Bactericidal Effects of Konjac Fluid on Several Food-Poisoning Bacteria

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ABSTRACT

In this study, the bactericidal effects of Japanese alkaline foods on food-poisoning bacteria were evaluated. Konjac is an alkaline food soaked in calcinated calcium (the pH of konjac fluid ranges from 11.42 to 12.53). Konjac fluids completely inactivated Escherichia coli, enterohemorrhagic E. coli O157:H7 and E. coli O26:H9, Salmonella Enteritidis, Vibrio parahemolyticus, and Staphylococcus aureus. The initial level of 6 log CFU/ml dramatically decreased after incubation with konjac fluid, and no viable gram-negative bacterium cells could be detected within 1 to 2 days and no viable S. aureus cells could be detected within 3 to 5 days. On the other hand, treatment with konjac fluid was also effective in reducing levels of spore-forming bacteria (Bacillus subtilis, Bacillus cereus, Clostridium perfringens, and Clostridium botulinum type E and type A). At least a 4-log reduction of spore-forming bacteria was obtained in konjac fluid within 7 to 14 days. Vegetative cells were more susceptible to konjac fluid than spores were. When the initial cell count was 6 log CFU/ml, a few surviving spores remained for 60 to 90 days, but no spores could be detected after 120 days. When the initial count of spore-forming bacteria was 3 to 4 log CFU/ml, the cells considered vegetative were completely inactivated within 1 to 3 days. Repeated treatment with konjac fluid caused complete inactivation of spores in less than 1 to 3 days. Our studies indicate that konjac fluid, which has a long history of use in food, will control food-poisoning bacterial contamination during the production or preservation of konjac and other foods and has a preventive effect on bacteria that can cause severe disease at uniquely low levels.

Konjac has been consumed as a part of traditional Japanese dishes for >1,000 years and has also been used for food preservation. Konjac glucomannan is a main component of the konjac flour produced from tubers of konjac root (konnyaku-imo, Amorphophallus konjac K. Koch) and is distributed in plant tissues as a cell component (21, 24, 37, 40). Konjac glucomannan is a fiber composed mainly of a polysaccharide chain of repeating units of 1,4-ß-linked glucose and mannose (21, 24, 37) and forms a gel. The biochemical (40) and rheological (29, 30) characteristics of glucomannan have been investigated. Konjac is a nonfat, low-calorie food with several health-promoting properties (14, 15, 46). When consumed with other foods, it reduces the speed of sugar intake, lowers cholesterol levels (3, 39, 40, 41), and improves vitamin balances (18). Konjac is also soaked in calcinated calcium (to produce konjac fluid) and serves as a dietary source of calcium. Other alkaline foods include salt water kansui (used for Chinese noodles and containing K2CO3, Na2CO3, Na3PO4, and Na4O7P2) and pidan (processed duck or chicken egg). However, alkaline foods are not common.

The contamination of food with pathogenic microorganisms continues to be a serious public health problem. Recent enterohemorrhagic Escherichia coli O157:H7 outbreaks linked to several foods have focused attention on the contamination of processed foods from environmental sources. The ability of E. coli O157:H7 to survive in low-pH synthetic gastric fluid has also been reported (2, 17, 42), suggesting that the survival of the organism as it passes through the human stomach may be an important determinant of infectivity. The bactericidal effect of acid or acidic foods (e.g., acetic acid (1, 10, 26) and lactic acid (11, 16, 19, 34, 47)) has been demonstrated for several foodborne pathogenic bacteria.

On the other hand, there are few observations regarding the ability of bacteria to survive when exposed to alkaline environments (5, 38). Given the alkaline nature of detergents and some of the chemical sanitizers used to clean and sanitize equipment and foods, information on the response of bacteria to alkaline treatment would be useful in designing interventions to prevent postprocessing contamination of foods. Alkaline chemicals, trisodium phosphate (23, 36), NaOH (12), KOH (12), Ca(OH)2 (44), and calcinated calcium (4, 20, 31), possess antibacterial activity against some food-poisoning bacteria and have been used to clean and disinfect the surfaces of eggs (8), meat (12, 13), and oranges (32).

