Research article

Evaluation of Bacterial Contamination Levels in Pickles Sold at Wet Market in Cambodia -Part 2- Detection of Several Food-poisoning Bacteria of 48 Samples from Phnom Penh

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Abstract Some pickles transported from the local province are sold in the wet markets of Phnom Penh, Cambodia. Therefore, products sold in Phnom Penh can be considered representative samples that reflect the approximate levels of bacterial contamination in the food supply chain of Cambodia. In this study, we conducted a microbiological inspection of the food samples from Phnom Penh to investigate the seriousness of the unsanitary conditions of food products. Eight kinds of pickles were purchased from three wholesale markets one week apart on two separate occasions. A total of 48 samples were used for microbial testing, and the values of Brix, pH, and salt content of these samples were measured. Although all food samples were purchased from the same markets and vendors, several pickles showed different results in the measured values between the first and second purchases. To determine the contamination level of the pickles, we targeted general viable bacteria, coliforms, fungi,

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Salmonella, *Staphylococcus aureus*, and *Bacillus cereus*. The total number of microorganisms detected by the aerobic plate count showed that 15 of 48 samples contained more than 7 $\log_{10}$ CFU/g of sample. Pickled onion and mustard showed relatively higher number of microorganisms compared to other products. Of the 48 pickles, 58.3% were coliform positive, 41.7% of the samples were *Salmonella* positive, and 83.3% and 37.5% were *S. aureus* and *B. cereus* positive samples, respectively. These results indicate that the products from the wholesale market of Phnom Penh are significantly contaminated with bacteria. In general, food contamination result from raw materials, cross-contamination, and poor water quality. Thus, it is important to educate food handlers regarding the practical knowledge to ensure proper food sanitation practices. The results of this study will be useful to reveal the cause of food contamination and to develop appropriate countermeasures.

Keywords food contamination, food poisoning bacteria, wet market, pickles

INTRODUCTION

Pickles are ready-to-eat (RTE) products and are very popular to Khmer people in Cambodia. However, due to the absence of heat sterilization prior to consumption, the safety of the products is a major concern. Our previous study had found that several pickles sold at the wet markets of Kampong Cham and Phnom Penh (PP), Cambodia were unsanitary. In addition, the hygiene practices of the local wet market were very poor, which subsequently lead to food contamination. We speculate that these unsanitary conditions of food and food-processing and the selling environment were derived from the food handlers who had limited awareness and knowledge on food hygiene.

The vegetables that are often the raw material for pickled foods can harbor pathogens such as *Escherichia coli* and *Salmonella* (Francis, 1999; Matthews, 2017; Skočková, 2013). Oliveira et al. (2011) reported that there were *Salmonella* and thermotolerant coliforms in RTE vegetables. Further, there are several kinds of food spoilage microbes and foodborne pathogens that account for the majority of foodborne diseases. The World Health Organization reported that the leading causes of foodborne deaths included *Salmonella* and enteropathogenic *E. coli* (WHO, 2015). *B. cereus* is a well-known food poisoning bacteria. *B. cereus* has caused food-borne outbreaks via a large variety of foods in many countries (Tewari, 2015). *S. aureus* is a ubiquitous bacterium that causes potentially fatal infections (Kadariya, 2014). To assess the safety and quality of food products in Cambodia, the information on the actual hygiene practices for food products served to local Khmer people is very important. However, scientific reports on food safety in regards to the pickle products in Cambodia are nearly absent except for one report by Chrun et al. (2017). Therefore, it is necessary to accurately assess the microbiological safety of food products in Cambodia. In order to determine the food hygiene level in Cambodia, we investigated the major foodborne pathogens and food spoilage microbes in pickled products. The level of microbe contamination in samples we tested reflect the sanitary conditions of the wet market. In this study, we investigated the pickle products sold in PP. Because some products are transported from the local province to PP, the products sold in PP represent a model of the food supply chain in Cambodia. Due to the large population of PP, microbial food contamination leads to foodborne disease outbreaks.

OBJECTIVE

The objectives of this study were 1) to analyze the current status of food and microbiological quality of pickles sold in PP, 2) to assess the substantial food hygiene problem in Cambodia.

