

Prevalence of foodborne pathogens in ready-to-eat foods in the markets in Khon Kaen, Thailand

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Abstract:

The aims of this research were to evaluate consumer behavior regarding the consumption of ready-to-eat foods and to evaluate the prevalence of pathogens in ready-to-eat food products obtained from the markets of Khon Kaen, Thailand. As to the consumer behavior survey, top three highly consumed ready-to-eat foods had been reported as fresh vegetable salads, followed by sandwiches and Thai fresh spring rolls, respectively. Subsequently, the microbial contamination in the selected ready-to-eat food products was collected, analyzed and reported as the prevalence of this study. The 54 samples were collected from two types of markets; open markets and supermarkets located in Khon Kaen. The presence of contaminated pathogenic bacteria; *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus* was investigated by selective media. Poor hygiene was indicated by total coliforms and the presence of *Escherichia coli*. The results revealed that *Salmonella* spp. was detected in 5 out of 27 (19%) samples in open markets and 5 out of 27 (19%) samples in supermarkets. *L. monocytogenes* was detected in 4 out of 27 (15%) samples in open markets and 3 out of 27 (11%) samples in supermarkets. *S. aureus* was detected in 8 out of 27 (30%) samples in open markets and 9 out of 27 (33%) samples in supermarkets. Total coliforms were contaminated in 27 out of 27 (100%) samples in open markets and 23 out of 27 (85%) samples in supermarkets. *E. coli* was contaminated in 2 out of 27 (7%) samples of open markets and 1 out of 27 (4%) samples of supermarkets. A contamination of *L. monocytogenes* in open markets was higher than that in supermarkets. A contamination of *S. aureus* in supermarkets was higher than that in open markets; however, a contamination of *Salmonella* spp. in supermarkets was equal to that in open markets. This data provided food safety information of ready-to-eat foods for consumption of both markets in Khon Kaen.

Keywords: Prevalence, ready-to-eat, foodborne pathogens, Thailand

Introduction

Ready-to-eat foods are very popular in Thailand [1]. As to SCRIBD website, Thailand is in the first ranking of top 10 markets which claim to purchase ready-to-eat foods frequently [2]. Thai consumers are the most frequent buyers of ready-to-eat foods in the world. Due to these foods are convenient, inexpensive and readily available in the markets.

Salmonella spp., *Listeria monocytogenes* and *Staphylococcus aureus* are commonly contaminated bacteria in ready-to-eat foods and caused foodborne diseases. Moreover, the presence of total coliforms and *Escherichia coli* in these foods are confirmed of poor hygienic practices [3]. The presence of these pathogens in ready-to-eat foods is also likely to be either post contamination or cross contamination in food products. If the level of contamination of foodborne pathogens is high, it may lead to public health issues. *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli* are normally found in soil, water, air as well as animal and human intestine. Therefore these pathogens can easily contaminate in foods. Moreover, the production of ready-to-eat foods regarding preparation, storage and distribution; some steps have been performed without any heating step that can involve the contamination of both pathogens. Thus, the aims of this study were to evaluate the frequency of ready-to-eat foods consumption and to estimate risk assessment or the probability of foodborne illness for the sample collections of ready-to-eat food products obtained in markets of Khon Kaen, Thailand.

Materials and methods

Sample Collection

According to consumer behavior survey, the questionnaire survey was performed to achieve the data. The questionnaire survey was collected, analyzed and interpreted of the experiences and behaviors of a group of people from a target population through the asking of questions. The questionnaire survey was divided into two parts to obtain the general information (gender, age, education, and occupation) and consumer behavior (types of ready-to-eat foods, frequency of consumption, places to buy and health issues when consuming ready-to-eat foods). A total of 100 persons participated in the questionnaire. The results from

consumer behavior survey were used as criteria of sample collections for the second part of the study. This consisted of 54 samples of ready-to-eat foods composing of 18 samples each of fresh vegetable salads, sandwiches, and Thai fresh spring rolls that were purchased in local markets and analyzed in the laboratory within 3 h [4].

