Evaluation and Control of the Risk of Foodborne Pathogens and Spoilage Bacteria Present in Awa-Uirou, a Sticky Rice Cake Containing Sweet Red Bean Paste

Naoki Okahisa,¹ Yasuhiro Inatsu,² Vijay K. Juneja,³ and Shinichi Kawamoto²

Abstract

The risk of food poisoning and growth of spoilage bacteria in Awa-Uirou, a sticky rice cake containing sweet red bean paste, was evaluated. Toxin-producing bacteria such as *Staphylococcus aureus* and *Bacillus cereus* are the main causes of food poisoning linked to this kind of food. The water activity in this product is in the range suitable for growth of *S. aureus*, *B. cereus*, and *B. subtilis*. The viable count of *S. aureus* or *B. cereus* spore cocktail was significantly reduced to 2.3 log colony-forming units (CFU)/g after 70 minutes steaming treatment at 100°C. However, the heat-resistant endospores of *B. subtilis* germinated during storage at 30°C to cause appreciable syneresis of the starch gel matrix in 4 days. The addition of 0.5% glycine before steaming treatment was found to effectively suppress the growth of *B. cereus* but was not effective in controlling *S. aureus* throughout the 7 days incubation period at 30°C. On the other hand, *S. aureus* and *B. cereus* could grow > 5.0 log CFU/g in an inoculated sample without glycine within 3 days when stored at 30°C. Moreover, addition of 0.5% glycine before the steaming process did not have any significant effect on color, texture, or taste of sticky rice cake. Therefore, results of this study demonstrated that the addition of 0.5% glycine before the steaming process could inhibit *B. cereus* and *B. subtilis* multiplication in the steamed rice confection which in turn may help reduce the risk of food poisoning or quality loss.

Introduction

Sticky steamed rice cakes such as Khanom Chan in Thailand, Banh da ion in Vietnam, Nian Gao in China, and Tteok in Korea are popular confections in East to Southeast Asia and consumed widely. These confections are basically a mixture of sticky or ordinary rice flours and sugar steamed to make a sticky starch gel. Japanese raw confections such as Dango (rice dumpling) or Mochi and Uirou (rice cake) are included in this food category. In addition to rice flour, other flours made from mung bean, cassava, and tapioca and ingredients such as fruits, sweet bean paste, or pandan leaves are used to enhance the texture, taste, and flavor of the confections. Awa-Uirou, is a kind of Uirou traditionally produced in Tokushima prefecture, Japan, and is characterized by mixing sweet red bean paste (Sarasi-An) with sticky rice flour and sugar before steaming at 100°C for 70 minutes.

The risk of food poisoning from raw sticky rice cake has been recognized because many of these steamed rice cakes are made in small household enterprises and typically vended on streets in the different countries (Notermans

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S. aureus is a gram-positive non–spore-forming bacterium. Around 25% of these bacteria isolated from food samples produced heat-resistant enterotoxin (Le Loir et al., 2003). Several outbreaks due to S. aureus have occurred due to steamed rice cake in Japan; for example, Kashiwa-Mochi (Kaneko et al., 1982), Tukimi-Dango (Ishii et al., 1988; Uematsu and Kaneko, 1989; Tsuji et al., 2003), Mitu-Dango, An-Dango, Daihuku (Houjyo et al., 2001), and Zunda-Mochi (Saito et al., 2001) outbreaks. All of these outbreaks involved sticky rice cakes except for Tsukimi-Dango and Mitsu-Dango, which contained sweet bean paste.

B. cereus, a gram-positive spore-forming bacteria, produces either an emetic (vomit-inducing) toxin in foods or diarrheal toxins (enterotoxins) in the intestine that induce acute symptoms between 8 and 16 hours after ingestion of the contaminated foods (Drobniewski, 1993; Notermans and Batt, 1998; McKillip, 2000). B. cereus is ubiquitous in nature and frequently isolated from soil. As a common inhabitant of soil, this bacterium can be easily transmitted into vegetables or crops and hence into foods. B. cereus grows well at room temperature in starchy foods to produce a heat-resistant emetic toxin (Agata and Mori, 1997; Agata et al. 2002). It is becoming apparent that many incidents of B. cereus food poisoning involve consumption of starchy foods such as cooked rice, noodles, or sticky rice cakes (Shinagawa, 1990). In December 2001, 346 patients who ate sweet red bean paste covered with sticky rice cake (An-Iri-Mochi) in Kuma-moto prefecture in Japan suffered from food poisoning caused by cereulide, a cyclic dodecadepsipeptide (Matsuoka et al., 2003; Huruse et al., 2004).

