

Research Article

## Thermal resistance of local isolates of *Staphylococcus aureus*

Ratih Dewanti-Hariyadi<sup>1,2\*</sup>, Juli Hadiyanto<sup>2</sup> and Eko Hari Purnomo<sup>1,2</sup>

<sup>1</sup>Southeast Asia Food Agricultural Science and Technology (SEAFAST) Center, Bogor Agricultural University, Bogor 16002, Indonesia.

<sup>2</sup>Department of Food Science and Technology, Faculty of Agricultural Technology, Bogor Agricultural University, Bogor 16002, Indonesia.

\*Email: [ratihde@ipb.ac.id](mailto:ratihde@ipb.ac.id)

---

### Abstract

Currently, 40.87% of foodborne disease outbreaks in Indonesia are associated with homemade food. *Staphylococcus aureus* are probably important pathogens contributing to the food poisoning cases because they are natural microbiota that live in human body and could contaminate food due to poor sanitation and hygienic practices. Since most of Indonesian food is heavily heated, it is interesting to know whether *S. aureus* isolated from local food is more resistant to heat and whether common processing could actually inactivate a large number of these pathogens. The objective of this research is to evaluate the heat resistance of several isolates of *S. aureus* obtained from ready to eat (RTE) Indonesian traditional food. The study was conducted by inoculating 1 ml of late log phase *S. aureus* culture into 9 ml of heating menstruum (Trypticase Soy Broth) at 53, 54, 55, and 56°C for 5, 7, 10 and 15 minutes. *S. aureus* surviving from the heating process was enumerated on Baird Parker Agar (BPA) media containing egg yolk tellurite after incubation for 48 hours at 35°C. Thermotolerance parameters, i.e. D and Z values, were estimated using standard regression analysis based on log linier models. The result was used to estimate the adequacy of various cooking methods for several RTE Indonesian traditional food items. The D<sub>53</sub>, D<sub>54</sub>, D<sub>55</sub>, and D<sub>56</sub> values of local isolates of *S. aureus* were 19.47-64.59 min, 13.42-23.8 min, 6.59-14.3 min and 5.17-8.78 min, respectively. The thermal inactivation of *S. aureus* followed first order kinetics with r<sup>2</sup> values of 0.92-0.99. The Z values calculated in this study ranged from 3.37 to 6.06°C. These values were within the range of reported Z values for most non-spore forming bacteria (4-6°C). This study provided data on the thermal resistance of *S. aureus* isolated from Indonesia and validated that heating commonly applied in cooking of RTE traditional food could reduce *Staphylococcus aureus* to up to 6.9x10<sup>6</sup> log cycle. However, common practices following heating of certain food items may allow recontamination, thus handling of RTE food after cooking is very important for the management of this pathogen.

**Keywords:** food-borne disease, pathogens, D-value, Z-value, heat resistance, Indonesia.

---

## Introduction

*Staphylococcus aureus* is an important foodborne pathogen worldwide and has been linked to various foodborne disease outbreaks. The ability of the bacterium to grow in food containing salt up to 20% or  $A_w$  as low as 0.83, as well as its ability to produce different kinds of enterotoxins [1] have been thought to play a role in causing food toxication in processed food such as pasteurised milk, cream-filled bakery, etc.

In Indonesia, bacterial pathogens were the main cause of foodborne outbreaks between 2007-2010. Based on limited data, reports also suggest that ready-to-eat food produced in the home was accountable for 40% of the outbreaks [2]. Although *Staphylococcus aureus* was not singled out as the main causative agent of the outbreaks, it was very likely that *S. aureus* was responsible for some of the outbreaks because of poor implementation of sanitation and hygiene programs observed with various food vendors.

*S. aureus* has been reported to grow well in several ready-to-eat (RTE) Indonesian traditional food items such as chicken soup, stir fry green bean and rice cooked in coconut milk (*nasi uduk*) [3]. Dwintasari [4] and Apriyadi [5] reported that *S. aureus* can be isolated from hands of street vendors, *nasi uduk* and shredded chicken in several vendors around the Bogor area. Several factors can be attributed to the finding of these bacteria in such food. First, although most Indonesian traditional food is well cooked, post processing handling may lead to contamination. Secondly, common household practices to store ready-to-eat food at room temperature may support bacterial growth and subsequent toxin production. Thirdly, reheating which is a common practice, may be inadequate and or may not be effective since the heat stable enterotoxin may have already been produced.

