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EVALUATION OF THERMAL PROCESSES FOR REDUCTION OF SALMONELLA SPP. AND ESCHERICHIA COLI IN PORK SAUSAGE WRAPPED IN BANANA LEAVES

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This study was aimed to evaluate the thermal inactivation of *Salmonella* spp. and *Escherichia coli* in pork sausage wrapped in banana leaves heated to an internal temperature of 71.6 degrees C (161 degrees F) using boiling and steaming methods. Pork batter (200 g, n=9) inoculated with a five-strain mixture of *Salmonella Rissen* DMST 17365, *Salmonella Typhimurium* DMST 562 (ATCC 13311) และ *Salmonella Weltevredens* DMST 17375, *E. coli* DMST 4212 (ATCC 25922) and *E. coli* DMST 24373 at a concentration of 10^7 CFU/g were wrapped in a 3 layer of banana leaves to form a 3 cm diameter wide and 18 cm long cylinder and heated until the cold point temperature readings were 71.6 degrees C. The cooking time required for the sausage to reach 71.6 degrees C was 10.25 min for boiling and 19.35 min for steaming.Thermal process letghality (*F*-value) at 70 degrees C for both *Salmonella* spp. and *Escherichia coli* by boiling and steaming were not statistically different. Both heating methods were effective in reducing 7 log CFU/g of *Salmonellae* and 26 log CFU/g of *E. coli* in pork sausage wrapped in multilayer of banana leaves. The findings confirm that small local producers should be recommended to control the internal cooking temperature to at least 72 degrees C for product safety.

Keywords: Thermal process, Reduction, *Salmonella*, *Escherichia coli*, Pork sausage**.**

Introduction

Thai pork sausage or Moo- Yor is a regional food product of Northern and Northeastern regions of Thailand. At present, it is popular among consumers in all regions of Thailand and some southeast Asian countries because it is convenient for consumer to buy and it can also be served in many different dishes.They are produced by some large food factories, however a majority of the productions are done by many small local producers who use their inherited knowledge and experience in their production. The pork meat is grounded with fat, flour, salt, pepper, polyphosphate and ground ice until it turns into pork emusion.Traditionally, the pork emulsion will be wrapped in 3-5 layers of banana leaves and then heated by immersing in boiling water or by steaming. The producers have been recommended to apply heat treatment to the products until the internal temperature reach 72˚C (161.6°F) (Department of Livestock Development, 2017) to ensure the product safety from food pathogens. However, Thai pork sausage had

been reported to be contaminated with Salmonella around 6.25% (Bangtrakulnont et al., 1999). Contamination of pork products during slaughtering or meat cutting at the slaughter house could promulgate *Salmonella* and *Escherichia coli* to human (Oliveria et al., 2012; Nongman and Sirijaroenchai, 2014; Bualert and Nimnuan, 2014). Further meat processing such as cutting, grinding and mincing of contaminated pork cuts may spread the pathogens into meat. Improper handling and storage can also increase the contamination level of the pathogens which will be a high risk for consumers. Ice and water used in the product preparation could also be a vehicle for transfer *Salmonella* and *E. coli* into the product (Prapasuwannakul, 1997). Therefore, the United States Department of Agriculture-Food Safety Inspection service (USDA-FSIS) has suggested that minimum internal temperature of 71.1˚C (160˚F) should be targeted for cooking ground pork to eliminate the risk of surviving pathogens. Recent study also revealed that cooking in pan grill might not ensure the final safety of beef patties contaminated with a high level of Salmonella and *E. coli* O157:H7 even if the internal cooking temperature was in accordance with the recommendation (71°C) and cooking in oven broiler was found to be more effective than that of pan grill (Manios and Skandamis, 2015). According to the above, we aimed to evaluate the effectiveness of heating processes for reduction of *Salmonella* spp. and *Escherichia coli* in pork sausage wrapped in banana leaves.