B. subtilis is also distributed widely in the environment and foods. Contamination with the organism and its rapid growth in starchy foods is associated with spoilage or quality loss (Thomas and Masters, 1988; Sorokulova et al., 2003). Rice flour and beans can be contaminated with Bacillus spores as a result of soil contamination (Watanabe and Hayano, 1993). B. subtilis from food ingredients can cause syneresis of the starch–sugar gel matrix in bean jam jelly (Mizuyoukan) or sticky rice cake (Uirou) resulting in quality loss (Nanba and Itho, 1997; Naito and Matsunaga, 2002).

Glycine is the simplest amino acid, and it has been used as an antibacterial agent in foods due to its low toxicity in animals. Although glycine is toxic to humans when it is given in large amounts, it also has antibacterial potential (Hammes et al., 1973). For example, Lactococcus lactis subspecies failed to grow in medium containing >2% glycine (Helge and Ingolf, 1989). Furthermore, glycine concentrations of 1.5–6% resulted in 70–90% reductions in growth of Enterococcus faecalis (Gary et al., 1991). Glycine is known to inhibit the synthesis of a peptido-glycan component of the bacterial cell wall (Hishinuma et al., 1969). Although the bacterial cell wall is thinner in gram-negative bacteria than in gram-positive bacteria, it is thought that the amount of glycine required to suppress bacillus proliferation is lower than that required to suppress gram-positive bacteria (Schwartz et al., 1979). Hops extracts possess antimicrobial activities against Streptococcus mutans and other streptococci (Bhattacharya et al., 2003), and salmon roe protein has been shown to have a certain degree of antimicrobial activity against gram-negative and gram-positive bacteria (Anonymous, 2008).

In this study, we evaluated the growth characteristics of B. subtilis, B. cereus, and S. aureus in Awa-Uirou to evaluate food safety and quality risks in traditional rice–bean confections. Heat-stable antimicrobial food additives—glycine, hop extract, and salmon roe protein—were evaluated for their ability to control the growth of B. cereus, S. aureus, and B. subtilis in artificially contaminated Awa-Uirou.

Materials and Methods

Measurement of physical parameters

Twenty-nine samples of sticky rice cake made locally with sweet red bean paste (Awa-Uirou) and destined for distribution in Tokushima prefecture, Japan, were collected. All samples were analyzed 2 days after production. The water activity (aw) was measured with AW-CENTER (Novasina Co. Ltd., Pfaffikon, Switzerland). The sugar content was measured by an Appel type
refractometer IN-2E (As One Co. Ltd., Tokyo, Japan) after reconstituting the samples in distilled water followed by centrifugation. The water content was measured by weighting the samples before and after drying at 105°C until the value reached a constant (5 hours). The rate of syneresis of uninoculated laboratory-prepared Awa-Uirou was calculated from the number of samples showing syneresis out of the total 60 samples.

**Inocula and enumeration**

The bacterial strains used for this study were: *B. subtilis* strains 740, U-1, U-18, S-26, and S-56 isolated from Awa-Uirou samples; *B. cereus* strains 1F03457 (origin unknown), S-8 (from Awa-Uirou), 734 (from rice flour), and IDC22, IDC23, and IDC24 (all from the swab of a food factory); *S. aureus* strains IF013726 (from a human lesion), JCM2179 (origin unknown), JCM2874 (from a wound), and 727 (from Awa-Uirou). A rifampicin-resistant spontaneous mutant of each strain was isolated by the enrichment cultivation method (Inatsu et al., 2004). Spores of *B. subtilis* and *B. cereus* were produced in Schaeffer’s sporulation medium (containing, per liter, 8 g of Bacto-nutrient broth, 10 mL of 10% KCl, 10 mL of 1.2% MgSO₄·7H₂O, 0.50 mL of 1 M NaOH, 1.0 mL of 1 M Ca(NO₃)₂, 1.0 mL of 0.010 M MnCl₂, 1.0 mL of 1 mM FeSO₄) shaken at 30°C for 4 days (150 rpm). Spores were harvested by centrifugation after lysozyme treatment (in case of *B. subtilis*) or self-lysis by 1 day at 4°C storage (in case of *B. cereus*) followed by repeated centrifugation and washing twice in sterile distilled water. Early stationary phase cells of *S. aureus* prepared in brain heart infusion broth (Nissui Co. Ltd., Tokyo) were used for the inoculation studies. A cocktail of spores or pure bacterial culture was used as inocula. Trypticase soy agar (Nissui Co. Ltd.) containing 50 μg/mL rifampicin (Wako Pure Chemical Co. Ltd., Tokyo) was used for the enumeration of inoculated samples. A standard plate count agar (Nissui Co. Ltd.) was used for the determination of aerobic bacterial counts of noninoculated samples.