*S. aureus* is a non-spore forming bacterium which could easily be killed by heat. Although data of thermal resistance of *S. aureus* has been reported worldwide, it is not known whether *S. aureus* isolated from RTE Indonesian traditional food which generally receives long cooking time has similar heat resistance.

The objectives of this study was to obtain information on the thermal resistance (D and Z values) of several *S. aureus* previously isolated from RTE Indonesian traditional food and use the information to evaluate thermal adequacy of several cooking methods commonly applied by food vendors.

## Materials and Methods

### *Inoculum preparation*

*S. aureus* strains used in this study have been confirmed using biochemical assays and consisted of AS2 (isolated from shredded chicken), NU3 (isolated from *nasi uduk*) obtained by Apriyadi [5] and ATCC 25923 as a control. Individual isolate was grown in Trypticase Soy Broth (TSB) at 35°C for 24 h to reach late log phase. The cultures containing ca.  $1.0 \times 10^8$  -  $1.0 \times 10^9$  CFU/ml were used as inocula to achieve the desired initial concentration in the heating menstruum.

### *Preparation of heating menstruum*

The heating menstruum was 9 ml TSB which was previously sterilised at 121°C for 15 minutes.

### *Thermal resistance testing*

Sets of glass tubes containing the heating menstruum were placed in different waterbaths set at 53, 54, 55 and 56°C. When the heating menstruum reached the desired temperatures, one milliliter of overnight culture of *S. aureus* was inoculated into the glass tubes containing the heating menstruum such that the initial counts were ca.  $1.0 \times 10^7$  -  $1.0 \times 10^8$  CFU/ml. The menstruum in the tubes was

heated for 5, 7, 10 and 15 minutes. Enumeration of *S. aureus* surviving the heating was carried out on Baird Parker Agar (BPA) containing egg yolk tellurite [6], after incubation at 35°C for 48 h. The number of *S. aureus* surviving were plotted against the heating times to yield a curve of rate of inactivation at four different temperatures, i.e. 53, 54, 55 and 56°C. Based on the curve, the D values, i.e. time (minutes) at certain temperatures to reduce the number of *S. aureus* by 1 log cycle can be calculated from the equation  $D = -1/\text{slope}$ . A Thermal Death Time (TDT) curve was made to establish the relationship between D (minutes) with temperatures (°C). The Z values, i.e. temperature intervals to reduce D value by 1 log cycle was also determined from the curve.

### ***Assessing thermal adequacy of several RTE Indonesian traditional food cooking methods***

Assessment of the thermal adequacy of various RTE food items was conducted through a survey to determine the common practice, cooking time generally applied by food vendors and measurement of product internal temperatures during cooking. The respondents were 16 food vendors surrounding Darmaga campus area, Bogor, who process and serve 16 different RTE types of food. The adequacy of thermal process applied in the surveyed food vendors was assessed by extrapolating the Z value equation to obtain  $D_T$  (D values at the cooking temperatures applied).

## **Results and Discussion**

### ***D values of local isolates of Staphylococcus aureus***

The data of heat resistance of local isolates of *S. aureus* are presented in Figure 1. The D values of *S. aureus* isolate AS2 originated from shredded chicken at 53, 54, 55 and 56°C were 19.47±1.33; 13.42±0.13; 6.59±0.85 and 5.17±0.26 minutes, respectively. *S. aureus* NU3, isolated from *nasi uduk* had D values of 64.59± 2.95, 23.83± 0.80, 14.3±0.78 and 8.78±0.92 minutes at 53, 54, 55 and 56°C, respectively. Meanwhile, the control ATCC isolate had D values of 22.00± 1.02, 15.31± 1.16, 11.12±0.52 and 7.53±1.76 minutes at the above temperatures. The data suggest that the heat resistance of the three isolates varied. The linear equation of the logarithmic decrease of the bacterial number had  $r^2$  values of 0.92-0.98.

The results of the study showed that *S. aureus* NU3 had  $D_{53}$ ,  $D_{54}$ ,  $D_{55}$ ,  $D_{56}$  values higher than those of AS2 and ATCC 25923. *S. aureus* NU3 was isolated from *nasi uduk*, a rice dish which was generally stored in rice thermos at 40-60°C for prolonged period [3]. Storage at the temperature range probably contribute to the increased heat resistance [7].

