Transportation via containers at ice temperature inhibits decay and maintains the quality of certain fresh produce

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ABSTRACT

The objective of this study was to determine the differences in quality of fresh produce when exported at ice temperature and general temperature. Twenty-eight different types of fresh produce were exported from Japan to Singapore simultaneously in a precise temperature-controlled reefer container set at 0°C and a regular reefer container set at 5°C. In Singapore, we used instrumental analysis and sensory evaluation to compare the quality of fresh produce. The growth of molds on figs and grapes ("Shine Muscat") that were transported at 0°C was inhibited. Additionally, the growth of aerial mycelia on the shafts of shiitake mushrooms was inhibited. Consequently, the saleable quality rate increased upon transportation increased at 0°C. The comparison of appearance by sensory evaluation revealed that nine items, including leafy vegetables and mushrooms, transported at 0°C were preferred over those transported at 5 °C.

1. Introduction

The total value of global food exports in 2020 was USD 1,581 billion, which is more than three times the value in 2000 (UNCTAD, 2022). These values are expected to continue to increase in the future, thus, the importance of food logistics is increasing annually, and research is being conducted on this topic (Ait Hou et al., 2015; Tanrattanaphong et al., 2020). However, despite the fact that fresh produce is essential for daily life, the volume of export is not very high because of its short shelf life and strict regulations imposed regarding quarantine for pests and pesticide residues. The export value of agricultural, forestry, fishery, and food products from Japan in 2020 was JPY 922.3 billion (Ministry of Finance Japan, 2021), among which the export of fresh fruits, vegetables, and nuts accounts for JPY 45.7 billion. These values correspond to USD 8.6 billion and 427 million, respectively, calculated at the annual exchange rate for the year (Mitsubishi UFJ Research and Consulting, 2022). Although the amount of exported fresh produce is not high, a significant upward trend has been observed in recent years. In descending order of export value, the exported fresh produce are apples, grapes, strawberries, potatoes, oranges, peaches, pears, and citrus fruits. Among them, the export of grapes, strawberries, and citrus fruits have increased by more than 20% compared to the previous year (2019) (Ministry of Finance Japan, 2021), and further expansion of exports is expected in the future. In recent years, the export of soft fruits with short shelf lives, such as strawberries and grapes, has increased. To expand the export of fresh produce, studies have been conducted focusing on the improvement in transportation technology for exports (Ikegaya et al., 2019, 2021).

Approximately 80% of food products exported from Japan are assumed to be transported by sea and the

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remaining by air (Shikoku Regional Development Bureau, Ministry of Land, Infrastructure, Transport and Tourism Japan, 2019). In addition, approximately 40% of the products transported by sea are kept in reefer containers, which are temperature-controlled containers with attached refrigerators (Shikoku Regional Development Bureau, Ministry of Land, Infrastructure, Transport and Tourism Japan, 2019). Sea transportation is expected to become increasingly important for fresh produce owing to its low cost and high volume. However, sea transport requires a very long time and results in greater deterioration of the quality of the produce compared with air transport (Ikegaya et al., 2019). Therefore, it is necessary to properly control the temperature, humidity, gas composition, and other factors that have a significant impact on the quality of fresh produce inside containers. Optimal storage conditions for fresh produce vary with the item (Gross et al., 2018). Low temperatures inhibit the transpiration and respiration of fresh produce (Caleb et al., 2012), thus preventing the reduction of water, sugars, and organic acids (Ikegaya et al., 2020; Nunes & Emond, 2007). Therefore, except a minor possibility of chilling injury (Pan et al., 2017; Vithana et al., 2018), transportation at the lowest possible temperature without freezing is the most suitable for maintaining shelf life. In fact, many studies have shown that 0°C and slightly above is the optimal storage temperature for fresh produce (Gross et al., 2018). However, unlike incubators used for research, reefer containers generally have temperature irregularities of ±2°C or more, depending on the position and the operation of the refrigerator (Laguerre et al., 2002; Rodríguez-Bermejo et al., 2007). Therefore, the temperature is often set to at least 5°C for export, considering the risk of freezing. However, in recent years, technological innovation has led to the development of precise temperature-controlled reefer containers with uniform temperatures. We expected that this reefer container would enable practical export at 0°C and actually exported 28 types of fresh produce from Japan to Singapore and examined their quality upon arrival. A standard reefer container set to 5°C was used as the control.

2. Materials and methods

2.1. Fresh produce

Twenty-eight types of fresh produce produced in Japan were used for the export test, which were selected based on the interests expressed by produce buyers in Singapore. The selected items, producers, and packaging are listed in Table 1 and Figure 1. In this study, "Akiabe", "Shinano Gold" and "Shinano Sweet" apples; fig; "Shine Muscat", "Pione", and "Kyoho" grapes; greenhouse melon; "Nitta" and "Nansui" Japanese pear; Japanese persimmon; Satsuma mandarin; and yuzu were classified as fruits, and all other products were classified as vegetables.

2.2. Test plan for export by sea

An outline of the export test is shown in Figure 2. This method is a partial modification of a previously reported method (Ikegaya et al., 2019, 2021). The produce was shipped from each production area in Japan on 9 October 2019, and arrived at a refrigerated warehouse set to 5°C near Shimizu Port the next morning. In the warehouse, fresh produce was removed from the boxes, photographed, weighed, and repackaged. One ethylene absorbent sachet, consisting mainly of potassium permanganate and activated carbon (Green Keeper Sachets, Sagueny Group, Alexandria, Egypt), was placed in each box of apples. Extra packaging material (Adfresh M10, 0.05 × 1000 × 1000 mm, Mitsui Chemicals, Inc., Tokyo, Japan) was then placed over the entire outer box. This process protects other fresh produce from the excessive ethylene produced by apples (Pathak et al., 2017). This treatment was not administered to other products.

