Research article

Terd Improvements in Preservation Properties of Homemade-Style Pickled Cucumber in Cambodia

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Abstract Pickled cucumbers selling at wet markets in Cambodia are popular among local people. It has been reported that pickles using homemade recipes are at a high risk of spoiling and causing food poisoning, as there are no heating steps in the production process. Therefore, it is necessary to improve the hygienic conditions of the food supply to make safe pickles in Cambodia. Heat treatments using hot pack filling and pasteurization lead to microbiologically

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safe products and extended shelf life. This study aimed to examine the effect of heat treatments (hot pack filling and pasteurization) on preservative properties and to evaluate the acceptability of the pickled cucumbers prepared with the heating process. Pickled cucumbers prepared with and without the heating process were made using a traditional Cambodian recipe and stored at 30 °C for 7 days. The presence of total viable bacteria, coliforms, *Escherichia coli*, yeasts, and molds in the pickled products was tested during the storage periods. The samples without the heating process resulted in contamination with coliforms, E. coli, yeasts, and molds and were in an initial putrefactive state because of the number of total viable bacteria. On the other hand, coliforms, E. coli, yeasts, and molds were not detected in the samples prepared with the heating process from Day 0 to Day 7. This result indicated that the pasteurization treatment killed many spoilage microbes, including foodborne pathogens. To investigate the acceptability of the pickled cucumbers prepared with the heating process, sensory evaluation tests were performed. From the results of the sensory evaluation and questionnaire survey, there was the possibility of acceptance of the pickled cucumbers prepared with the heating process made either in Japan or Cambodia by the Cambodian panelists; however, further improvement in the softness of the pickles was commented upon.

Keywords cucumber, homemade-style pickle, pasteurization, sensory evaluation, acceptability

INTRODUCTION

Pickled cucumbers are popular pickles in Cambodia. Nearly all Cambodian people like to eat pickles in their daily diet. These pickles are mostly produced at home using traditional recipes and are usually sold at wet markets in Cambodia. The Ministry of Health in Cambodia reported that after eating these homemade-style pickled cucumbers, many people suffer from symptoms of food poisoning, including diarrhea, abdominal pain, fever, and nausea (MoH, 2016). A review report has described that important foodborne pathogens have been detected in the food supply in Cambodia (Thompson et al., 2021). Studies by Muramatsu et al. (2020a, 2020b) and Chrun et al. (2017) showed that some microorganisms could cause food spoilage and food poisoning in homemade pickled products sold at wet markets. The current preparation method for homemade-style pickles is generally as follows. Fresh vegetables are washed with tap water. Then, they are submerged in boiled water or normaltemperature water along with seasonings. The pickles are then soaked for two to four days at room temperature. The room temperature ranges from 25 to 35 °C according to the tropical climate of Cambodia. Homemade pickles are commonly stored at room temperature at approximately 30 °C during and after the production of pickles. Muramatsu et al. (2022) prepared three kinds of homemade-style pickles using traditional Cambodian recipes and evaluated the microbial quality of those pickles. The report revealed that microbial growth was observed in all pickles starting at Day 0, which poses a high risk of food spoilage and food poisoning. Therefore, we focused on the production process for obtaining microbiologically safe products. Therefore, this study aimed to examine the effect of thermal preservation methods on the microbial quality of pickled cucumbers. The heating step is considered to affect the taste of the pickled cucumber, and therefore we also investigated the acceptability by Cambodian people.

Pickled cucumbers were prepared with and without the heating process following the Cambodian homemade-style recipes. For the purpose of providing safe pickle products in Cambodia, we aimed to investigate the effect of the heating process (hot pack filling and pasteurization) on the microbial quality of cucumber pickles. The acceptability of these pickled cucumbers was examined based on sensory evaluation in Cambodia.

OBJECTIVE

The objectives of this study were 1) to examine the effect of the heating process (hot pack filling and pasteurization) on the preservative properties of the pickled cucumbers and 2) to evaluate the acceptability among Cambodian people of the pickled cucumbers prepared using the heating process.