However, the bactericidal effects of alkaline foods on food-poisoning bacteria have not been investigated because such foods are not common. Also, the effects of alkaline foods on spore-forming bacteria have not been elucidated explicitly. The objective of this study was to determine the bactericidal effects of alkaline konjac fluid on the survival of several food-poisoning bacteria, including spore-forming bacteria.
**MATERIALS AND METHODS**

**Bacterial strains.** The strains used in this study are listed in Table 1. Strains of enterohemorrhagic *E. coli* O157:H7 (strains S778, S902, and S909), *E. coli* (ATCC 25922 and an isolate from a patient), *Salmonella* Enteritidis, *Vibrio parahemolyticus*, and *Staphylococcus aureus* were used (22). *E. coli* O157:H7 and *E. coli* O26:H9 strains were originally isolated from patients at the Sakai City Institute of Public Health in association with an outbreak in Sakai City, Osaka, in 1996 and 1997 and produce Shiga-like toxin I and/or Shiga-like toxin II. The strains of *Salmonella* Enteritidis, *V. parahemolyticus*, and *S. aureus* were originally isolated from patients at Wakayama Medical College (Wakayama, Japan) and stocked in our laboratory. Strains of *B. subtilis* subsp. *subtilis* (NRBC13719, and an isolate from food), *B. cereus* (strain S6), *C. perfringens* (strain S4), and *C. botulinum* type E (strain S1) and type A (strain S6), were used in this experiment. *B. cereus*, *C. perfringens*, and *C. botulinum* type E and type A were originally isolated from patients at the Sakai City Institute of Public Health in association with foodborne illnesses. Stock cultures were prepared from a subculture of the initial isolate in brain heart infusion (BHI) broth.

**TABLE 2. Effects of konjac fluid on the viability of several nonspore bacteria**

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Treatment</th>
<th>Count (CFU/ml) after storage (incubation) time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>PBS</td>
<td>$4.8 \times 10^6$</td>
</tr>
<tr>
<td></td>
<td>Konjac fluid</td>
<td>$40$</td>
</tr>
<tr>
<td><em>O157:H7</em></td>
<td>PBS</td>
<td>$5.3 \times 10^6$</td>
</tr>
<tr>
<td></td>
<td>Konjac fluid</td>
<td>$20$</td>
</tr>
<tr>
<td><em>O157:H7</em></td>
<td>PBS</td>
<td>$4.0 \times 10^6$</td>
</tr>
<tr>
<td></td>
<td>Konjac fluid</td>
<td>$23$</td>
</tr>
<tr>
<td><em>O26:H9</em></td>
<td>PBS</td>
<td>$4.8 \times 10^6$</td>
</tr>
<tr>
<td></td>
<td>Konjac fluid</td>
<td>$14$</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>PBS</td>
<td>$5.3 \times 10^6$</td>
</tr>
<tr>
<td></td>
<td>Konjac fluid</td>
<td>$17$</td>
</tr>
<tr>
<td><em>V. parahemolyticus</em></td>
<td>PBS</td>
<td>$3.9 \times 10^6$</td>
</tr>
<tr>
<td></td>
<td>Konjac fluid</td>
<td>$36$</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>PBS</td>
<td>$4.4 \times 10^6$</td>
</tr>
<tr>
<td></td>
<td>Konjac fluid</td>
<td>$3.5 \times 10^4$</td>
</tr>
</tbody>
</table>

*a Bacterial cells (at initial cell counts of about 6 log CFU/ml) were treated with konjac fluid at pH 12.50 and incubated at room temperature for the indicated number of days.

*b See Table 1 for abbreviations.

c PBS (pH 7.3) was used as a control.

d Values are means for two plates. NT, not tested.

e Immediately (5 to 10 min) after treatment.
FIGURE 1. Effects of konjac fluid on the viability of food-poisoning bacteria. The initial cell levels of each food-poisoning bacterium were 5 to 6 log_{10} CFU/ml (a), 4 to 5 log_{10} CFU/ml (b), and 3 to 4 log_{10} CFU/ml (c). The cells were treated with konjac fluid at pH 12.50 (the same konjac fluid was used to obtain the data presented in Tables 2 and 3). Each sample was incubated at room temperature for the indicated number of days. Bacteria used were E. coli O157:H7 (*), O26:H9 (△), E. coli (○), Salmonella Enteritidis (●), V. parahaemolyticus (□), S. aureus (■), B. subtilis (△), B. cereus (○), C. perfringens (▲), C. botulinum type A (△), and C. botulinum type E (×).