Subsequently, one set of fresh produce was prepared and loaded onto the pallet. The fresh produce was loaded onto the pallet in the ascending order of its weight, and the loading procedure comprised five steps. A second identical pallet was then prepared. After the fresh produce was arranged, both palettes were wrapped in a net to prevent collapse and stored in the same warehouse. Another set of fruits was prepared and used for instrumental analysis, as described in Section 2.5. After clearing customs on 10 October 2019, the two pallets were loaded onto a normal reefer container (SeaCube container leasing Ltd., Woodcliff Lake, NJ, USA) and a precise temperature-controlled reefer container (Fresh Keeping Device “futecc,” Denso Corp., Aichi, Japan) set to 5°C and 0°C, respectively. The refrigeration unit of the precise temperature-controlled reefer container consists of two compressors and two inverters, enabling more precise temperature control than that possible with ordinary containers. In addition, it is designed to allow air to flow along the container walls and floor without directly hitting the cargo (Denso Corp., 2022).

The containers were loaded onto a cargo ship (NYK Vesta), which left Shimizu Port on 16 October 2019, and arrived in Singapore on 28 October 2019. After clearing the customs on 29 October 2020, the containers were transported to a refrigerated warehouse near Singapore Port, where the produce was removed from them. Subsequently, the produce was transported in a refrigerated truck to the Research and Development Center of Mitsui Chemicals, Singapore (hereafter referred to as R&D Center).

Immediately upon arrival, the appearance of the fresh produce was visually checked to calculate the saleable quality rate, and their weights were determined. The details of these methods are shown in Section 2.4. In addition, fruit quality was assessed using instrumental analysis described in Section 2.5. Subsequently, the vegetables were stored at approximately 25°C, and the fruits were refrigerated at 4°C for 24 h. After 24 h of storage, the appearance was again examined visually to calculate the saleable quality rate. In addition, sensory evaluation of fresh produce that had been stored for 24 h after arrival was performed using the methods described in Section 2.6.

2.3. Measurement of temperature and relative humidity during transport

During transport, the temperature and relative humidity were measured using temperature, relative humidity, and shock recorders (G-MEN20, SRIC Corp., Nagano, Japan), which were placed inside the box of Satsuma mandarin during export for both containers. The Satsuma mandarin box was placed at the bottom of the load on the pallet.
Table 1. Producers and packaged form of fresh produce.

| Item                  | Scientific name                          | Producer                                      | Packaging form                                      |
|-----------------------|------------------------------------------|----------------------------------------------|-----------------------------------------------------|
| Fruit                 |                                          |                                               |                                                     |
| Apple ‘Aikabé’        | Malus domestica                          | JA Nagano, Nagano, Nagano                    | Packed 36 per cardboard box with buffer material.  |
| Apple ‘Shinano Gold’  | Malus domestica                          | Shimoina Growers Association, ltd, Nagano    | Packed 40 per cardboard box with buffer material.  |
| Apple ‘Shinano Sweet’ | Malus domestica                          | Shimoina Growers Association, ltd, Nagano    | Packed 40 per cardboard box with buffer material.  |
| Fig                   | Ficus carica                             | JA Ooigawa, Fujieda, Shizuoka                |                                                     |
| Grapes ‘Kyo’ho’       | Vitis spp.                               | JA Nagano, Nagano, Nagano                    | Placed in plastic trays, with 350 g, and batches of four trays were placed in a cardboard box. |
| Grapes ‘Pion’         | Vitis spp.                               | JA Nagano, Nagano, Nagano                    | Covered with buffer material individually and placed in a cardboard box, with eight bunches per box. |
| Grapes ‘Shine Muscat’ | Vitis spp.                               | JA Nagano, Nagano, Nagano                    | Covered with buffer material individually and placed in a cardboard box, with nine bunches per box. |
| Greenhouse melon      | Cucumis melo                             | Melon Growers Association Clown              | Packed six per cardboard box with buffer material. |
| Japanese pear ‘Nansui’| Pyrus pyrifolia var. culta               | Shimoina Growers Association, ltd, Nagano    | Packed 16 per cardboard box with buffer material.  |
| Japanese pear ‘Nitaka’| Pyrus pyrifolia var. culta               | JA Niigata-Nankan, Sanjo, Niigata            |                                                     |
| Japanese persimmon    | Diospyros kaki                           | JA Topia-Hamamatsu, Hamamatsu, Shizuoka      | Wrapped individually in a film, and packed 8 per cardboard box with buffer material. |
| Satsuma mandarin      | Citrus unshiu                            | JA Shizukuashi, Shizuoka, Shizuoka           | Placed in cardboard boxes, and 20 boxes per cardboard box. |
| Yuzu                  | Citrus junos                             | Fujinokuni Kaneve Yuzu Growers Association, Kawanenhocho, Shizuoka | Placed in plastic box, with 3 pieces per box, and batches of four trays were placed in a cardboard box. |
| Vegetable             |                                          |                                               |                                                     |
| Cucumber              | Cucumis sativus                          | JA Fueluki, Fueluki, Yamanashi               | Packed in plastic bags, with 5 kg per plastic bag, and one plastic bag per cardboard box. |
| Eggplant ‘Orido-nasu’ | Solanum melongena                        | JA Shimizu, Shizuoka, Shizuoka               | Packed 11 per cardboard box with buffer material. |
| Eggplant ‘Senyo-nigou’| Solanum melongena                        | JA Fueluki, Fueluki, Yamanashi               |                                                     |
| Enoki-dake mushroom   | Flammulina velutipes                     | JA Tokamachi, Tokamachi, Niigata             | Wrapped individually in a film, with 30 pieces per cardboard box. |
| Green Shiso leaves    | Perilla frutescens var. crispa           | Higasi-Mikawa onshitu-engei                  | Placed in plastic boxes, with 10 bundle per box, and 20 boxes per cardboard box. |
| Green soy beans       | Glycine max                             | JA Shizuku, Shizuoka, Shizuoka               | Packed in plastic bags, with 200 g per bag, and 30 bags per cardboard box. |
| Japanese mustard spinach| Brassica rapa var. perviridis           | Morishima farm, Hamamatsu, Shizuoka          | Packed in plastic bags, with 200 g per bag, and 25 bags per cardboard box. |
| Paprika               | Capsicum annuum                          | Manushin Shibamoto Seicha Ltd., Makinohara, Shizuoka | Packed in plastic bags, with 5 kg per plastic bag, and one plastic bag per cardboard box. |
| Potato ‘Danchaku’     | Solanum tuberosum annuum                 | JA Biho-ro-Kouki-ren, Biboro, Hokkaido       | Packed 10 kg per cardboard box.                     |
| Potato ‘May Queen’    | Solanum tuberosum                        | JA Memuro, Memuro, Hokkaido                  | Packed 10 kg per cardboard box.                     |
| Shiitake mushroom     | Lentinula edodes                         | JA Minami-Uonuma, Minami-Uonuma, Niigata     | Placed in plastic trays, with 170 g and covered with film, in addition, batches of 20 trays were placed in a cardboard box. |
| Shimeji mushroom      | Hyphizygus marmoreus                     | JA Minami-Uonuma, Minami-Uonuma, Niigata     | Wrapped individually in a film, with 30 pieces per cardboard box. |
| Spinach               | Spinacia oleracea                        | Mikakuya Growers Association, Kami-shizuoka | Packed in plastic bags, with 200 g per bag, and 25 bags per cardboard box. |
| Sweet potato ‘Beni’   | Ipomoea batatas                          | JA Mishima-Kannami, Mishima, Shizuoka        | Packed 5 kg per cardboard box.                      |
| Sweet potato ‘Beni’   | Ipomoea batatas                          | Unagi-Imo Growers Association, Hamamatsu, Shizuoka | Packed 5 kg per cardboard box. |