Materials and Methods

Bacterial Cultures and Inocula Preparation

A three- strain composite of *Salmonella spp.*consisted of S. Rissen DMST 17365, S. Typhimurium DMST 562 (ATCC 13311), S. Weltevreden DMST 17375 and a two-strain composite of *Escherichia coli* consisted of *E. coli* DMST 4212 (ATCC 25922), *E. coli* DMST 24373 obtained from the Culture Collection Center of the Department of Medical Sciences, Ministry of Public Health , Thailand were used in this reaearch. Each strain was activated separately by transferring a single colony into 10 ml trypticase soy broth (TSB, Difco, USA) at 37°C for 24 hr. and followed by a second enrichment in TSB at 37°C for 18 hr. The culture suspensions were centrifuged at 3600 rpm at 4°C for 10 min. The supernatant liquid was decanted and the pellet cells were mixed equally and resuspend in 100 ml phosphate buffer (Manios snd Skandamis, 2015). The level of the strain composite inoculum was 1-1.5×10^9 CFU/ml.

Inoculation of Pork Sausage

Lean pork meat and fat were purchased from a local butcher shop on the day of each experiment trial. The sausage was prepared using the following formula: 62% lean pork, 12% fat, 18% ground ice, 3.0% tapioca flour 1.4% salt, 1.4% pepper, 2.0% sugar, and 0.12% sodium tri polyphosphate). Portions (200 g) of meat batter was placed into a sterile stomacher bag and was inoculated with 1.5 ml of the strain composite of *Salmonella* and *E. coli* and was mixed thoroughly by hand-message for 10 mim, then two inoculated portions were mixed together and were inoculated with additional 1.0 ml of the strain composite and were mixed thoroughly by hand-message for 10 min.The repetition of inoculations were done until 1,600 g of meat batter was inoculated with 20 ml. of the strain composite in order to yield and initial ionoculation level of approximately 7.0 log CFU/g. Each portion (200 g) of inoculated meat batter was wrapped in a 3 layers of sterile banana leaves (cleaned with 70% ethanol) to form a constant cylindrical sausage (diameter 3 cm × length 18 cm). All inoculated samples were stored at 4°C for 24 hr. in order to allow the cultures to adhere with the meat tissues.

Thermal Process of Pork Sausage

The samples were taken out from the refrigerator and left at the room temperature until the temperature at the geometrical center of the samples reached 10°C before heat treatments were applied. The heating

process either boiling or steaming was ceased when the internal temperature at the cold point of the samples reached 71.6°C (161°F). The heat-treated samples were immediately immersed into a sterile cold water bath after the thermal exposure and were stored at refrigeration temperature until survival cell enumeration were performed within 2 h. Changes in temperature during cooking were monitored using a time-temperature data logger (Xplorer GLX-PS-2002, Pasco Scientific, California, USA) equipped with thermocouples type K (PS-2125, Pasco Scientific, California, USA). Each thermocouple was inserted through layers of banana leaves from one side to the geometrical center of the sausage during heating. The *F*-value of each treatment were calculated using the eqauation (Murphy et al., 2004):

$$F = \int_0^t 10^{(T(t\ ) - T(ref)/Z}\, dt$$

With *T* the temperature in the center of the pork sausage at t=0 to t=t_i and *T(ref)* the reference temperature, which is a theoretical temperature at which decimal reduction time (*D* value) should be known. In the present study, the thermal resistance constants (Z values) in pork patties (Osali et al, 2007) were used for *Salmonella* (Z=6.2) and *E. coli* (Z=5.4), while *T(ref)* was determined at 70°C. This parameter was used to describe mathematically the effect of each thermal process on the reduction of the pathogens. The final *F*-value was calculated as the mean of nine values derived from nine independent samples.

Microbiological Analysis

Microbiological analysis were performed at three stages during the experimental procedure; (i) before inoculation, to determine the contamination level in raw fresh meat, (ii) after inoculation, to evaluate the inoculation level, (iii) after heating, to evaluate the effect of heating method on the reduction of the pathogens. Pathogen enumerations were performed by the most probable number (MPN) dilution technique using a three tubes per dilution according to Bacteriological Analytical manual, USDA. At each stage of sampling, 25 g of sample was mixed with 225 ml Buffered Peptone Water (BPW) in a stomacher for 2 min. Following decimal reduction, *Salmonellae* population was enumerated by first and secondary enrichment in Rappaport-Vassiliadis (RV, Difco) and Tetrationate broth (TT, Difco) respectively, direct isolated on xylose deoxycholate agar (XLD, Merck) and Hektoen enteric agar (HE, Merck) and positive colonies confirmed by biochemical test on Triple sugar iron agar (TSI) and Lysine iron agar (LIA) and serological test. *E. coli* population were enumerated by presumtive enrichment in Lauryl tryptose broth (LST, Difco), confimed by selective enrichment in EC broth (Difco), and complete tested by direct isolated on Levine's eosin-methylene blue (L-EMB, Difco) agar and biochemical IMViC testing (BAM, USDA).

