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## **Microbiological Hazard Contamination in Fermented Vegetables Sold in Local Markets in Cambodia**

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**Fermented vegetables are common part of Cambodian diet. The food safety status for these foods has not been investigated. This study was conducted to evaluate the microbiological hazards that contaminated fermented vegetables. A total of 68 samples of fermented vegetables were purchased randomly from five wet markets in Phnom Penh. The conventional culture methods for microbiological analysis were used. Coliform bacteria (*Escherichia coli*, *Cronobacter sakazakii*, and *Enterobacter* spp.), opportunistic non-*Enterobacteriaceae*, *Enterococcus* spp., *Staphylococcus* spp., and *Listeria* spp. were found in these fermented foods. The highest contamination rate of *Enterococcus* spp. was 34% of total fermented vegetable samples, followed by *Bacillus* spp. coliform bacteria and *E. coli* (31%, 24% and 10%, respectively). The potential foodborne pathogen, *C. sakazakii*, was identified in one sample. Fermented mixed vegetables showed higher contamination rate of coliform bacteria (50%) than fermented single-type vegetables (13%). The results showed that fermented vegetables sold in wet market are poor in hygiene. The stage in the processing chain where contamination occurred should be identified and basic sanitary practice should be enforced to improve the food safety of fermented vegetables in Cambodia.**

**Key words :** Fermented vegetables / Bacterial contamination / Food safety.

Vegetable play an important role in daily dish for the nutrient perspectives. However, the availability of certain fresh vegetables is not stable through the year. Fermentation is one of the oldest methods of food preservation with improving the nutritional value, organoleptic properties and digestibility of food raw material and can remove anti-nutritional factors to make the food safe to eat (Smid and Hugenholtz, 2010; Jägerstad et al., 2005). Therefore, fermented vegetables have an important role in providing foods for the world's population (Battcock and Azam-Ali, 1998).

With the popularity of traditional fermentation, many kinds of fermented Cambodian vegetables have emerged, depending on varieties of domestic raw vegetables, such as cucumbers, mustard greens, carrots, bean sprouts, cabbages, African cabbages, daikon radish, shallots,

papaya etc. Fermentation of vegetables produced in Cambodia generally occurs "spontaneously" by the natural lactic bacterial in surface microflora. These foods are usually prepared by soaking in brine, 5-6% salt along with single-type vegetable or mixed with other vegetables or ingredients such as ginger, chili, and garlic. It takes only 2-3 days at room temperature (25-35°C) for fermentation. The obtained goods are sold as soon as possible at wet markets. Some kinds of fermented vegetables, such as fermented salty daikon radishes with high salt concentration (20-25%), have been dried for few days before storage for long time. The Cambodian fermented vegetables range in pH from 3.6-6.5, depending on the raw materials and the recipe of fermentation.

The consumption of such lactic acid fermented vegetables helps to enhance human nutrition, to promote the growth of healthy intestinal flora as well as the positive health image of probiotics (Yamano et al., 2006). However, improper handling and contamination of raw

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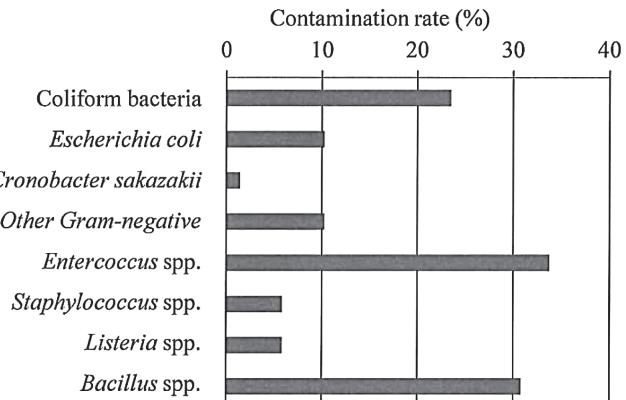
materials with pathogenic bacteria during marketing could pose a potential risk for the consumers (Inatsu et al., 2004). Foodborne outbreaks are commonly reported in Cambodia, but the scientific reports on food safety status for these foods has not been well documented. Therefore, this study aimed to evaluate microbiological hazards by contaminated fermented vegetables, which were produced and sold in five different open markets in Phnom Penh, Cambodia.