Temperature and relative humidity readings were recorded every minute during transport.

2.4. Appearance and fresh weight

The weight and appearance of produce were evaluated following delivery to the warehouses at Shimizu Port and then to the R&D Center by two experienced Japanese assessors. The assessors visually inspected the occurrence of blemishes, microorganisms, decay, and water-soaking. Items with any amount of mold, spoilage, or other abnormalities were classified as “unsaleable,” and the saleable quality was calculated as the percentage of unsaleable units with respect to the total number of units (Ayalza-Zavaleta et al., 2004). In addition, the assessors checked and recorded the changes in the appearance of the fresh produce, which were difficult to determine using instrumental analysis or photography. When calculating fresh weight loss, the pre-export weight was considered 100%. Vegetables were stored at room temperature; fruits were stored in a refrigerator at 4°C, and appearance was evaluated again 24 h after arrival to determine whether abnormalities would occur after display in the store (Ozkaya et al., 2009).
2.5. Instrumental analysis of fruits

Fruits that arrived at the R&D Center were allowed to reach the room temperature, and their quality was investigated using instrumental analysis. Six fruits from both the 0°C and 5°C groups were assessed, except for grapes for which six bunches were assessed. Five berries of grape were collected from the center of a bunch and analyzed using the same methods as described for other fruits. The average value for five fruits was taken as the value for one bunch. This was because the quality of the grapes in the center of the top and shoot has been shown to be similar to the value measured for one bunch (Hagiwara et al., 1987). In addition, we determined the berry drop rate of the grapes.

The color of the fruit surface at the center was measured using a colorimeter (CR-13; Konica Minolta Japan, Inc., Tokyo, Japan). The colors were presented using the L*a*b* color system formulated by the International Commission on Illumination (1976). Fruit surface and flesh were assessed to
determine firmness along the center to the peduncle and apex, with a fruit firmness meter (KM-1, Fujiwara Factory, Tokyo, Japan) equipped with a conical plunger of 1 cm in diameter. The fruits were then divided into two equal parts along the equator between the peduncle and apex, and the firmness of the flesh was measured at the cross section. Color and firmness were measured thrice for each piece of fruit, and the mean value was determined.

Total soluble solids (TSS) were measured in the resulting juices using a Brix-accuracy meter (PAL-BX ACID FS; Atago Co. Ltd., Tokyo, Japan). To determine juice production, all edible parts of each fruit were removed, individually wrapped in gauze, and processed in a juicer (Hand Juicer, Ito Seisakusho Co. Ltd., Mie, Japan). All five grapes were processed together.

The quality of the fruits before export was analyzed for comparison. When fruits arrived at Shimizu Port on 10 October 2019, an additional batch was prepared, which was analyzed using the same instruments and methods at the Shizuoka Prefectural Research Institute of Agriculture and Forestry.

2.6. Sensory evaluation

2.6.1. Sensory evaluation of vegetables
The appearance of vegetables shipped in a normal reefer container set to 5°C and a precise temperature-controlled reefer container set to 0°C was compared using a two-sample preference test (Meilgaard et al., 2019; Sato, 1985). In this test, the items were examined by a panel comprising 28 judges (15 females and 13 males), of whom 6 were aged 20–29 years, 9 were aged 30–39 years, 9 were aged 40–49 years, and 4 were aged 50 years or older. The judges were not given any specific training on food assessment. Each judge determined the sample that “looked better.” Each sample, coded with three random digits, was provided to the judges in a box, and the judges were able to evaluate the content freely. Decayed samples were excluded from the test because unsellable samples could have a large impact on the assessment.

2.6.2. Sensory evaluation of fruits
The same panel, described in Section 2.6.1, examined the fruits. The fruits shipped in a normal reefer container set to 5°C and a precise temperature-controlled reefer container set to 0°C were compared using a two-sample preference test (Meilgaard et al., 2019; Sato, 1985) for both appearance and taste. To evaluate the appearance, the judges were provided with five fruits each of apples, fig, greenhouse melons, Japanese pears, Satsuma mandarins, and Yuzus, and three bunches of grapes. Each judge determined the sample that “looked better.”

After comparing the appearance, the judges evaluated taste using the following samples: apples and Japanese pears were peeled and cut into eight radial slices; individual grapes were removed from a bunch; greenhouse melons were cut into eight radial slices, which were then cut in half; Japanese persimmons were cut into eight radial slices, and Satsuma mandarin was divided in two by hand. The judges compared texture, sweetness, flavor, and overall preference by selecting the most preferable samples according to a two-sample preference test (Meilgaard et al., 2019; Sato, 1985).