METHODOLOGY

Materials, Production Process, and Procedure for Samples Testing

The pickled cucumbers were prepared with and without a heating process according to traditional Cambodian recipes (Muramatsu et al., 2022) as samples for the test. All ingredients and seasonings were purchased at a supermarket in Tokyo, Japan. The seasoning mixture was prepared using 30.6 grams of salt and 700 ml of water. The ingredients used to make the samples were 200 g (5 pieces) of cut fresh cucumbers, 4.5 g (1 piece) of garlic, and 1.0 g (2 pieces) of chili. All ingredients were mixed with the seasonings. Figure 1 shows the production process of pickled cucumbers with the heating process. Cucumbers were washed with tap water and wiped with a paper towel. Cucumbers with diameters of 2.2-3.2 cm were cut into lengths of 7 cm using a knife. The garlic was peeled and sliced. A piece of chili was sliced into rings. The liquid seasoning was heated to boiling. The heated liquid seasoning, 120 ml, a piece of cucumber, and a few slices of garlic and chili were placed into a glass bottle up to 3-5 mm from the bottle lid. After closing the lid of the bottle (sealing), the bottle was turned upside down. The hot glass bottle filled with seasoning and cucumber was submerged in hot water at a temperature of 80 °C. Then, the hot water was heated to boiling in a pot. After coming to a boil, the glass bottle was maintained in boiling water for 10 minutes to raise the center temperature of the cucumber to above 75 °C. After this pasteurization, the glass bottle was cooled at room temperature in an inverted state for a few hours.



Fig. 1 Production process of pickled cucumber prepared with the heating process

The pickled cucumbers without a heating process were prepared using the same seasoning and ingredients as the heated sample shown in Fig. 1. After placing the ingredients into the glass bottle, the liquid seasoning at room temperature was added. The lid of the glass bottle was closed.

Preparation of the Samples with the Heating Process for Examination of Preservability

To examine the preservability of cucumber pickles, the samples were observed for 7 days, as the vendors who sold pickles at wet markets reported that the shelf life of their products was approximately 2-4 days according to our survey and that the products were kept for 3-4 days during preparation by the traditional recipe.

The food properties of the samples during the storage periods, the Brix values, salt concentrations, and pH of the pickle liquid seasoning, were measured by using a Brix refractometer (Atago, Tokyo, Japan), a salt meter (Horiba, Kyoto, Japan), and a pH meter (Horiba), respectively.

To investigate the safety of the samples, microbiological tests were conducted by detecting total viable bacteria, coliforms, *E. coli*, yeasts, and molds. In total, 10 g of solid and liquid pickled cucumber was mixed with 90 ml of phosphate-buffered saline (PBS) (ELMEX, Tokyo, Japan) and placed in a sterilized storage bag. To mix well, samples were crushed using a hammer. After mixing, 1 ml of the sample suspension was mixed with 9 ml of PBS. A series of diluted samples were used for the detection of total viable bacteria, coliforms, *E. coli*, yeasts, and molds in the samples. Commercial sheet-shaped, dry, and ready-made microorganism detection media with nonwoven fabric, MC-media PadTM (JNC Corporation, Tokyo, Japan), were used for the detection of microorganisms. One milliliter of sample solution at each dilution was spread onto each specific MC-

media PadTM. After spreading, the MC-Media PadTM "Aerobic Count (AC)", MC-Media PadTM "EC", and MC-Media PadTM "YM" were incubated at the set temperature of 35 °C for 48 hours, 24 hours, and 25 °C for 72 hours, respectively for assessment of total viable bacteria, coliforms, *E. coli*, yeasts, and molds. After incubation, the log_{10} CFU/g was calculated using media on which there were 30-300 colonies available to count. Three repetitions were carried out for the detection.

Sensory Evaluation of the Pickled Cucumber Made in Japan and Cambodia

For sensory evaluation, we prepared pickled cucumber samples with two pasteurization times, 10 and 20 minutes, similar to the samples prepared for the examination of preservability, as shown in Fig. 1. It was assumed that a longer pasteurization provided a higher killing effect on the microbes but that the texture might be unfavorably affected. Therefore, two kinds of pickled samples were evaluated. The pickled cucumbers pasteurized for 10 minutes raised the center temperature to above 75 °C, and those pasteurized for 20 minutes raised the center temperature to above 80 °C.

