Thermal Resistance of Local Isolates of *Staphylococcus aureus*

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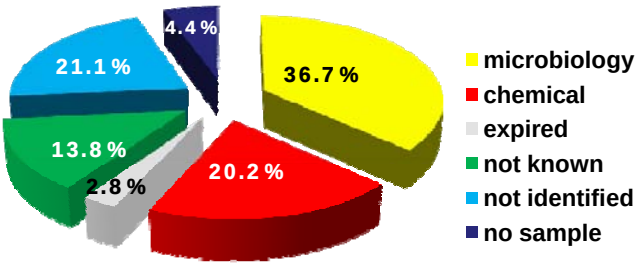
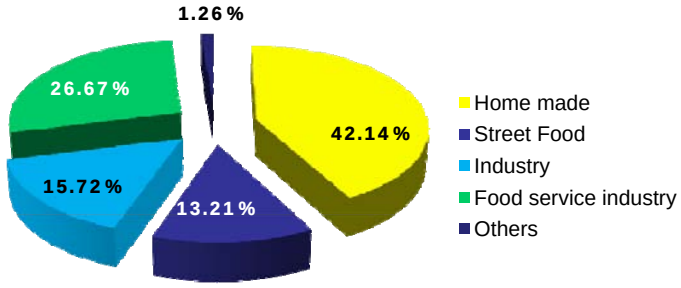
Foodborne Diseases in Indonesia 2009



n = 119

*Suratmono, 2010

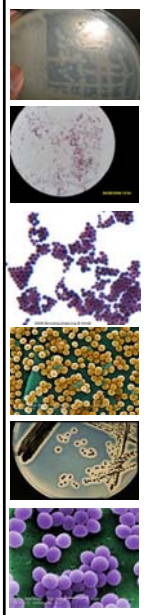
Foodborne Diseases in Indonesia 2009



n = 119

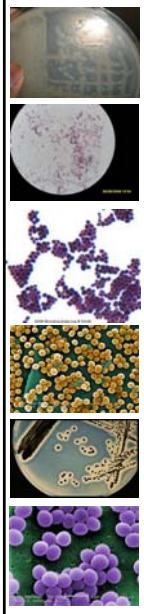
Foodborne Intoxication

- **In Indonesia**
  Causative agent of outbreaks is rarely reported
  Pathogens causing infection are identified though clinical specimens : most frequently isolated : *Shigella flexneri, V. cholerae, Salmonella, Campylobacter* (Oyofo,2005)
  Pathogen causing food intoxication/poisoning?
- **World wide *:***
  Staphylococcus aureus is an important pathogen of foodborne intoxication and has been linked to pasteurized milk, cream-filled bakery, picnic sandwiches outbreaks.



S. aureus in RTE Foods

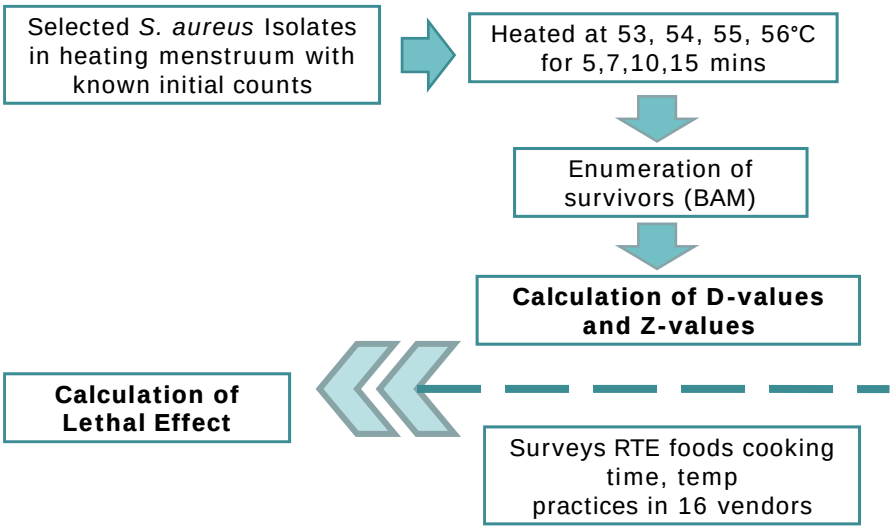
- *S. aureus* has been isolated from RTE in Indonesia, i.e. rice dish, rice cooked in coconut milk *(nasi uduk),* shredded chicken etc.; also from hands of workers in street vendors
- Sporadic outbreak due to *S. aureus*: snacks made from glutinous rice (2007), rice dish (2009)
- *S. aureus* is a non-spormer bacterial pathogen
  - easily be killed by heat
  - some are enterotoxigenic, produce heat-stable SEA, SEB, SEC, SED, SEE, SEH, TSST
  - grow in food with salt up to 20%; Aw 0.83
- *S. aureus* in RTE foods
  - post processing handling
  - storage of RTE foods at rom temperature
  - inadequate reheating