2.7. Statistical analysis
Each value is expressed as the mean ± standard deviation (SD). The results of fresh weight loss were compared using Student’s t-test. The results of other instrumental analyses were evaluated using analysis of variance, and the means were compared using Tukey’s multiple range test. Angle conversion was applied in advance for the fresh weight test and berry drop rate, thereby eliminating heteroscedasticity. The results of the sensory evaluations were analyzed using a two-tailed test (Meilgaard, 2019; Sato, 1985). All tests were performed at a significance level of p < 0.05.

3. Results

3.1. Temperature and humidity
Changes in temperature and humidity inside the normal reefer container set to 5°C and the precise temperature-controlled reefer container set to 0°C during the export test are presented in Figures 3 and 4. During export by sea, both containers exhibited fairly high temperature stability during shipping and maintained the set temperature. Regarding the relative humidity, it was gradually increased in the normal reefer container and maintained at approximately 90% during transportation, and it was gradually increased to approximately 85% in the precise temperature-controlled reefer container. Hence, the relative humidity of the normal reefer container remained higher than that of the precise temperature-controlled reefer container by approximately 5% during the transportation.

3.2. Appearance and saleable quality rate
The saleable quality rate based on the assessment of the product appearance is presented in Table 2. The vegetables were classified into leafy vegetables, fruit vegetables, flesh legumes, starchy vegetables, and mushrooms for the presentation of the results. Except for fig, grapes “Shine Muscat,” green Shiso leaves, cucumber, eggplant “Orido-nasu” and “Senryo-nigou,” and shiitake mushrooms, all products were determined to be of saleable quality after transport at both 0°C and 5°C and 24 h storage. Figure 5 shows the condition immediately after the arrival of the items that were not available for sale in their entirety. Some figs were softened and dried in transportation at both 0°C and 5°C; moreover, only some figs transported at 5°C had mold. Regarding the “Shine Muscat” grapes, all grapes maintained saleable quality after transportation at 0°C, but some of the grapes had mold after transportation at 5°C. Regarding green Shiso leaves, the color of some leaves changed to reddish. The assessors visually confirmed that the degree of discoloration was clearly higher for leaves transported at 5°C than transported at 0°C. Most of the cucumbers transported at 0°C maintained a good enough quality to be sold immediately after transportation; however, pitting occurred in all the samples and some of them were decayed after leaving them at room temperature for 24 h. Additionally, pitting had already occurred in all samples during transportation at 5°C, and some had decayed. Eggplant samples of any variety could not be sold at any transportation temperature because the surface color changed from dark purple to brown. The symptoms observed in cucumber and eggplant were inferred to be caused by chilling injury (Gross et al., 2018). However, the degree of discoloration was greater in
eggplants that were transported at 0°C than those transported at 5°C. Some of the shiitake mushrooms that were transported at 5°C had aerial mycelia growing on the shaft, whereas no such growth was found in those transported at 0°C.

### 3.3. Loss of fresh weight after transport

The loss of fresh weight following sea exports is shown in Table 3. The weight loss of 17 items was significantly greater after transportation at 0°C than at 5°C. Loss of fresh weight during transportation at 0°C was high for fig, spinach, shiitake mushroom, and “Senryu-nigou” eggplant at 11.7%, 4.9%, 4.2%, and 4.0%, respectively. Only figs exhibited a weight loss of 4% or more when transported at 5°C. All other items lost less than 4% fresh weight during transportation at 0°C and 5°C.

### 3.4. Assessment of fruit quality by instrumental analysis

The fruit quality based on instrumental analysis is shown in Table 4. Following transportation at both 0°C and 5°C, TSS significantly increased in fig and Japanese persimmon. TSS in the green house melon and Satsuma mandarin increased during transportation at 5°C and 0°C, respectively.

Regarding the fruit surface firmness, the fruits with thin skin except for melon, mandarin oranges, and yuzu may have been affected by the firmness of the flesh as well as the firmness of the skin. The fruit surface firmness of “Akibae” apples, three varieties of grapes, two varieties of Japanese pears, Satsuma mandarin, and yuzu decreased significantly following transportation at both 0°C and 5°C. The fruit surface of figs softened due to transportation at 5°C, but this was suppressed by transportation at 0°C. The firmness of “Akibae” and “Shinano Gold” apples, “Pione” and “Shine Muscat” grapes, and two varieties of Japanese pears decreased significantly following transportation at both 0°C and 5°C. The flesh firmness of apples was significantly reduced only when transported at 0°C. The flesh of fig, Satsuma mandarin, and yuzu was too soft; hence, their firmness could not be measured.

Regarding the color index, “Shinano Gold” apples, figs, all varieties of grapes, greenhouse melon, and two varieties of Japanese pears had significantly lower L* values after transport at 0°C and 5°C. In addition, the L* value of “Shine Muscat” grapes and greenhouse melons was significantly lower after transportation at 5°C than at 0°C. For “Niitaka” Japanese pear and Satsuma mandarins, the L* value increased only after transportation at 5°C. Many items showed changes in a* and b* values after transportation, but the values did not differ depending on transport temperature.
Table 2. Saleable quality rate of fresh produce immediately after transportation and storage for 24h.

Tabla 2. Índice de calidad vendible de los productos frescos inmediatamente después del transporte y del almacenamiento durante 24 horas.