Fermented vegetables were randomly purchased from different wet markets within the city of Phnom Penh, Cambodia. Sixty eight samples were collected in March and July, 2014. The samples were composed of two categories, in which 48 samples were fermented single-type vegetable and the other 20 were fermented mixed vegetables. The purchased samples were put in sterile zippered plastic bags and kept less than 5 days in 5°C until the experiments.

Referring the methods used for the surveillances of Thai raw foods (Ananchaipattana et al. 2012), *E. coli*, *Salmonella* spp., *C. sakazakii*, *Enterococcus* spp., *Staphylococcus* spp., *Bacillus* spp. and *Listeria* spp. were isolated from each of collected samples by conventional culture methods. Chromocult Coliform agar, MLCB agar, Chromocult Enterobacter Sakazaki agar, EF agar, Mannitol Salt agar, NGKG agar and Oxford Agar were used for isolating each of bacteria listed above. These selective media had been purchased from Nissui Co., Ltd., Tokyo, Japan and Merck, Darmstadt, Germany.

Typical colonies developed on the suitable agar plates for the objected bacteria were purified twice on nutrient agar plates and subjected to brief identification by microscopic observation after Gram-staining and oxidase / catalase activities testing. Biochemical analysis including the use of API test kits (BioMérieux, Marcy l'Etoile, France), the immunological test including the use of NH Immunochromato Listeria (Cosmo Bio Co., Ltd., CA, USA), PS latex (Eiken Chemical C. Ltd. Tokyo, Japan) and polymerase chain reaction (PCR)-based methods including the use of *Bacillus cereus* (CRS gene) PCR Detection Kit (Takara bio Co. Ltd., Kyoto, Japan) were used for further identification of the bacteria. API 20 E, API Staph, API Strep, API Listeria and API 50 CHB test kits were used for identification of coliform bacteria, *Staphylococcus* spp., *Enterococcus* spp., *Listeria* spp. and *Bacillus* spp., respectively. U.S. food and Drug Administration's Bacteriological Analytical Manual (BAM) was used for references of the obtained results by these test kits.

The contamination rate of each of bacteria in samples was defined as the number of positive samples in the (category of) entire samples. The significance of the differences of contamination rate of bacteria between the one of fermented single-type vegetables and the one of



**FIG. 1.** Contamination rate of spoilage or pathogenic bacteria in collected 68 fermented vegetable samples

mixed vegetables were expressed as the P-values of odds rates calculated by SPSS version 16.0 (IBM Co. Ltd. New York, USA).

Collected 68 fermented vegetable samples could be divided into two categories. The first one consisted of 48 fermented single-type vegetables (mustard greens, cucumbers, shallots, salty daikon radish, salty sweet daikon radish and green papaya). The another consisted of 20 fermented mixed vegetables (cucumber-bean sprout, African cabbage-bean sprout, cucumber-carrot-papaya, bean sprout-chili and bean sprout-cucumber-chili-lotus root). All of these samples were qualitative analyzed for hazardous bacteria and some specific spoilage bacteria. The average salt concentration of the each category of samples was 5.6 w/w% (for fermented single-type vegetables) and 3.0 w/w% (for fermented mixed vegetables), respectively. The average pHs of the each category of samples were 4.28 and 4.44, respectively. No significant difference of salt concentration or pH of collected samples was observed ( $P>0.05$ ).

The contamination rate of isolated bacteria in all fermented vegetable samples is shown in Fig.1. The major contamination rate of *Enterococcus* spp. was 34% of total fermented vegetable samples, followed by *Bacillus* spp., coliform bacteria and *E. coli* (31%, 24% and 10%, respectively). The minor contamination rate which was 10% or below of total fermented vegetables were opportunistic Non-Enterobacteriaceae (10%), *Staphylococcus* spp. (6%: no coagulase positive *S. aureus* was found.), *Listeria* spp. (6%). and *C. sakazakii* (1%). None of isolated strains were confirmed as *Salmonella* spp. However, one sample from salty sweet daikon was contaminated with *C. sakazakii*, which is a potential pathogen. Neither cereulide synthesis (CRS) gene harboring *B. cereus* nor enterotoxin producing *B. cereus* was isolated.

