Thermal Inactivation of Foot-and-Mouth Disease Virus Strains A and Asia1 in Pork Products

P. Youeaimyut[1*], R. Udon[2], P. Thongtha[2], R. Thongtha[2], S. Nuanualsuwan[1]
[1]Veterinary Public Health, Veterinary Science, Chulalongkorn University, Bangkok, 10330 Thailand
[2]Regional Reference Laboratory for FMD in Southeast Asia, National Institute for Animal Health, Nakhon Ratchasima, 30130 Thailand
*Corresponding author
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Introduction

Foot-and-mouth disease virus (FMDV) is classified within the genus Aphthovirus, family *Picornaviridae* (1). Seven serotypes of FMDV are namely O, A, C, SAT1, SAT2, SAT3, and Asia1. FMDV is etiological agent of foot-and-mouth disease (FMD), which is the most contagious disease of cloven-hoofed animals and can cause severe economic loss (10). FMD is on the OIE List A, because it has the great potential for rapid spread across countries and can cause impact to international trade in animals and animal products (1). OIE had a regulation for treating animal products from FMD-infected country by thorough cooking, also meat shall be subjected to heating so that an internal temperature of 70°C or greater is maintained for minimum of 30 minutes (9).

Thermal inactivation is a function of time and temperature to predict the heat resistance of virus. The decimal reduction time (*D*-value) is the time at a constant temperature to reduce the population of microorganisms by 90% (or one log cycle) (7), and it can indicates the heat resistance or rate of microbial inactivation in each specific condition (6). Many published studies present the heat resistance of FMDV but not included strains in Thailand (2-4, 8). The objective of this study was to investigate the heat resistance of FMDV strains A and Asia1 in Thailand in pork products at 50, 60, 70, and 80°C, in terms of *D*-value.

Materials and Methods

Virus and cell culture: FMDV serotypes A (strain A118) and Asia1 (strain AS1), isolated from epidemics in Thailand, were obtained from the Regional Reference Laboratory for FMD in Southeast Asia, Pakchong, Nakhon Ratchasima, Thailand. The viruses were propagated in baby hamster kidney (BHK-21) cell line in growth medium, that was Modified Eagle's Medium (MEM) supplemented with 10% normal bovine serum, 1% of stock 2.92% L-glutamine, 1% antibiotic (kanamycin 100 mg/ml, streptomycin 100 mg/ml, and penicillin 1.173 mg/ml), and 3% of stock 7% sodium bicarbonate. Maintenance medium consisted of growth medium with no serum and 1.5% of stock 7% sodium bicarbonate.

Virus titrations: Samples were serially diluted 10-fold in virus diluents (maintenance medium). A volume (200 µl) of each virus dilution was inoculated with BHK-21 cells suspension in growth medium (500 µl) on each well of 24-well plate. The control cells were inoculated with maintenance medium 200 µl. These plates were placed in an incubator providing 5% CO_2 in air at 37°C for 48 h to observe cytopathic effect. Virus titer was recorded as the 50% tissue culture infective dose ($TCID_{50}$) according to the method of Spearman-Karber.

Thermal inactivation: Measurement of pH for minced pork was done by pH indicator paper strips (Merck®). Adding 7% sodium bicarbonate to minced pork was to adjust pH up to 7-8. Five cm. in diameter and 2 cm. thick of 60 g. minced pork was set. Minced pork, that prepared to cooking tonkatsu, ware added with 0.8% sodium chloride and 400 ppm sodium tripolyphosphate. Preheated pork in three cooking methods; deep-fried, roasted, and tonkatsu (Japanese breaded deep-fried pork) until core temperatures reached 50, 60, 70, and 80°C. Then FMDV stock was added into the core of pork and the required core temperatures were maintained during sampling time, after that immersed pork immediately into ice bath (2, 5). The metal probe from thermocouple was put in one piece of pork at each temperature that acted as a reference temperature piece. To reduce error of the experiment, three times of experiments were done.

Virus isolation: One gram of pork was sampled to prepare 10% stock virus suspension. Pork was grounded with a small volume of 0.04M phosphate buffer saline (PBS), further PBS should be added to have final volume of nine times of pork. Pork suspensions were centrifuged at 2,000 rpm for 20 minutes. Supernatants, suspected to contain FMDV, were filtered and inoculated onto cell cultures.

Calculation of D-values: D-values (rate of FMDV inactivation) were calculated from the negative reciprocal of the slope of the inactivation curve by plotting log of survival counts and their corresponding heating times using Microsoft Excel (6).

Statistical analyses: The analysis of variance (ANOVA) was used to determine the statistical significance of differences in heat resistance of FMDV strains across temperatures and cooking methods.

