Fundamental Toxicological Sciences (Fundam. Toxicol. Sci.)
Vol.7, No.4, 167-170, 2020

The Japanese Society of Toxicology

Fundamental Toxicological Sciences
URL : http://www.fundtoxicolsci.org/index_e.html

Letter

Bacteriological evaluation of feasibility of food poisoning from long-term stored "pack-cooked" meals

Toshihiro Kobayashi and Harumi Sakuda

Faculty of Human Sciences, Kobe Shoin Women's University,
1-2-1, Shinohara-obanoyama-cho, Nada-ku, Kobe 657-0015, Japan

(Received April 22, 2020; Accepted May 3, 2020)

ABSTRACT — Recently, a "pack cooking" method, which uses heat-resistant plastic bags, has attracted attention for being simple and safe for emergencies such as natural disasters. One serving is filled in a pack. It uses less kitchenware and clean water, so "pack cooking" will particularly be a powerful tool for soup runs. However, its risk for food poisoning has not been given attention. It is important to consider the safety of "pack-cooked" meals, because medical shortage is as much a problem as food shortage during natural disasters. We conducted a bacteriological evaluation of curry and rice as typical "pack-cooked" meals, assuming that they were stored at room temperature for a long time. "Pack-cooked" meal samples were stored at 25°C and 4°C for 24 hr, and their homogenates were used as sample for bacteriological analysis. Obvious standard plate count bacteria were found in "pack-cooked" curry, which was stored at 25°C for 24 hr, whereas those stored at 4°C for 24 hr did not. Furthermore, there was a sample that was stored at 25°C for 24 hr in which coliform bacteria were detected. Since 25°C is equivalent to room temperature, our results demonstrate that the "pack-cooked" curry stored at room temperature for 24 hr could cause food poisoning. While "pack cooking" is a useful method, it is important to understand that prolonged storage at room temperature of "pack-cooked" meals should be avoided.

Key words: Pack cooking, Heat-resistant plastic bag cooking, Food poisoning, Soup runs, Standard plate count

INTRODUCTION

During times of disasters such as typhoons, floods, earthquakes, or volcanic eruptions, safe meals without using much water, electric cooker, city gas, or clean tableware, are needed. If people's housing is damaged, evacuation shelters may be set up. There, by means of soup runs, a lot of food is consumed in spite of poor sanitary conditions. Thus, boiled or stewed dish is preferred to soup runs, because hot meals can be served to a large number of people in a short time, and also contaminated bacteria in foods are expected to be killed by heating. Recently, a plastic bag cooking method, so-called "pack cooking," has been featured, because it is considered to satisfy both sanitary conditions and convenience. "Pack cooking" uses heat-resistant plastic bags, and all foodstuffs are packed and enclosed in a bag before cooking. After cooking, "pack-cooked" meals are never opened until people eat them. Therefore, clean water is not necessary for boiling. People can open the bag and eat, so all they need is a spoon and they do not have to wash dishes. As long as they are sufficiently heated and eaten immediately, "pack-cooked" meals are very safe from a food microbiology perspective. However, the safety of "pack-cooked" meals eaten hours after cooking had not been studied. If people are worried about not having access to enough food they may store "pack-cooked" meals, which appear to be vacuum-packed, for longer periods of time. We, therefore,

Correspondence: Toshihiro Kobayashi (E-mail: kobayapi@shoin.ac.jp)

focused on bacteriological safety of “pack-cooked” meals that are left at room temperature. “Pack-cooked” meals were left at room temperature and analyzed for bacterial growth. Similarly, refrigerated ones were also analyzed.

MATERIALS AND METHODS

Preparation of food samples

Heat-resistant bag

The “pack cooking” method requires high-density polyethylene (HDPE) bags for heat-resistant foods. HDPE bags were purchased at a commercial supermarket, and heat resistance was labeled as 110°C.

Rice

Akitakomachi was purchased at a grocery store, and 80 g was weighed and placed in a HDPE bag. Tap water 120 g was added through activated carbon filter. Air was expelled. The bag was tightly closed and let to stand for 30 min to so the rice could soak up the water.