All species of Gram-negative and -positive bacteria

isolated from collected samples were listed in Table 1. Isolated coliform bacteria were identified as *Citrobacter freundii*, *Enterobacter cloacae* and *Klebsiella pneumonia*. Other gram-negative bacteria (opportunistic non-Enterobacteriaceae) were identified as *Aeromonas salmonicida*, *Rhizobium radiobacter* and *Shewanella putrefaciens* group. Gram-positive coccus such as *Enterococcus faecalis*, *E. faecium*, *E. durans* and *Aerococcus urinae* were isolated. Most of opportunistic non-Enterobacteriaceae was isolated from fermented daikon radishes and shallots. *E. coli* and *Staphylococcus* spp. (*S. lentus* and *S. xylosus*) were frequently isolated from fermented mustard greens. *E. coli* contaminated also some fermented vegetables mixed with bean sprouts such as fermentation of African cabbage-bean sprout and bean sprout-chili. No *Listeria*

*monocytogenes* was isolated from 68 collected samples, however, *Listeria grayi* (non-pathogenic bacteria) was isolated from fermented shallot, cucumber, papaya and African cabbage samples.

In comparison between two categories of fermentation recipe, fermented mixed vegetables showed higher contamination rate of coliform bacteria (50%), *Enterococcus* spp. (50%) and *E. coli* (20%) than those in fermented single-type vegetables (13%, 27% and 6%, respectively) (Table 2).

In this study, we evaluated the possibility of the contamination of several potent food poisoning bacteria and spoilage bacteria in fermented vegetables sold in local markets in Cambodia (Fig.1). A high contamination rate of *E. coli* or *Enterococcus* spp. in fermented foods can indicate direct or indirect contamination with feces; it may also indicate the potent contamination risk of pathogenic bacteria such as *E. coli* O157:H7 (Keeratipibul et al., 2010). The isolation of *E. coli*, *Citrobacter* spp. and *Enterobacter* spp. from foods is also generally regarded as the possibility of fecal contamination that may cause food poisoning sometime (Leus et al., 2002). In Japan, Ministry of Health, Labor and Welfare reported that *E. coli* was isolated from 262 (12.4%) of 2118 commercially purchased fermented vegetables during 2005 to 2015; no *Salmonella* spp. nor enterohemorrhagic *E. coli* was isolated from these samples. It may require careful consideration to interpret the results of contamination rates of the indicating bacteria.

The minimum pH for growth of most Enterobacteriaceae is 3.8 (Baylis et al. 2012). However, most of obtained pH values collected fermented vegetable samples were higher than this value, permit causing contaminated coliforms and *E. coli* to grow or survive in these products. *Enterobacter aerogenes* and *E. cloacae* are the most

**TABLE 1.** Isolated bacterial species from total fermented vegetable samples

Gram negative bacteria	Gram positive bacteria
<i>Citrobacter freundii</i>	<i>Enterococcus faecium</i>
<i>Cronobacter sakazakii</i>	<i>Enterococcus durans</i>
<i>Ewingella americana</i>	<i>Enterococcus faecalis</i>
<i>Enterobacter cloacae</i>	<i>Aerococcus urinae</i>
<i>Escherichia. coli</i>	<i>Staphylococcus lentus</i>
<i>Klebsiella pneumoniae</i>	<i>Staphylococcus xylosus</i>
<i>Moellarella wisconsensis</i>	<i>Listeria grayi</i>
<i>Rahnella aquatilis</i>	<i>Bacillus cereus</i>
<i>Serratia plymuthica</i>	
<i>Aeromonas salmonicida</i>	
<i>Rhizobium radiobacter</i>	
<i>Shewanella putrefaciens</i> group	

**TABLE 2.** Comparison of contamination rate of bacterial strains between fermented single-type and fermented mixed vegetables