Preparation of alkaline solutions. Commercially packed konjac provided by Miyuki Fujimoto (Miyukiya Co., Hiroshima, Japan) was used in this study. Packed konjac was opened aseptically. For microbial analysis, 1 ml of konjac fluid was placed on an agar plate with a loop over the surface and incubated for 48 h at 35°C. A Standard Method agar plate (0.25% [wt/vol] yeast extract, 0.1% peptone, 1.5% agar; Nissui) was used for aerobic bacteria and peptone, soytone, protease peptone, yeast extract, beef extract, liver extract, glucose, starch, L-tryptophan, L-cysteine, thioglycolate, L-arginine, vitamin K, hemin, sodium chloride, agar, and other reducing agents were compared) and a clostridia count agar under anaerobic conditions with the use of the AnaeroPack Kenki kit (Mitsubishi Gas Chemical, Japan). PBS treatment under same conditions as the konjac experiments was used as a control. Experiments were replicated at least twice, and typical data are presented. Each replication involved duplicate plating. Results were recorded in terms of log CFU per milliliter.

RESULTS

Bactericidal effects of konjac fluid on food-poisoning bacteria. Konjac fluids sold in stores exhibited pHs of 11.42 to 12.53. No aerobic or anaerobic microbes were found in any of the 33 fresh konjac fluids we obtained. Food-poisoning bacteria were treated with konjac fluid to determine their survival. Each sample was incubated at room temperature (15 to 18°C) for an appropriate period. In general, treatment with konjac fluid for 5 to 10 min resulted in the inactivation of the bacterial cells. At least 5-log reductions of the gram-negative bacteria E. coli, E. coli (W), O157:H7, O157:H7 (S902), O157:H7 (S909), O26: H9, Salmonella Enteritidis, and V. parahaemolyticus were obtained with konjac fluid after 1 day. When the initial level of cells in konjac fluid at pH 12.50 was 6 log CFU/ml, no surviving gram-negative bacterial cells could be detected.
TABLE 3. Effects of konjac fluid on the viability of spore-forming bacteria

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Control</th>
<th>Konjac fluid pH 12.50</th>
<th>Konjac fluid pH 11.00</th>
<th>Konjac fluid pH 10.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis</td>
<td>0.5 log CFU/ml</td>
<td>2 log CFU/ml</td>
<td>4 log CFU/ml</td>
<td>4 log CFU/ml</td>
</tr>
<tr>
<td>B. cereus</td>
<td>0.5 log CFU/ml</td>
<td>2 log CFU/ml</td>
<td>4 log CFU/ml</td>
<td>4 log CFU/ml</td>
</tr>
<tr>
<td>C. perfringens</td>
<td>0.5 log CFU/ml</td>
<td>2 log CFU/ml</td>
<td>4 log CFU/ml</td>
<td>4 log CFU/ml</td>
</tr>
<tr>
<td>C. botulinum A</td>
<td>0.5 log CFU/ml</td>
<td>2 log CFU/ml</td>
<td>4 log CFU/ml</td>
<td>4 log CFU/ml</td>
</tr>
<tr>
<td>C. botulinum E</td>
<td>0.5 log CFU/ml</td>
<td>2 log CFU/ml</td>
<td>4 log CFU/ml</td>
<td>4 log CFU/ml</td>
</tr>
</tbody>
</table>

A bacterial cells (at initial cell counts of about 6 log CFU/ml) were treated with konjac fluid at pH 12.50 and incubated at room temperature for the indicated number of days. Values are means of two plates, NT, not tested. Bacterial cells were detected after 120 days even when the initial level of B. subtilis or B. cereus was 6 log CFU/ml (Table 3). Incubation in konjac fluid at pH 12.50 effectively reduced the initial cell level by 4 to 5 log CFU/ml, but bacterial cells survived for at least 7 days (Fig. 1a and 1b). However, when the initial level of cells was 3 log CFU/ml, konjac fluid completely inactivated bacterial cells within 3 days (Fig. 1c). In general, anaerobic bacteria treated with konjac fluid were more sensitive than aerobic bacteria. PBS treatment (control) produced only a minor reduction in bacterial populations (Tables 2 and 3).