Objectives

- To obtain thermal resistance parameters (D and z values) of several *S. aureus* previously isolated from RTE Indonesian traditional foods
- To use the information to assess thermal adequacy of cooking and handling practices commonly applied by food vendors

Methods



Methods

Isolates

- *S. aureus* AS2 (shredded chicken,enterotoxigenic)
- *S. aureus* NU3 (*nasi uduk*,enterotoxigenic)
- S. aureus ATCC 25923 (nontoxigenic)

Inoculum Preparation

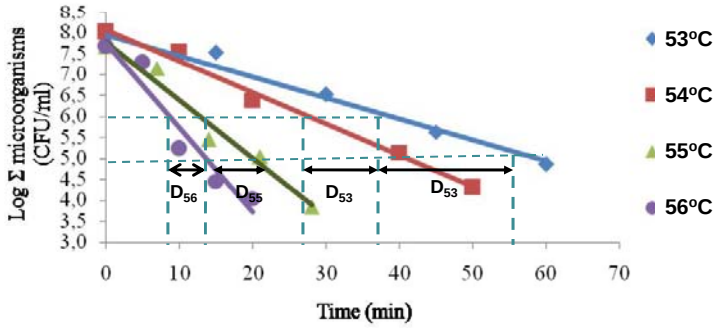
- Isolate grown in TSB to reach late log phase

Heating Menstruum

- Trypticase Soy Broth

Results

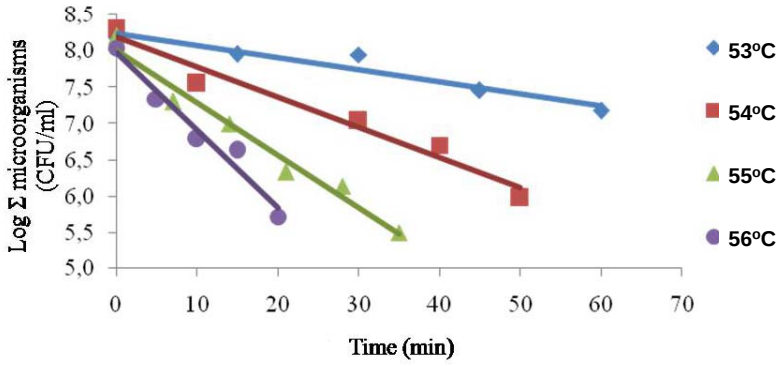
Thermal Death Time curve of *S. aureus AS2*



D values, i.e. time (min) = -1 / slope

Results

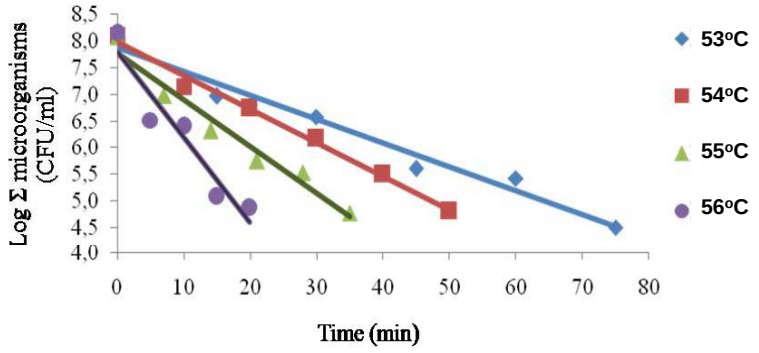
Thermal Death Time of *S. aureus NU3*



D values, i.e. time (min) = -1 / slope

Results

Thermal Death Time of *S. aureus ATCC 25923*



D values, i.e. time (min) = -1 / slope

Thermal Resistance of Local Isolates of *S. aureus* (D-values)