Bacterial strains	Fermented single-type vegetables		Fermented mixed vegetables		P-value of odds ratio
	Positive / Total	(%)	Positive / Total	(%)	
Coliform bacteria	6 / 48	13	10 / 20	50*	*0.05
<i>Escherichia coli</i>	3 / 48	6	4 / 20	20	0.11
<i>Cronobacter sakazakii</i>	1 / 48	2	0 / 20	0	0.88
Opportunistic non-Enterobacteriaceae	6 / 48	13	1 / 20	5	0.40
<i>Enterococcus</i> spp.	13 / 48	27	10 / 20	50	0.10
<i>Staphylococcus</i> spp.	4 / 48	8	0 / 20	0	0.35
<i>Listeria</i> spp.	3 / 48	6	1 / 20	5	0.84
<i>Bacillus</i> spp.	17 / 48	35	4 / 20	20	0.22

The superscript \* represents a statistically significant difference in the detection rate ( $P<0.05$ ) between fermented single vegetable and fermented mixed vegetables.

frequently encountered human pathogens among the genus *Enterobacter* (Sanders et al., 1997).

*E. faecium* and *E. faecalis* have been used as an indicator of fecal contamination and an index of processing sanitation (Obaidat and Funs, 2005). A previous study showed that *Enterococcus* spp. was detected from 73% of Thai fermented meats and fishes (Ananchaipattana et al., 2012), while in this study contamination rate of *Enterococcus* spp. in total fermented vegetables was 34%. Although some reports showed that some members of *Enterococcus* genus have been used as probiotics in food for human health promoting properties (Sivieri et al., 2008), their role in these products has not been fully elucidated. In contrast, it is recognized that *E. faecium* and *E. faecalis* exhibit widespread multiantibiotic resistance (Ananchaipattana et al., 2012) and occurrence of virulence factors (Valenzuela et al., 2008).

*B. cereus* is a spore forming bacterium, which is commonly found in the environment (e.g. soil) as well as a variety of foods. In general, most of *B. cereus* is harmless. However, some of *B. cereus* can cause two types of foodborne illness, emetic (vomiting) and diarrhoeal syndromes (Schoeni and Wong, 2005; Senesi and Ghelardi, 2010). Previous study reported that *B. cereus* was the most prevalent in fermented meat and fish samples in Thailand (Ananchaipattana et al., 2012). In this case, fermented vegetables were contaminated also at high level rate of *B. cereus* (31%). The main causes of contamination in vendor foods were the use of improper methods in the preparation, inappropriate holding temperature and poor personal knowledge for hygiene of food handlers (Barro et al., 2006). The presence of *Staphylococcus* spp. (10%) and *Listeria* spp. (10%) may also point unhygienic food handling procedures, inadequate sanitary processing places, and keeping temperature abuse over a period of time to allow for bacterial growth.

All of the vegetables used for fermentation excepting for bean sprouts were cultivated on / in soil. Even bacterial counts of bean sprouts tend to be higher than ones of the other kinds of vegetables; we could find out only a small difference of bacterial contamination rates that related to the addition of bean sprouts. The obtained results suggest that fermented mixed vegetables containing bean sprouts have significant ( $P<0.05$ ) higher risk of coliforms contamination than that of fermented single-type vegetables (Table 2). Although the contamination rates of *E. coli* (20%) and *Enterococcus* spp. (50%) in fermented mixed vegetables were also higher than in fermented single-type vegetables, the difference was not statistically significant ( $P>0.05$ ). That may due to the small number of collected samples. The result of coliforms may reflect the chance of cross contamination during mixing several kinds of vegetables under unhy-

gienic food handling because the more varieties of vegetables are used; the more food handlers are required. The food selling style of both kinds of fermented vegetables were exposed to the flies and dust that can be effective vectors of foodborne illness and should be considered as a risk to food safety (Hinrichs et al., 2014).

This study may provide the first scientific information about the hygienic situation about fermented vegetables distributed in Cambodia. Inatsu et al. (2016) have revealed the difficulty in disinfecting vegetable surface attached *E. coli* O157 or contaminated *E. coli* O157 into lightly fermented vegetables. The stage in the processing chain where contamination occurred should be identified and basic sanitary practice should be enforced to improve the food safety of fermented vegetables for consumers.

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