Consumer Behavior Survey towards the Consumption of ready-to-eat Foods

Part 1 General Information

1. Gender

Male

Female

2. Age

16-25 years old

26-40 years old

41-65 years old

3. Education Level

Undergraduate less than bachelor/ high school

Bachelor degree

Master degree

Doctoral degree

4. Occupation

Government officer

Private company employee

State enterprise employee

Student

Business owner

Others

Part 2 Consumer behavior

5. How often do you eat ready-to-eat foods per week?

Less than twice a week

2-4 times per week

6-8 times per week

10-12 times per week

More than 12 times per week

6. Which of the following ready-to-eat food do you often eat?

Hamburger

Sandwich

Salad

Thai fresh spring roll

Other

7. Where do you usually buy ready-to-eat foods?

Open market

Supermarket

Convenience store

8. Do you have any health issues when eating ready-to-eat foods? (e. g. diarrhea, vomiting, headache, fever)

Yes

Never

Not sure

Microbiological Analysis

Salmonella spp.

A sample of 25 g was added to 225 ml of lactose broth [5] and incubated at 35 °C for 24 h ± 2 h [4]. Then, the 0.1 ml of an overnight sample was transferred to 10 ml of an enrichment broth and broth was incubated for 24 ± 2 h at 42 ± 0.2°C. After incubation, RV medium was streaked on xylose lysine deoxycholate (XLD) agar and incubated 24 ± 2 h at 35°C. Suspected colonies were submitted to Triple Sugar Iron (TSI), lysine iron agar (LIA) slants and biochemical test.

Listeria monocytogenes

A 25 g sample was added to 225 ml of Half Fraser broth and incubated at 37°C for 48 h. A 0.1 ml portion of broth was transferred to 10 ml of Fraser Broth and incubated for 24 h at 35°C. A loopful of overnight culture was streaked on Oxford agar (OXA) and PALCAM and incubated at 35°C for 24-48 h [5]. Suspected colonies from each selective agar were streaked on purity to Trypticase soy agar with 0.6% yeast extract (TSAYE) and incubated at 30° C for 24-48 h [5]. All the isolates were subjected to biochemical tests and β-hemolytic activity.

Staphylococcus aureus

A 25 g sample was added to 225 ml Butterfield's phosphate-buffered water. Decimal dilutions were prepared by 10 ml from previous dilution plus 90 ml sterile dilution water. Each 1 ml of dilution was transferred to sample suspension and each dilution was spread onto three plates of Baird-Parker agar and incubated at 35-37°C for 45-48 h [5]. Suspected colonies were confirmed by the coagulase test with Rabbit plasma.

Total coliforms and Escherichia coli

A 25 g sample was added to 225 ml Butterfield's phosphate-buffered water. Decimal dilutions were prepared by 10 ml from previous dilution plus 90 ml of sterile dilution water. Each 1 ml portions of each dilution were transferred to three Lauryl Tryptose Broth (LST) tubes and incubated at 35°C ± 0.5°C for 24 ± 2 h. The positive result was a gas production. For negative result, tubes were re-incubated and examined again at 48 ± 2 h. The test was confirmed on all presumptive positive (gas) tubes [5]. A loopful of each gassing LST tube was transferred to EC broth and incubated at 45.5 °C for 24 ± 2 h. The positive result was gas production. As to the negative result, broth was re-incubated and examined again at 48 ± 2 h. Results of this test were calculated the numbers of fecal coliform as MPN/g [5]. A loopful of EC tube was transferred and culture was streaked for isolation on L-EMB agar plate and incubated at 35°C±0.5°C for 18-24 h.

Statistical Analysis

The data was analyzed using descriptive analysis. The chi-square test was used to test for differences. All statistical analysis was performed using SPSS program Version 19 or Microsoft Excel 2010.

