October 2013. Assuming that each store disposes of 10 kg of food as waste per day, simple arithmetic suggests a wastage of 500 tons of food every day. It is easy to imagine that the high food waste rate in Japan partly results from this large quantity of food wasted at CVSs. While our food self-sufficiency rate is only 40% based on calories, the wastage rate is reaching 25%.

One effective measure to reduce the disposal loss of boxed lunches and ready-made dishes at CVSs is to extend their consume-by dates. There are generally two ways to accomplish this. Firstly, a storage test can be conducted to re-evaluate the current consume-by date and determine whether it should stand or if there still is room for extension. Secondly, some technical innovations can be made to extend the consume-by date. By developing novel containers and packaging techniques, utilizing improvements in storability, and upgrading food distribution systems and cold chains, we may be able to extend the consume-by date for boxed lunches and ready-made dishes.

As already stated, while the shelf-life for boxed
lunches and ready-made dishes sold at CVSs is relatively short (about two days), there is only a limited information on their bacteriological levels (Ikegame et al., 1994). Consumers have no choice but trust the quality of the product and purchase it using the consume-by date printed on it as an indicator. Consumers also seem to think that the products are still edible even after the expiry of the consume-by date if not too much time has passed.

In the present study, we investigated cooked rice products sold at CVSs for their actual bacteriological levels. We used rice balls (Onigiri), one of the most popular rice products, as a representative product and evaluated the adequacy of their consume-by dates. The results are herein reported.

MATERIALS AND METHODS

Study samples
The study samples were obtained from two CVSs near our university. Those CVSs belong to Company A and Company B, respectively. The sample rice balls were purchased immediately after their delivery to the respective CVS and brought back to our laboratory. The samples consisted of a total of 240 rice balls, including 60 canned tuna and mayonnaise rice balls (hereinafter abbreviated as Tunamayo) and 60 pickled plum rice balls (hereinafter abbreviated as Plum) from each CVS.

Storage conditions
Sixty samples of Tunamayo and 60 samples of Plum from the two CVSs were divided into 4 groups (15 each) and subjected to a microbiological examination starting immediately after the delivery till several h after the expiry of the consume-by date. The products of Company A were tested at 1, 6, 19 and 24 h and that of Company B were tested at 1, 7, 19 and 27 h after delivery. Throughout this paper, the h indicated the time after delivery of the samples to the respective CVS. Nineteen h after delivery was almost the end of the consume-by date for the products of both companies. For Company A products, samples for 1 and 19 h after delivery belonged to the same lot, and those for 6 and 24 h after delivery belonged to another lot. For Company B products, samples for 1 and 19 h after delivery belonged to the same lot, and those for 7 and 27 h after delivery belonged to another lot. The samples were stored at the same temperature as the storage temperature of each CVS, namely 20°C for Company A products and 13°C for Company B products.

Viable cell counts
The samples were diluted in standard method agar (Eiken Chemical Co., Ltd.) and incubated at 35°C for 48 h.

Coliform and Escherichia coli counts
The samples were diluted in XM-G agar (Nissui Pharmaceutical Co., Ltd.) (Ogihara et al., 2004) and incubated at 35°C for 24 h. The colonies with blue or violet-blue color were determined to be E. coli and those with pink or red color were determined to be coliforms other than E. coli.

Staphylococcus spp. counts
The samples were streaked onto Nissui mannitol salt agar with egg yolk (MSEY) plates (Nissui Pharmaceutical Co., Ltd.) and incubated at 35°C for 48 h.

Simplified identification for Gram-negative bacteria
Since the majority of Gram-positive bacteria detected in the storage test were Staphylococcus spp., a simplified identification method was employed for Gram-negative bacteria. After the Gram staining for standard method agar plates, those determined to be Gram-negative were subjected to an oxidase test using Bactident Oxidase (Merk). The simplified identification was carried out on the oxidase-negative and oxidase-positive bacteria using API20E and API20NE, respectively (both reagents from SYSMEX bioMerieux).