The  $D_{53}$ ,  $D_{54}$ ,  $D_{55}$  and  $D_{56}$  values of *Staphylococcus aureus* AS2, NU3 and ATCC 25923 were higher than that reported by Walker and Harmon [8] who concluded that the heat resistance of *S. aureus* S-18 and B-120 in phosphate buffer was lower than those in milk. They found that the isolates had lower D values in the phosphate buffer, possibly due to protection by nutrients in milk. Jay [7] also stated that nutrients such as carbohydrate, proteins, fat, soluble solid, as well as  $A_w$  and pH, strongly influence cell damage during heating. In general, carbohydrate, proteins, fat and soluble solid protect bacterial cells from heating. Heat resistance increases with increase in the above nutrient content. Since this study was conducted in TSB, it is thought that the nutrient protection is similar to milk. Additionally, the difference in the heat resistance of isolates used in this study and those of Walker and Harmon [8] could also be attributed to strain difference [7].

The  $D_{55}$  values of AS2 and ATCC 25923 isolates were lower than the  $D_{55}$  value of *S. aureus* cocktail reported by Kennedy, *et al* [9] i.e. 13.0 minutes. Parente and Mazzatura [10] also reported on the heat resistance of *S. aureus* BP3 and *S. aureus* 237 isolated from goat milk. *S. aureus* BP3 also had a lower  $D_{55}$  value (3.30 minutes), while isolate 237 had similar  $D_{55}$  (10.60 minutes) to those of NU3, AS2 and ATCC 25923.