The survival of bacterial cells in other konjac fluids at pHs of 11.50, 12.06, and 12.46 and konjac fluids artificially adjusted to pHs of 7.00, 10.00, 11.00, and 12.70 was studied (Fig. 2). Generally, larger reductions in cell counts were achieved at higher pHs. Similarly, cells were inactivated more rapidly at higher pHs. When O157:H7, V. parahaemolyticus, and S. aureus were suspended in konjac fluid adjusted to pHs of 10.00 and 11.00, initial cell counts of 5 log CFU/ml decreased by 1 to 2 log units after 1 to 3 days (Fig. 2a through 2c). Like the konjac fluid at pH 12.50, the konjac fluids at pHs of 11.50, 12.06, and 12.46 completely inactivated O157:H7 and V. parahaemolyticus within 1 to 3 days (Fig. 2a and 2b). Similar results were obtained for E. coli, E. coli (W), and Salmonella Enteritidis (data not shown). Very few S. aureus cells survived in konjac fluids at pHs of 11.50 and 12.06 for 5 days (Fig. 2c). However, S. aureus cells were completely inactivated within 5 days in another experiment by konjac fluids at pHs of 11.50 and 12.06 (data not shown) and at pH 12.46 (Fig. 2c), as well as at pH 12.50 (Table 2). On the other hand, when B. subtilis was suspended in konjac fluids adjusted to pHs of 10.00 and 11.00, cell counts decreased by 1 to 2 log units after 30 days (Fig. 2d) and after 120 days (data not shown), respectively. At pHs of 11.50, 12.06, and 12.46, 4-log reductions in bacterial cell counts were achieved, but cells survived at levels of only 5 to 50 CFU/ml for 30 days. However, bacterial cells could not be detected after 90 to 120 days (data not shown). When initial bacterial cell counts were 3 log CFU/ml, cells were completely inactivated even in konjac fluids at pHs of 11.50 and 12.06 within 1 to 3 days (data not shown). Also, within 7 to 14 days, no B. subtilis cells could be detected in konjac solutions adjusted.
FIGURE 2. Effects of pH on food-poisoning bacteria. The initial cell counts for O157:H7 (a), V. parahemolyticus (b), S. aureus (c), B. subtilis (d), B. cereus (e), and C. botulinum type E (f) were $5.1 \times 10^5$, $4.1 \times 10^5$, $5.1 \times 10^5$, $3.3 \times 10^5$, $4.3 \times 10^5$, and $5.1 \times 10^5$ CFU/ml, respectively. These cells were treated with konjac fluid at pHs of 10.00 (●), 11.00 (▲), 11.50 (▲), 12.06 (△), 12.46 (■), and 12.70 (□). Konjac fluid adjusted to pH 7.00 with BHI medium was used as a control (×). Each sample was incubated at room temperature for the indicated number of days.

Effects of storage conditions on bacterial cells. The survival of bacterial cells in konjac fluid after storage at 5, 15, 25, and 35°C was monitored at pH 12.53. The bactericidal activity of the fluid against O157:H7, E. coli, Salmonella Enteritidis, V. parahemolyticus, and S. aureus was not affected by temperature in the range studied. The O157: H7, V. parahemolyticus and S. aureus cells (at initial levels of 5 log CFU/ml) were inactivated in konjac fluid at 5, 15, 25, and 35°C (Fig. 3a through 3c, respectively). On the other hand, when the initial B. subtilis cell count in konjac fluid was 5 log CFU/ml, cells stored at 15, 25, and 35°C were more sensitive than cells stored at 5°C (Fig. 3d). Storage in konjac fluid for 1 to 7 days at 15, 25, and 35°C resulted in 3- to 4-log reductions in cell counts. Storage for 21 days at 35°C and for 30 days at 15°C and 25°C led to the complete elimination of viable cells. However, storage for 30 days at 5°C resulted in the survival of 1 to 2 log CFU/ml. Similar results were obtained for B. subtilis (W) (data not shown), B. cereus (Fig. 3e), and C. botulinum type E (Fig. 3f).

