Research article

Evaluation of Bacterial Contamination Level in Pickles Sold at Wet Market in Cambodia - Part 1- In Case of the Samples from Kampong Cham and Phnom Penh

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Received 15 January 2020 Accepted 23 April 2020 (*Corresponding Author)

Abstract Agriculture is the main industry in Cambodia and home-made agricultural products for local Khmer people are commonly sold at wet markets. However, food poisoning and food-related diseases frequently occur due to these home-made products. The improper handling of food by vendors, inadequate storing conditions, and cross-contamination risk while on display, were observed when inspecting wet markets. Sanitary conditions in the wet markets were poor and the food handlers seemed to treat food based on their personal experience. In this study, we aimed to investigate the hygiene level of food products sold in wet markets in Cambodia. In Kampong Cham, a typical small town, processed foods are consumed in the local area and are also transported to large cities, like Phnom Penh. Pickled vegetables, which are a popular ready-to-eat food in Cambodia, were selected and assessed for microbial contamination. A total of 17 pickles were collected from the local market in Kampong Cham and we measured the Brix value, salt concentration, and pH in these samples. Adenosine triphosphate (ATP) concentration was also measured, because the amount of ATP

in a food sample indicates the approximate microbial contamination level. The five samples with the highest ATP values were used for a quantitative evaluation of microorganism contamination. In addition, five other samples with relatively low ATP concentrations were tested for comparison. An assessment of colony number and morphology indicated that nine of the samples showed initial putrefaction or putrefaction. There was one coliform-positive sample. Similarly, three pickles purchased in Phnom Penh showed putrefaction and two samples from these three pickles were coliform-positive. These results indicated that food sanitary conditions are unsafe and there is a critical need for improving food safety at wet markets in Cambodia.

Keywords food safety, contamination, wet market, pickles

INTRODUCTION

Agriculture is the main industry in Cambodia and home-made agricultural products for local Khmer people are commonly sold at wet markets. However, food poisoning and food-related diseases occur frequently due to these home-made products. Many consumers are not aware of the likelihood of spoilage of the purchased product when it is stored at an unsuitable temperature for a long period (Sanlier, 2009; Odeyami et al., 2019). Therefore, food handlers and service employees have a responsibility to provide safe food products. However, the lack of personal hygiene consciousness is a common cause of foodborne illness (Taulo et al., 2009). There are several reports describing a low level of knowledge regarding food hygiene among food handlers (Osaili et al., 2013; Baluka et al., 2014; Emmanuel et al., 2015). In Cambodia, the major reason for foodborne illness is that the vendors have little knowledge of food hygiene and safety. Large-scale foodborne outbreaks are monitored by event-based surveillance in Cambodia, but there are no formal statistics regarding individual food poisoning cases (Vandy et al., 2012). In addition, most foodborne diseases that occur at home are under-diagnosed and/or under-reported (Redmond et al., 2003; Keegan et al., 2009; Vrbova et al., 2012). Therefore, the current status of hygiene conditions in Cambodia requires clarification. Here, we aimed to evaluate the hygiene conditions of the food sales environment and food products used by the local Khmer people. These inspections will lead to practical ways of improving food safety.

Pickles are a very common ready-to-eat food in Cambodia. There are many types of vegetables used to make pickles. The basic procedure for making pickles is to wash the vegetables and then add sugar, salt, and other ingredients, such as fish sauce, soy sauce, garlic, chili, and rice. Most pickles stored in jars are placed in the sun or in a room at ambient temperature for 1 to 4 days. Some of these pickles are fermented spontaneously by lactic acid bacteria (Chrun et al., 2017). Although fermentation increases the shelf-life and the nutritional value of the product, the raw materials can be sources of microbial contamination. Therefore, these home-made fermented foods are often risky (Anal et al., 2020) and food poisoning incidents are often caused by these pickled products. Since these pickled products are consumed daily, they can be a critical hazard if not prepared hygienically. Investigating the quality of pickled products requires the local Khmer people to change their way of thinking about food safety.

Kampong Cham (KPC) is a typical small town and the food products produced there are consumed in the local area and also transported to large cities like Phnom Penh (PP). Pickled vegetables, which are a popular ready-to-eat food in Cambodia, were selected and examined for microbial contamination.

OBJECTIVE

The objectives of this study were: 1) to investigate the safety levels and simple chemical properties of pickles sold in the wet market in Cambodia, 2) to discuss the cause of the unsanitary conditions of these samples.

