Pathogenic Ability of *Salmonella* spp. Isolated from Pork Products Retailed in Sakon Nakhon Province, Thailand

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***Abstract*—Safety of food consumption especially pork product is important for consumer in Thailand. This study aims to estimate the pathogenic ability of *Salmonella* Enteritidis and *Salmonella* Kentucky isolated from pork products by evaluation heat resistance and survival of these bacteria when exposed to simulated gastrointestinal system. D-value at 50 ℃ of *S*. Enteritidis and *S*. Kentucky were 30.49 and 21.55 min, respectively. D_{55} of these 2 strains were decreased to 6.49 and 8.22 min, respectively. While D_{60} were 2.79 and 2.62 min, respectively. To evaluate the survival of these bacteria in simulated gastrointestinal system, *S*. Enteritidis and *S*. Kentucky were exposed to the acid broth system (pH 2.0) and the simulated small intestinal system (bile broth system, pH 6.5). After 110 min of incubation time in acid broth system, the survival rates of *S*. Enteritidis and *S*. Kentucky were 80.29% and 82.11%, respectively. Furthermore, the survival rate of *S*. Enteritidis and *S*. Kentucky in bile broth system demonstrated that these bacteria were able to resist in the simulated small intestinal system (pH 6.5) due to they showed high survival rate as 86.72% and 102.51%, respectively. As these results, if these bacteria contaminate in food products, it will increase the risk of foodborne disease to consumer. To prevent the risk from *Salmonella* spp. in these products, the food safety during handling and the appropriate thermal process for these products should be concerned.**

***Index Terms*—*salmonella* Enteritidis, *salmonella* Kentucky, heat resistance, simulated gastrointestinal system, food safety**

I. Introduction

Pork and pork products are recognized as one of general ingredient of food in Sakon Nakhon province, Thailand. However, increasing of small and medium food industry and street vendor in Sakon Nakhon province which food hygienic practice and food safety awareness are not efficiently implemented also caused of increasing foodborne outbreak. During processing of pork products (such as pork ball, pork sausage, and Vietnamese sausage), cutting, mincing, and mixing can spread *Salmonella* contamination into meat products. Thermal process is a combination of temperature and time required to eliminate microorganism during food processing and also in house cooking. The decimal reduction time (D-value) is time necessary to inactivate 90% or 1 log cycle of microbial population at individual temperature which calculated from the slope of the survival curve [1].

The thermal process is required for safety of food products consumption. However, there were 276 cases caused from salmonellosis in 2015 in Thailand [2].

Therefore, the thermal process in food processing is always defined as critical control point at which the achievement of an adequate heating and avoidance of post contamination are both essential to product safety. Although heat treatment, chilling process, and other processing techniques can reduce contamination of foodborne pathogens in food, some of foodborne pathogenic bacteria can survive due to mild heat treatment, inadequate cooking, or inappropriate storage which caused increasing of risk of pathogenic potential from the foodborne pathogens [3]. Moreover, the sensitivity of bacteria to heat depends on the condition to which the cell has been exposed before heating process and the components of the food in which the cell is heated [4]. For example, *Salmonella* Typhymurium DT104 increased ability to survive in simulated gastric and intestinal condition, although, it was confronted with modified atmosphere environment and chilling process [5].

The aim of this present study was to evaluate the pathogenic potential of *S*. Enteritidis and *S*. Kentucky which were isolated from pork products by evaluation heat resistance and survival of these bacteria when exposed to simulated gastrointestinal tract. *Salmonella* spp. was isolated from pork ball and pork sausage retailed in Sakon Nakhon province, Thailand. Then they were determined heat resistance in liquid medium. Pathogenic potential of *S*. Enteritidis and *S*. Kentucky in simulated gastrointestinal system including acid broth system (pH 2.0) and bile broth system (pH 6.5) were determined.

II. Materials and Methods

A. Bacterial Strains

Salmonella Kentucky and *Salmonella* Enteritidis were isolated from pork products retailed in the markets in

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Muang district, Sakon Nakhon province, Thailand. Each strain was kept in Tryptic soy agar (TSA) slant at 4 ℃ as stock culture. When the culture wanted to be examined, it was activated in Tryptic soy broth (TSB) and incubated at 35 ℃ for 18 h two times before used.

B. Heat Resistance of Salmonella spp. in Tryptic Soy Broth

To evaluate heat resistance of *Salmonella* Kentucky and *Salmonella* Enteritidis, 90 ml of TSB in 250 ml Erlenmeyer flask was allowed to equilibrate at 50 ℃, 55 ℃, and 60 ℃ in shaking water bath. Ten ml of each strain of *Salmonella* culture was inoculated into flask. During the various incubation periods, 3 ml of samples were taken periodically and put into another sterilized test tube and cooled in ice bath for 30 sec. The survival cell were enumerated by spread plate technique on TSA plate and incubated at 35 ℃, 24 h.