Comparison of effects on spores and effects on vegetative cells. The effects of konjac fluid on spores and vegetative cells of B. subtilis were investigated. Bacterial cells inactivated in konjac fluid (pH 12.50) over 60 days were heated at 70°C for 10 min to completely kill the vegetative cells (33). Cells found to be almost solely spores (>90%) as observed by microscopy were used. After their fresh cultivation, the vegetative cells (at initial counts of $4.5 \times 10^4$ CFU/ml) were easily inactivated in konjac fluid at pH 12.18, but the spores (at initial counts of $4.2 \times 10^4$ CFU/ml) were more resistant (Fig. 4a).

Konjac solution containing almost solely spores was
adjusted to a pH of 7.00 with BHI broth and incubated at 35°C for 1 day to germinate the spores. The dilution effect dealt with in the enumeration of CFU per milliliter was revised by a calculation based on the volume of the additional BHI medium. Following readjustment to a pH of ca. 12.00 to 12.50 through the addition of the appropriate weight of powder to calcinated calcium or CaO (final concentration, 0.5 to 1.0% [wt/vol]), alkali-treated viable vegetative cells could not be detected within 7 days (Fig. 4b). This alkali shock treatment was more effective in reducing cell counts when it was repeated three times (Fig. 4b). After the third treatment, no vegetative cells could be detected within 1 day. Similar results were obtained for B. cereus and C. botulinum type E under these conditions (data not shown). Similarly, following centrifugation, the removal the supernatant, and the addition of the same volume of fresh konjac fluid to the germinated spores (vegetative cells), the same result was obtained (data not shown). However, we observed decreases in cell counts due to centrifugation, so these results were not consistent.

Effects of kansui and alkaline solutions. The same extent of inactivation achieved with konjac fluid was observed for O157:H7, V. parahemolyticus, S. aureus, B. subtilis, B. cereus, and C. botulinum type E was with kansui solution (pH 12.58) under similar experimental conditions (Fig. 5). Treatment with kansui solution was also sufficient to produce the desired 4- to 5-log reduction. Alkaline so-
FIGURE 4. Comparison of effects on vegetative cells and effects on spores. (a) Vegetative cells (at initial counts of $4.5 \times 10^4$ CFU/ml (○) and spores (at initial counts of $4.2 \times 10^4$ CFU/ml (□) of B. subtilis were treated with konjac fluid at pH 12.18. As a control, PBS (pH 7.3) was used for vegetative cells (×) and spores (*). (b) Konjac solution containing primarily spores was adjusted to pH 7.00 with BHI broth (long arrow) and incubated at 35°C for 1 day. Counts (CFU/ml) were adjusted to take into account dilution with BHI medium. The sample was readjusted to about pH 12.00 with calcinated calcium (short arrow) (the powder was added to yield a final concentration of ca. 0.5% [wt/vol]) and incubated at room temperature (●). This adjustment was repeated one more time (□). Samples whose pHs were not adjusted (○), as well as PBS (×), were used as controls. After the third treatment, no vegetative cells could be detected within 1 day.

Solutions of Ca(OH)$_2$, NaOH, and KOH adjusted to pH 12.50 resulted in the same effect produced by konjac fluid at pH 12.46. The same extent of inactivation achieved with konjac fluid for other bacterial strains was observed with these solutions under similar experimental conditions (data not shown).

DISCUSSION

These studies demonstrate for the first time that konjac fluid, which has a long history of use in food preservation, will inactivate food-poisoning bacteria. In our preliminary study, even when the initial cell counts were 7 log CFU/ml, the konjac fluids (pH 12.50) completely inactivated O157:H7, E. coli, Salmonella Enteritidis, and V. parahemolyticus within 1 to 3 days. The U.S. Food and Drug Administration recently mandated a warning statement on packaged foods that have not been treated to reduce target pathogen levels by 5 log units. In this study, treatment with konjac fluid at pH 12.5 for 5 or 10 min was found to be sufficient to produce the desired 5- to 6-log reductions of these bacteria. The pH of konjac fluid is usually between 11.5 and 12.5 and is most likely an important factor in the severity of the treatments needed to achieve 5-log reductions in bacterial cell counts.