Curry stew

Vermont Curry (House Foods, Tokyo, Japan) was purchased at a grocery store, and all other ingredients were purchased from Nichibei Cook (Osaka, Japan). Potatoes were cut into 2-cm pieces. Carrots were cut into about 1-cm pieces. Onions were round sliced into 3-mm thick slices. White sausage was cut into 1-cm thick rings. The amount of foodstuffs and water per bag is shown in Table 1. All foodstuffs were placed in a HDPE bag, and the bag was closed the same way as the rice described above.

Procedures of “pack cooking”

All closed bags, except unheated ones, were placed in boiling water for 30 min. The boiled bags were picked up after 30 min and divided into three groups as follows: quickly cooled in ice water and stored immediately at −80°C in a freezer, cooled to 4°C in cold water and stored at 4°C in a fridge, and cooled to 25°C in tap water and stored at 25°C in an incubator. The first group corresponds to eating immediately after cooking. The next group corresponds to storing in a refrigerator as soon as possible after cooking. The last group corresponds to leaving the food at room temperature (i.e., 25°C) after cooking. Each of the three bags was picked after 24 hr, and each of the three bags of curry was picked after 2, 6, and 24 hr, from the 4°C fridge and 25°C incubator. The unheated ones were also assigned three bags each. All bags were stored at −80°C in the freezer until the bacterial test.

Table 1. Amount of foodstuffs per curry bag.

Foodstuff	g /pack
Curry roux (Vermont Curry)	19
Potato	50
Carrot	20
Onion	30
White sausage	20
Tap water (activated carbon filtered)	60

One pack is meal for one.

Bacterial test

All bacterial tests were performed in accordance to “Standard Methods of Analysis in Food Safety Regulation” (Japan Food Hygiene Association, 2018). For bacterial testing, the frozen bags were thawed in a 40°C-water bath for 15 min. The bags were rubbed to mix the contents and then the outside part was wiped with 70 w/w% ethanol. Then the bags were handled aseptically in a clean bench. Twenty-five g food samples were weighed and put into sterile stomacher bags, and 225 mL sterilized 0.1% peptone salt solution were added. The contents of the bags were blended by Stomacher 400 Lab Blender (Seward Medical, London, UK) for 1 min at middle speed and used for bacterial testing. For the measurement of standard plate count (SPC) bacteria, we used the pour plate method. In brief, the test solution was decimal diluted, and pipette 1 mL of each dilution was placed in a sterile petri dish, followed by 20 mL of sterile dissolved SPC agar (Standard Methods Agar; Nissui, Tokyo, Japan) keeping at 50°C was added. After the agar solidified, the petri dishes were inverted and incubated for 48 hr at 35°C. Bacterial counts were determined by counting the petri dishes with 30–300 colonies per plate and multiplying the average value of these by the dilution factor. The petri dishes for counting coliform bacteria were prepared the same way as SPC. The dishes were added 20 mL of dissolved deoxycholate agar (Nissui, Tokyo, Japan) that kept at 50°C, followed by incubation at 35°C. After 22 hr incubation, coliform bacteria colonies were counted. For *Bacillus cereus* detection, pipette 0.1 mL of diluted test solution, and was plated on egg yolk-added NaCl Glycine Kim Goepfert (NGKG) agar plate (Atect, Shiga, Japan). The agar plates were incubated for 48 hr at 32°C, and only lecithinase-positive colonies were counted, which indicate typical *Bacillus cereus*. For *Clostridium perfringens* testing, pipette 10 mL of the test solution into AnaeroPack anaerobic pouch (A-99; Mitsubishi Gas Chemical Company, Inc., Tokyo, Japan) and poured 15 mL of modified Handford agar (mHFA; Eiken Chemical, Tokyo, Japan), which had been sterilized and cooled to 50°C. The

Table 2. Results of bacterial growth in “pack-cooked” rice.

Time	SPC bacteria	Coliform bacteria	*Bacillus cereus*	*Clostridium perfringens*
Unheated	5×10^2	$< 3 \times 10^2$	ND	ND
Just after cooking	$< 3 \times 10^2$	ND	ND	ND
4°C, 24 hr	$< 3 \times 10^2$	ND	ND	ND
25°C, 24 hr	$< 3 \times 10^2$	ND	ND	ND

ND: not detected (i.e., no colony was found for 1:10 dilution)
$< 3 \times 10^2$: less than lower limit of detection (i.e., < 30 cfu/g for 1:10 dilution)

Table 3. Results of bacterial growth in “pack-cooked” curry.

