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Thermal Resistance of Local Isolates of *Staphylococcus aureus*

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Abstract

Food poisoning is an important indicator for food safety status in any country. Report from The National Agency for Drugs and Food Control of Republic of Indonesia (BPOM) showed that 40.87% of food poisoning occurred in Indonesia was associated with homemade food. *Staphylococcus aureus* probably is an important pathogen contributing to the food poisoning cases in Indonesia, because this pathogen is a natural flora that lives in human body and could contaminate food due to poor sanitation and hygienic practices. Generally, growth of *S. aureus* can be prevented by temperature modification such us refrigeration and heating. Since most of Indonesian foods are heavily heated, it is interesting to know whether most processing could actually inactivate a large number of *S. aureus* and whether the pathogens isolated from local sources are tolerant to heat. This is important because the risk of having *S. aureus* surviving in cooked food is even worse since they could eventually produce enterotoxins. The goal of this research is to evaluate the heat resistance of several *S. aureus* isolated from ready to eat (RTE) Indonesian traditional foods. The study was conducted by inoculating 1 ml of a late log phased *S. aureus* culture into 9 ml of heating menstruum (Trypticase Soy Broth) at 52, 53, 54, and 56^{o}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^{o}C. Thermo tolerance 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 foods. The D_{53}, D_{54}, D_{55}, and D_{56} 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^2 values of 0.92-0.99. The Z values calculated in this study ranged from 3.37 to 6.06^{o}C. These values were within the range of reported Z values for most non-spore forming bacteria (4 – 6^{o}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 foods could reduce *Staphylococcus aureus* to up to $6.9x10^{6}$ log cycle. However, common practices following heating of certain foods may allow recontamination, thus handling of RTE foods after cooking is very important for the management of this pathogen.

Keywords: Staphylococcus aureus, D-value, Z-value, thermal resistance

Introduction

Staphylococcus aureus is an important foodborne pathogen worldwide and has been linked to various foodborne disease outbreaks. The ability the bacterium to grow in food containing salt up to 20% or Aw as low as 0.83 as well as its ability to produce different kinds of enterotoxins (Adams and Moss 2005) have been thought to play a role in causing food intoxication in processed food such as pasteurized milk, cream-filled bakery etc.

In Indonesia, bacterial pathogens were the main cause of foodborne outbreaks between 2007-2010. Based on limited data, the report also suggested that ready-to-eat food produced in home was accountable for 40% of the outbreaks (BPOM 2010). Although *Staphylococcus aureus* was not singled out as the main causative agent in the outbreaks, it was very likely that the pathogen was responsible for some of the outbreak because of poor implementation of sanitation and hygiene program observed in various food vendors. *S. aureus* has been reported to grow well in several ready-to-eat (RTE) Indonesian traditional foods such as chicken soup, stir fry green bean and rice cooked in coconut milk *(nasi uduk)* (Dewanti-Hariyadi and others 2008). Dwintasari (2010) and Apriyadi (2010) reported that *S. aureus* can be isolated from hands of workers in street vendors, *nasi uduk* and shredded chicken in several vendors around Bogor area. Several factors can be attributed to the finding of these bacteria in such foods. First, although most Indonesian traditional foods are wll cooked, post processing handling may lead to contamination. Secondly, common household practice to store ready-to-eat at rom temperaturee may support bacterial growth and subsequent toxin producton. Thirdly, reheating which is a comon practice may be inadequate and or may not be effective since the heat stable enterotoxin may have already been produced.

S. aureus in non-spormer bacteria which could easily be killed by heat. Although data of thermal resistance of *S. aureus* have neen reported worldwide, it is not known whether *S. aureus* isolated from RTE Indonesian traditional foods which generally receive 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 foods and use the information to evaluate thermal adequacy of several cooking methods commonly applied in food vendors.

Materials and Methods

Inoculum preparation *S. aureus* used in this study were AS2 (isolated from shredded chicken), NU3 (isolated from nasi uduk) obtained by Apriyadi (2010) and ATCC 25923 as a control. Individual isolate was grown in Tryptose Soy Broth (TSB) at 35°C for 24 h to reach late log phase. The culture containing ca. $1.0x10^8$ -$1.0x10^9$ CFU/ml was used as inoculum to achieve the desired initial concentration in the heating menstruum.