S. aureus Isolates	D values (min) at 53°C	54 °C	55°C	56 ˚C
AS2	19.47 ± 1.33	13.42 ± 0.13	6.59 ± 0.85	5.17 ± 0.26
NU3	64.59 ± 2.95	23.83 ± 0.80	14.3 ± 0.78	8.78 ± 0.92
ATCC	22.00 ± 1.02	15.31 ± 1.16	11.12 ± 0.52	7.53 ± 1.76

MS 149[a] (milk)	-	32,30	[a]Thomas, 1966
237[b] (goat milk)	-	17,68	[b]Parente and Mazzatura, 1991
Cocktail[e] (TSB)		28,51	[e]Kennedy, 2005

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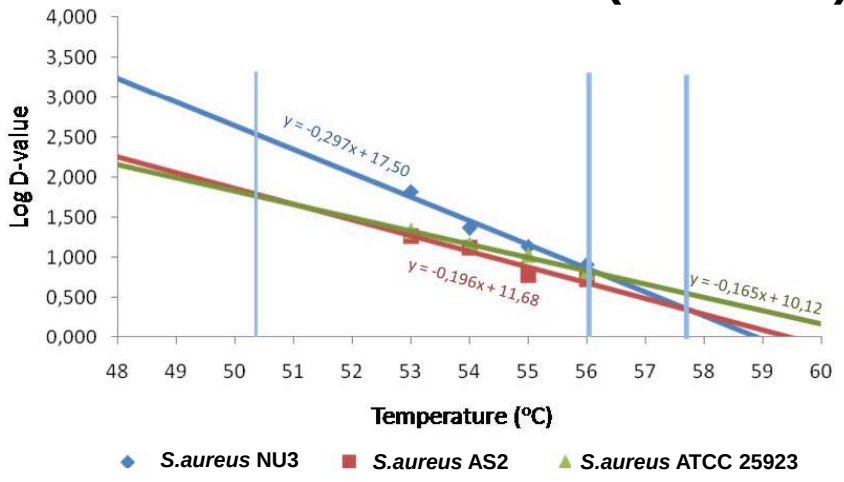
Temperature Dependence of *S. aureus* Inactivation (Z-values)



Temperature Dependence of *S. aureus* Inactivation (Z-values)

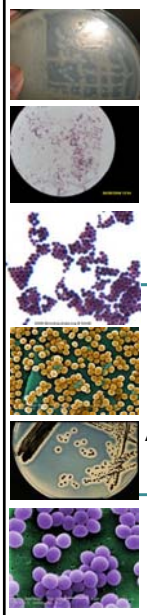
- The sensitivity of D values to temperature changes, i.e. changes of temperatures to change D value by 1 log cycle or 90% (Toledo 1991)
- **The Z values of *S. aureus* isolates**
  AS2 : 4.40˚C
  NU3 : 3.53˚C
  ATCC 25923 : 5.80˚C

Various Z Values

Microorganisms	Heating menstruum	Z (˚C)
Staphylococcus aureus AS2	TSB	4.40
Staphylococcusaureus NU3	TSB	3.53
Staphylococcus aureus ATCC 25923	TSB	5.80
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
Vitamine B12		50

Survey on Common Cooking and Handling Practices in Food Vendor

Vendor	Name of Food	Cooking Methods	Temperature
1	*Semur jengkol*	boiling for 1 h, storage at RT	92 º C
2	Beef soup	boiling beef for 3 h, storage at RT, cutting into cubes, mixing with hot/warm broth	92 º C
3	Meatball soup	boiling for 1 h, storage RT, reheat as needed	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, storage RT, reheat as needed	95 º C
7	Vermicelli (rice noodle) salad	boiling of vermicelli for 1 min, 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, storage RT	73 º C
12	Fried coconut chicken	frying in shredded coconut till brown, RT	95 º C
13	Fried tempe	frying for 2-3 min, storage at RT	98 º C
14	Fried potato	frying for 2-3 min, storage at RT	98 º C
15	Stir fried green bean	stirfrying for 5 min, storage at RT	73º C
16	Stir fried eggplant	stirfrying for 5 min, storage at RT	73º C



Thermal Resistance of Local Isolates of *S. aureus* at Different Temperatures

Isolate	D_{73} (min)	D_{92} (min)
NU3	0,0002	$1,62x10^{-9}$
AS2	0,001	$1,25\ x10^{-7}$
ATCC 25923	0,006	$1,93\ x10^{-6}$