C. Survival of Salmonella spp. in Simulated Gastrointestinal System

The acid broth system was used as simulated gastric fluid system. It contained 12 ml of 0.1 mol/L HCl:KCl buffer pH 2 containing 500 U/ml pepsin A and 1 g/L bacteriological peptone. The broth was sterilized by filter sterilization (0.22 μm). The *Salmonella* spp. isolates were inoculated into the broth at an initial cell population of approximately 7 Log CFU/ml. The sample was incubated for 110 min at 35 ℃ in a water bath. Samples were taken for enumeration immediately upon inoculation and then every 10 min. Samples, serially diluted, were plated in duplicate onto TSA plates containing 1 g/L pyruvate.

The bile broth system was used as simulated small intestinal system. It contained 12 ml of 1 g/L phosphate buffer pH 6.5 containing 3 g/L oxbile. The broth was sterilized by filter sterilization (0.22 μm). The *Salmonella* spp. isolates were inoculated into the broth at an initial cell population of approximately 7 Log CFU/ml. The sample was incubated for 3 h at 35 ℃ in a water bath. Samples were taken immediately upon inoculation and then every 15 min and were enumerated by plating in duplicate onto TSA plates containing 1 g/L pyruvate [6].

D. Statistical Analysis

The results are presented as means ± SD. The survival of *Salmonella* spp., expressed as $\log_{10}$ CFU/ml, was presented against time (min). The decimal reduction time (D-value) was calculated from the slope of simple regression line for each survival curve [7].

III. Results and Discussion

A. Heat Resistance of Salmonella spp. in TSB Medium

The 2 strains of *Salmonella* spp. were determined heat resistance at 50 ℃, 55 ℃, and 60 ℃ in TSB medium. At 50 ℃, the initial population of *S*. Enteritidis and *S*. Kentucky were 8.14 and 8.03 Log CFU/ml, respectively. Survival cells of *S*. Enteritidis and *S*. Kentucky decreased slowly to 7.36 and 7.27 Log CFU/ml, respectively at 20 min of heating time. After 50 min of heating period, survival cells of *S*. Enteritidis and *S*. Kentucky reduce to 6.58 and 5.46 Log CFU/ml, respectively "Fig. 1".

On the contrary, survival cells of *S*. Enteritidis and *S*. Kentucky rapidly decreased at 55 ℃. The initial population of *S*. Enteritidis was 8.90 Log CFU/ml and *S*. Kentucky was 8.85 Log CFU/ml. Survival cells of *S*. Enteritidis and *S*. Kentucky rapidly decreased to 1.72 and 2.06 Log CFU/ml, respectively at 50 min of heating period "Fig. 2".

When *S*. Enteritidis and *S*. Kentucky were exposed to higher temperature as 60 ℃ the survival cell of these 2 strains decreased rapidly from 8.66 and 8.69 Log CFU/ml at the initial of heating period to 1.57 and 1.20 Log CFU/ml, respectively after 20 min of heating period "Fig 3".

D-value of *S*. Enteritidis and *S*. Kentucky in TSB medium were calculated from slope of linear regression of the survival curve of each strain. At 50 ℃, *S*. Enteritidis and *S*. Kentucky showed D_{50} with 30.49 and 21.55 min, D_{55} were 6.49 and 8.22 min, and D_{60} were 2.79 and 2.62 min, respectively. The results showed that increase temperature of heating decrease timing of destruction of *Salmonella* spp. Therefore, the D-value at higher temperature was lower than D-value at low temperature.

These results indicated that higher temperature as 60 ℃ showed higher ability to eliminate *Salmonella* cells than 55 ℃ or 50 ℃. However, in food environment, there are factors affecting to heat resistance of bacterial cell, such as temperature, bacterial strain, and food composition.

Therefore, in food stuff that contained nutrients as protein, fat and carbohydrate can protect *Salmonella* cells from heat better than in broth medium [7], [8]. Besides, it can increase heat resistance of the cells higher than in broth medium. In Sakon Nakhon province, Thailand, pork products as pork sausage, pork ball, and Vietnamese sausage are directly consumed or treated with mild thermal process as grilling or blanching with short time cooking. Moreover, these products contain high content of protein and fat which can protect bacterial cells from high temperature. For example, D-value of *Salmonella typhimurium* DT 104 increased when fat content in ground beef increase from 7% to 24% [9]. Consequently, if the temperature for cooking these products before eating is not appropriated to destroy *Salmonella* spp. the higher risk of contamination of *Salmonella* spp. is needed to be considered.