Results and discussion

Consumer Behavior Surveys

According to the consumer behavior survey questionnaires, the questionnaires survey was focusing on the consumer's behavior in Khon Kaen. The results of 100 questionnaires have revealed 6-8 times of ready-to-eat foods consumption per week (32%). The open market was the most favorite location to purchase ready-to-eat foods (65%) followed by supermarket (28%) and convenience store (7%), respectively. These results are similar to the findings of other authors that showed the 76.29% of consumers in Nigeria, they would rather buy ready-

to-eat food products from open markets than supermarkets. Their reasons would be open markets were close to their resident [7]. Ready-to-eat fresh vegetable salad was the most consumed, followed by sandwiches and Thai fresh spring rolls, respectively. As to the questions regarding health issues, 60% of consumers say they never experienced any health issues when consuming ready-to-eat foods. However, the results revealed that 29% of the consumers in Khon Kaen felt sick after food consumption and 11% of the consumers probably felt sick when consuming the ready-to-eat food products.

Table 1 Socio-Demographic Characteristics of the Sample (n=100)

General information	% of Total
Gender	
Male	32%
Female	68%
Age group	
16-25 years old	33%
26-40 years old	33%
40-65 years old	34%
Educational level	
Undergraduate less than bachelor/high school	23%
Bachelor's degree	67%
Master's degree	9%
Professional degree or PhD.	1%
Occupation	
Government officer	30%
Private company employee	8%
State enterprise employee	9%
Student	36%
Business owner	9%
Others	8%

Prevalence of Foodborne Pathogens

Open markets

From Table 2, total coliforms were the majority of the contamination in ready-to-eat foods (100%), followed by *S. aureus* (30%), *Salmonella* spp. (19%), *L. monocytogenes* (15%) and *E. coli* (7%), respectively. The number of total coliforms and *E. coli* was ranged from 110

to >11,000 and 30 to >750 MPN/g, respectively. *S. aureus* was detected in fresh vegetable salads (44%), Thai fresh spring rolls (33%) and sandwiches (11%). *Salmonella* spp. was detected in Thai fresh spring rolls (44%), fresh vegetable salads (11%) but not detected in sandwiches. *L. monocytogenes* was detected in Thai fresh spring rolls (33%), fresh vegetable salads (11%) and not detected in sandwiches. *E. coli* was detected in fresh vegetable salads (22%), but not detected in Thai fresh spring rolls and sandwiches. Total coliforms were detected in fresh vegetable salads, Thai fresh spring rolls and sandwiches (100%).

Supermarkets

From Table 2, the most contamination bacterium was total coliforms (85%), followed by *S. aureus* (33%), *Salmonella* spp. (19%), *L. monocytogenes* (11%) and *E. coli* (4%), respectively. The number of total coliforms was ranged from <30 to >11,000 MPN/g. *E. coli* was reported as 30 MPN/g. *S. aureus* was detected in sandwiches (67%) and Thai fresh spring rolls (33%) but not detected in fresh vegetable salads. *Salmonella* spp. was detected in fresh vegetable salads (33%) and Thai fresh spring rolls (22%) but not detected in sandwiches. *L. monocytogenes* was detected in sandwiches (33%) but not detected in both of Thai fresh spring rolls and fresh vegetable salads. *E. coli* was detected in sandwiches (11%) but not detected in both of Thai fresh spring rolls and fresh vegetable salads. Total coliforms were detected in Thai fresh spring rolls (100%), sandwiches and fresh vegetable salads (78%), respectively.

The study compared products from two types of markets (n=54); *S. aureus* was the most contaminated pathogen in ready-to-eat foods (31%), followed by *Salmonella* spp. (19%) and *L. monocytogenes* (13%), respectively. According to poor hygiene indicators, total coliforms were detected in ready-to-eat foods (93%) and *E. coli* was detected in ready-to-eat foods (6%). From these results, *Salmonella* spp. and *S. aureus* were reported as two out of top 3 pathogens that caused the illness in Thailand [6]. *S. aureus* was positively found in all ready-to-eat food products. Because its resources from human body. It can present in the hair, nasal and skin of vendors. Moreover, environmental conditions as humid weather in Thailand and products were stored under room temperature due to lack of refrigeration in open markets. Furthermore, ready-to-eat foods were handled extensively during preparation and there was no further processing such as cooking to inactivate potential pathogens [8]. As for the fresh vegetable salads tested in this study, *S. aureus* was detected in 22%. This result was higher than those of a similar study conducted by Hasan et al., which detected *S. aureus* in

