Research article

Microbial Behavior in Cambodian Homemade-style Pickles

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Abstract Pickled products made using vegetables are popular in Cambodia. Many types of homemade pickles are sold at wet markets. Our previous study showed that pickles are seriously contaminated by microbes, including food poisoning bacteria. Such contamination may depend on several factors. The objectives of this study were to investigate the levels of contaminating microbes and their growth patterns in pickles prepared according to traditional Cambodian recipes and to suggest an improved method to ensure the microbial safety of homemade pickles. Three kinds of pickles, namely cucumber pickles with or without fish sauce and bok choy pickles, were prepared following the methods used by local people. Five sample bottles for each kind of pickle were prepared to observe changes in their characteristics over time. The Brix values, salt concentrations, and pH of the samples were measured. Next, we conducted microbiological examination of the samples by testing the presence of total viable bacteria, coliforms, and fungi. More than 5.00 $\log_{10}$ CFU/g of total bacteria were detected in all pickle samples at day 0; moreover, the number of bacteria increased until day 4. A similar concentration of coliforms was observed. Some samples tested positive for fungi. In conclusion, all samples were contaminated by harmful

microorganisms that may cause food spoilage. The results of this study revealed that microbial growth occurred in all tested pickles, resulting in a high risk of food spoilage and food poisoning. We suggest that an additional step of pasteurization would help provide microbiologically safe products. Moreover, use of food additives that do not alter the taste of pickles may allow maintenance of low levels of microorganisms.

Keywords pickles, microorganisms, contamination, microbial quality

INTRODUCTION

Pickled products made using vegetables are popular in Cambodia. Many types of homemade pickles are sold at wet markets. In our previous study, we showed that pickles are seriously contaminated by microbes, including food poisoning bacteria (Muramatsu, 2020a, b). Despite the seriousness of this issue for local people in Cambodia, there are few reports on the food safety of homemade products. In particular, lack of surveys on food poisoning cases in the local population makes it hard to grasp the current state of food safety in Cambodia.

Food safety is a pivotal issue for all countries. Indeed, food safety and/or food sanitation is indispensable for human quality of life and promotes not only good health but also economic growth (Fung, 2018). Nevertheless, the World Health Organization (WHO) reported approximately 600 million cases of foodborne illness and more than 400,000 cases of death from foodborne illness in 2010 (WHO, 2015). Foodborne illness is a serious threat in both developing and developed countries. Most outbreaks of illnesses associated with food consumed at home have been under-diagnosed and/or under-reported (Redmond and Griffith, 2003; Keegan, 2009; Vrbova, 2012). In particular, it is estimated that less than 1% of such cases are reported in developing countries (Satcher, 2000). Especially in developing country, food poisoning incidents are underestimated (Bhaskaran et al., 2020; Rusnan et al., 2020; Le et al., 2021). Thus, investigating only food poisoning incidents that have been reported is not sufficient to ensure public health.

Contamination of homemade-style pickles may have several causes. For instance, microbial behavior during the preparation of pickled products is plausibly associated with food contamination. Therefore, in this study, we focused on microbial quality of homemade pickles and highlight existing issues in cooking methods and types of ingredients and seasoning used for their preparation.

In this study, three kinds of pickles, namely cucumber pickles with and without fish sauce, and bok choy pickles were prepared following the methods used by local people. The samples prepared in this study showed a highly risk to food poisoning. We aimed to investigate the microbiological quality of homemade pickles and suggest an improved method to obtain safe homemade products.

OBJECTIVE

The objectives of this study were 1) to determine the microbial quality and nutritional properties of homemade-style pickles, and 2) to suggest an improved method to obtain safe homemade products.

METHODOLOGY

Materials and Pickle Production Process

Three kinds of pickles were prepared according to recipes used by local Cambodians. The ingredients and seasonings of the pickles are shown in Table 1. Sugar, salt, soy sauce, and fish sauce were purchased in Cambodia and used for seasoning. The other materials used were purchased in Japan. Bok choy was used instead of mustard because of unavailability of the latter. Bok choy belongs to the *Brassica* genus similar to mustard. Cucumbers were washed with tap water and pat dried with a paper towel. The cucumbers used for sample A were rubbed with salt, compressed using a weight, and left to stand for 60 minutes. Then, the salt on the surface of the