We have also demonstrated that 4- to 5-log reductions in the spore-forming organisms B. subtilis, B. cereus, C. perfringens, and C. botulinum (at initial cell counts of 5 to 6 log CFU/ml) can be achieved with konjac fluid, although the exact proportions of spores and vegetative cells were undetermined. The initial cell populations were considered primarily vegetative cells because of their age and the method of cultivation. Very few viable cells survived for 21 to 90 days after treatment with konjac fluid. The remaining cells were found to be primarily spores by microscopic observation. In a preliminary study, when the initial inoculum level was higher (7 log CFU/ml), a few spores survived for longer periods. However, even when the initial cell counts were 5 to 6 log CFU/ml, no surviving cells could be detected after 120 days. Konjac fluid also completely inactivated bacterial cells within 7 days when the initial levels of cells (considered vegetative cells) were 3 to 4 log CFU/ml. The spore-forming bacteria might be completely inactivated if cells were present at <4 log CFU/ml in the early stages in processed food contaminated from environmental sources.

Our results suggest that the pH that allowed the survival of O157:H7, E. coli, Salmonella Enteritidis, and V. parahemolyticus was ca. 10.5 and that the pH that allowed the survival of S. aureus was ca. 11.0. On the other hand, the levels of viable cells of spore-forming bacteria decreased by 1 to 2 log units when these cells were suspended in konjac fluid adjusted to pHs of 10.0 and 11.0. Cell count reductions of 4 to 5 log units were achieved at pHs of 11.5 to 12.5, although a small number of spores survived. Bacterial spores at initial levels of 3 log CFU/ml were completely inactivated even in konjac fluids at pHs of 11.5 and 12.0 within 7 days. Our results indicate that the maximum pH for the survival of gram-positive bacteria was higher than that for the survival of gram-negative bacteria. The resistance of bacteria to pH stress is strongly influenced by other aspects of the environment, so different environments. 

lutions of Ca(OH)$_2$, NaOH, and KOH adjusted to pH 12.50 resulted in the same effect produced by konjac fluid at pH 12.46. The same extent of inactivation achieved with konjac fluid for other bacterial strains was observed with these solutions under similar experimental conditions (data not shown).

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FIGURE 5. Effects of kansui and other alkaline solutions on food-poisoning bacteria. The initial cell counts for O157:H7 (a), V. parahemolyticus (b), S. aureus (c), B. subtilis (d), B. cereus (e), and C. botulinum type E (f) were $2.0 \times 10^5$, $3.0 \times 10^5$, $3.3 \times 10^5$, $1.3 \times 10^5$, $1.9 \times 10^5$, and $3.3 \times 10^6$ CFU/ml, respectively. The cells were treated with konjac fluid (pH 12.46) (●), kansui solution (pH 12.58) (○), $\text{Ca(OH)}_2$ (pH 12.50) (▲), NaOH (pH 12.50) (△), and KOH (pH 12.50) (■). Each sample was incubated at room temperature for the indicated number of days.

Reduced survival of E. coli O157:H7 in acidic foods at higher temperatures has also been observed in studies involving apple juice and cider (26, 42, 48). We found that konjac fluid’s bactericidal activity against O157:H7, E. coli, Salmonella Enteritidis, V. parahemolyticus, and S. aureus was not affected by incubation temperature. In contrast, the survival of spores in konjac fluid was related to storage temperature. Interestingly, konjac fluid at 5°C enhanced the survival of spores compared with konjac fluid at 15, 25, and 35°C. Storage for 30 days at 15 to 35°C led to complete inactivation. However, storage for 30 days at 5°C resulted in the survival of a few spores. These data confirm that spores have the ability to survive in alkaline media at refrigeration temperature. Refrigeration is an essential step in extending the shelf lives of foods; however, if spores are present in foods, refrigeration will enhance their survival.

Spores can cause spoilage or even health risks after germination and subsequent outgrowth. The difference in konjac fluid’s efficiency in inactivating vegetative cells and its efficiency in inactivating spores was investigated. Vegetative cells were readily inactivated in konjac fluid, but spores were more resistant to this agent. Konjac fluid is not able to completely inactivate spores when the initial spore count is $>4$ log CFU/ml. However, repeated alkali treatment with konjac fluid completely inactivated spores. Our results may indicate that after incubation at 35°C for 1 day at pH 7.00, the spores germinated to produce vegetative cells and were easily killed by alkali treatment. After the third treatment, no viable cells could be detected within 1 day. Tyndallization (fractional sterilization) sometimes is
used with the aim of sterilizing certain heat-labile materials and is based on the assumption that materials so treated are able to support the germination and outgrowth of any spores they may contain. The material is heated to 80 to 100°C for periods of up to ca. 1 h on each of three successive days and is incubated at room temperature or 35°C, for intervening periods. Our konjac fluid investigation was based on this principle. The first period of treatment with konjac fluid is intended to kill all vegetative cells initially present in the materials. Incubation during the intervening periods is intended to permit germination of any spores that may be present; vegetative cells arising from such spores are killed during subsequent periods of konjac fluid treatment. In a preliminary study, the sensitivity of spores having undergone nutrient-induced germination to konjac fluid treatment was examined for different phases of germination. Susceptibility to konjac fluid seemed to arise immediately after the germination process was initiated.