Preparation of Heating Menstruum

The heating menstruum was 9 ml TSB which was ppreviously sterilized at 121°C for 15 minutes**Thermal Resistance Testing**Sets of glass tubes containing the heating menstruum were placed in different waterbath set at 53, 54, 55, dan 56°C. When the heating menstruum reached the desired temperatures, one mililiter of overnight culture of *S. aureus* was inoculated into the glass tubes containing the heating menstruum such that the

initial counts were ca. $1{,}0x10^7$ -$1{,}0x10^8$ CFU/ml. The menstruum in the tubes was allowed to be 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 (Bennet and Lancette 2001) 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, dan 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/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 by 1 log cycle was also determined from the curve.

Assessing Thermal Adequacy of Several RTE Indonesian Traditional Foods

Assessment of the thermal adequacy of various RTE foods was conducted through a survey to determine the common practice and time of cooking generally applied by food vendors and measurement of product internal temperatures during cooking. The respondents were 16 food vendors surrounding Darmaga campus area, Bogor. 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 at the cooking temperatures applied).

Results and Discussions

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. 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.

S. aureus NU3 had D_{53}, D_{54}, D_{55}, D_{56} values higher than AS2 and ATCC 25923. This strain was isolated from nasi uduk which was generally maintained in rice thermos at 40-60°C (Dewanti-Hariyadi and others 2008). Storage at the temperature range probably contribute to the increased heat resistance (Jay 2000).

The D_{53}, D_{54}, D_{55}, dan D_{56} values of *Staphylococcus aureus* AS2, NU3, dan ATCC 25923 were higher than that reported by Walker and Harmon (1966) who concluded that the heat resistance of *S.aureus* S-18 and B-120 in phosphate buffer was lower than those in milk. They concluded that the isolates had lower D values in the phosphate buffer, possibly due to protection by nutrient in milk. Jay (2000) also stated that nutrient such as carbohydrate, proteins, fat, soluble solid as well as aw and pH strongly influence cell damage during heating. In general carbohydrate, proteins, fat and soluble solid protect bacterial cell from heating. Heat resistance increases with increase in the above nutrient content. Since this study was conducted in TSB, it is thought that the nutrent protection is similar to milk. Additionally, the difference in the heat resistance of isolates used in this study and those of Walker and Harmon (1966) could also be attributed to strain difference (Jay, 2006).