cucumbers was wiped off with a paper towel. The cucumbers were cut to fit into a glass bottle and used until the stem end and the tip. For bok choy, the end of each bunch was first cut off, and leaves were separated from the stalks. The leaves were then washed with tap water and pat dried with a paper towel. Garlic and a small onion were peeled and sliced. A piece of chili was sliced into rings. After placing the mixed ingredients and liquid seasoning into glass bottles, these were kept at 30°C for 4 days until the products were ready (Fig. 1). Since the original homemade-style process includes keeping the mixture under the sun for 4 days, in this study, the pickles were kept at 30°C under conditions close to those of the traditional method. Five samples for each kind of pickle were prepared in order to observe changes in their characteristics over time. A mixed sample was kept at 30°C for 30 minutes and used as the sample at day 0. Another sample was kept at 30°C for 20-24 hours and considered the sample at day 1, and samples at days 2-4 were prepared in a similar manner. The samples were stored at 4°C for subsequent analysis.

Table 1 Ingredients and seasonings used in the three kinds of pickles

Ingredients and seasonings	Sample A	Sample B	Sample C
	Cucumber with fish sauce	Cucumber without fish sauce	Bok choy
Cucumber (g)	500	500	-
Bok choy (g)	-	-	500
Steamed rice (g)	-	-	18.0
Sugar (g)	80.0	-	6.0
Salt (g)	70.0	17.5	16.0
Soy sauce (mL)	48.0	-	-
Fish sauce (mL)	48.0	-	-
Hot water (mL)	300	-	-
Water (mL)	-	400	400
Garlic (g)	15	18.75	-
Small onion (g)	5	-	-
Chili (piece)	1	1	-

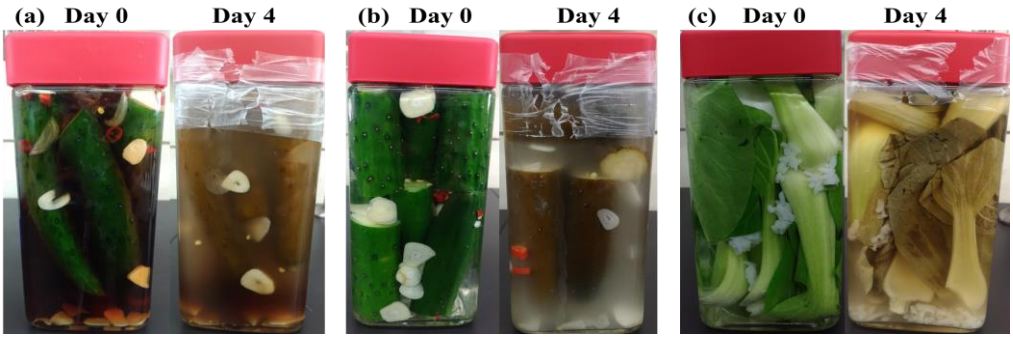

Fig. 1 Appearance of pickles at day 0 and day 4
(a) sample; cucumber with fish sauce, (b) sample B; cucumber without fish sauce, and (c) sample C; bok choy

Measurement of Food Properties

Since the sugar and salt contents and pH of foods affect microbial growth, their values can be good indicators of the preservability of food. Therefore, we measured the Brix sugar content (soluble solids content), salt content, and pH of the pickled liquid of the samples using a Brix refractometer (Atago, Tokyo, Japan), a salt meter (Horiba, Kyoto, Japan), and a pH meter (Horiba), respectively, for all prepared samples.

Detection of Microbes in the Samples

After collecting all samples, several microbial detection assays were performed. Ten grams of each sample containing pickled ingredients and seasoning liquid was mixed with 90 mL of Maximum Recovery Diluent (Merck KGaA, Darmstadt, Germany) solution in a sterilized storage bag. To obtain homogeneous suspensions, samples were homogenized using a Stomacher blender. After stomaching, 1 mL of sample suspension was mixed with 9 mL of Maximum Recovery Diluent solution in a sterile tube. A series of diluted samples was used for the detection of total viable bacteria, coliforms, and fungi. Standard agar medium (AS ONE, Osaka, Japan) was used for the detection of total viable bacteria. Desoxycholate agar medium (AS ONE) was used for the detection of coliforms. Solid plates were incubated at 35 ℃ for 48 hours. For the detection of fungi, plates with Potato Dextrose Agar medium (AS ONE) were incubated at 30 ℃ for 5 days.

RESULTS AND DISCUSSION

Nutritional Properties of Pickles Prepared According Traditional Recipes

The nutritional properties of the final pickled products are shown in Table 2. Sample A showed the highest Brix value and salt concentration. The measured pH values varied slightly among replicates. The pH value could be affected by the microbes present in the samples; therefore, differences in the identity of contaminating microorganisms would result in variations in pH.