Results and Discussion

The heat resistance of FMDV strains A and Asia1 in Thailand in cooking pork products was calculated with respect to *D*-values that were obtained by linear regression. *D*-values of FMDV strain A118 (Table 1) were not statistically significant differences (p>0.05) among temperatures for the same cooking methods except deep fried and tonkatsu at 80°C. For the same temperatures among different cooking methods, *D*-values of FMDV strain A118 were not significantly different (p>0.05), except at 80°C there were significantly different between deep fried and tonkatsu.

D-values of FMDV strain AS1 (Table 2) were not significantly different (p>0.05) among temperatures for the same cooking methods except roasted pork, that were significantly different (p<0.05) at 50°C, 60°C, and 70°C. But 80°C and other temperatures were not significantly different (p>0.05). For the same temperatures across cooking methods, *D*-values of FMDV strain AS1 were not significantly different (p value > 0.05)

However, *D*-values of FMDV strain A and Asia1 were not significantly different (p>0.05) at the same temperatures and the same cooking methods (Table 3). Therefore, difference of *D*-values of FMDV strain A and Asia1 may be attributed to the effect of different ingredient batches that conducted heat.

The previous study (6) reported *D*-values of FMDV strains in Thailand in phosphate-buffered saline (PBS). *D*-values of FMDV strain A118 at 50, 60, 70, and 80°C were 851 s, 18.36 s, 8.87 s, and 3.81 s, respectively. And *D*-values of FMDV strain AS1 at 50, 60, 70, and 80°C were 1,275 s, 32.83 s, 9.24 s, and 5.42 s, respectively. Because the *D*-value was specific to the property of condition, so comparison of *D*-value of FMDV must be based on the same content. Nevertheless, *D*-values of FMDV in pork products in this study seem to be greater than those of FMDV in suspension due to the higher concentration of protein (4). This study showed the temperatures and times that should inactivate FMDV in pork products and be useful for commercial food processing improvement.

Table 1 *D*-values of FMDV strain A118 at 50, 60, 70, and 80°C in various cooking methods.

Temp (°C)	*D*-value(s)[a] in cooking method		
	Deep fried	Roasted	Tonkatsu
50	3,916 [A, a]	6,469 [A, a]	2,867 [A, a]
60	123 [A, a]	182 [A, a]	183 [A, a]
70	51.99 [A, a]	67.30 [A, a]	37.55 [A, a]
80	2.42 [B, a]	8.68 [A, a, b]	5.80 [B, b]

[a] Values are means of three replications.

In the same column, mean values with different letters imply that there are statistically significant differences (p<0.05) among the different temperatures for the same cooking methods (letters A and B) In the same row, mean values with different letters imply that there are statistically significant differences (p<0.05) among the different cooking methods for the same temperatures (letters a and b)

Table 2 *D*-values of FMDV strain Asia1 at 50, 60, 70, and 80°C in various cooking methods.

Temp (°C)	*D*-value(s)[a] in cooking method		
	Deep fried	Roasted	Tonkatsu
50	2,092 [A, a]	2,225 [A, a]	4,490 [A, a]
60	143 [A, a]	67.94 [B, a]	82.95 [A, a]
70	26.27 [A, a]	20.76 [C, a]	17.08 [A, a]
80	4.56 [A, a]	6.98 [A, B, C, a]	5.86 [A, a]

[a] Values are means of three replications.

In the same column, mean values with different letters imply that there are statistically significant differences (p<0.05) among the different temperatures for the same cooking methods (letters A through C) In the same row, mean values with different letters imply that there are statistically significant differences (p<0.05) among the different cooking methods for the same temperatures (letters a and b)

Table 3 *D*-values of FMDV strains in cooking pork at 50, 60, 70, and 80°C.

Cooking method	Temp (°C)	*D*-value(s)[a] in FMDV strain	
		A118	Asia1
Deep fried	50	3,916[a]	2,092[a]
	60	123[a]	143[a]
	70	51.99[a]	26.27[a]
	80	2.42[a]	4.56[a]
Roasted	50	6,469[a]	2,225[a]
	60	182[a]	67.94[a]
	70	67.30[a]	20.76[a]
	80	8.68[a]	6.98[a]
Tonkatsu	50	2,867[a]	4,490[a]
	60	183[a]	82.95[a]
	70	37.55[a]	17.08[a]
	80	5.80[a]	5.86[a]

[a] Values are means of three replications.

In the same row, mean values with different letters imply that there are statistically significant differences (p<0.05) among the different FMDV strains for the same temperatures and cooking methods

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