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Effect of Common Cooking and Handling Practices in Food Vendors on *S. aureus*

	Temp	Descriptive analysis
Vermicelli salad	89°C	boiling of vermicelli for 1 minute, mixing with fresh chilli-peanut sauce Reduction of *S. aureus* : 4.3 x 10^6 Problems : mixing with fresh sauce after boiling
Grilled fish	73°C	grilling for 10 min Log reduction of *S.aureus* : 10^4 Problems : pre and post grilling storage at room temperature

Conclusions

- *S. aureus* isolated from RTE Indonesian traditional foods had thermal resistance similar to those reported from *S. aureus* isolated elsewhere
- The Z values of *S. aureus* isolates ranged from 3,53 to 5.80°C : within the range of reported Z values for non-spore forming bacteria, lower than those for vit. B12
- Heating commonly applied in cooking of RTE Indonesian foods significantly eliminates *S. aureus*.
- Pre and post cooking practices (storage) became very critical because possible toxin production than can not be eliminated by normal cooking practices.
- Addition of fresh ingredients after cooking allows recontamination to occur

Thank You
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Methods

Calculation of D and z values

- *S. aureus* surviving were plotted against heating times to yield a curve of rate of inactivation at four different temperatures, i.e. 53, 54, 55, dan 56˚C.
- D values, i.e. time (minutes) at certain temperature to reduce the number of *S. aureus* by 1 log cycle was calculated from the equation D = -1/slope
- A Thermal Death Time (TDT) curve was made to establish the relationship between D (minutes) with temperatures (ºC)
- The Z values, i.e. temperature intervals to reduce D by 1 log cycle was also determined from the curve.

Methods

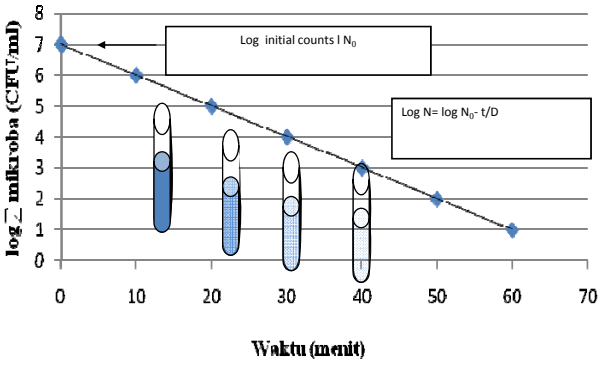
Thermal Resistance Testing

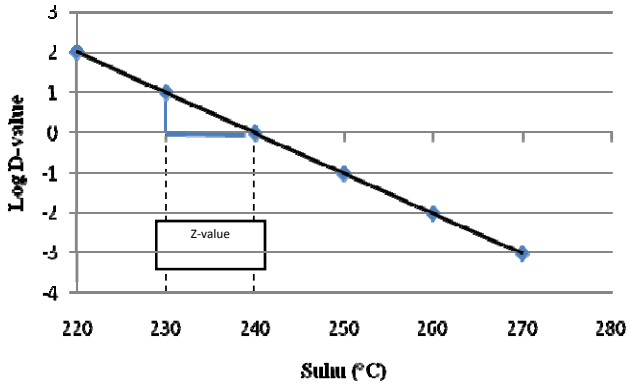
- Sets of glass tubes containing HM in waterbath set at 53, 54, 55, 56°C
- When HM reached the desired temperatures, culture of *S. aureus* was inoculated at initial counts ca. $1,0x10^7$ -$1,0x10^8$ CFU/ml
- HM allowed to be heated for 5,7,10,15 mins
- *S. aureus* before & after heating was enumerated on BPA+eggyolk tellurite incubated at 35°C 48 h

Methods

Assessing Thermal Adequacy of Several RTE Indonesian Traditional Foods

Survey in 16 food vendors surrounding Darmaga campus area, Bogor to determine :
- common practice
- time of cooking
-internal temperatures during cooking
-Adequacy of thermal process was assessed by extrapolating the Z value equation to obtain D_T (D values at at the cooking temperatures applied).


log ∑ mikroba (CFU/ml)
Log initial counts l N_0
Log N= log N_0- t/D
Waktu (menit)


Log D-value
Z-value
Suhu (°C)