Statistical Analysis

The experiment was conducted in triplicate with three independent samples analyzed per repetition (n=9). Microbial populations of the pathogens were converted to log CFU/g before statistical analysis. The populations of pathogens and *F* values were subjected to analysis of varience (ANOVA) and the means were compared by Duncan multiple range test, with $P<0.05$.

Results

The microbial counts of *Salmonella* and *E. coli* in fresh pork meat were 4.8±0.2 log CFU/g and 4.7±0.1 log CFU/g as shown in table 1 indicated that the pork meat sold in general retail shop might be generally contaminated during preliminary breeding, slaughtering, cutting, handling or storage. The results was agreed with those reported by Oliveria et al., 2012; Nongman and Sirijaroenchai, 2014; Bualert and Nimnuan, 2014.The heating process either boiling or steaming process applied to the inoculated samples

with high load of *Salmonella* and *E. coli* around 7 log CFU/g could reduce all of the inoculated pathogens (Table1). These findings agreed with the issue guidelines, where it is suggested that cooking of ground pork mixtures at an internal temperature of 72°C (161.6°F) could sufficiently ensure product safety from pathogens since in this study we stopped the heat treatment at 71.6°C (161°F). Steaming process required significantly higher time compared to boiling process. This indicated that the rate of heat transfer and the way that heat was transferred to the geometrical center of sausage was slower in steaming process, however, the calculated thermal process letghality ($F_{70°C}$) of both processes were not significantly different (Table2). In this study, Z values and D values for *Salmonella* and *E.coli* used for calculation of *F* value and logarithamic readuction were those found in a study of Osali et al (2007) in bread pork patties. A minimum $F_{70°C}$ =2.13±0.09 min and 2.17±0.22 min of boiling process and steaming process could be sufficiently reduce the populations of *Salmonella* 7.34±0.31 log CFU/g and 7.48±0.75 log CFU/g respectively. Therefore if the contamination level of Salmonella in the raw product is greater than 7 log CFU/g, the heat treatment shoul be held at 72 °C for a few minutes to ensure food safety. *E. coli* is more sensitive to heat than *Salmonella*, therefore, *E. coli* can be reduced up to 25.97±1.18 log CFU/g and 26.99±2.73 log CFU/g (Table 2).

Table 1. Mean population (log CFU/g) ± standard deviation of *Salmonella* spp. and *E. coli* in pork meat, inoculated raw sausage and sausage after heating

Pathogen	Microbial counts (log CFU/g)			
	Pork meat	Inoculated raw sausage	Sausage after heating	
			boiling	steaming
Salmonella spp.	4.8±0.2	7.1±0.3	<0.47	<0.47
Escherichia coli	4.7±0.1	7.0±0.1	<0.47	<0.47

Table 2. Mean values of heating time and thermal process lethality ($F_{70°C}$) of *Salmonella* spp. and *Escherichia coli* in pork sausage which were heated by boiling and steaming

Heating method	Heating time (min)	*F*- value (min)		Logarithamic reduction	
		Salmonella spp.	*Escherichia coli*	*Salmonella spp.*	*Escherichia coli*
		(Z =6.2˚C)	(Z =5.4˚C)	(D=0.29 min)	(D=0.08 min)
Boiling	10.25±0.17^{B}	2.13±0.09	2.08±0.09	7.34±0.31	25.97±1.18
Steaming	19.35±0.25^{A}	2.17±0.22	2.16±0.22	7.48±0.75	26.99±2.73

Different superscripts in the same column indicate the significant difference ($p<0.05$).

Conclusions

The thermal processes that use moist heat regardless of boiling or steaming process which targets to reach the internal cooking temperature at 71.6˚ C can reduce 7 log cycles (10^7) of *Salmonella* population and 26 log cycles (10^{26}) of *Escherichia coli* in pork sausage wrapped in banana leaves. Therefore small local producers and consumers should be advised to control the internal cooking temperature of pork sausage at least 72 ˚C for reducing the risk of food pathogens. Further study on D value and Z value of foodborne pathogenic bacteria in pork sausage could be useful for food producers to establish appropriate thermal process schedules for pork sausage wrapped in banana leaves.

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