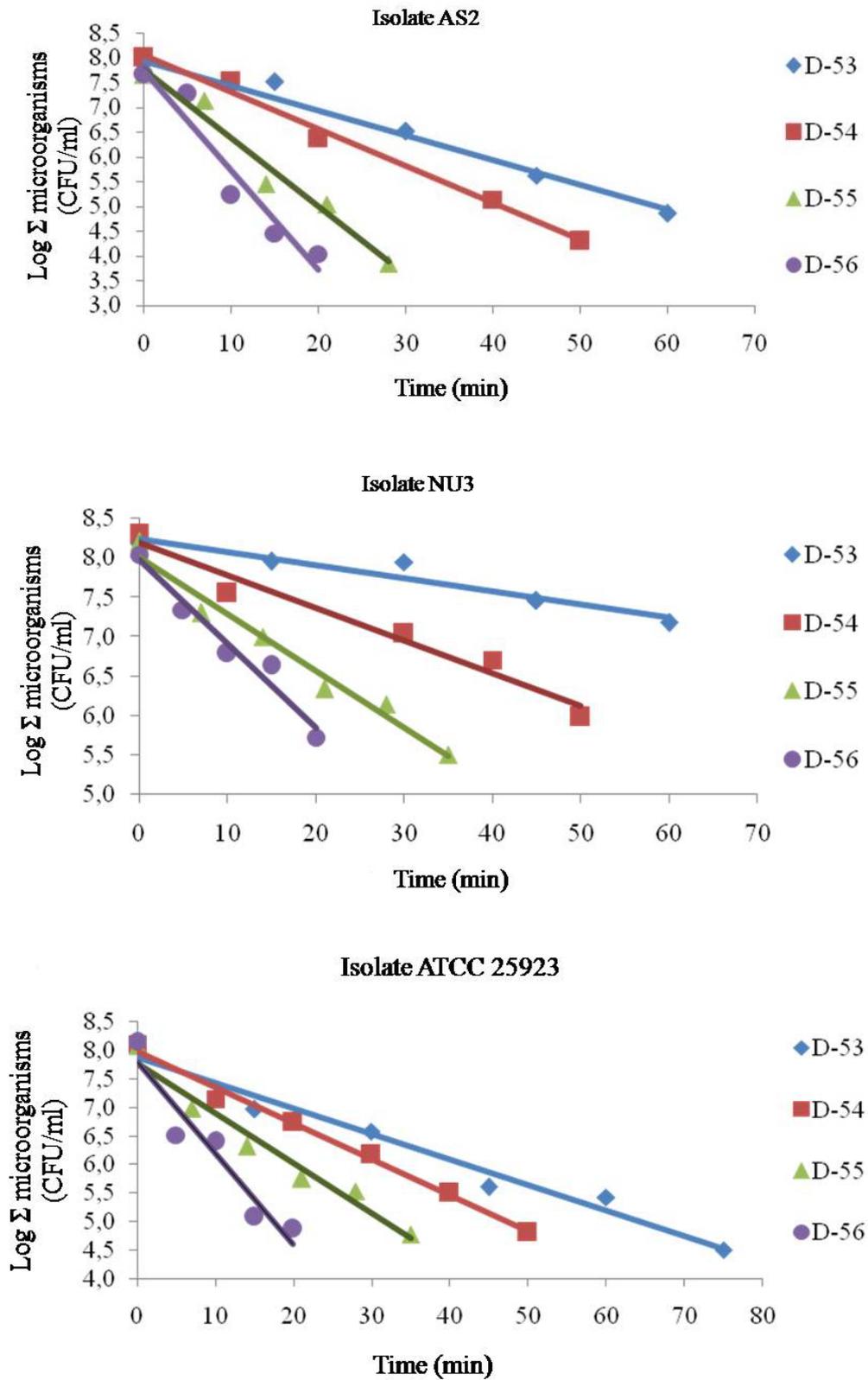


Figure 1. Log decrease of the number of *S. aureus* heated at 53, 54, 55, 56°C.

### Z values of *Staphylococcus aureus* AS2, NU3 and ATCC 25923

The sensitivity of D values to temperature changes is expressed as Z-value, i.e. changes of temperature to change D value by 1 log cycle or 90% [11]. The Z values of *S. aureus* AS2 were 4.74-5.10°C, *S. aureus* NU3 were 3.37-3.7°C, while ATCC 25923 were 5.59-6.06°C (Figure 2). The results showed that *S. aureus* NU3 had the lowest Z thus the D values of the isolate were more sensitive to temperature changes than that of AS2 or ATCC 25923. Figure 2 also suggests that *S. aureus* NU3 was more heat resistant than AS2 and ATCC 25923 at temperatures of less than 56°C. However, this pattern does not apply when heating temperature changes. Analysis of the Z values suggested that the intercept between Z curves of NU3, AS2 and ATCC 25923 occurred at 57.6°C, 55.9°C and 50.3°C. Two microorganisms have the same heat resistance at the interception of the Z curve due to the same D-values [11]. Our results suggest that NU3 and AS2 isolates have the same heat resistance at 57.6°C. At temperatures below 57.6°C, NU3 isolate is more heat resistant than AS2; however at temperatures above 57.6°C, NU3 isolate becomes less heat resistant than AS2.

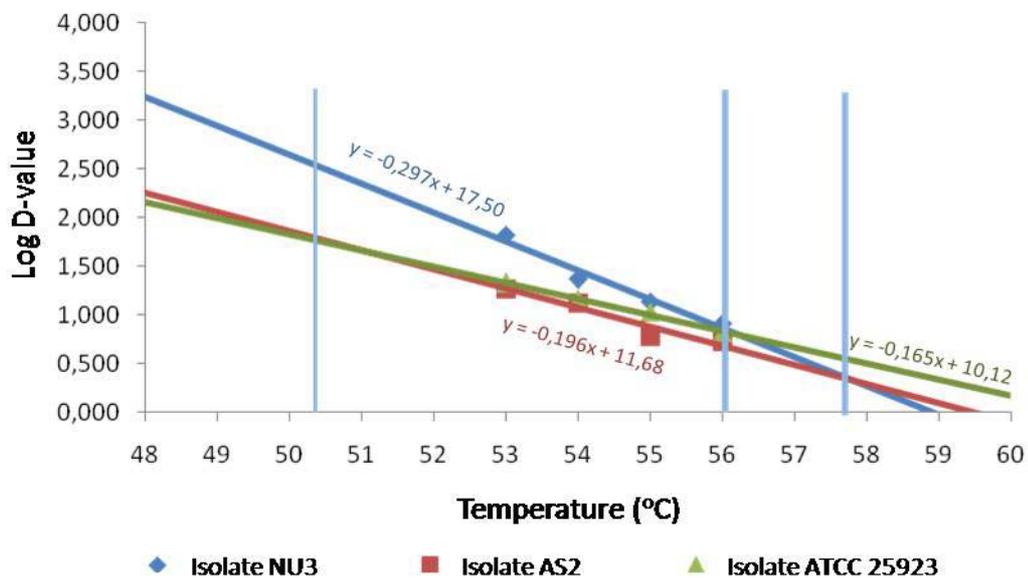


Figure 2. Z-value curves of *S. aureus* AS2, NU3 and ATCC 25923.