RESULTS AND DISCUSSION

Detection of bacteria in samples
TABLE 1 summarizes the detection of bacteria in the Tunamayo and the Plum samples of the two CVSs, based on the results obtained from the storage test. At 1 h after delivery, the viable cell counts for the Tunamayo of both companies A and B were distributed between 10¹ - 10² CFU/g indicating a generally good bacteriological quality. As the time elapsed after the delivery, the center of viable cell count distribution shifted towards a higher level. At 19 h after delivery, the viable cell counts for the Tunamayo of Company A were between 70 - 270,000 CFU/g (data not shown) and the dispersion also increased with the storage time. At the same testing point, the viable cell counts for the Tunamayo of Company B were between 410 - 4,700 CFU/g, indicating an overall tendency for a lower value than that for Company A (data not shown). This was thought to be the result of the bacterial growth suppression in the products because a lower storage temperature was maintained at Company B (13°C). In many CVSs, the storage temperature for cooked rice products is set at 20°C to avoid the problem of decline in the quality of rice. However, solely from a
TABLE 1. Viable cell counts, coliform counts, *E. coli* counts and *Staphylococcus* spp. counts in two kinds of rice balls produced by Company A and Company B

### Viable cell counts

| Order (CFU/g) | <10 | 10<sup>1</sup> | 10<sup>2</sup> | 10<sup>3</sup> | 10<sup>4</sup> | 10<sup>5</sup> | 10<sup>6</sup> |
|---------------|-----|----------------|----------------|----------------|----------------|----------------|----------------|
| **Tuna and mayonnaise** |     |                |                |                |                |                |                |
| Company A      | 1 h | 0              | 7              | 8              | 0              | 0              | 2              |
|                | 6 h | 1              | 4              | 8              | 2              | 0              | 13             |
|                | 19 h| 0              | 1              | 6              | 6              | 0              | 2              |
|                | 24 h| 0              | 0              | 0              | 4              | 7              | 4              |
| **Pickled plum**|    |                |                |                |                |                |                |
| Company A      | 1 h | 0              | 5              | 10             | 0              | 0              | 0              |
|                | 7 h | 0              | 0              | 0              | 14             | 0              | 2              |
|                | 19 h| 0              | 0              | 0              | 11             | 0              | 1              |
|                | 27 h| 0              | 0              | 0              | 0              | 14             | 1              |
| **Company B**  |    |                |                |                |                |                |                |
| **Coliform counts** |     |                |                |                |                |                |                |
| Order (CFU/g) | <10 | 10<sup>1</sup> | 10<sup>2</sup> | 10<sup>3</sup> | 10<sup>4</sup> | 10<sup>5</sup> | 10<sup>6</sup> |
| **Tuna and mayonnaise** |     |                |                |                |                |                |                |
| Company A      | 1 h | 13             | 2              | 0              | 0              | 0              | 15             |
|                | 6 h | 6              | 2              | 3              | 4              | 0              | 15             |
|                | 19 h| 9              | 1              | 2              | 1              | 1              | 15             |
|                | 24 h| 2              | 1              | 2              | 3              | 5              | 2              |
| **Pickled plum**|    |                |                |                |                |                |                |
| Company A      | 1 h | 15             | 0              | 0              | 0              | 0              | 13             |
|                | 7 h | 15             | 0              | 0              | 0              | 0              | 13             |
|                | 19 h| 15             | 0              | 0              | 0              | 0              | 13             |
|                | 27 h| 15             | 0              | 0              | 0              | 0              | 15             |
| **Company B**  |    |                |                |                |                |                |                |
| **E. coli counts** |     |                |                |                |                |                |                |
| Order (CFU/g) | <10 | 10<sup>1</sup> | 10<sup>2</sup> | 10<sup>3</sup> | 10<sup>4</sup> | 10<sup>5</sup> | 10<sup>6</sup> |
| **Tuna and mayonnaise** |     |                |                |                |                |                |                |
| Company A      | 1 h | 15             | 0              | 0              | 0              | 0              | 15             |
|                | 6 h | 15             | 0              | 0              | 0              | 0              | 15             |
|                | 19 h| 15             | 0              | 0              | 0              | 0              | 15             |
|                | 24 h| 15             | 0              | 0              | 0              | 0              | 15             |
| **Pickled plum**|    |                |                |                |                |                |                |
| Company A      | 1 h | 15             | 0              | 0              | 0              | 0              | 15             |
|                | 7 h | 15             | 0              | 0              | 0              | 0              | 15             |
|                | 19 h| 15             | 0              | 0              | 0              | 0              | 15             |
|                | 27 h| 15             | 0              | 0              | 0              | 0              | 15             |
| **Company B**  |    |                |                |                |                |                |                |
| **Staphylococcus spp. counts** |     |                |                |                |                |                |                |
| Order (CFU/g) | <10 | 10<sup>1</sup> | 10<sup>2</sup> | 10<sup>3</sup> | 10<sup>4</sup> | 10<sup>5</sup> | 10<sup>6</sup> |
| **Tuna and mayonnaise** |     |                |                |                |                |                |                |
| Company A      | 1 h | 15             | 0              | 0              | 0              | 0              | 15             |
|                | 6 h | 11             | 4              | 0              | 0              | 0              | 15             |
|                | 19 h| 11             | 3              | 1              | 0              | 0              | 15             |
|                | 24 h| 5              | 7              | 2              | 1              | 0              | 14             |
| **Pickled plum**|    |                |                |                |                |                |                |
| Company A      | 1 h | 14             | 1              | 0              | 0              | 0              | 15             |
|                | 7 h | 15             | 0              | 0              | 0              | 0              | 15             |
|                | 19 h| 15             | 0              | 0              | 0              | 0              | 15             |
|                | 27 h| 15             | 0              | 0              | 0              | 0              | 15             |

