

HEAT RESISTANCE OF LOCAL ISOLATE OF VT1 AND VT2 GENES-BEARING *Escherichia coli* O157:H7 IN HEATED MILK AND COOKED BEEF

DAYA TAHAN PANAS ISOLAT LOKAL *Escherichia coli* O157:H7 PEMBAWA GENA VT1 DAN VT2 DALAM SUSU DAN DAGING CINCANG

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ABSTRACT

Heat resistance, stated as D value of local isolate of VT1 and VT2 genes-bearing *Escherichia coli* O157 was investigated in milk and minced beef. Local isolate, B-33, and control strain O157:H7 were first regenerated from a stock culture into Sorbitol MacConkey (SMAC) agar, pH 7.4. Colonies of *E. coli* from the SMAC was inoculated into 5 ml tryptouse soy broth (TSB), and incubated at 37°C for 24 h. This inoculum was used as a 10% seed into a fresh TSB medium. This culture was incubated at 37°C for 14 h. The cells were harvested by mean of centrifugation and washed twice with physiologic NaCl. The *E. coli* cell suspension was serially diluted and inoculated into a plate count (TSA) agar to calculate the cell concentration. Fresh milk samples were aliquoted into sterile test tubes, 9 ml each, and sterilized by autoclaving. After autoclaving the milk tubes were cooled in refrigerator before being used as heating medium. Beef sample was first proximate analyzed to measure the concentration of fat, ash and protein. The other minced beef samples were wrapped in aluminium foil, autoclaved for 15 min. and cooled in refrigerator. The heat resistance of *E. coli* in milk was carried out by inoculating 1 ml of 10^x CFU *E. coli* cell suspension into 9 ml milk test tubes. Two hours post inoculation the tubes were then heated into 60°C, 65°C and 70°C at time interval 5, 10, 15, 20, 25, and 30 minutes. After the treatment the viable cells were calculated, based on the CFU values. The heat resistance of *E. coli* in beef was carried out by inoculating the bacteria into minced beef in an erlenmeyer flask and heated up to 100°C. The *E. coli* viable cells were calculated 2, 4, 6, 8, 10, and 12 minutes after heat treatment. The *E. coli* heat resistance response was expressed from the number of viable bacteria (CFU/g) converted into D value and analyzed in a linear regression. The results show that the D values of the local isolate in milk were 69.8812, 11.466 and 10.939 at 60, 65 and 70°C, respectively. In beef, the D value was found to be 2.305 min at 100°C. It was also revealed that local isolate is more heat resistant than the US isolate.

Key words : D values, *E. coli* O157:H7, milk, beef

ABSTRAK

Penelitian ini bertujuan mengetahui daya tahan panas isolat lokal *E. coli* O157 pembawa gena VT1 dan VT2 dalam susu dan daging cincang. Isolat lokal, B33, dan galur kontrol O157:H7 diperoleh dari biakan induk dan berturut-turut dibiakan dalam agar sorbitol-McConkey, Tryptose soy broth. Suspensi sel dicuci dengan larutan garam faal steril 2x, dan dihitung konsentrasinya sebagai CFU pada agar trypticase soy. Sampel susu mentah ditempatkan ke dalam tabung reaksi steril 9 ml setiap tabung, dan disterilkan dalam otoklaf 15 menit, lalu didinginkan. Sampel daging dibungkus dalam aluminium foil, diotoklaf, dan didinginkan dalam almari es. Sebanyak 1 ml suspensi 10^x. *E. coli* dimasukkan ke dalam setiap tabung susu. Dua jam setelah inokulasi, tabung dipanaskan pada 60°C, 65°C, dan 70°C selama 5, 10, 15, 20, 25 dan 30 menit. Pada daging, suspensi bakteri yang sama diinokulasikan pada daging cincang dalam Erlenmeyer, dan dipanaskan 100°C. Jumlah sel yang hidup dihitung sebagai CFU pada TSA. Jumlah CFU dikonversikan sebagai harga D dan dianalisis dalam regresi linier. Hasilnya menunjukkan bahwa harga D isolat lokal dalam susu adalah berturut-turut 69.8812, 11,466 dan 10.939 menit pada pemanasan 60, 65 dan 70°C. Pada daging cincang, harga ini adalah 2,305 menit pada 100°C. Juga diperlihatkan, bahwa isolat lokal ini lebih tahan panas daripada isolat Amerika yang digunakan sebagai kontrol.