The Z values of *S. aureus* isolates in this study vary, but are similar to previous studies reported by Stumbo [12] for *S. aureus* in pasteurised food i.e. 4.6-6.7°C. Isolates AS2 and ATCC 25923 had Z values within the range of those reported in that study. However, the Z value of NU3 (3.3-3.37°C) was lower than that reported. Eden, *et al* [13] reported a Z value of 9.46°C, while Kennedy, *et al* [9] concluded that a *Staphylococcus aureus* cocktail had Z values ranging from 7.70 to 8.0°C. The Z values reported in this study were a lot lower than those reported by [9], suggesting that these local isolates had a higher sensitivity toward heat. However, the Z values of local isolates of *S. aureus* were similar to other non-spore forming bacteria in protein-rich heating menstruum such as chicken broth, TSB etc, i. e. 5°C. Table 1 shows the Z values reported in this study as compared to other pathogens.

**Table 1. Z values of *Staphylococcus aureus* AS2, NU3 and ATCC 25923 as compared to other pathogens in protein-rich heating menstruum.**

Microorganism	Heating menstruum	Z (°C)
<i>Staphylococcus aureus</i> AS2	TSB	4.74-5.10
<i>Staphylococcus aureus</i> NU3	TSB	3.37-3.7
<i>Staphylococcus aureus</i> ATCC 25923	TSB	5.59-6.06
<i>Campylobacter jejuni</i> <sup>a</sup>	chicken broth	5.81
<i>Salmonella</i> <sup>b</sup>	chicken broth	5.35
<i>Listeria monocytogenes</i> <sup>b</sup>	chicken broth	5.11
<i>Salmonella typhimurium</i> <sup>c</sup>	chicken broth	5.80
<i>Salmonella enteritidis</i> <sup>c</sup>	chicken broth	5.86
<i>Yersinia enterocolitica</i> <sup>d</sup>	minced beef	5.1
<i>S. epidermidis</i> <sup>e</sup>	chicken broth	7.46
<i>Escherichia coli</i> O-157 <sup>f</sup>	breaded pork patties	5.43

<sup>a</sup>[14], <sup>b</sup>[15], <sup>c</sup>[16], <sup>d</sup>[17], <sup>e</sup>[18], <sup>f</sup>[19].

### **Evaluation of thermal process adequacy of several RTE Indonesian traditional food vendors**

The results of the survey of 16 food types from 16 food vendors is presented in Table 2. In general traditional RTE food was heated at temperatures above 70°C. The food was either boiled, steamed, grilled, fried, stir-fried or received a combination of two cooking methods.

Using the equation obtained in the Z curves, extrapolation was carried out to determine the D<sub>73</sub> and D<sub>92</sub> values. Extrapolation at 92°C was conducted to simulate boiling, while that at 73°C was used to simulate stir-frying and grilling. The results of extrapolation were used to assess the thermal process adequacy.

Table 3 shows the extrapolated D<sub>73</sub> and D<sub>92</sub> values for each isolate used in this study. The extrapolated values suggest that cooking food at 73°C for 0.00006-0.011 minutes could reduce *Staphylococcus aureus* by one log cycle. A similar effect could also be obtained by cooking at 92°C for 1, x10<sup>-10</sup> – 1.93 x10<sup>-6</sup> minutes.

Boiling food at 92°C for an hour could reduce 6.9x10<sup>6</sup> log cycle of the bacteria, meanwhile stir-frying at 73°C for 5 minutes decreases 454.5 log cycle of *S. aureus*. Using an assumption that the initial count of *nasi uduk* is 1.0x10<sup>3</sup> CFU/g [20], a serving size of *nasi uduk* of 100 g would contain 1.0x10<sup>5</sup> CFU *S. aureus*. Therefore, boiling at 92°C for 1 hour or stir-frying at 73°C for 5 minutes could reduce *S. aureus* to very low numbers (<1/10<sup>449.5</sup>). The results suggested that the likelihood of *S. aureus* being present in RTE Indonesian traditional food after cooking was very low and compliance with various guidelines that called for a maximum *S. aureus* of 1x10<sup>2</sup> CFU/gr [21] or 0-5x10<sup>3</sup> CFU/g (BPOM 2009) is easy to achieve.