METHODOLOGY

Evaluation of the Quality of Pickle Products

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Eight kinds of common pickle products were purchased from three major wholesale markets of PP. The popular pickle products were selected based on interviews with Khmer people. They were collected in March, 2019. In the following week, the same pickles were purchased from the same shops. In total, 48 samples were collected and used in this study (Table 1). The samples were stored at 4°C for one day. The values of Brix, salt concentration, and pH of the samples were measured.

Table 1 Pickles sample used in this study

Market	Sample	Product name	Sample	Product name
A / B / C	A	Fermented papaya (sliced)	E	Pickled mustard (small)
	B	Fermented cucumber	F	Pickled cucumber
	C	Pickled young onion	G	Sweet salty radish
	D	Pickled mustard (big)	H	Kong chay

Microbiological Examination of Pickle Products

Ten grams of the food sample was mixed with 90 mL of Maximum Recovery Diluent (Merck KGaA, Darmstadt, Germany) solution in a sterilized storage bag. To make a homogeneous suspension, the sample was homogenized by a stomacher. After stomaching, 1 mL of the sample suspension was mixed with 9 mL of Maximum Recovery Diluent solution in a sterile tube. The serial dilution samples were prepared and used for testing. For the detection of *S. aureus* and *Salmonella*, Buffered Peptone Water (Merck KGaA, Darmstadt, Germany) solution was used for sample preparation. The standard agar medium (AS ONE, Osaka, Japan) was used for the general viable count. Desoxycholate agar medium (AS ONE, Osaka, Japan) was used for the detection of coliform. Potato Dextrose Agar medium (AS ONE, Osaka, Japan) was used for fungi. CHROMagar™ Salmonella medium (KANTO CHEMICAL, Tokyo, Japan) was used for *Salmonella*. Mannitol salt agar medium with egg yolk (KANTO CHEMICAL, Tokyo, Japan) was used for *S. aureus*. BACILLUS CEREUS AGAR BASE (KANTO CHEMICAL, Tokyo, Japan) with Oxoid Polymyxin B Supplement (KANTO CHEMICAL, Tokyo, Japan) and egg yolk was used for *B. cereus*. 10 μL of the dilution samples aliquoted onto plates. For general viable counts and the detection of coliform, *S. aureus*, plates were incubated at 35°C for 48 hours. For the detection of *Salmonella* and *B. cereus*, plates were incubated at 35°C for 24 hours. For the detection of fungi, plates were incubated at 30 ℃ for 5 days. All experiments were conducted three times independently.

RESULTS AND DISCUSSION

Evaluation of the Quality Variation of Pickle Products

Eight kinds of popular pickle products for local Khmer people were selected and purchased from three major wholesale markets. These 24 food samples were the first samples used in experiments. To examine the quality variation of the products, the same products from the same shop were purchased in the following week. In total, 48 samples were collected and used for the experiments.

To evaluate the quality of pickle products, Brix, salt concentration, and pH value were measured for the pickle products. Three samples were more than three times different in Brix values between the samples purchased on the first versus second weeks. Seven samples were more than 1% different in salt concentration values. Three samples varied in pH values greater than 1.0. The fact that the different values were obtained between the same pickle products showed the quality of pickle products were unstable and/or the way of food processing was varied (data not shown).

Contamination and Spoilage Level of Foodborne Pathogen and Microbe in Pickle Products

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All samples were subjected to microbiological investigation. We investigated the generally viable microbes, coliform, fungi, *Salmonella*, *S. aureus,* and *B. cereus* to determine the food contamination and spoilage levels. The total number of generally viable microbes of 48 samples is shown in Table 2. Fifteen samples were over 7 $\log_{10}$ CFU/g, indicating initial putrefaction. The samples from market B and C had relatively higher total CFU than those from market A. The sample C, pickled young onion, sample D and E, and pickled mustard showed a higher number (6 $\log_{10}$ to 8 $\log_{10}$) of CFU (data are not shown).