B. Survival of Salmonella spp. in Acid Broth System (pH 2.0)

The resistance of *S*. Enteritidis and *S*. Kentucky in acid broth system (pH 2.0) was showed in "Fig. 4". It was indicated that *S*. Enteritidis and *S*. Kentucky resisted to simulated gastric fluid system. When *S*. Enteritidis and *S*. Kentucky were exposed to acid broth condition the initial cell gradually decreased from 6.80 and 6.93 Log CFU/ml to 5.46 and 5.69 Log CFU/ml, respectively, after 110 min of exposure time. The survival rate of *S*. Enteritidis and *S*. Kentucky were 80.29 % and 82.11 %, respectively, at 110 min of exposure time. There was reported that *S*. Typhimurium can grow in broth medium as low pH as 5

then viability rapidly decrease when pH dropped to 4.3, 3.8, and 3.3 [10].

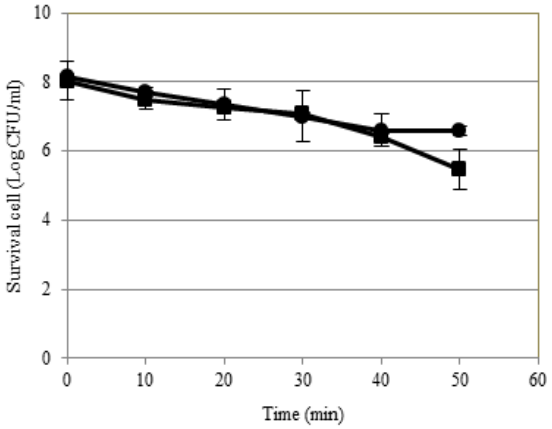

Figure 1. Survival cell of *S*. Enteritidis (•) and *S*. Kentucky (■) in TSB at 50 ℃.

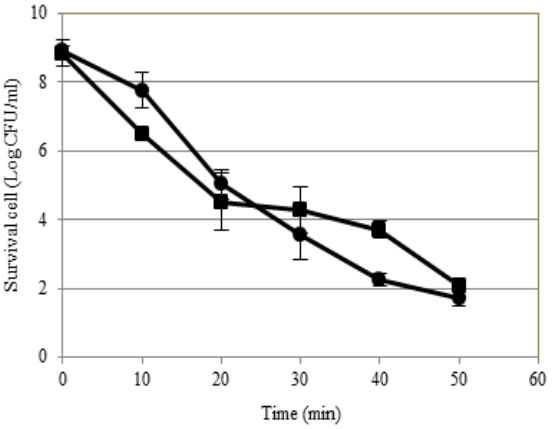

Figure 2. Survival cell of *S*. Enteritidis (•) and *S*. Kentucky (■) in TSB at 55 ℃.

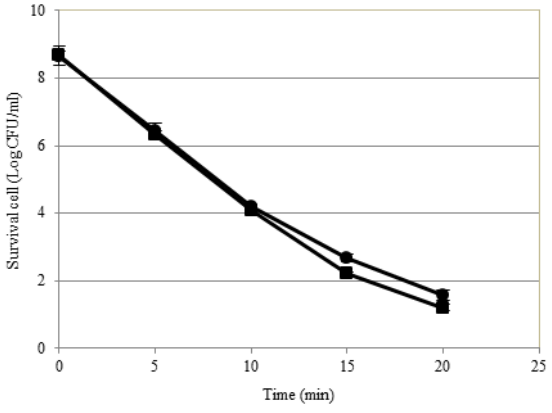

Figure 3. Survival cell of S. Enteritidis (•) and *S*. Kentucky (■) in TSB at 60 ℃.

The importance of acid condition in human gastric fluid is to defense against foodborne pathogenic bacteria. In another study, the population of *S*. Typhimurium DT104 reduced about 50% after 100 sec exposure to gastric fluid pH 1.5 [11]. However, the contrasting results could be depend on bacterial strain because the present study estimated on *Salmonella* spp. isolated from pork products in Sakon Nakhon province, Thailand. Moreover, the study of survival rate of *Salmonella* Typhimurium DT104 in simulate gastric fluid (pH 3.5) after sequential incubation in soil, lettuce, fresh-cut lettuce under modified atmosphere condition, and control treatment were 85% - 92% [5]. As same as the effect of modified atmosphere package for fresh-cut lettuce storage on acid resistance of *Escherichia coli*, the acid resistance ability of wild-type *E. coli* strain was induced after exposed to low oxygen storage atmosphere. In contrast, the *rpoS*-mutant strain could not induce acid resistance. It indicated that *rpoS* gene is significant regulatory gene for expression of some environment resistance [12]. Additionally, it was reported that during and after consumption of meal, the pH in stomach increase in a short time to 4-7 as the buffering effect of food [13]. As the results, it could be explained that the resistance of *S*. Enteritidis and S. Kentucky against to simulated gastric system increase risk of foodborne illness from these 2 strains.