Kata kunci : harga D, *E. coli* O157, susu, daging

INTRODUCTION

Strain O157:H7 of *Escherichia coli* is known as the cause of serious illnesses in humang being. The research is aimed to find out the heat resistance of local isolate of *E. coli* O157:H7 in heated milk and cooked beef because they were very often associated with an outbreak of colibacillosis. Ahmed *et al.* (1995) reported that the D-value for *E. coli* at 60°C in beef was 0.45-0.47, indcating that there is a potential hazard of *E. coli* O157:H7.

MATERIALS AND METHODS

Preparation of Inoculum

Escherichia coli O157 of local isolate B33 (Drastini *et al.*, 2002) proven to possess VT1 and VT2 genes, and O157:H7 (Sijabat-Tambunan and Bensink, 1997) as control were used throughout the study. This latter strain was initially isolated from a human case in the United State (US)

The isolates were first regenerated from a stock

other minced beef samples were wrapped in aluminium foil, autoclaved for 15 min. and cooled in a refrigerator.

Heat Resistance Test

The heat resistance of *E. coli* in milk was carried out by inoculating 1 ml of 10⁸ CFU *E. coli* cell suspension into 9 ml milk test tubes. Two hours post inoculation the tubes were then heated into 60°C, 65°C and 70°C at time interval of 5, 10, 15, 20, 25, and 30 minutes. After the treatment, the viable cells were calculated based on the CFU values. The heat resistance of *E. coli* in beef was carried out by inoculating the bacteria into minced beef in an erlenmeyer flask and heated up to 100°C. The *E. coli* viable cells were calculated 2, 4, 6, 8, 10, and 12 minutes after heat treatment.

Data Analysis

The *E. coli* heat resistance response was expressed from the number of viable bacteria (CFU/g) converted into D value and analyzed in a linear regression.

Table 1. Mean Log CFU/ml of local isolate *E. coli* O157 in milk heated at 60°C, 65°C, and 70°C for 5,10, 15, 20, 25 and 30 minutes

Temperature	Heating Time					
	5	10	15	20	25	30
60°C	10.101 ±	9.926 ±	10.037 ±	9.764 ±	9.811 ±	9.726 ±
	0.402	0.484	0.385	0.513	0.297	0.488
65°C	9.827 ±	9.568 ±	9.470 ±	9.405 ±	9.195 ±	9.143 ±
	0.524	0.315	0.249	0.212	0.170	0.160
70°C	10.000 ±	9.863 ±	10.110 ±	9.694 ±	9.420 ±	9.084 ±
	0.526	0.657	0.417	0.466	0.460	0.117

culture into Sorbitol MacConkey (SMAC) agar, pH 7.4. Colonies of *E. coli* from the SMAC was inoculated into 5 ml tryptouse soy broth (TSB), and incubated at 37°C for 24 h. This inoculum was used as a 10% seed into a fresh TSB medium, and incubated at 37°C for 14 h. The cells were harvested by mean of centrifugation and washed twice with physiologic NaCl. The *E. coli* cell suspension was serially diluted and inoculated into a plate count (TSA) agar to calculate the cell concentration. The bacterial cell concentration was calculated as the colony forming unit (CFU) value.

Preparation of Heating Medium

In this experiment, milk and beef were used as the medium or carrier of *E. coli* cells during the heating treatment. A fresh milk sample was aliquoted into sterile test tubes, 9 ml each, and sterilized by autoclaving. After autoclaving the milk tubes were cooled in refrigerator a before being used as heating medium. Beef sample was first proximate analyzed to measure the concentration of fat, ash and protein. The

RESULTS AND DISCUSSIONS

Milk

The temperatures used in this experiments were those of pasteurization temperature range. The result of local isolate is presented in Table 1. Table 2 shows the results of control bacteria.