**Food additives**

Glycine (Wako Pure Chemical Co. Ltd.), the hop extract *Hoprex* (Mitsubishi Chemical Foods Co. Ltd., Tokyo), and protein from salmon soft roe (Asama Chemical Co. Ltd., Tokyo) were used for experiments. All of these additives were dissolved in water to make solutions of different concentrations as shown in Table 1.

**Preparation of Awa-Uirou and inoculation tests**

Awa-Uirou was prepared from commercial rice flour, sugar, and red beans. Raw sweet red bean paste (Nama-Ann) was prepared from red beans and sugar and was used as a filler in Awa-Uirou. Awa-Uirou was prepared from commercial rice flour, sugar, and red beans. Raw sweet red bean paste (Nama-Ann) was prepared from red beans and sugar and was used as a filler in Awa-Uirou.

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**Table 1. Effectiveness of Food Additives for Inhibiting *B. subtilis* in Uirou-Tane Before the Steaming Process**

<table>
<thead>
<tr>
<th>Additive</th>
<th>Concentration (mg/g sample)</th>
<th>0 day</th>
<th>3 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>0</td>
<td>2.7 ± 0.1</td>
<td>6.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>2.7 ± 0.1</td>
<td>5.3 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>2.9 ± 0.0</td>
<td>2.8 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.9 ± 0.0</td>
<td>2.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>2.8 ± 0.1</td>
<td>2.7 ± 0.1</td>
</tr>
<tr>
<td>Hop extract</td>
<td>0</td>
<td>2.9 ± 0.1</td>
<td>6.8 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>0.125</td>
<td>2.7 ± 0.1</td>
<td>6.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>2.8 ± 0.0</td>
<td>6.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>2.9 ± 0.0</td>
<td>6.3 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>2.7 ± 0.0</td>
<td>5.4 ± 0.1</td>
</tr>
<tr>
<td>Salmon roe protein</td>
<td>0</td>
<td>2.8 ± 0.1</td>
<td>7.2 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>2.7 ± 0.0</td>
<td>7.0 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>2.8 ± 0.0</td>
<td>7.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>2.8 ± 0.0</td>
<td>6.9 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>2.8 ± 0.0</td>
<td>7.1 ± 0.2</td>
</tr>
</tbody>
</table>

Data represent mean log value of the viable cell counts from three independent experiments as described in Methods.
beans as follows: 100 g of red beans was soaked in 300 mL of tap water for 16 hours at 25°C. The swollen beans were then boiled in 250 mL of tap water for 1 hour and the excess water was drained off, followed by a second wash with tap water. The beans were boiled again with 500 mL of tap water for 1 hour until softened.

Softened beans were mashed by using a food processor and then pureed with 400 mL of water. The supernatant was discarded after precipitation and the puree was resuspended with 500 mL of water. This washing step was repeated again to purify the bean paste. To reduce the water content to 65–68%, the bean paste was dewatered by wringing in a sterile closed vessel.

Sarashi-An (100 g) and 70 g of granulated sugar were mixed well, then 36 g of rice flour (Jo-Yo-Ko) was added. Finally, the sugar content was adjusted to 45 degrees Brix by adding water. Different concentrations of glycine, hop extract, and protein from salmon soft roe was mixed with this Awa-Uirou paste (Uirou-Tane).