Samples were stored at 20°C for Company A products and at 13°C for Company B products, respectively. Fifteen samples were examined for each storage period. The shaded columns indicate the counts shared by more than five samples.

bacteriological point of view, the importance of a lower storage temperature was confirmed. At 24 h (Company A) and at 27 h (Company B) after delivery, the products of both companies showed a viable cell count
counts were distributed centering around $10^2$ CFU/g. As for the Plum of Company A, the viable cell counts were distributed centering around $10^1$ at 1 h after delivery, indicating a favorable quality. At 6 h after the delivery, 13 samples showed cell counts lower than the value obtained at 1 h. This was thought to be caused by a variation in the initial viable cell counts between two different production lots which we employed for the sake of test efficiency. In contrast to the Tunamayo, the Plum showed no tendency for the center of viable cell counts to shift towards a higher level with the passage of time. Even at 24 h after delivery, the cell count distribution centered around $10^2$ CFU/g. At 1 h after delivery, the viable cell counts for the Plum of Company B were between $10^2$ - $10^3$ CFU/g (actual values were 90 - 5,600 CFU/g) which were slightly higher than that for the Plum of Company A (data not shown). As was the case for the Plum of Company A, the viable cell counts for the Plum of Company B showed no tendency to shift towards a higher level with the passage of time. Even at 27 h after delivery, the center of distribution was between $10^1$ - $10^2$ CFU/g. As previously stated, there was no large increase in the cell counts for the Plum of both companies A and B compared with that for the Tunamayo. The reason was attributed to the antibacterial action of citric acid in pickled plums (Alonso-Hernando et al., 2013). Viable cell counts in two kinds of rice balls are summarized in TABLE 2.

As for the Plum of Company A, the viable cell counts for Staphylococcus spp. were distributed centering around $10^2$ CFU/g. At 1 h after delivery, Staphylococcus spp. was detected in most of the Plum of Company B and the counts were distributed between $10^1$ - $10^2$ CFU/g. This was thought to be due to salt tolerance of Staphylococcus spp. (Nagamachi et al., 1985). At 7 and 27 h after delivery, the Staphylococcus spp. counts for all 15 samples were $<10$ CFU/g. This was also attributed to the variation in the initial viable cell counts between two production lots. At 19 h after delivery, the values were distributed centering around $10^3$ CFU/g. The "Health-code for boxed lunches and ready-made dishes" stipulates that viable cell counts in precooked foods should be no more than $1.0 \times 10^5$ CFU/g. Ikegame et al. (1994) conducted a bacteriological test on grilled salmon rice balls sold at CVSs and reported that 26.6% of the samples had $10^5$ CFU/g or more viable cell counts immediately after purchase. The ratio increased up to 51.1% of the samples at the end of the consume-by date. In our present study, the number of samples with $10^5$ CFU/g or more viable cell counts at 19 h after delivery was 2 (13.3%) for the Tunamayo and the Plum of Company A, 0 for the Tunamayo of Company B, and 1 (6.7%) for the Plum of Company B.