| Item                  | 0 °C Immediately after arrival | 5 °C Immediately after arrival | 0 °C Stored for 24 h | 5 °C Stored for 24 h |
|-----------------------|-------------------------------|-------------------------------|--------------------|--------------------|
| Fruit                 |                               |                               |                    |                    |
| Apple ‘Akibae’        | 100                           | 100                           | 100                | 100                |
| Apple ‘Shinano Gold’  | 100                           | 100                           | 100                | 100                |
| Apple ‘Shinano Sweet’ | 100                           | 100                           | 100                | 100                |
| Fig                   | 65                             | 60                             | 35                 | 25                 |
| Grapes ‘Kyoho’        | 100                           | 100                           | 100                | 100                |
| Grapes ‘Pione’        | 100                           | 100                           | 100                | 100                |
| Grapes ‘Shine Muscat’ | 100                           | 100                           | 78                 | 78                 |
| Greenhouse melon      | 100                           | 100                           | 100                | 100                |
| Japanese pear ‘Nansui’| 100                           | 100                           | 100                | 100                |
| Japanese pear ‘Niltaka’| 100                          | 100                           | 100                | 100                |
| Japanese persimmon    | 100                           | 100                           | 100                | 100                |
| Satsuma mandarin      | 100                           | 100                           | 100                | 100                |
| Yuzu                  | 100                           | 100                           | 100                | 100                |
| Vegetable             | Leafy vegetable               |                               |                    |                    |
| Green Shiso leaves    | 95                             | 85                             | 45                 | 45                 |
| Japanese mustard spinach | 100                      | 100                           | 100                | 100                |
| Spinach               | 100                           | 100                           | 100                | 100                |
| Fruit vegetable       | Cucumber                      | 98                             | 0                  | 0                  |
| Eggplant ‘Orido-nasu’  | 0                             | 0                             | 0                  | 0                  |
| Eggplant ‘Senryo-nigou’| 0                             | 0                             | 0                  | 0                  |
| Paprika               | 100                           | 100                           | 100                | 100                |
| Fresh legume          | Green soy beans               | 100                           | 100                | 100                |
| Starchy vegetable     | Potato ‘Danshaku’            | 100                           | 100                | 100                |
| Potato ‘May Queen’    | 100                           | 100                           | 100                | 100                |
| Sweet potato ‘Beni-azuma’ | 100                      | 100                           | 100                | 100                |
| Sweet potato ‘Beni-haruka’ | 100                       | 100                           | 100                | 100                |
| Mushroom              | Enokidake mushroom            | 100                           | 100                | 100                |
| Shiitake mushroom     | 100                           | 100                           | 100                | 100                |
| Shimeji mushroom      | 100                           | 100                           | 100                | 100                |

a: Fruits were stored at 4°C, vegetables were stored at room temperature (about 25°C). Saleable quality rate was assessed by two Japanese assessors with experience evaluating the quality of fruits and vegetables.

Figure 5. Appearance of fresh produce that were assessed to be unsalable.

Figura 5. Aspecto de los productos frescos evaluados como no vendibles.

3.5. Sensory evaluation of fruits

The comparison of sensory evaluation based on the appearance and taste of fruits transported at 0°C and 5°C is shown in Table 5. Figs were excluded from the taste evaluation because of their high incidence of decay and insufficient samples for evaluation. Yuzu was also excluded from the taste evaluation because it has a very strong sour taste and is generally not consumed raw. The appearances of fig and “Nansui” Japanese pear were found to be significantly superior after transportation at 0°C. In contrast, “Shinano Gold” apples, “Pione” and “Shine Muscat” grapes, and Japanese persimmon were found to be significantly superior after transportation at 5°C. In terms of taste, “Akibae” apples transported at 0°C were evaluated to be
Table 3. Fresh weight loss after transport at 0°C and 5°C.

| Item          | 0°C | 5°C |
|---------------|-----|-----|
| Fruit         |     |     |
| Apple 'Akiabae' | 0.0 | ± 0.1 | 0.0 | ± 0.2 | NS |
| Apple 'Shinano Gold' | 0.0 | ± 0.1 | 0.0 | ± 0.1 | NS |
| Apple 'Shinano Sweet' | 0.0 | ± 0.1 | 0.0 | ± 0.1 | NS |
| Fig           | 11.7 | ± 0.2 | 5.0 | ± 0.7 | *** |
| Grapes 'Kyoho' | 1.1 | ± 0.1 | 0.3 | ± 0.0 | ** |
| Grapes 'Pione' | 2.2 | ± 1.1 | 0.5 | ± 0.1 | ** |
| Grapes 'Shine Muscat' | 0.8 | ± 0.2 | 0.3 | ± 0.0 | *** |
| Greenhouse melon | 1.0 | ± 0.1 | 0.4 | ± 0.1 | *** |
| Japanese pear 'Nansui' | 1.1 | ± 0.2 | 0.2 | ± 0.1 | *** |
| Japanese pear 'Niitaka' | 0.6 | ± 0.1 | 0.4 | ± 0.1 | *** |
| Japanese persimmon | 0.0 | ± 0.0 | 0.0 | ± 0.1 | NS |
| Satsuma mandarin | 1.3 | ± 0.2 | 0.9 | ± 0.2 | *** |
| Vegetable     |     |     |
| Leafy vegetable | 1.7 | ± 0.5 | 1.0 | ± 0.3 | * |
| Green Shiso leaves | 0.4 | ± 0.4 | 0.3 | ± 0.3 | NS |
| Spinach       | 4.9 | ± 2.6 | 0.9 | ± 0.4 | * |
| Fruit vegetable | 0.9 | ± 0.5 | 0.2 | ± 0.3 | * |
| Cucumber      | 2.6 | ± 1.0 | 2.1 | ± 0.2 | NS |
| Eggplant 'Orido-nasu' | 4.0 | ± 0.9 | 2.4 | ± 0.8 | ** |
| Paprika       | 0.0 | ± 0.1 | 0.0 | ± 0.1 | NS |
| Fresh legume  |     |     |
| Green soy beans | 2.9 | ± 0.9 | 1.5 | ± 0.5 | * |
| Starchy vegetable | 0.0 | ± 0.1 | 0.0 | ± 0.2 | NS |
| Potato 'Danshaku' | 0.0 | ± 0.1 | 0.0 | ± 0.0 | NS |
| Potato 'May Queen' | 2.1 | ± 0.1 | 0.9 | ± 0.5 | *** |
| Sweet potato 'Beni-azuma' | 0.6 | ± 0.1 | 0.4 | ± 0.1 | ** |
| Mushroom      |     |     |
| Enokiade mushroom | 0.1 | ± 0.2 | 0.2 | ± 0.0 | NS |
| Shiitake mushroom | 4.2 | ± 0.2 | 3.3 | ± 0.2 | *** |
| Shimeji mushroom | 0.0 | ± 0.0 | 0.0 | ± 0.1 | NS |

Mean ± SD (n = 6). * ** *** and NS indicate significant differences at P < 0.05, 0.01, 0.001, and not significant respectively. Cucumber, eggplant, and okra were excluded from the results of weight loss, because all samples were decayed.