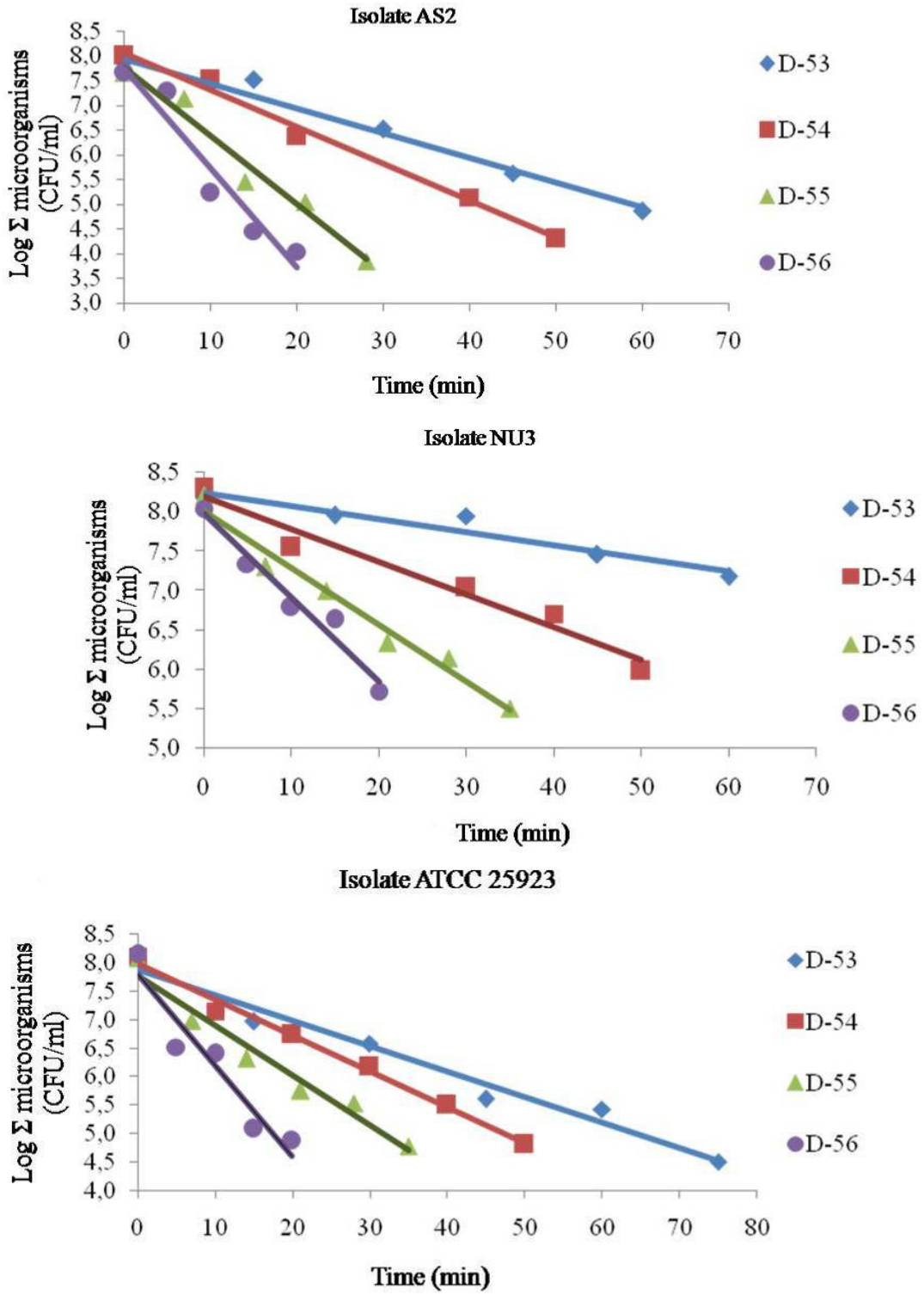

Figure 1 Log decrease of the number of S. aureus heated at 53, 54, 55, 56 °C.

The D_{55} values of AS2 and ATCC 25923 isolates were lower than the D_{55} value of *S. aureus* cocktail reported by Kennedy and others (2005) i.e.13.0 minutes. Parente and Mazzatura (1991) also reported the heat resistance of *S. aureus* BP3 and *S. aureus* 237

isolated from goat milk. Isolate BP3 had also 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.**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 temperatures to change D value by 1 log cycle or 90% (Toledo 1991). The Z values of *S. aureus* AS2 were 4.74-5.10˚C, *S. aureus* NU3 were 3.37-3.7˚C whileATCC 25923 were 5.59-6.06˚C (Figure 2). The results showed that *S. aureus* NU3 had the lowest Z thus the D value of the isolate was more sensitive to temperature changes than that of AS2 or ATCC 25923. Figure 2 suggests that *S. aureus* NU3 is more heat resistant than AS2 and ATCC 25923 at temperatures less than 56°C. However, this is not always happen when heating temperature changes. Analysis of the Z values suggested that the intercept between Z curves of NU3, AS2 and ATCC 25923 occured 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 (Toledo 1991). Our results suggests 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 that of AS2; however at temperatures above 57.6°C, NU3 isolate is less heat resistant than that of AS2.