Ten grams of prepared Uirou-Tane paste was packed into a sterile plastic bag. The paste was inoculated with spores of *B. cereus*, *B. subtilis*, or vegetative cells of *S. aureus* for "presteaming inoculation" tests. The tightly sealed bags were steamed at 100°C for 70 minutes. For "post-steaming inoculation" tests, the bags were opened after steam treatment, the inoculum was added, and the bags were sealed again. The inoculated and noninoculated (control) samples were stored at 30°C for 3 days.

Each bag containing Awa-Uirou was then sanitized with 70% ethanol and opened with sterile scissors in a laminar airflow biological cabinet. The Awa-Uirou was transferred into a stomacher bag, 90 mL of phosphate-buffered saline was added, and the bag contents were homogenized for 1 minute in a stomacher. Ten-fold serial dilutions were made and samples were plated on selective and nonselective agars for bacterial enumeration. All the inoculation studies were repeated four times. The logarithmic values of viable cell counts were used for statistical analysis.

Rate of syneresis

Twenty bags of Uirou-Tane without inoculation of any bacteria, prepared by the above method, were stored at 30°C. The syneresis caused by natural bacterial spore contamination was evaluated visually. This experiment was repeated three times. The rate of syneresis was calculated as the percentage of samples showing signs of syneresis out of the total 60 samples examined.

**Sensory evaluation**

Several packs of 100 g of noninoculated Awa-Uirou samples with glycine (2.5 or 5.0 mg/g sample) or without glycine were prepared. The color (appearance), taste, and texture were evaluated by a visual analogue scale method (Har-Zion et al., 2004). Testing was conducted in the sensory laboratory at the National Food Research Institute. Twenty-one trained panelists (12 men and 9 women) were informed about the nature of the study. The test area was free of extraneous odors and sound, and panelists were instructed not to talk during testing. Panelists evaluated samples and marked score sheets in individual booths. Panelists were instructed to evaluate samples in the order of presentation and to clear their palates between samples with the crackers and water. The presentation order of the samples was balanced among panelists to avoid a position error bias. Three 15-cm horizontal lines with two anchors corresponding to the opposite (worst and best) attributes for each quality at 1.5 cm from each end of the line were shown to the panelists. The panelists marked points on the lines corresponding to the value of their evaluation. The values corresponding to each quality were calculated by using the formula: value = length between left side (corresponding to "worst") to the mark (cm)/15 (cm)×10. The Tukey–Kramer test was used to determine the statistical significance.

**Statistical analysis**

All experiments were done in triplicate with duplicate samples being analyzed at each sampling time. Data were subjected to analysis of variance (ANOVA) using the Microsoft (Redmond, WA) Excel program. Significant differences in plate count data were established by least significant difference at the 5% level of significance.
Results

Physical parameters of commercial Awa-Uirou

The average values of the sugar content, water content, and water activity \((a_w)\) of the 29 locally made samples were 56.7 ± 4.1 degrees Brix, 41.4 ± 4.0%, and 0.924 ± 0.010, respectively. The ranges of these values were 48.0 to 64.0 degrees Brix, 32.1-48.7%, and 0.900-0.949, respectively. A highly positive relationship \((R^2 = 0.84)\) between the value of water content and water activity was observed (data not shown). A clear negative relationship \((R^2 = 0.91)\) between the value of water content and sugar content was also exhibited (data not shown). Therefore, the sugar content was inversely proportional to the water activity (Fig. 1). The shelf life on the samples set by manufacturers was 7.3 ± 4.6 days (range, 1–16 days). No clear relationship was observed between shelf life and sugar or water content (data not shown).

Evaluation of the natural microflora in Awa-Uirou

The growth of bacteria naturally present in laboratory-prepared uninoculated Awa-Uirou was evaluated. The heat-resistant bacteria grew rapidly to reach 5.0 log colony-forming units (CFU)/g within 3 days and reached maximum level (6.4 log CFU/g) at day 6 and remained constant throughout the incubation period (Fig. 2). The rate of syneresis increased proportionally to the growth of bacteria and reached 28% at day 7 at 30°C storage.