Table 2 Nutritional properties of final pickled products

Measured item	Sample A	Sample B	Sample C
	Cucumber with fish sauce	Cucumber without fish sauce	Bok choy
Brix	11.2	4.4	4.7
pH	4.55	4.5	4.88
Salt (%)	5.1	2.3	2.4

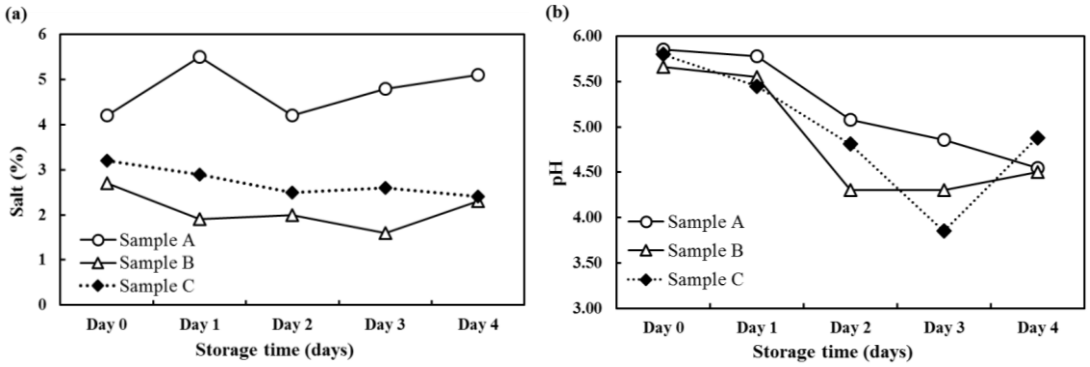

Fig. 2 Changes in chemical properties of the three kinds of pickles (a) salt concentration and (b) pH

The Brix values of all pickles did not change much from day 0, just after pickling, to day 4, and were approximately constant (data not shown). The lack of decreasing Brix values indicates that the microorganisms in samples A and C were unlikely to utilize sugar. During the 4-day storage period, the salt concentrations in samples B (cucumber without fish sauce) and C (bok choy) decreased slightly, whereas that in sample A (cucumber with fish sauce) remained almost constant (Fig. 2(a)). All samples first displayed pH values of approximately 6, but these gradually decreased over time (Fig. 2(b)). Decreasing pH values indicated that the microbes in the samples produced acids. *Clostridium botulinum*, which can trigger deadly diseases, can grow at a minimum pH of 4.6. Improper home canning of vegetables and improperly fermented and processed foods have caused outbreaks in many countries (Matthews, 2017). In particular, pickles in sealed containers with pH higher than 4.6 seem to have considerable potential to cause severe food poisoning. Moreover, the Brix value and salt concentration of pickles are presumably not sufficient

to inhibit the growth of most bacteria and yeasts; therefore, molds grow regularly in pickles (Matthews, 2017).

Microbial Behavior in Homemade Pickles

A total of five samples (from day 0 to day 4) for each of the three kinds of pickles were used to observe changes in microbial composition over time. We tested the presence of total viable bacteria, coliforms, and fungi (Fig. 3). More than 5.00 $\log_{10}$ CFU/g of total bacteria were detected in all pickle samples at day 0; also, the number of bacteria increased until day 4 (Fig. 3(a)). Coliforms were detected at a level similar to that of total viable bacteria. The initial number of coliforms in samples B and C was almost 5.00 $\log_{10}$, and sample A also tested positive for these bacteria. The concentration of coliforms increased until day 4 (Fig. 3(b)). Coliforms are considered indicators of fecal contamination; however, they have been recently acknowledged to be able to grow in non-fecal sites such as food, water, and waste (Matthews, 2017). Therefore, contamination by coliforms, as shown in Fig. 3(b), does not directly imply insanitary conditions. Izumi (2010) reported that vegetables dipped in tap water showed a total bacterial count of approximately 5 $\log_{10}$. This suggests that only washing vegetables with tap water does not eliminate harmful microorganisms.