**TABLE 2. Viable cell counts for each storage period in two kinds of rice balls produced by Company A and Company B**

| Company | Time after delivery (h) | Tuna and mayonnaise | Pickled plum |
|---------|-------------------------|---------------------|-------------|
| A       | 1                       | 2.10 ± 0.36         | 1.48 ± 0.58 |
|         | 6                       | 3.01 ± 0.91         | 1.36 ± 0.68 |
|         | 19                      | 4.43 ± 1.00         | 2.69 ± 0.98 |
|         | 24                      | 5.15 ± 0.72         | 2.23 ± 1.06 |
| B       | 1                       | 2.16 ± 0.34         | 3.21 ± 0.52 |
|         | 7                       | 3.26 ± 0.20         | 2.36 ± 0.90 |
|         | 19                      | 3.29 ± 0.31         | 4.18 ± 0.97 |
|         | 27                      | 4.59 ± 0.23         | 2.22 ± 0.99 |

*Mean value ± standard deviation of the fifteen samples.*
Since the rice balls examined were not the same type and most likely produced at different vendor factories, a categorical comparison is not possible. Nevertheless, compared with the results of Ikegame et al., our present results indicated a generally better bacteriological quality of rice balls.

**Correlation between the elapsed time after delivery and viable cell counts**

For each storage period, the rice balls with the highest viable cell counts were examined for a correlation to the elapsed time after delivery (FIG. 1). There was a good correlation observed for both products of Company A with a correlation coefficient of 0.9845 for the Tunamayo and 0.8867 for the Plum. The slope of the correlation curve was less steep for the Plum than that for Tunamayo, indicating a more gradual increase in the viable cell counts, which in turn, was attributed to the antimicrobial activity in pickled plums.

There was also a good correlation found for the Tunamayo of Company B. The correlation factor was 0.9214 and the slope of the curve was more gradual compared with the Tunamayo of Company A. On the other hand, no correlation was found for the viable cell counts for the Plum of Company B. The counts did not increase with the elapsed time after delivery. The highest bacterial count was observed in the Plum at 19 h after delivery. As mentioned previously, it was due to a variation in the initial viable cell counts between the two production lots examined in the current study.

Except for the Plum of Company B, there was a good correlation between the viable cell counts and the elapsed time after the delivery. The obtained regression equations varied depending on the type of rice balls and their storage temperature.

**Evaluation of the adequacy of the consume-by date**

From the above results, the adequacy of the consume-by date was bacteriologically evaluated. As previously described, “Health-code for boxed lunches and ready-made dishes” stipulates that the viable cell counts in precooked foods should be no more than $1.0 \times 10^5$ CFU/g. From the regression equation obtained in FIG 1, the time after delivery required to reach this value would be 16 h for the Tunamayo of Company A, 55 h for the Plum of Company A, and 30 h for the Tunamayo of Company B (TABLE 3). Bacteriologically speaking, the Plum of Company B could be stored for an extended period of time.

The samples used in the present storage test were cooked rice products purchased at CVSs. These products went through the actual distribution chain from the production at the vendor factory, shipping and delivery to each CVS by delivery vehicles. In order to validate the storability of a newly-developed product and to determine the consume-by date (or best-before date), the manufacturer must conduct a preservation test. However, this test usually does not include a consideration of the actual distribution chain. On the other hand, our present study model could determine a more realistic consume-by date.