Artificial inoculation before steaming operation, evaluation, and control

The survival and growth of cells or spores in Awa-Uirou artificially inoculated with \(S.\) aureus, \(B.\) cereus, and \(B.\) subtilis before steaming operation were evaluated. Each of 3.5, 4.5, and 4.8 log CFU/g of \(B.\) subtilis, \(B.\) cereus, and \(S.\) aureus spores or cells, respectively, were inoculated into 10 g of Uirou-Tane (paste) and steamed at 100°C for 70 minutes. The residual viable \(S.\) aureus cell and \(B.\) cereus spores were less than 2.3 log CFU/g after the steaming operation and subsequent incubation for 7 days at 30°C (data not shown). However, the 2.9 log CFU/g of residual \(B.\) subtilis spore started to germinate rapidly and reached 6.6 log CFU/g within 3 days when stored at 30°C (Table 1). The effectiveness of glycine, hop extract, and salmon soft roe protein in presteamed Uirou-Tane inoculated with \(B.\) subtilis is shown on Table 1. The hop extract and salmon soft roe protein did not show any antimicrobial effect against \(B.\) subtilis at the concentrations recommended by the company (Mitsubishi Chemical Food Co. Ltd., Tokyo, Japan). However, the addition of > 2.5 mg/g glycine effectively suppressed the growth of inoculated \(B.\) subtilis. The growth patterns of \(B.\) subtilis in Awa-Uirou supplemented with 2.5 and 5.0 mg/g of glycine and stored at 30°C are shown in Fig. 3. The addition of 2.5 mg/g of glycine was found to suppress the growth of inoculated bacteria by 2 days and thereafter, the bacteria grew rapidly and reached 6.0 log CFU/g level within 5 days. However, the

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**FIG. 1.** Relationship of water activity and sugar contents of Awa-Uirou \((n = 29)\).

**FIG. 2.** The growth of heat-resistant spores in non-inoculated Awa-Uirou (●) and the rate of syneresis (▲).
addition of 5.0 mg/g of glycine suppressed the growth of *B. subtilis* throughout the incubation period at 30°C.

**Artificial inoculation after the steaming operation, evaluation, and control**

The growth of bacteria in Awa-Uirou artificially inoculated with *S. aureus* and *B. cereus* after the steaming operation was evaluated (Fig. 4). *S. aureus* grew rapidly to reach 7.0 log CFU/g level in 2 days under 30°C storage condition. However, *B. cereus* grew slowly and reached 4.0 log CFU/g within 3 days and remained constant and/or increased slightly throughout the incubation period at 30°C. The addition of 5.0 mg/g of glycine did not affect the growth of *S. aureus* throughout the 7-day incubation period at 30°C (data not shown), but the growth of *B. cereus* was suppressed throughout the 7-day incubation period at 30°C.

**Sensory evaluation**

The sensory quality of Awa-Uirou supplemented with glycine (2.5 and 5.0 mg/g) or without glycine was evaluated by 21 trained panelists according to a visual analogue scale method. As shown in Table 2, no significant influence on color (appearance), taste, or texture was observed after the addition of glycine up to 5.0 mg/g.

**Discussion**

Enterotoxin-producing *B. cereus* and *S. aureus* are recognized as potential hazards from raw confections (Anunciacao et al., 1995; Agata and
Mori, 1997; Agata et al., 2002). Sticky rice cakes commonly consumed in Japan and other Far East and Southeast Asian countries have been associated with several outbreaks of food poisoning (Kaneko et al., 1982; Ishii et al., 1988; Uematsu and Kaneko, 1989; Anunciacao et al., 1995; Houjyo et al., 2001; Saito et al., 2001; Matsuoka et al., 2003; Niwa et al., 2003; Huruse et al., 2004). To evaluate or control the risk of food poisoning from this kind of food, we chose Awa-Uirou and surveyed its physical parameters relating to bacterial growth and investigated the sterilization efficacy of the steaming operation, growth characteristics of the bacteria, and effectiveness of antimicrobial compounds to control the growth of bacteria.

The water activity of commercial Awa-Uirou was found to be 0.900 to 0.949 (Fig. 1) and the pH was between 6.0 and 7.0, which suggests that these products could have a potential risk to cause food poisoning. According to the FDA’s definition of potentially hazardous foods (FDA, 2001), those foods which have pH and aw over 5.0 and 0.88, respectively, are potentially hazardous foods.