Tukey's HSD for 60°C revealed no difference between heating times for both local and US isolate. This is understandable, because 60°C is still in the range of optimum temperature for bacterial growth. At 65°C, differences were found between 5 and 25 minutes, and between 5 and 30 minutes for local isolate. For US isolate, differences were found between 5 and 25 minutes, and between 10, 15 and 25 minutes. This difference demonstrates that the local isolate seems to be more heat resistant than the control. At 70°C, there was significant difference of log CFU

Table 2. Mean Log CFU/ml of isolate of control *E. coli* O157H7 in milk heated at 60°C, 65°C, and 70°C for 5, 10, 15, 20, 25 and 30 minutes

Temperature	Heating Time					
	5	10	15	20	25	30
60°C	9.846 ± 0.424	9.814 ± 0.411	9.831 ± 0.229	9.756 ± 0.467	9.583 ± 0.427	9.147 ± 0.419
65°C	10.284 ± 0.496	9.926 ± 0.417	9.930 ± 0.265	9.817 ± 0.351	9.384 ± 0.298	9.505 ± 0.318
70°C	10.428 ± 0.400	10.382 ± 0.085	10.131 ± 0.540	10.031 ± 0.535	9.729 ± 0.119	9.351 ± 0.274

between 5 and 25 minutes, and between 5, 10, 25 and 30 minutes of heating time. Whereas US isolate shows highly significant differences between 5, 10 and 25 minutes and between 5, 10, 15, 20 and 30 minutes. Again, this demonstrate that local isolate must be more resistant than that of the control isolate.

Beef

The heating temperature used in this experiment (100°C) reflects the habit of Indonesian households in cooking beef. The heating times used (2, 4, 6, 8, 10 and 12 minutes) correspond to the type of cooking times carried out for different menus. The mean of log CFU/g of the bacteria upon heating at 100°C is shown in Table 4.

Table 3. D values of VTEC in milk heated at 60°C, 65°C and 100°C

Temperature	Isolate	Slope	Y Intercept	Coefficient	D value
60°C	Local	-0.0143	10.145	87.69	69.881
	US	-0.0084	8.812	9.722	119.332
65°C	Local	-0.0914	9.708	73.34	10.939
	US	-0.1029	10.941	84.91	9.717
70°C	Local	-0.0872	10.539	96.47	11.466
	US	-0.448	11.856	84.35	6.904

The results of regression analysis on local and US isolates reveal that the D value of local isolate (69.8812 min) is less than that of US isolate (119.332 min) at 60°C. However, at 65°C and 70°C, local isolate is more heat resistant than the US isolate. The D values of local isolate were 11.466 min and 10.939, respectively, while for those temperatures, the D values of the control were 9.717 minutes and 6.904 min. Table 3. shows these figures.

Compared to the heat resistance of other enterotoxigenic *E. coli* at 50°C-70°C (Batish, 1988), both isolates indicates greater resistance to heat.

Statistical analysis revealed no significant difference between local and US isolates. Both isolates, however, show similar trend in the decrease of the number of CFU. The number decreased rapidly during initial heating and more slowly at later time.

The regression analysis revealed the D value of both isolate at 100°C as shown in Table 5.

The figures are much higher than those of Ahmed *et al.* (1995) showing 0.45-0.47 min at 60°C. This could be due to the strain used, or the recovery medium used for culturing the injured organism. The medium used in this experiment (TSA) gives more

Table 4. Mean of log CFU/g of VTEC in ground beef heated at 100°C for 2, 4, 6, 8, 10, and 12 minutes

ISOLATE	HEATING TIME					
	2	4	6	8	10	12
Local						
Mean	10.520	10.092	10.068	9.912	9.936	9.660
SD	0.789	0.295	0.107	0.196	0.227	0.134
US						
Mean	10.196	10.208	10.158	10.232	10.032	9.836
SD	0.557	0.508	0.360	0.241	0.210	0.332

favourable environment for the recovery of injured *E. coli* (McCarthy, 1998), than other medium, such as phenol red sorbitol agar used by Ahmed *et al.* (1995).

Product Composition. *J. of Food Science*, 60:606-610.

Table 5. D value of local and US isolate of VTEC in ground beef heated at 100°C

Temperature	Isolate	Slope	Y Intercept	Coefficient	D value
100oC	Local	-0.4337	11.37113	0.9738	2.3058
100oC	US	-0.1406	9.67713	0.6119	7.1118

The figures show that in beef, too, local isolate is more resistant than that of US isolate. It is recommended, therefore, the cooking should use longer time to be saved from VTEC infection.

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