Table 2 Total number of general viable microbes in pickle products

$\log_{10}$ CFU/g	Market					
	A		B		C	
	1st sample	2nd sample	1st sample	2nd sample	1st sample	2nd sample
< 3	1	1	1	2	0	1
4	2	1	1	0	0	1
5	3	3	2	1	1	1
6	0	2	3	1	3	2
7	1	1	1	3	2	2
8	1	0	0	1	2	1

Next, we investigated major food spoilage microbes and food pathogens. Results are shown in Table 3. Coliform is an indicator of whether or not the food was handled in a proper manner. Coliform inhabit the human intestinal tract, the presence of them means likely fecal contamination (Madigan, 2019). As a result, the total contamination rate of coliform was 58.3%. According to Ceuppens (2014), the existence of coliform was correlated to the presence of *E. coli* and a sufficient amount of *E. coli*. More than half of the pickles were coliform positive and had a significant risk of including pathogenic *E. coli* such as EHEC.

Microbial spoilage of fresh vegetables is often caused by fungi (Madigan, 2019) that are able to survive at lower a_w conditions. So, the contamination by fungi was examined. The total rate of contamination was 68.8%.

Salmonellosis is very common for bacterial food infection. This disease is typically caused by *Salmonella* and *Salmonella*-contaminated food and animals. Of the first 24 samples, two samples were *Salmonella* positive and in the second batch of samples, 18 samples were positive. In total, 41.7% of pickles were contaminated by *Salmonella*. Combined with the wet market conditions shown in our previous study, *Salmonella* contamination was most likely caused by cross-contamination of chicken and egg products and by *Salmonella*-carrying animals such as dogs that come into contact with the food.

S. aureus cause food poisoning and the produced toxins by *S. aureus* cause gastrointestinal symptoms. These toxins can be superantigens that lead to lethal toxic shock syndrome. The presence of *S. aureus* (83.3%) was surprisingly high in our tested samples. Chrun et al. (2017) reported that the contamination rate of fermented vegetables collected from the wet market in Phnom Penh was less than 10%, but the samples used in this study exceeded 80%. *S. aureus* can grow in both aerobic and anaerobic conditions and in many foods. *S. aureus* is often carried by humans. The interior of nostrils and skin are associated with *S. aureus* and a pus-forming wound becomes a frequent cause (Madigan, 2019; Matthews, 2017). In our previous study, we observed that the vendors handled their products with bare hands and utensils that were left out. Most staphylococcal contamination is caused by humans during food preparation (Matthews, 2017). It is assumed that the inappropriate manner of food handling increases the chance of food contamination.

B. cereus is widespread in nature and is frequently isolated from growing plants and soil. So, this organism is easily spread if there is no equipment to prevent dust and dirt from the outside. Foodborne illness by *B. cereus* is of two types—the emetic form results in vomiting and the diarrheal form results in diarrhea. Our results showed that 37.5% of the total samples were *B. cereus* positive. Upon microbiological examination, there were large variations in between different pickle products

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and even within the same pickle products. Some pickles were considered to contain enough sugar and salt to prevent spoilage; however, most products had some kind of microbiological hazard. Our previous study revealed that the wet market was in a condition where cross-contamination can easily occur. The cause of bacterial contamination could be from raw materials and cross-contamination. The water used for food processing and cleaning may also be a source of foodborne pathogens (Steel, 2004).

Table 3 The rate of microbiological contamination in pickle products

Microbial sp.	Number of positive / Number of total		
	First sample	Second sample	Total rate (%)
Coliform	14/ 24	14 /24	58.3
Fungi	13 / 24	20 / 24	68.8
Salmonella	2 / 24	18 / 24	41.7
S. aureus	19 / 24	21 / 24	83.3
B. cereus	12 / 24	6 / 24	37.5

CONCLUSION

In this study, we investigated the level of food spoilage and the rate of food contamination of pickle products. The results indicated that significantly poor sanitary conditions and unsafe food products were provided at the wholesale market in PP. It is necessary that food handlers as well as consumers receive practical knowledge on food hygiene to prevent the spread of bacterial food contamination. In the future, we will focus on the cause of food contamination and give countermeasures against it.

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