The addition of 0.07 to 0.4% (4) or 1% (44) calcinated calcium or shell calcium (31) to foods has been shown to inactivate test microorganisms. Konjac fluid contains about 0.1 to 0.2% calcinated calcium or shell calcium (31) to foods has been shown to inactivate test microorganisms. Konjac fluid contains about 0.1 to 0.2% calcinated calcium and this concentration is consistent with a pH of ca. 11.5 to 12.6. A 0.4% calcinated calcium solution is almost saturated and is consistent with a pH of about 13.00. This solution completely inactivated spores and vegetative cells. These findings suggest that konjac fluid containing calcinated calcium might be safe and useful for the control of most bacterial contamination during the production of foods.

Similarly, the inactivation of spores in kansui solution (pH 12.58) was studied. The same extent of inactivation achieved with konjac fluid was observed with kansui solution under similar experimental conditions. Treatment with kansui solution was sufficient to produce the desired 5-log reduction. Small et al. (35) showed that the inclusion of KOH rather than NaOH in broth used to stress *E. coli* resulted in an increase in the survival rate from 0.06 to 50% at pH 10.2. Higher rates of entry of Na+ occur at an alkaline pH (7), and Na+ may have damaged *Listeria monocytogenes* cells and sensitized them to heating. In our study, alkaline solutions of Ca(OH)₂, NaOH, and KOH adjusted to a pH of 12.00 showed the same effects produced by konjac fluid at pH 12.06. These findings suggest that alkaline pH enhances the susceptibility of bacteria rather than some special materials in konjac fluid that produce the bacterial effects. The *rpoS* gene, which regulates acid tolerance in *O157:H7*, has an influence on survival (9, 43). A regulation mechanism that is operative on *E. coli* cells at pH 10 has also been demonstrated (27, 45), but it is not clear whether it is operative at pHs of >10. These previous reports suggest that a high-alkaline-pH regulation or tolerance gene is present, but further study is required to confirm this hypothesis.

In general, gram-negative bacteria were found to be more sensitive to konjac fluid than gram-positive bacteria were. Mendonca et al. (25) examined the morphology of alkal-stressed cells with scanning electron microscopy and transmission electron microscopy. Interestingly, the exposure of bacterial cells to alkaline pHs caused the cytoplasmic membrane to bulge against the cell wall. At pH 12, gram-negative test cells appeared to be collapsed and showed evidence of lysis, while gram-positive *L. monocytogenes* cells did not. These researchers concluded that the presence of a thick rigid peptidoglycan layer in gram-positive bacteria probably prevents the cytoplasmic membrane from expanding and bursting. It was concluded that the destruction of gram-negative foodborne pathogens by high pHs involved the disruption of the cytoplasmic membrane.

No aerobic or anaerobic microbes could be found in any of 33 fresh konjac fluids sold in stores. Our findings suggest that konjac fluid is a microbiologically safe food, may be useful for controlling food-poisoning bacterial contamination during the production or preservation of konjac or other foods, and may help to prevent infection by bacteria that can cause severe disease when present at uniquely low levels.

Konjac fluid was found to be useful for the preservation of food products in this study. However, it may be possible that contact with foods is likely to reduce the pH of the fluid and hence its bactericidal effect. In a preliminary study, the effectiveness of washing treatments with konjac fluid and Ca(OH)₂ in decontaminating soybean and azuki bean surfaces was evaluated. The alkaline washing treatment inactivated the contaminated microorganism by at least 3 to 4 log CFU/ml. Repeated treatments were found to be more effective than a single treatment. Further study is necessary to examine the bactericidal effects of konjac and other alkaline solutions on food and products artificially contaminated with food-poisoning bacteria.

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