We aimed to show that the pickles made in Japan following Japanese sanitation guidelines were also able to produce in Cambodia, especially by using household cooking utensils. Besides, varieties of cucumber for pickles are assumed to be different between Cambodia and Japan. And the difference may affect the texture of the pickles, especially with heating samples. It was also important that how acceptable for Cambodian people both pickles made in Cambodia and Japan. Therefore, we prepared pickles made in Cambodia and Japan using local products for sensory evaluation.

The samples made in Japan were prepared using ingredients and seasonings purchased in Japan. The samples made in Cambodia were prepared using ingredients and seasonings purchased in Cambodia. All sensory was conducted in Cambodia. The panelists evaluated 2 samples with heating for 10 and 20 minutes. Twenty panelists evaluated the samples made in Japan at the Royal University of Agriculture (RUA) in Phnom Penh, and another 20 panelists evaluated the samples made in Cambodia at a wet market in Prey Nop district in Preah Sihanouk. The purpose of sensory evaluation in this study is not a comparison pickles made in Cambodia and Japan. We aimed to investigate the acceptability of respective pickles. Therefore, no particular problem occurs when the panels were different between the products in Cambodia and Japan.

The age of the evaluating panelists was 21-28 years (average: 23 years) at RUA and 22-60 years (average: 31 years) at the wet market. The color, sweetness, sourness, saltiness, flavor, texture, and overall evaluation of each sample were quantified using a five-point hedonic scale (1: strongly dislike, 2: dislike, 3: neutral, 4: like, and 5: strongly like) for the sensory test (preference type).

RESULTS AND DISCUSSION

Examination of the Preservability of the Pickles Prepared with the Heating Process

To investigate the effects of the heating process on the quality of the samples, the nutritional properties, and microbial quality were examined. During the storage period of 7 days, the Brix value, salt concentration, and pH values were measured (Table 1). The average Brix values of the pickled cucumbers prepared with and without the heating process were 4.9 and 4.2, respectively. There is a significant difference in the value of Brix. But the difference in Brix values between pickles with heating and without heating is assumed not to affect the preservation stability of the samples. The average salt concentrations in the pickles prepared with and without heating were 4.9 and 4.3, respectively. The Brix value and salt concentration were generally constant throughout the storage period.

On the other hand, the pH value of the sample prepared with heating was 5.3 on Day 0 and 5.3 on Day 7, and that of the sample without heating was 5.5 on Day 0 and 4.3 on Day 7 (data not shown). While the pH values of the heated samples were stable, those of the nonheated samples decreased over time. This indicated that the pickled cucumber prepared without the heating process contained microorganisms producing acids in the samples.

Measured item	Brix (%)	Salt (%)	pH
Pickles with heating	4.9 ± 0.25 *	4.9 ± 0.20	5.3 ± 0.18
Pickles without heating	4.2 ± 0.10 *	4.3 ± 0.41	4.6 ± 0.35

Table 1 Nutritional properties of the pickles prepared with and without heating

(Mean \pm standard deviation), the symbol (*) means a significant difference (p < 0.05).

Next, we examined the microbial quality of the pickled cucumbers by measuring the number of total viable bacteria, coliforms, *E. coli*, yeasts, and molds. The total viable bacteria on Day 0 were more than 5.00 \log_{10} and over 7.00 \log_{10} after Day 3. Coliforms were detected in samples from Day 0 to Day 7, and there were samples positive for *E. coli*. Yeasts and molds were detected in some samples. A similar result was reported by Muramatsu et al. (2022). From the result of the experiment for measuring the microbial quality of the pickles without heating, the pickles contained unacceptable numbers of total viable bacteria on Day 3 for eating. Besides, coliforms, *E. coli*, yeast, and mold were detected. Yeast and mold can cause putrefaction. Contamination of *E. coli* implies a risk of the presence of other enteric pathogens. Enterohemorrhagic *E. coli* is classified as a severe hazard, which is life-threatening for the general population, based on International Commission on Microbiological Specifications for Foods (Matthews et al., 2017). Taking this into consideration, the pickled cucumbers without a heating process in this study were not safe for consumption.