Although the cooking processes of all RTE Indonesian traditional food provides adequate heating for inactivation of *S. aureus*, several practices may allow recontamination of the food. For beef soup for example, prolonged storage and cutting the beef into cubes may allow the re-entry of *S. aureus* or other pathogens as well as spoilage bacteria. Vegetable and vermicelli for the salad are boiled, however mixing with fresh chilli-peanut sauce may introduce bacteria, including *S. aureus*. Grilled chicken also needs precaution such that storage time after boiling prior to grilling is short (<2 h) to avoid growth, although the grilling should be able to decrease the bacterial number substantially.

**Table 2. Survey of cooking practices and temperature of several RTE traditional food items during cooking.**

Vendor	Food Item	Cooking Methods	Temp. of product during heating
1	<i>Semur jengkol</i>	boiling for 1 h	92° C
2	Beef soup	boiling beef for 3 h, storage at RT, cutting into cubes, mixing with broth	92° C
3	Meatball soup	boiling for 1 h	96° C
4	Steamed coconut milk-rice ( <i>nasi uduk</i> )	boiling for 30 min, steaming for 30 min, storage at 50° C	82° C
5	Steamed rice	boiling for 30 min, steaming for 25 min, storage at RT	83° C
6	Chicken <i>opor</i>	boiling for 1 h	95° C
7	Vermicelli salad	boiling of vermicelli, mixing with fresh chilli-peanut sauce	89° C
8	Cooked vegetable salad	boiling of vegetable, mixing with fresh chilli-peanut sauce	89° C
9	<i>Siomay</i>	steaming continuously	86° C
10	Grilled chicken	boiling for 2 h, storage at RT, grilling 5-6 minutes	73° C
11	Grilled fish	grilling for 10 min	73° C
12	Fried coconut chicken	frying in shredded coconut until brown	95° C
13	Fried tempe	frying for 2-3 min	98° C
14	Fried potato	frying for 2-3 min	98° C
15	Stir fried green bean	stir frying for 5 min	73° C
16	Stir fried eggplant	stir frying for 5 min	73° C

**Table 3. Extrapolated D<sub>73</sub> and D<sub>92</sub> values of *S aureus* AS2, NU3 and ATCC 25923.**

Isolate	D <sub>73</sub> (min)	D <sub>92</sub> (min)
NU3 (1)	0.0002	1.62x10 <sup>-9</sup>
NU3 (2)	0.00006	1.5x10 <sup>-10</sup>
AS2 (1)	0.001	1.25 x10 <sup>-7</sup>
AS2 (2)	0.002	4.4 x10 <sup>-7</sup>
ATCC 25923 (1)	0.006	1.93 x10 <sup>-6</sup>
ATCC 25923 (2)	0.011	8.70 x10 <sup>-6</sup>

**Table 4. Inactivation of *S. aureus* due to cooking commonly practiced for RTE traditional food.**

Cooking method	Temperature	Time	Decrease in <i>S. aureus</i> number (log cycle)	Notes
Boiling	92° C	1 h	6.9 x 10 <sup>6</sup>	Boiling is effective in reducing <i>S. aureus</i> , however some products were mixed with fresh sauce or stored at RT for prolonged period prior to serving thus permit recontamination
Stirfrying/grilling	73° C	5 min	454.5	Stir frying is effective in reducing <i>S. aureus</i> , recontamination may occur during storage at RT

## Conclusion

This study found that *S. aureus* isolated from RTE Indonesian traditional food had thermal resistance similar to that reported from *S. aureus* isolated elsewhere, with  $D_{53}$ ,  $D_{54}$ ,  $D_{55}$  and  $D_{56}$  values of 19.47- 64.59 min, 13.42 – 23.8 min, 6.59 – 14.3 min and 5.17-8.78 min, respectively. The  $Z$  values of *S. aureus* isolates ranged from 3.37 to 6.06°C, which were within the range of reported  $Z$  values for most non-spore forming bacteria. The study also reported that heating commonly applied in cooking of RTE Indonesian traditional food could significantly eliminate *Staphylococcus aureus*. Therefore, post cooking practices become very critical because recontamination may occur.