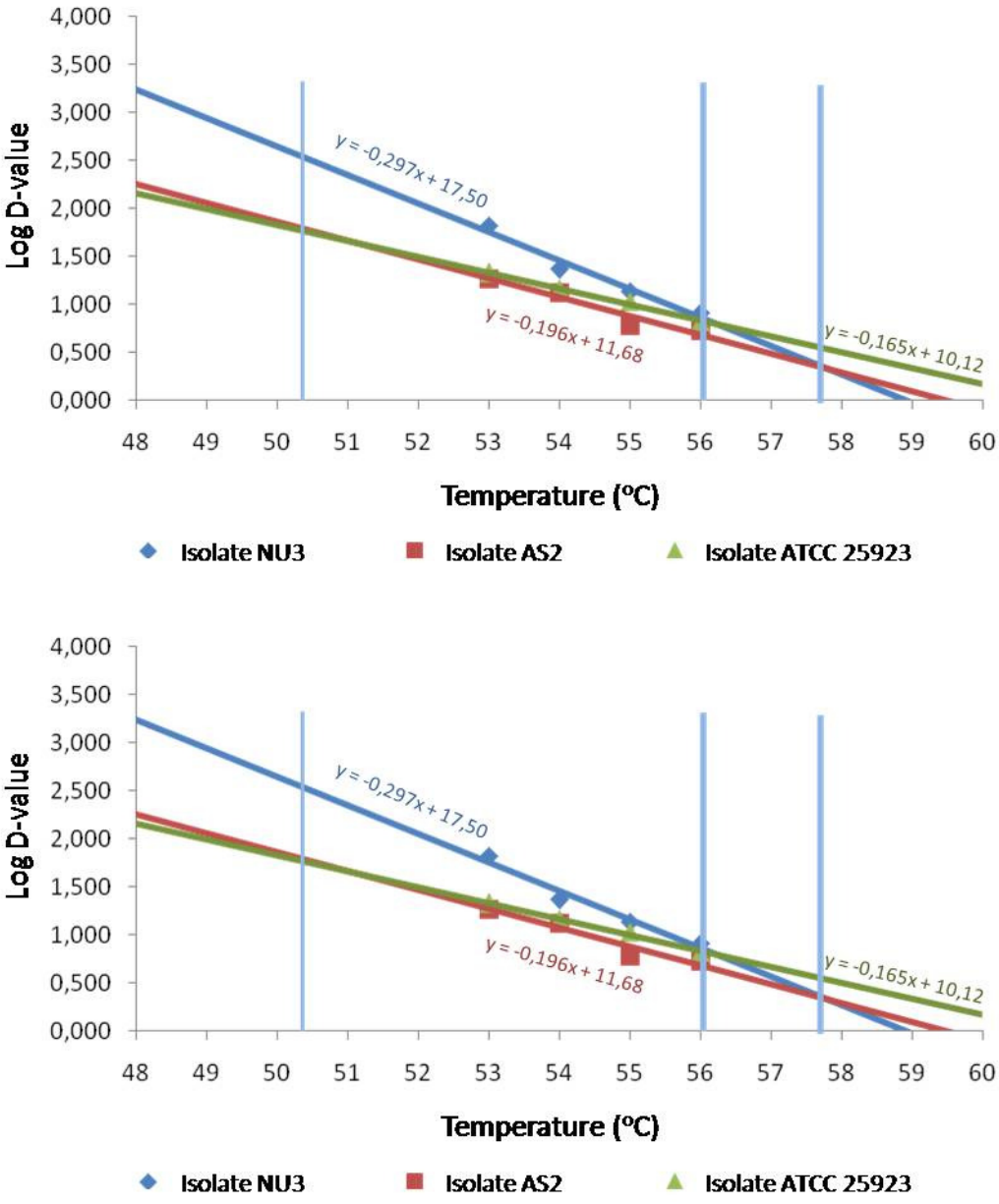

Figure 2 Z-value curves of *S. aureus* AS2, NU3, dan ATCC 25923

The Z values of *S.aureus* isolates in this study varies and similar to previous studies reported by Stumbo (1973) for *S. aureus* in pasteurized food i.e. 4.6-6.7˚C. Isolates AS2 and ATCC 25923 had Z values within the range of those reported by Stumbo (1973). However, the Z value of NU3 (3,3-3,37˚C) was lower than that of Stumbo (1973). Eden and others (1977) reported a Z value of 9.46˚C, while Kennedy and others (2005) concluded that a *Staphylococcus aureus* cocktail had Z value ranging from 7.70 to 8.0˚C. The Z values reported in this study were a lot lower than those reported by Kennedy and others 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 nonsporming bacteria in protein-rich heating menstruum such as chicken broth, TSB etc, i. e. 5°C. Table 1 showed the Z values reported in this study as compared to other pathogens (Table 1).