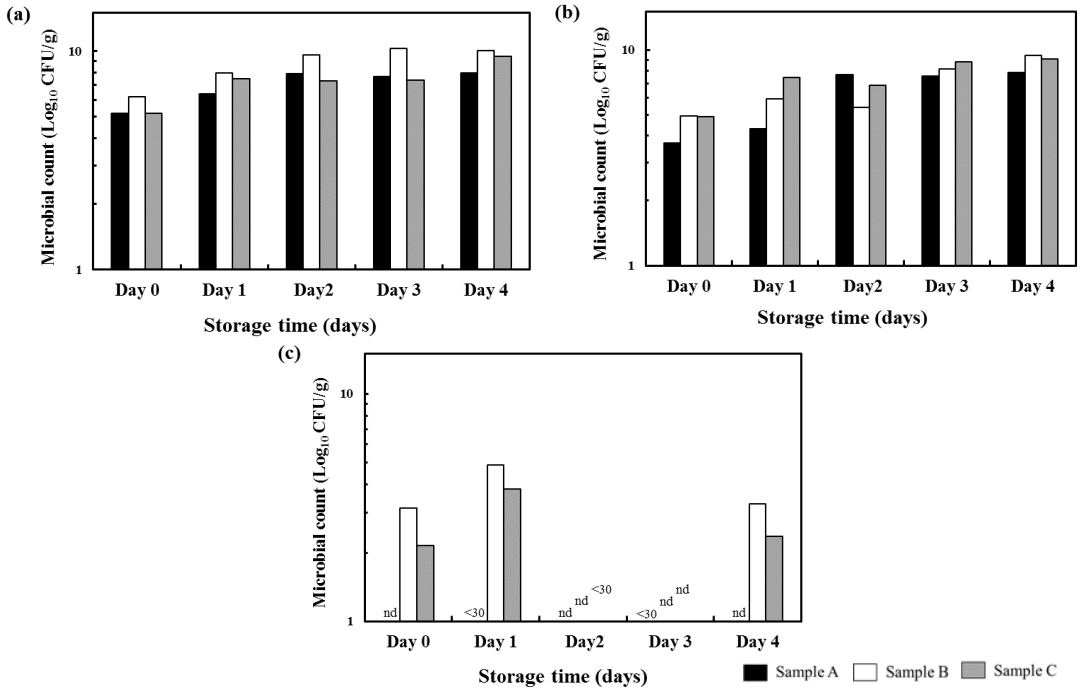

Fig. 3 Changes in the number of microbes in the three kinds of pickles (a) total viable bacteria, (b) coliforms, and (c) fungi

Colony observation on agar plates revealed that many samples were contaminated by dangerous microorganisms that could cause food spoilage. Thus, none of the prepared pickles were safe to eat. Fungi were not detected on day 0 in sample A, and some samples tested negative or positive for fungi on days 1-4. Conversely, samples B and C showed fungal contamination on days 0, 1, and 4. We believe that continuous growth was not observed because each sample for microbial counting was placed in an independent bottle in order to prevent contamination of the sample once opened for analysis. The microflora of vegetables is generally composed by 80% bacteria and 20% fungi (Izumi, 2008). This observation suggests that bacteria remained in all samples prepared by washing with tap water, but fungi were eliminated from some samples. The results of this study suggest that microbial growth occurred in all three kinds of pickles, resulting in a high risk of food spoilage and food poisoning. Therefore, the current production process needs to be modified to improve microbial quality. The addition of a pasteurization step and/or the use of

food additives is an effective and practicable solution. Similar cucumber pickles containing a similar amount of salt, after storage at 30°C for 2 days, were reported to carry 8 $\log_{10}$ CFU/g of total viable bacteria and maintain this initial concentration for 8 days at 4°C (Miyao, 2004). Moreover, some trials of pickle production showed that the products were of uneven quality. This implies that the traditional methods and ingredients used in this study do not allow spontaneous desirable fermentation into safe products.

In this study, pickles were prepared following homemade-style recipes and analyzed for their microbial quality. We suggest that pasteurization is required to obtain microbiologically safe products. Moreover, the use of food additives such as glycine, which does not alter the taste of pickles, sorbic acid, or alcohol during preparation seems to be effective in controlling the concentration of microorganisms.

CONCLUSION

In this study, three kinds of pickles were prepared following local traditional methods. To observe changes over time, five pickle samples were prepared for days 0 to 4. The Brix value, salt concentration, and pH of the pickling liquids were measured, and microbial detection assays were performed. More than 5.00 $\log_{10}$ CFU/g of total bacteria was detected in all the samples at day 0; also, the number of bacteria increased until day 4. Coliforms showed concentrations similar to those of total viable bacteria. Some samples were positive for fungi. In conclusion, all samples were contaminated by harmful microorganisms that could cause food spoilage.

The results of this study revealed that microbial growth occurs in all pickles, posing high risk to food spoilage and food poisoning. We suggest that pasteurization would help obtain microbiologically safe products. Moreover, use of food additives that do not alter the taste of pickles may allow maintenance of low levels of microorganisms.

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