On the other hand, coliforms, *E. coli*, yeasts, and molds were not detected in any of the samples subjected to the heating process. In addition, total viable bacteria were almost not detected throughout the storage time. The heating time for pasteurization was decided based on the effect of heating on the texture of the cucumber. Due to this, the bactericidal effect of the heated samples seemed to change depending on this condition. However, the number of CFU/g among the samples in which some total viable bacteria were detected was at most $5.00 \log_{10}$. These results indicate that the heat treatment was effective in killing microbes, but this heating step was barely effective for some microorganisms. For food preservation, a sufficient amount of salt and/or a lower pH value is supposed to be used. Since the samples with the heat treatments, namely, the hot pack filling and 10 minutes of pasteurization, maintain their qualities sufficiently until Day 7, the process of adding heat treatments may be a suitable procedure to make safe products that do not change the traditional taste.

Sensory Evaluation of the Samples Made in Japan and Cambodia

To investigate the acceptability characteristics of pickled cucumbers during the heating process, sensory evaluation tests were performed. Fig. 2 shows the results of the sensory evaluation tests of samples (a) made in Japan and (b) made in Cambodia. The average values of each sensory item for the pickled cucumbers pasteurized for 10 min and 20 min made in Japan ranged from 2.8-3.6 and 2.4-3.8, respectively (Fig. 2a). In the overall evaluation, both the 10- and 20-minutes pasteurization samples scored approximately 3.0. There was no significant difference in accordance with the paired t-test (5% significance level) between all characteristics, except for texture. The average value of texture for the samples with 10 min of pasteurization was higher than that of the sample with 20 min of pasteurization, which was 3.4 and 2.4. This result showed that longer pasteurization affected the texture of the cucumber. However, the overall evaluation scores were both approximately 3.0. This is a representative evaluation of whether or not the sample was considered acceptable. The results indicated that the samples made in Japan were considered acceptable in the Cambodian market. Figure 2b shows the results of the evaluation of pickled cucumbers with the heating process made in Cambodia. The average scores of all evaluated items ranged from 2.6-3.6 and 2.3-4.0, respectively. Except for color and texture, there were no significant differences between the evaluated items according to a paired t-test (5% significance level).

Because the average score for color was close to 4.0, Cambodian panelists preferred the color of the sample with pasteurization for 20 min made in Cambodia. The texture of the sample with pasteurization 20 min was given a lower rating score of 2.3. The heating treatment presumably caused

a change in the texture of the cucumbers. Although the average values of sourness and flavor for both samples did not exceed 3.0, the overall evaluation score given by the panelists was nearly 3.0. Based on the results, the samples pasteurized for 10 min and 20 min made in Cambodia may also be acceptable in the Cambodian market. In addition, the t-test for each characteristic used the average score between the samples made in Japan and Cambodia with 10 and 20 minutes of pasteurization. There were no significant differences. Since a similar evaluation was obtained, there did not seem to be differences in quality.



The symbol (*) means a significant difference (p < 0.05).

Fig. 2 Sensory evaluation of pickled cucumbers with pasteurization for 10 min and 20 min: (a) samples made in Japan and (b) samples made in Cambodia

Most Cambodian panelists commented that there was a lack of sourness for these pickled cucumbers. Some panelists commented that sweetness and saltiness were lacking. If these characteristics were to be improved, the overall evaluation would receive a higher rating. The heating treatments, hot pack filling, and pasteurization were effective in killing microorganisms and were recommended for pickle production. Although a longer heating process affected the texture (softness) of the pickled cucumbers, they received a nearly normal score on the overall evaluation from the Cambodian panelists. From the results of the sensory evaluation and questionnaire survey, there was the possibility of acceptance of the pickled cucumbers prepared with pasteurization made either in Japan or Cambodia by the Cambodian panelists; however, further improvement in the softness of the pickles was commented upon. Regarding improvements for the product, some food additives are recommended as they do not affect the softness of pickles and are effective in keeping microbiological safety.

CONCLUSION

To investigate the effect of the heating process on the preservative properties of pickled cucumber, pickles using a traditional Cambodian were prepared. While the products that did not undergo the heating process were highly contaminated by microbes, the products prepared with the heating process did not show the presence of coliforms, *E. coli*, yeasts, and molds for 7 days at 30 °C. This result showed that the heating process killed many spoilage microbes, including foodborne pathogens. The heating process is considered to be effective in providing safe products and extending shelf life. Sensory evaluation tests were conducted in Cambodia. From the results, there was the possibility of acceptance of the pickled cucumber prepared with the heating process by the Cambodian people. There were comments from panelists that further improvement in the softness of the pickles was needed.

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