There are two possibilities for bacterial contamination of Awa-Uirou: 1) cells or spores that survive after steaming and 2) surface contamination of the product by hands, cooking instruments, or the environment. However, during our experiments, S. aureus cell and B. cereus spore contamination of Uirou-Tane was not detected even after 7 days of storage at 30°C (data not shown). The decimal reduction time at 58°C (D58) of S. aureus cells in buffer solution at pH 7.0 was reported as 0.32 ± 0.33 (0.09–1.1) minutes (Thomas and Masters, 1988). Dufrenne et al. (1994, 1995) reported the D90 value of B. cereus to be 23.0 ± 41.6 (2.2 to >200) minutes. In our experiment, we found that the initial level of contaminated cells or spores was <3.0 log CFU/g Uirou Tane (Fig. 2). From this viewpoint, 70 minutes of steaming operation at 100°C was thought to be sufficient to kill S. aureus cells and most of the B. cereus spores. However, extremely heat-resistant B. cereus spores can survive the steaming operation.

After steaming, contaminating S. aureus cells and B. cereus spores are thought to grow rapidly at 30°C (Fig. 4). Agata et al. (2002) reported that the accumulation of emetic toxin by B. cereus reached <10 ng/g when the viable B. cereus cell counts were >6.0 log CFU/g in boiled rice. The average value of B. cereus viable cells in Awa-Uirou in most cases was found to be >5.0 log CFU/g after 7 days of storage at 30°C and some samples exhibited close to 6.0 log CFU/g (Fig. 4). Interestingly, the addition of 5.0 mg/g of glycine could suppress the growth of B. cereus significantly, suggesting the usefulness of such antimicrobials (Fig. 4).

A starch matrix such as rice flour gel is a good medium for the growth of S. aureus and enterotoxin production (Shinagawa et al., 1982). According to several reports of outbreaks related to sticky rice cakes in Japan (Ishii et al., 1988; Houjyo et al., 2001; Tsuji et al., 2003), 7.0 log CFU/g S. aureus could produce 2.4 to 5.1 ng/g of enterotoxins, which is thought to be sufficient to cause foodborne disease. Based upon these observations and our experimental results (Fig. 4), post-process contamination of Awa-Uirou over 2 days at 30°C without any bacterial control may contribute to the risk of food poisoning by S. aureus enterotoxins.

The syneresis of starch gel matrix is a major cause of quality loss of Uirou. As shown in Fig. 2, with natural contamination, bacterial spores grew rapidly to cause syneresis of Awa-Uirou samples when stored at 30°C. With artificial contamination, B. subtilis spores, added before steaming, exhibited similar growth patterns (6.0 log CFU/g) (Fig. 3) and resulted in syneresis of the samples. B. subtilis is known to produce several kinds of extracellular enzymes. The destruction of the sugar chain of starch by amylase or decomposition of sucrose by levan sucrase is thought to increase or decrease the water-holding capacity of the starch gel matrix and consequently cause syneresis of Awa-Uirou (Naito and Matsunaga, 2002).
It is not feasible to store ordinary rice flour gel foods such as Uirou at low temperature or freezing to prevent the growth of bacteria because the product easily loses its texture during aging due to disruption of the starch gel matrix. pH control is not suitable because it changes not only the taste and texture but also the color. However, the addition of 5.0 mg/g of glycine was found to effectively suppress the growth of \textit{B. cereus} (Figs. 3 and 4) without changing the sensory characteristics of Awa-Uirou (Table 2). These results suggested that glycine could be useful for reducing the risk of food poisoning by \textit{B. cereus} enterotoxins and preventing quality losses caused by \textit{B. subtilis}.

In conclusion, the results of this study demonstrated that the addition of 0.5\% glycine before the steaming process could reduce the microbial risk of the steamed rice confection and prevent the risk of food poisoning and quality loss. Therefore, addition of glycine along with good manufacturing and handling practices could be an effective means for reducing the risk of contamination of Awa-Uirou.

Acknowledgment

This work was done as part of “Food Project” with financial support by the Ministry of Agriculture, Forestry and Fisheries. We would like to thank Mitsubishi Chemical Food Co. Ltd. and Asama Chemical Co. Ltd. for kindly providing the food additives. We are grateful to Ms. Mari Mochida and Ms. Tomoko Kitagawa for their technical assistance and support.

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