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

Micrroorganism	*Heating menstruum*	Z (˚C)
Staphylococcus aureus AS2	TSB	4,74-5,10
Staphylococcusaureus NU3	TSB	3,37-3,7
Staphylococcus aureus ATCC 25923	TSB	5,59-6,06
Campylobacter jejuni[a]	chicken broth	5,81
Salmonella[b]	chicken broth	5,35
Listeria monocytogenes[b]	chicken broth	5,11
Salmonella typhimurium[c]	chicken broth	5,80
Salmonella enteritidis[c]	chicken broth	5,86
Yersinia enterolitica[d]	*minced beef*	5,1
S. epidermidis[e]	chicken broth	7,46
Escherichia coli O-157[f]	*breaded pork patties*	5,43

[a](Blankenship and others 1982), [b](Murphy and others 2004), [c](Jenuja and other 2001), [d](Bolton and others 2000), [e](Bertolatti and others 2001), [f](Osaili and others 2007)

Evaluation of Thermal Process Adequacy of Several RTE Indonesian Traditional Foods Sold in Food Vendors

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

Using the equation obtained in the Z curves, extrapolation was carried out to determine D_{73} and D_{92} values. Extrapolation at 92°C was conducted to simulate boiling, while 73°C was used to simulate stir friyng and grilling. The results of extrapolation was used to assess the thermal process adequacy.

Table 2 Survey of cooking practices and temperature f several RTE traditional foods during cooking

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

Table 3 shows the extrapolated D_{73} dan D_{92}. The extrapolated values suggest that cooking food at 73°C for 0,00006-0,011 minute could reduce *Staphylococcus aureus* by one log cycle. Similar effect could also be obtained by cooking at 92°C for $1{,}5x10^{-10}$ - $1{,}93\ x10^{-6}$ minute .

Table 3 Extrapolated D_{73} and D_{92} values of *S. aureus* AS2, NU3, and ATCC 25923

Isolate	D_{73} (min)	$D_{92\ (min)}$
NU3 (1)	0,0002	$1{,}62x10^{-9}$
NU3 (2)	0,00006	$1{,}5x10^{-10}$
AS2 (1)	0,001	$1{,}25\ x10^{-7}$
AS2 (2)	0,002	$4{,}4\ x10^{-7}$
ATCC 25923 (1)	0,006	$1{,}93\ x10^{-6}$
ATCC 25923 (2)	0,011	$8{,}70\ x10^{-6}$

Boiling food at 92°C for an hour could reduce $6{,}9x10^{6}$ log cycle of the bacteria, meanwhile stir-frying at 73°C for 5 minutes decreases 454,5 log cycle of *S. aureus*. Assuming that the initial count of *nasi uduk* is $1{,}0x10^{3}$ CFU/g (Hartini 2001), a serving size of *nasi uduk* of 100 g would contain $1.0x10^{5}$ CFU *S. aureus* and boiling at 92°C for 1 hour or stir frying at 73°C for 5 minutes could reduce *S. aureus* to very low number ($<1/10^{449,5}$). The results suggested that the likelihood of *S. aureus* to present in the RTE Indonesian traditional food after cooking was very low and compliance with various guideline that called for a maximum *S. aureus* of $1x10^{2}$ CFU/g (Shapton and Shapton 1993) or $0\text{-}5x10^{3}$ CFU/g(BPOM 2009) are easy to achieve.

Although the cooking process of all RTE Indonesian traditional foods provide an adequate heating for inactivation of *S. aureus*, several practices may allow recontamination of the foods. For beef soup for examples, 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. 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 bacterial number substantially.

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

Cooking method	Temperature	Time	Decrease in *S. aureus* number (log cycle)	Note
Boiling	92° C	1 h	6.9 x 10^6	Boiling is effective in reducing S. aureus, 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 *S. aureus*, recontamination may occur during storage at RT

Conclusion

This study found that *S. aureus* isolated from RTE Indonesian traditional foods 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 foods could significantly eliminate *Staphylococcus aureus*. Therefore, post cooking practices became very critical because recontamination may occur.

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