## References

1. Adams M.R. and Moss M.O. (2005). Food Microbiology 2<sup>nd</sup> Edition. United Kingdom: The Royal Society of Chemistry.
2. BPOM. (2010). Annual report of foodborne disease outbreaks 2009. Unpublished data. The National Agency for Drug and Food Control (NADFC), Republic of Indonesia.
3. Dewanti-Hariyadi, R., Rawendra, R. and Dewi, S.P. (2008). Growth of *S. aureus* in Indonesian Traditional Dishes at Storage Temperatures Practiced by Households. Poster Presentation at Conference of Asian Food and Nutrition Safety, Cebu, Philippines, November 5-6, 2008, ILSI-SEA.
4. Dwintasari, V. (2010). Growth of *Staphylococcus aureus* in shredded chicken and its correlation with cleanness of employee and handling practices in vendors. Skripsi. Available from: Faculty of Agricultural Technology, Bogor Agricultural University (in Bahasa Indonesia).
5. Apriyadi, T.E. (2010). Risk of *Staphylococcus aureus* in ready to eat Indonesian traditional food and evaluation of its presence in *nasi uduk*. Skripsi. Available from: Faculty of Agricultural Technology, Bogor Agricultural University (in Bahasa Indonesia).
6. Bennett, R.W. and Lancette, G.A. (2001). *Staphylococcus aureus*. In Bacteriological Analytical Manual. Association of Official Analytical Chemists, Arlington, VA.
7. Jay, J.M. (2000). Modern Food Microbiology. 6th Edition. Gaithersburg, Maryland: Aspen Publishers, Inc.
8. Walker, G.C. and Harmon, L.G. (1966). Thermal resistance of *Staphylococcus aureus* in milk, whey and phosphate buffer. *Applied Microbiology*, 14: 584-590.
9. Kennedy, K., Blair, I.S., McDowel, D.A. and Bolton, D.J. (2005). An investigation of the thermal inactivation of *Staphylococcus aureus* and the potential for increased thermotolerance as a result of chilled storage. *Journal of Applied Microbiology*, 99: 1229–1235.
10. Parente, E. and Mazzatura, A. (1991). Growth and heat resistance of *Staphylococcus aureus* in goat milk. *Italian Journal of Food Science*, 3: 27-37.
11. Toledo, R.T. (1991). Fundamentals of food process engineering 3rd. Georgia: Springer.
12. Stumbo, C.R. (1973). Thermobacteriology in food processing. New York: Academic Press.

13. Firstenberg-Eden, R., Rosen, B. and Mannheim, C.H. (1977). Death and injury of *Staphylococcus aureus* during thermal treatment of milk. ***Canadian Journal of Microbiology***, 23, 8 pp 1034-37.
14. Blankenship, L.C. and Craven, S.E. (1982). *Campylobacter jejuni* survival in chicken meat as a function of temperature. ***Journal of Applied Environmental Microbiology***, 44: 88-92.
15. Murphy, R.Y., Osaili, T., Duncan, L.K. and Marcys, J.A. (2004). Thermal inactivation of *Salmonella* and *Listeria monocytogenes* in ground chicken thigh/leg meat and skin. ***Journal of Poultry Science***, 83:1218-1225.
16. Jenuja, V.K., Eblen, B.S. and Ransom, G.M. (2001). Thermal inactivation of *Salmonella* spp. in chicken broth, beef, pork, turkey and chicken: Determination of D- and Z-values. ***Journal of Food Science***, 66:1.
17. Bolton, D.J., McMahon, C.M., Doherty, A.M., Sheridan, J.J., McDowell, D.A., Blair, I.S. and Harrington, D. (2000). Thermal inactivation of *Listeria monocytogenes* and *Yersinia enterocolitica* in minced beef under laboratory conditions and in sous-vide prepared minced and solid beef cooked in a commercial retort. ***Journal of Applied Microbiology***, 88: 626-632.
18. Bertolatti, D., Steven, J.M., Warren, B.G. and Colin, W.B. (2001). Thermal inactivation of antimicrobial resistant gram-positive cocci in chicken meat: D and Z value determinations. ***International Journal of Environmental Health Research***, 11:257– 266.
19. Osaili, T.M., Griffin, C.L., Martin, E.M., Beard, B.L., Keener, A.E. and Marcy, J.A. (2007). Thermal inactivation of *Escherichia coli* O157:H7, *Salmonella* and *Listeria monocytogenes* in breaded pork patties. ***Journal of Food Science***, 7:2.
20. Hartini, P.B. (2001). Study on microbiological safety of food sold in campus canteen. Skripsi. Available from: Faculty of Agricultural Technology, Bogor Agricultural University (in Bahasa Indonesia).
21. Shapton, D.A. and Shapton, N.F. (1993). Principles and practices for the safe processing of food. Oxford, Great Britain: Butterworth-Heineman Ltd.