Table 3. Pérdida de peso en fresco tras el transporte a 0°C y 5°C.

significantly desirable. In contrast, "Kyoho" grapes, "Niitaka" Japanese pears, and Satsuma mandarins transported at 5°C were evaluated to be desirable. However, the results for apples, grapes, and Japanese pears differed with the variety, and did not show a clear trend. Similarly, no clear trend was observed for sweetness, sourness, flavor, or texture.

3.6. Sensory evaluation of vegetables

The comparison of sensory evaluation based on the appearance of vegetables transported at 0°C and 5°C is shown in Table 6. Three types of leafy vegetables (green Shiso leaves, Japanese mustard spinach, and spinach), "Danshaku" potatoes, and three types of mushrooms (enokiade, shiitakem, and shimeji) were evaluated to be of superior quality when transported at 0°C. Conversely, paprika, green soy beans, and three types of starchy vegetables ("May Queen" potato, and "Beni-azuma" and "Beni-haruka" sweet potatoes) were evaluated to be superior when transported at 5°C.

4. Discussion

The temperatures of precise temperature-controlled reefer and regular reefer containers, set at 0°C and 5°C, respectively, were maintained nearly the same as the set value during transportation (Figure 3). The regular reefer container set at 5°C showed high temperature stability because it was made in 2018 and was very new; although it was not a special product, it showed high performance. Therefore, the effect of temperature during transportation on the quality of fresh produce was clearly observed. However, in general, reefer containers are a property of the shipping company, and cargo owners cannot specify which container to use. Some of them are very old or perform poorly; therefore, care should be taken when using them commercially. In addition, this study found no particular problems with customs clearance procedures or ship operations. Although the conditions related to exports differ each time, the results of this study were considered reasonably reproducible.

The fresh weight of 17 of the 28 items was significantly lower after transportation at 0°C than at 5°C. Generally, when moisture loss exceeds 4–6% of the total fresh weight, visible wilting or wrinkling of the surface occurs in most produce (Kays, 1997). However, not much weight loss was observed under either transport condition. Low temperatures inhibit respiration (Barrios et al., 2014; Caleb et al., 2012) and transpiration in fresh produce (Nunes & Emond, 2007); thus, the loss of fresh weight should be more inhibited in items transported at 0°C than those transported at 5°C (Paniagua et al., 2014). The reason for this was thought to be the different cooling methods used in the containers. The precise temperature-controlled reefer container used in this study has a large amount of cold air circulating at a temperature slightly lower than the set temperature to improve temperature stability. This container is designed to allow cold air to crawl under the floor of the container and blow out along the sidewalls, thus preventing the cold air from directly hitting the products inside. Although the airflow did not directly impact the fresh produce, the high airflow caused low humidity inside, and the fresh weight decreased significantly (Figure 4, Table 3). Therefore, it is desirable to devise packaging for fresh produce to prevent water loss when using this container (Ikegaya et al., 2021; Wambrauw et al., 2020).

The comparison of appearance after arrival revealed that transportation at 0°C inhibited the development of molds on figs and “Shine Muscat” grapes. It also inhibited the growth
Table 4. Determination of quality of fruits that were transported at 0°C and 5°C by instrumental analysis.

| Item             | Condition          | Transport temperature | Total soluble solid (% of dry weight) | Firmness (N) | Color index | Berry drop rate (%) |
|------------------|--------------------|-----------------------|---------------------------------------|--------------|-------------|---------------------|
|                  |                    |                       | Surface                               | Flesh        | L*          | a*                  |
| Apple ‘Akihara’  | Before exporting   | 0°C                   | 13.2 ± 0.4                            | 7.4 ± 0.5    | 6.2 ± 0.4   | 32.4 ± 6.0          |
|                  |                    | 5°C                   | 13.6 ± 1.0                            | 7.4 ± 0.3    | 5.9 ± 0.3   | 34.2 ± 7.5          |
|                  | After arrival      | 0°C                   | 15.0 ± 0.5                            | 7.4 ± 0.3    | 5.9 ± 0.3   | 34.2 ± 7.5          |
|                  |                    | 5°C                   | 14.5 ± 1.2                            | 7.1 ± 0.2    | 5.1 ± 0.6   | 66.7 ± 0.5          |
|                  | Apple ‘Shinano Gold’ | Before exporting   | 15.0 ± 0.5                            | 7.4 ± 0.3    | 5.9 ± 0.3   | 28.9 ± 3.3          |
|                  |                    | After arrival         | 0°C                                  | 7.0 ± 0.2    | 6.5 ± 0.4   | 67.5 ± 1.1          |
|                  | Apple ‘Shinano Sweet’ | Before exporting | 14.3 ± 1.0                            | 7.0 ± 0.2    | 6.5 ± 0.4   | 54.9 ± 6.6          |
|                  |                    | After arrival         | 0°C                                  | 7.0 ± 0.2    | 6.5 ± 0.4   | 49.6 ± 5.8          |
| Fig              | Before exporting   | 12.7 ± 0.7            | 2.5 ± 0.6                             |              | 40.0 ± 6.4  |                      |
|                  | After arrival      | 0°C                   | 14.6 ± 1.1                           | 2.0 ± 0.3    | 31.2 ± 3.4  |                      |
| Grapes ‘Kyoho’   | Before exporting   | 16.3 ± 0.8            | 4.6 ± 0.4                             |              | 29.5 ± 1.2  |                      |
|                  | After arrival      | 0°C                   | 16.9 ± 0.9                           | 3.7 ± 0.3    | 20.2 ± 1.7  |                      |
| Grapes ‘Pione’   | Before exporting   | 17.0 ± 0.5            | 3.5 ± 0.1                             |              | 20.5 ± 0.5  |                      |
|                  | After arrival      | 0°C                   | 17.7 ± 2.1                           | 3.4 ± 0.3    | 21.9 ± 2.8  |                      |
| Grapes ‘Shine Muscat’ | Before exporting | 16.5 ± 0.6            | 4.9 ± 0.1                             |              | 43.8 ± 0.8  |                      |
|                  | After arrival      | 0°C                   | 17.5 ± 0.6                           | 3.9 ± 0.4    | 40.2 ± 0.6  |                      |
| Greenhouse melon | Before exporting   | 13.7 ± 0.3            | 7.9 ± 0.1                             |              | 66.6 ± 1.0  |                      |
|                  | After arrival      | 0°C                   | 14.0 ± 0.6                           | 8.1 ± 0.1    | 63.2 ± 1.5  |                      |
| Japanese pear ‘Nansui’ | Before exporting | 14.3 ± 0.3            | 7.2 ± 0.2                             |              | 55.4 ± 1.9  |                      |
|                  | After arrival      | 0°C                   | 14.7 ± 0.4                           | 6.8 ± 0.3    | 55.1 ± 1.7  |                      |
| Japanese pear ‘Niitaka’ | Before exporting | 12.9 ± 0.5            | 7.9 ± 0.2                             |              | 57.2 ± 1.0  |                      |
|                  | After arrival      | 0°C                   | 12.8 ± 0.7                           | 7.2 ± 0.2    | 58.3 ± 1.0  |                      |
| Japanese persimmon | Before exporting  | 16.6 ± 0.1            | 7.4 ± 0.2                             |              | 56.1 ± 0.9  |                      |
|                  | After arrival      | 0°C                   | 17.5 ± 0.9                           | 6.8 ± 0.6    | 55.9 ± 0.7  |                      |
| Satsuma mandarin | Before exporting   | 10.0 ± 0.4            | 5.5 ± 0.5                             |              | 59.2 ± 1.3  |                      |
|                  | After arrival      | 0°C                   | 12.3 ± 0.4                           | 5.0 ± 0.2    | 59.6 ± 2.7  |                      |
| Yuzu             | Before exporting   | 8.2 ± 0.5             | 7.5 ± 0.3                             |              | 33.0 ± 1.0  |                      |
|                  | After arrival      | 0°C                   | 7.9 ± 0.2                            | 5.5 ± 0.1    | 31.2 ± 2.4  |                      |