Time	SPC bacteria	Coliform bacteria	*Bacillus cereus*	*Clostridium perfringens*
Unheated	1.1×10^7*	1.7×10^6*	4×10^4	ND
Just after cooking	3×10^2	ND	ND	ND
4°C, 2 hr	3×10^2	ND	ND	ND
4°C, 6 hr	$< 3 \times 10^2$	ND	ND	ND
4°C, 24 hr	$< 3 \times 10^2$	ND	ND	ND
25°C, 2 hr	$< 3 \times 10^2$	ND	ND	ND
25°C, 6 hr	$< 3 \times 10^2$	ND	ND	ND
25°C, 24 hr	1.9×10^5*	4×10^2#	ND	ND

ND: not detected (i.e., no colony was found for 1:10 dilution)
$< 3 \times 10^2$: less than lower limit of detection (i.e., < 30 cfu/g for a 1:10 dilution)
* Only when 100 or more colonies per petri dish are counted, the valid number is two digits.
# Detected in one of the three bags although in the other two bags it was ND.

contents were mixed well. The air was expelled from the pouch and sealed. The pouches were kept at 46°C in an incubator and checked to see if *Clostridium perfringens* colonies were grown or not after 24 hr incubation.

RESULTS AND DISCUSSION

Under disastrous conditions, infrastructures such as electric power and water and gas supply sometimes go down. In case of a catastrophic incident, infrastructures are often damaged, and they take a long time to repair. Therefore, it becomes difficult for people to prepare meals. Municipalities and volunteer groups will be providing meals in public facilities. The menu for these meals varies and may be simple emergency meals, while hot meals such as rice balls, soup, or curry may be served. Because these meals are assumed to be eaten immediately after being served, the safety of eating them on a later time had not been considered. In a disaster shelter, it is likely that people will stock the meals for a while, because there is not always a sufficient supply of foods, so the risk for food poisoning would be high. If people get food poisoned by meals contaminated with bacteria, insufficient medical resources also prevent successful treatment, which can lead to severe disease. This concept convinced us that food safety is more important in times of disasters than in normal times, so we analyzed bacterial contamination in meals that are “pack-cooked” and left at room temperature. Also, meals stored in a refrigerator for the same period were also analyzed in the same way, and bacterial counts were compared.

Table 2 shows the results of bacterial growth in “pack-cooked” rice after the aforementioned period. Unheated packs contained a small level of bacteria (i.e., 5×10^2 cfu/g). However, the number of SPC bacteria in the packs after cooking was less than the lower limit of detection (i.e., 3×10^2 cfu/g) in all bags, including samples stored at 25°C for 24 hr. In addition, a few coliform bacteria colonies were found only in the unheated packs but no colonies were found in all packs after cooking. *Bacillus cereus* and *Clostridium perfringens* were undetected in all packs. These results indicate that, at least for the bacteria we analyzed, no significant bacterial growth was observed in our study on “pack-cooked” rice.

Table 3 shows the results of bacterial growth in the “pack-cooked” curry after the aforementioned period. The unheated curry contained 1.1×10^7 cfu/g SPC bacteria, whereas just after cooking it contained only 3×10^2 cfu/g. According to the “Food Sanitation Act” and “hygiene standards” of the Ministry of Health, Labour and Welfare of Japan, the critical limit for SPC bacteria in heat-cooked side dish products is 1.0×10^5 cfu/g. Thus, we judged that our cooking method, which included boiling for 30 min, was enough to decrease SPC bacteria and to satisfy the public standards.

When we analyzed for SPC bacteria after keeping "pack-cooked" curry under both conditions, wherein the storage temperature was 4°C and 25°C, it remained less than the lower limit of detection for up to 6 hr. After 24 hr of storage, SPC bacteria remained at a safe level at 4°C although with significant growth at 25°C. This result shows that sufficiently disinfected "pack-cooked" curry could be kept within the safety level of SPC bacteria for 24 hr at 4°C preservation but could not be kept at 25°C for the same period.

