, in those instances where a 90 % reduction still results in numbers approximating an infective dose for the susceptible organism under study, danger does exist. From the graph of decimal-reduction time at different temperatures, Fig. 3, we find a 50% reduction in time between 40 and 60 $^{\circ}\text{C}$ . However, even this information does not tell the investigator whether an infective dose exists after such a heat treatment, unless he knows the initial concentration of cells; a situation rarely encountered in practice. But, by employing D value alone, any arbitrary degree of destruction can be achieved irrespective of the initial concentration. For example, if  $D = 90\%$  reduction, then a process of  $2D = 99\%$  reduction, etc. Assuming an exaggerated situation in which  $1 \times 10^7$  *S. aureus* per gram of food are present, a process equivalent to  $6D$  would yield a 99.9999 % reduction in numbers, or  $10^1$  *S. aureus* per gram. However, an extended exposure of this type may not be practical.

As destruction time decreases with increase in temperature as shown in the Figure 3, prolonged holding at 60 $^{\circ}\text{C}$  for around 32.5 minutes would lead to 90% destruction of *S. aureus*.

**CONCLUSION**

To design an effective temperature treatment regime, it is imperative to have an understanding of the effects of heat on micro-organisms. This result of this work is

adequate to recommend practically cooking foods at around 60°C for ~30 minutes to render the food almost free from staphylococcal contamination. The simple classical method for determining heat survival can be a valuable tool for biochemical and genomic profiling. We recommend detailed study on reduction in virulence affecting infective dosage upon exposure to heat.

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