Mean ± SD (n = 6). Mean values of each column with different letters are significantly different by Tukey’s multiple range test at P < 0.05. NS indicate not significant.
of aerial mycelia on the shafts of shiitake mushrooms. As a result, the saleable quality rate increased due to transportation at 0°C. Although it is not possible to draw conclusions based on the results of this study alone, transportation at 0°C may be effective in maintaining the freshness of soft fruits which are prone to decay and mushrooms. Of the 28 items transported in this study, four items (figs, “Shine Muscat” grapes, green Shiso leaves, and shiitake mushrooms) had a higher saleable quality ratio when transported at 0°C than at 5°C. For cucumber and the two types of eggplants, all samples were not of saleable quality, regardless of transport temperature. However, the remaining 21 items were exported in a standard reefer container at 5°C without any special effort, and all of them were of saleable quality. Customs clearance and sea transportation took 18 days in this export test from Japan to Singapore, and it became clear that a wide variety of fruits and vegetables could be exported even in a standard reefer container set at 5°C.

Nevertheless, even if the products were assessed as being of saleable quality, the level of quality varied. Instrumental analysis was performed on the fruits; however, the results were unclear. Therefore, we aimed to clarify the differences in these qualities using sensory evaluation.

Regarding the appearance, two types of fruits were preferred to be transported at 0°C, while four types were preferred to be transported at 5°C. Respiration and transpiration of fruit are inhibited at low temperatures; thus, the fruits

Table 5. Comparison of appearance and taste of fruits that were transported at 0°C and 5°C.

| Item                | Appearance | Taste         |
|---------------------|------------|---------------|
|                     | Significance | Significance | Significance | Significance | Significance | Significance |
|                     | 0°C | 5°C | 0°C | 5°C | 0°C | 5°C | 0°C | 5°C | 0°C | 5°C | 0°C | 5°C |
| Fruit               |              |               |               |               |               |               |               |               |               |               |               |               |               |               |               |
| Apple ‘Akibae’      | 17           | 11            | NS            | 16           | 12            | NS            | 22           | 6            | **            | 21           | 7            | *             | 17           | 11            | NS            | 21           | 7            | *             |
| Apple ‘Shinano Gold’| 6            | 22            | **            | 18           | 10            | NS            | 9            | 19           | NS            | 17           | 11            | NS            | 15           | 13            | NS            | 18           | 10           | NS            |
| Apple ‘Shinano Sweet’| 11           | 17            | NS            | 14           | 14            | NS            | 14           | 14           | NS            | 11           | 17            | NS            | 15           | 13            | NS            | 11           | 17           | NS            |
| Fig                 | 25           | 3             | ***           |              |               |               |              |               |               |              |               |               |              |               |               |              |               |               |
| Grapes ‘Keiho’      | 19           | 9             | NS            | 5            | 23            | ***           | 14           | 14           | NS            | 7            | 21            | *             | 8            | 20            | *             | 7            | 21            | *             |
| Grapes ‘Pione’      | 6            | 22            | **            | 15           | 13            | NS            | 11           | 17           | NS            | 12           | 16            | NS            | 13           | 15            | NS            | 14           | 14            | NS            |
| Grapes ‘Shine’      | 1            | 27            | **            | 11           | 17            | NS            | 13           | 15           | NS            | 11           | 17            | NS            | 6            | 22            | **            | 9            | 19            | NS            |
| Muscat’ Greenhouse melon | 12           | 16            | NS            | 12           | 16            | NS            | 12           | 16            | NS            | 11           | 17            | NS            | 13           | 15            | NS            | 12           | 16            | NS            |
| Japanese pear ‘Narumi’ | 21           | 7             | *             | 17           | 11            | NS            | 16           | 12            | NS            | 17           | 11            | NS            | 17           | 11            | NS            | 16           | 12            | NS            |
| Japanese pear ‘Niiaka’ | 18           | 10            | NS            | 17           | 11            | NS            | 19           | 9            | NS            | 20           | 8             | *             | 16           | 12            | NS            | 7            | 21            | *             |
| Japanese persimmon | 9            | 19            | *             | 14           | 14            | NS            | 15           | 13            | NS            | 10           | 18            | NS            | 12           | 16            | NS            | 12           | 16            | NS            |
| Satsuma mandarin    | 10           | 18            | NS            | 10           | 18            | NS            | 13           | 15            | NS            | 7            | 21            | *             | 10           | 18            | NS            | 8            | 20            | *             |
| Yuzu                | 13           | 15            | NS            |              |               |               |              |               |               |              |               |               |              |               |               |              |               |               |