Next, we checked for coliform bacteria, which are also used as hygienic indicator because of their heat sensitivity (Read *et al*., 1961), in the same packs. The unheated curry packs contained 1.7×10^6 cfu/g of coliform bacteria, although the packs were coliform bacteria-negative just after cooking (Table 3). The packs stored in a 4°C fridge were coliform negative for 24 hr. However, coliform bacteria were detected in one of the three bags that were corresponded to be left at room temperature for 24 hr, although the other two bags were coliform negative. This might be because "pack cooking" usually uses a large pot and boils lots of packs at the same time, so uneven heating may occur and rarely a very small number of coliforms may remain.

Bacillus cereus is an aerobic spore-forming bacterium in the environment, particularly in soil and river water, so it is often found in a variety of foods (Kim *et al*., 1971). This bacterium is important in the field of food hygiene because unsanitary handling of food can cause food poisoning. In our study, *Bacillus cereus* was detected only in the unheated packs (i.e., 4×10^4 cfu/g) but was not found in the cooked packs even if these packs were left for 24 hr at 25°C. However, *Bacillus cereus* growth is possible when stored at room temperature after cooking, since heat-tolerant spores are viable during the cooking process (Gilbert *et al*., 1974, Fogele *et al*., 2018). It is not desirable to leave a pack cooked at the optimum temperature for the growth of *Bacillus cereus* (e.g., 30°C–37°C) for a long period of time.

We also analyzed whether *Clostridium perfringens* multiplied or not in "pack-cooked" meals, but its colony was not observed in all the packs including those that were unheated. Since no *Clostridium perfringens* colonies were detected in the packs before heating, we speculate that the curry roux we used in this study did not contain any *Clostridium perfringens* and therefore did not grow in any or all of the packs. However, a previous study has shown that commercially available curry roux may sometimes contain bacteria (Fujisawa *et al*., 2001). Further research is needed to determine the safety of *Clostridium perfringens* if the ingredients that are used in "pack cooking" methods contain it.

"Pack cooking" is a method that is both simple and safe and is one of the powerful tools in times of disasters. There is no risk of bacterial contamination after cooking, as the servings are pre-packed and cooked. However, "pack-cooked" meals are not sterilized like retortable pouch foods, so long preservation is not guaranteed. Our study revealed that, from a food hygiene standpoint, "pack-cooked" meals are inadequate for long-time storage without a cooling chamber.

In our study, although bacterial growth was only observed in the packs stored at 25°C for only 24 hr, "pack-cooked" meals should be eaten as quickly as possible if they could not be stored in refrigeration during a natural disaster.

ACKNOWLEDGMENTS

We thank Ms. Chikae Ishikawa and Ms. Ikuko Edazawa for cooking assistance.

Conflict of interest---- The authors declare that there is no conflict of interest.

REFERENCES

Fogele, B., Granta, R., Valciņa, O. and Bērziņš, A. (2018): Occurrence and diversity of *Bacillus cereus* and moulds in spices and herbs. Food Control, **83**, 69-74.

Fujisawa, T., Aikawa, K., Takahashi, T., Yamai, S. and Ueda, S. (2001): Occurrence of clostridia in commercially available curry roux. Shokuhin Eiseigaku Zasshi, **42**, 394-397.

Gilbert, R.J., Stringer, M.F. and Peace, T.C. (1974): The survival and growth of *Bacillus cereus* in boiled and fried rice in relation to outbreaks of food poisoning. J. Hyg. (Lond.), **73**, 433-444.

Japan Food Hygiene Association. (2018): Standard methods of analysis in food safety regulation-Microbiology (Revised 2nd edition).

Kim, H.U. and Goepfert, J.M. (1971): Enumeration and identification of *Bacillus cereus* in foods. I. 24-hour presumptive test medium. Appl. Environ. Microbiol., **22**, 581-587.

Read, R.B. Jr., Schwartz, C. and Litsky, W. (1961): Studies on the thermal destruction of Escherichia coli in milk and milk products. Appl. Environ. Microbiol., **9**, 415-418.