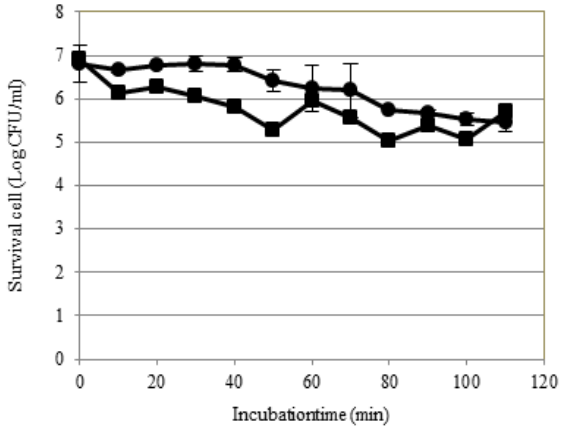

Figure 4. Survival cell of *S*. Enteritidis (•) and *S*. Kentucky (■) in acid broth system (pH 2.0).

C. Survival of Salmonella spp. in Bile Broth System (pH 6.5)

The survival of *S*. Enteritidis and *S*. Kentucky in bile broth system (pH 6.5) was showed in "Fig. 5". *S*. Enteritidis resisted to simulated intestinal condition as it showed high survival rate at 86.72% after 150 min of exposure time. The initial cell was 7.23 Log CFU/ml then decrease to 6.27 Log CFU/ml at the end of exposure time. Conversely, after exposure into bile broth system *S*. Kentucky slightly increased from 7.18 Log CFU/ml to 7.36 Log CFU/ml. The survival rate of *S*. Kentucky was 102.51%. Therefore, with both strains the survival rate after expose to bile broth system was significantly higher compare to the acid broth system ($P<0.05$). The resistance of both strains of *Salmonella* in bile broth system due to the system contained mild acid condition (pH 6.5) which is normally suitable for growth condition of *Salmonella* spp. [14].

Moreover, there were many reports that resistance of foodborne pathogen in bile broth system is not uncommon. For instance, *S*. Typhimurium DT104 resisted to simulated intestinal system after exposed in soil and lettuce [5]. *Escherichia coli* in combination with

Lactobacillus curvatus LTH1174(bac+) and 1174(bac-) showed high resistance to dynamic model of the intestinal tract [15]. It could be explained that resistance of foodborne pathogens in simulated intestinal system is necessary to increase of cells population for the ability of pathogenicity.

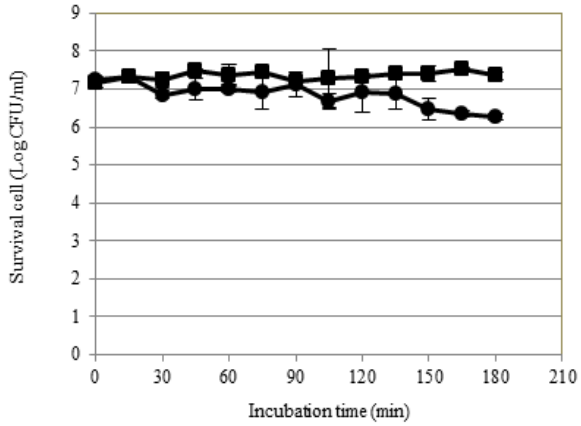

Figure 5. Survival cell of *S*. Enteritidis (•) and *S*. Kentucky (■) in bile broth system (pH 6.5).

IV. Conclusion

Salmonella Enteritidis and *S*. Kentucky showed higher heat resistance to mild thermal process as 50 ℃ and 55 ℃ than moderate temperature as 60 ℃. Moreover, they also showed the resistance to simulated gastrointestinal system. According to this study, contamination of *S*. Enteritidis and *S*. Kentucky isolated from pork products in Sakon Nakhon province, Thailand leaded to increase the higher risk of foodborne disease to consumer. The results from present study provided information on pathogenic potential which could be used for quantitative risk assessment. Additionally, to prevent the risk from consumption these products from *Salmonella* spp., the food safety during handling should be concerned and the appropriate thermal process for these products are required before eating.

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