In the food industry, a consume-by date (or best-before date) is determined by multiplying the storage test result by 0.7 or 0.8 as a safety factor. The actual value for a safety factor is frequently indicated as a guideline by the trade organization of each food industry. With this in mind, we measured the time for

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**TABLE 3.** Calculated consume-by dates for rice balls produced by Company A and Company B

| Company | Product         | Hours to reach $10^5$CFU/g (h) | $(A) \times 0.8$ (h) | $(A) \times 0.7$ (h) |
|---------|-----------------|-------------------------------|---------------------|---------------------|
| A       | Tuna and mayonnaise | 16                             | 13                 | 11                  |
|         | Pickled plum     | 55                             | 44                 | 38                  |
| B       | Tuna and mayonnaise | 30                             | 24                 | 21                  |
|         | Pickled plum     | NAa                            | NA                 | NA                  |

The consume-by dates for each product were calculated by inserting $10^5$ CFU/g in each equation for regression. “Health-code for boxed lunches and ready-made dishes” stipulates a value for viable cell counts in precooked foods to be no more than $10^6$ CFU/g.

*NAa: Not applied.
the viable cell counts of the samples to reach $1.0 \times 10^8$ CFU/g and multiplied the result by a safety factor of 0.8 or 0.7 to set a consume-by date (TABLE 3). With a stricter safety factor of 0.7, the consume-by date should be 11 and 38 h for the Tunamayo and the Plum of Company A, respectively, and 21 h for the Tunamayo of Company B. We made an inquiry to the companies A and B regarding the consume-by date of the rice balls examined in our study. It was between 17 - 20 h after the delivery for the Company A and 20 h for the Company B according to their internal standards. While the current consume-by date was adequate for the Plum of both companies and the Tunamayo of Company B, it was not the case for the Tunamayo of Company A. The product failed to meet the standard before the consume-by date set by the company and there seems to be a need for improvement in the quality of raw materials and the sanitary management of the vendor factory.

**Gram staining of isolated microbes**

Using Gram staining, we examined the microbes which were isolated from the samples at 19 h after delivery and which grew on a standard method agar. The number of isolated strains was as follows: 16 for the Tunamayo of Company A, 15 for the Plum of Company A, 15 for the Tunamayo of Company B, and 16 for the Plum of Company B.

The breakdown of 16 strains found in the Tunamayo of Company A showed 11 Gram-negative bacilli (68.8%), 2 Gram-negative short bacilli and 2 Gram-positive cocci (12.5% each), and 1 Gram-positive short bacillus (6.3%). All 15 strains found in the Plum of Company A were yeast. The breakdown of 15 strains found in the Tunamayo of Company B showed 7 Gram-positive cocci (46.7%), 6 Gram-positive bacilli (40.0%), and 2 Gram-positive short bacilli (13.3%). There were 15 Gram-positive cocci (93.8%) and 1 Gram-negative bacilli (6.3%) among 16 bacterial strains found in the Plum of Company B, and it was indicated that even for the same type of rice balls, the flora was different for each CVS. The flora was also different for the type of rice balls even they were produced by the same Company (data not shown).

Finally, 14 strains that were determined to be Gram-negative were subjected to simplified identification using API (TABLE 4). As a result, 10 (76.9%) out of 13 strains found in the Tunamayo of Company A and 1 strain (100%) found in the Plum of Company B were identified as *Serratia* sp.. In particular, there were a total of 9 samples which had *S. marcescens* including 8 Tunamayo samples of Company A and 1 Plum sample of Company B.

*Serratia* sp. has been isolated from soil, sewage and the fishery products (Mace et al., 2013). The representative species *S. marcescens* is known to possess a strong proteolytic activity and plays a role in the spoilage of fish meat, beef, milk and Kamaboko (minced and steamed fish meat) (Adham, 2003; Nabetani et al., 1974). In the present study, *Serratia* was found in a product (Plum) which does not fall into the above category. It would be that *Serratia* sp. such as *S. marcescens* inhabits the vendor factories.

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| COMPANY : PRODUCT | MICROORGANISMS | NO. OF ISOLATES |
|-------------------|---------------|----------------|
| **COMPANY A : TUNAMAYO** |             |               |
| *Serratia marcescens* | 8            |               |
| *Raoultella terrigena* | 2            |               |
| *Serratia liquefaciens* | 1            |               |
| *Serratia odorifera* | 1            |               |
| *Pseudomonas aeruginosa* | 1            |               |
| **COMPANY B : PULB** |             |               |
| *Serratia marcescens* | 1            |               |
| **TOTAL** | **14**     |               |

Gram-negative bacteria were identified by using API 20E or 20NE.
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