METHODOLOGY

In this study, we assessed the safety conditions of food and performed bacterial contamination inspections of pickles sold at wet markets in Cambodia.

Market Inspection

The market inspection was conducted through observation of equipment, facilities, and displaying and storage condition of products. The results of the evaluation of these conditions were ascertained by asking to seller.

Food Samples Used in This Study

Popular pickled and fermented vegetables were collected from local wet markets in KPC in August, 2018. The products we selected by randomly but only the products from the vendors who are able to tell the detailed information such as raw materials, shelf life and the process from the production to selling. In total, 17 food samples were purchased from four vendors at three different wet markets. In PP, three food samples were purchased at a wholesale market. The food samples used in this study are shown in Table 1. The properties of all food samples were measured within 2 hours after purchasing. Ten KPC samples selected for bacterial inspection were placed in a sterilized storage bag and kept at 4°C for 7 days until examination. PP samples were kept for 6 days under the same conditions as the KPC samples.

Measurement of Food Properties

The sugar and salt content and pH of foods affect microbial growth and these values can be indicators of the preservability of the food. We measured Brix sugar content (soluble solids content), salt content, and pH of food samples using a Brix refractometer (Atago, Tokyo, Japan), a salt meter (Horiba, Kyoto, Japan), and a pH meter (Horiba), respectively. ATP content is an approximate indication of the microbial contamination level of food samples. The standard ATP content value required to satisfy the sanitary conditions of food-handling rooms and equipment is 36 RLU. Therefore, samples showing greater than 100 RLU were selected as candidates for microbial investigation. Some samples with lower ATP content were also investigated for comparison. ATP content was measured using a luminometer and an ATP assay kit (SystemSURE Plus; Nitta, Osaka, Japan).

Sample Preparation and Bacterial Detection by Pour Plate Method

Ten grams of food sample was mixed with 90 mL of saline containing 0.1% peptone in a sterilized storage bag. To make a homogeneous suspension, the sample was homogenized using a stomacher. A series of sample dilutions was used for the detection of general viable bacteria, using standard agar medium and coliform bacteria, using desoxycholate agar medium. The solidified plates were incubated at 35 for 48 hours.

RESULTS AND DISCUSSION

Inspection of Wet Markets in Kampong Cham

To evaluate the hygiene level of food eaten on a daily basis in Cambodia, we inspected wet markets in KPC in August, 2018. Most food products were stored at room temperature and therefore, those products that do not have stable preservability at room temperature were susceptible to deterioration. In the wet markets, retailers displayed their products close together and nearly all pickled products were stored in a plastic bin at room temperature. The celling and the lights of the market were partly covered with dust and some unattended raw garbage was observed in the passage. Because pickles

were displayed without a lid, flies swarmed around them. From these inspection results, the wet market did not appear to have appropriate conditions for storing food products.

When most vendors served the pickles into a takeout bag, they did not usually use gloves, but served by bare hand. The risk of cross-contamination was also considered to be high for various reasons. For example, cross-contamination could occur between pickled products and other raw products and utensils, which can harbor microbes. The accumulation of waste and the manner in which it was disposed and stored may also result in the transport microbes and may lead to food product contamination. Although vendors decided on an approximate expiration date, unsold products were stored at room temperature. The hygiene conditions of the wet market meant that the food being sold was highly susceptible to microbial contamination.

From the inspection of the wet market, we concluded that the food products being sold were not handled in a hygienic manner. Therefore, we performed an assessment of food contamination levels in pickle products from the wet market.

Chemical Properties of Pickles from KPC and PP Markets

Popular pickle products were selected and examined for quality and safety. Seventeen pickle samples were purchased at the local wet markets in KPC and three pickle samples were purchased from a wet market in PP. These samples were measured for Brix value, salt concentration, pH, and ATP content immediately after purchase. The samples included several types of pickles. The Brix values ranged from 5.7 to 49.5 °Bx and salt concentration ranged from 1.4 to >25%. The pH values ranged from 3.6 to 5.2 (Table 1).