12% of vegetable salads. *Salmonella* spp. was detected in 22% of the tested salads, which is higher than the results of the study of Mediterranean salads by Zeki et al., which detected 14% [9], but lower than the 41.8% found in the study of salads by Anderson et al. [10]. *L. monocytogenes* was detected in 6%, which is not detected in Mediterranean salads [9]. Moreover, *L. monocytogenes* in sandwiches was detected in 17%. While the findings of a study by Moustafa et al. showed that *L. monocytogenes* was detected in 14% [11]. In a comparison between the two types of markets; the prevalence of *L. monocytogenes* in open markets was higher than in supermarkets. The contamination of *S. aureus* in supermarkets was higher than the open markets, and *Salmonella* spp. was equally found in both markets. These results differ from another study that showed a prevalence of contamination of *Salmonella* and *L. monocytogenes* in supermarkets was higher than in open markets [4]. The results of the consumer behavior survey showed the highest frequency of ready-to-eat foods consumption as 6-8 times per week (32%), and contaminated foodborne pathogens were found in all of ready-to-eat foods samples. It might be assumed that if one serving of ready-to-eat food products is weighed about 200 g, the consumer who had consumed the ready-to-eat food very often (6-8times/week) had much more chances to have foodborne illness than the other group of consumers.

Conclusions

According to consumer behavior survey questionnaires, the results showed that the most favorite location to purchase ready-to-eat food products of consumers in Khon Kaen was an open market. Consumers in Khon Kaen were purchased ready-to-eat food products 6-8 times per week. The results also revealed that fresh vegetable salad, followed by sandwich and Thai fresh spring roll were top three of ready-to-eat foods consuming in Khon Kaen, Thailand. As to the prevalence of foodborne pathogens in ready-to-eat foods of the markets in Khon Kaen, the results showed that *Salmonella* spp., *L. monocytogenes*, and *S. aureus* had been found in these food products. The indicators of poor hygienic practices; total coliforms and *E. coli* in ready-to-eat foods also had been positive. The contamination of foodborne pathogenic bacteria in ready-to-eat foods in Khon Kaen possibly implied that the ready-to-eat food products in open market and supermarket possibly caused foodborne illness for the Khon Kaen consumers. The results in this study can be provided the awareness of ready-to-eat food

consumption in Khon Kaen for the consumers and also the guidance for local producers to apply safety standards such as GMP, HACCP and personal hygiene for preparing foods.

Table 2 Prevalence of Foodborne Pathogens and Poor Hygiene Indicators in Ready-to-Eat Foods in Markets in Khon Kaen, Thailand

Sample	Isolation	OM*	Prevalence (%)	SM*	Prevalence (%)	p-Value **
Salad	<i>Salmonella</i> spp.	1/9	11%	3/9	33%	0.169
	<i>L. monocytogenes</i>	1/9	11%	0/9	0%	0.347
	<i>S. aureus</i>	4/9	44%	0/9	0%	0.035
	<i>E. coli</i>	2/9	22%	0/9	0%	0.169
	Total coliforms	9/9	100%	7/9	78%	0.169
Sandwich	<i>Salmonella</i> spp.	0/9	0%	0/9	0%	
	<i>L. monocytogenes</i>	0/9	0%	3/9	33%	0.081
	<i>S. aureus</i>	1/9	11%	6/9	67%	0.013
	<i>E. coli</i>	0/9	0%	1/9	11%	0.347
	Total coliforms	9/9	100%	7/9	78%	
Thai fresh spring roll	<i>Salmonella</i> spp.	4/9	44%	2/9	22%	0.169
	<i>L. monocytogenes</i>	3/9	33%	0/9	0%	0.081
	<i>S. aureus</i>	3/9	33%	3/9	33%	
	<i>E. coli</i>	0/9	0%	0/9	0%	
	Total coliforms	9/9	100%	9/9	100%	

*Number of positive samples/total of samples in the open market (OM) and the supermarket (SM)

** Significant at $p < 0.05$ (2-sided) based on comparison between supermarket and open market

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