Table 1 Chemical properties of pickles from KPC and PP markets

Region	Vendor	Sample No.	Product name	ATP (RLU)	Brix	pH	Salt (%)
Kampong Cham	A	a-1	Young Pickled Onion	5	33.5	3.7	8.1
		a-2	Slice Fermented Cucumber	4	32.6	4.2	5.5
		a-3	Fermented Cucumber	5	30.8	4.6	12
		a-4	Young Pickled Cucumber	378	10.7	4.3	3.8
		a-5	Salty-sweet Raddish	94	16.0	5.2	5.6
	B	b-1	Young Pickled Onion	21	31.8	3.6	7.8
		b-2	Young Pickled Cucumber	542	40.9	4.3	1.4
		b-3	Salty-sweet Radish	91	25.9	5.2	5.8
		b-4	Fermented Cucumber	2	43.0	4.0	13
		b-5	Fermented Green Mustard	184	16.9	3.6	3.0
		b-6	Fermented Ginger	4	31.4	4.0	8.3
		b-7	Fermented Papaya	400	49.5	4.9	9.1
		b-8	Fermented Mustard	64	5.90	3.9	-
	C	c-1	Fermented Papaya	148	48.3	4.3	9.6
		c-2	Fermented Small Mustard	212	9.80	4.2	3.2
	D	d-1	Fermented Sprout	5	14.3	3.9	4.4
		d-2	Fermented Cabbage	1	16.8	4.0	3.8
Phnom Penh	E	p-1	Fermented Cucumber	1	40.5	4.1	7.2
	F	p-2	Fermented Green Mustard	1,882	5.70	4.0	>25
	G	p-3	Fermented Green Mustard	201	6.60	-	>25

Generally, most bacteria required a minimum water activity (a_w) of 0.88-0.91, most yeasts require a minimum a_w of 0.88 and regular molds need an $a_w < 0.88$ (Matthews, et al., 2017). The

theoretical amounts of salt and sugar required to achieve an a_w value of 0.88 (salt) and 0.92 (sugar) are 17.8% and 62.6%, respectively. The minimum and maximum ATP content values were 1 and 1,882 RLU, respectively, with 55% of food samples showing ATP content values greater than 36 RLU.

Microbial Contamination Level of Food Samples from KPC and PP Markets

Of the 17 food samples from the KPC market, 10 were examined for general viable bacterial counts and for coliform bacteria (Table 2). Four samples from the KPC markets and two samples from the PP market had bacterial counts greater than 7.00 $\log_{10}$ colony-forming units (CFU)/g. These samples showed initial putrefaction and they were not suitable for consumption. Moreover, after observing the colonies on the detection plates, 12 samples were found to contain various unwanted microbes. One sample from the KPC market and two samples from the PP markets were found to be coliform-positive. Generally, coliform bacteria are not able to grow at pH values less than 4.0 (Besser et al., 1993; Zhao et al., 1994), but some *E. coli* strains are reported to grow even at pH 3.8 (Presser et al., 1997; Zhao et al., 1993). The pH values of the coliform-positive samples were 4.1 and 4.0 for samples p-1 and p-2, respectively. It was not clear if *E. coli* could proliferate under these pH conditions, but the contamination with coliform bacteria was considered to have occurred during the preparation of these products. The pickle samples with lower salt concentration, such as a-4, b-2, and c-2, tended to show higher $\log_{10}$ CFU/g values. However, other samples with high salt and sugar concentrations also contained unwanted microbes. The result of Table 2 showed that 55% of the sample from the KPC market and all samples from the PP market were not enough microbial quality to eat. Taking into consideration our inspection results, it is assumed that some steps lead to the contamination. The causes of contamination are presumed by raw materials, the process of food processing, storage condition. These steps might have contained complicated factors and there may multiple routes of the contamination.

Table 2 Microbial contamination level of food samples from KPC and PP markets

	Sample No.	$\log_{10}$ CFU/g	Coliform
Kampong Cham	a-1	3.36	Negative
	a-4	7.77	Negative
	b-2	8.00<	Negative
	b-3	3.30	Negative
	b-4	3.68	Negative
	b-7	5.48	Positive
	b-8	6.88	Negative
	c-1	6.27	Negative
	c-2	8.00<	Negative
	d-1	7.00	Negative
Phnom Penh	p-1	4.06	Positive
	p-2	7.18	Positive
	p-3	7.14	Negative

CONCLUSION

In this study, we evaluated the hygiene conditions of local wet markets in KPC and investigated the safety level and chemical properties of pickled products sold at wet markets in KPC and PP. The pickle samples were found to be highly contaminated with unwanted microbes and these products

were unsuitable for consumption. This result strongly suggested that the principle cause of food contamination is improper food handling. In Cambodia, food handlers and vendors seem to lack sufficient knowledge of food safety and do not have the opportunity to practice safe food handling. Food safety education is considered to be one of the challenges for the future to reduce the incidence of food poisoning.

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