The numbers in the table indicate the number of judges who found one of the two samples more preferable. For appearance and taste, judges made selections based on preference. (n = 28). *, **, and *** indicate significant differences at P < 0.05, 0.01, and 0.001, respectively. Fig was excluded from the evaluation of taste due to its high incidence of decay and insufficient samples to evaluate. Yuzu was excluded from the evaluation because it has a very strong sourness and generally is not eaten raw.

Table 6. Comparison of appearance of vegetables that were transported at 0°C and 5°C.

| Item                | Appearance | Significance |
|---------------------|------------|--------------|
|                     | Significance | Significance |
|                     | 0°C | 5°C | 0°C | 5°C |
| Vegetable           |              |               |               |               |
| Leafy vegetable     |              | Green Shiso leaves | 20 | 8 | * |
|                     |              | Japanese mustard spinach | 28 | 0 | *** |
|                     |              | Spinach | 23 | 5 | *** |
| Fruit vegetable     |              | Paprika | 4 | 24 | *** |
| Fresh legume        |              | Green soy beans | 3 | 25 | *** |
| Starchy vegetable   |              | Potato ‘Danshaku’ | 26 | 2 | *** |
|                     |              | Potato ‘May Queen’ | 4 | 24 | *** |
|                     |              | Sweet potato ‘Beni-azuma’ | 5 | 23 | *** |
|                     |              | Sweet potato ‘Beni-haruka’ | 3 | 25 | *** |
| Mushroom            |              | Enokitake mushroom | 21 | 7 | * |
|                     |              | Shiitake mushroom | 24 | 4 | *** |
|                     |              | Shimeji mushroom | 22 | 6 | *** |

The numbers in the table indicate the number of judges who found one of the two samples more preferable. For appearance, judges made selections based on preference. (n = 28). *, **, and *** indicate significant differences at P < 0.05, 0.01, and 0.001, respectively. Cucumber and eggplant were excluded from the evaluation because there was no products of quality that could be sold after transportation.
should remain fresh when transported at 0°C, expect for those that suffer from chilling injury. Among the fruits used in this study, chilling injury has been reported to occur in citrus and melon (Gross et al., 2018). Despite this, “Shinano Gold” apples, “Pione” and “Shine Muscat” grapes, and Japanese persimmon transported at 5°C were determined to be significantly superior in appearance. According to the judges who had experience in evaluating fresh produce for saleable quality rate, all fruits transported at 0°C were less ripened and maintained freshness compared to those transported at 5°C (Figure 6). Although marketers and logistics providers consider that less ripened fruits have a longer shelf life, consumers have different criteria for assessing the quality of fruits and often consider ripe fruits to be better than the fresh ones.

Five vegetables, namely paprika, green soy beans, one variety of potato, and two varieties of sweet potato, were found to be superior when transported at 5°C. Among these, chilling injuries have been reported in paprika and sweet potatoes (Gross et al., 2018). However, chilling injury in green soy beans has not been reported; thus, this could be due to greater water loss in vegetables transported at 0°C than those transported at 5°C (Table 3). For potatoes, the results differed depending on the variety, but the exact factor responsible for the differences is unknown.

In contrast, seven types of vegetables (three types of leafy vegetables, one variety of potato, and three types of mushrooms) were evaluated to be of superior quality when transported at 0°C. Of these, green Shiso leaves, spinach, and shiitake mushrooms were rated as having superior quality despite showing more weight loss. This study was intended for practical use; thus, the effects of factors other than temperature could not be eliminated. However, high quality was maintained despite the large decrease in moisture content, which is an extremely important factor for maintaining quality (Kays, 1997). This clearly demonstrated the effectiveness of transport at 0°C, especially for leafy vegetables and mushrooms. This study was planned assuming the practical conditions of such operations; therefore, the findings can be put to practical use immediately. Moreover, the necessity to control the decrease in water content by devising packaging became clear. We have previously conducted two export demonstrations using the same precise temperature-controlled reefer container at 0°C in mixed cargo shipments (Ikegaya et al., 2019, 2021). In each case, many items could be exported with practically saleable quality; however, the conditions and the selection of export items were mildly different in the two cases because of several variable factors involved in the export of fresh produce, such as logistics and market situations. Hence, in this study, attempted to conduct a simultaneous export demonstration using two containers. Here, the conditions are exactly the same except for the conditions in the container during transportation. Although this report is based on a single study, we believe that by examining it in conjunction with the two previous reports, it can be reproduced. In the future, we hope that research on the export of fresh produce will be conducted worldwide, and that the global distribution of these products will increase due to technological improvements.

5. Conclusion

In this study, we have demonstrated that transportation at 0°C was effective, especially for the export of leafy vegetables and mushrooms. This study was planned assuming the practical conditions of such operations; therefore, the findings can be put to practical use immediately. Moreover, the necessity to control the decrease in water content by devising packaging became clear. We have previously conducted two export demonstrations using the same precise temperature-controlled reefer container at 0°C in mixed cargo shipments (Ikegaya et al., 2019, 2021). In each case, many items could be exported with practically saleable quality; however, the conditions and the selection of export items were mildly different in the two cases because of several variable factors involved in the export of fresh produce, such as logistics and market situations. Hence, in this study, attempted to conduct a simultaneous export demonstration using two containers. Here, the conditions are exactly the same except for the conditions in the container during transportation. Although this report is based on a single study, we believe that by examining it in conjunction with the two previous reports, it can be reproduced. In the future, we hope that research on the export of fresh produce will be conducted worldwide, and that the global distribution of these products will increase due to technological improvements.

Acknowledgements

We would like to express our sincere gratitude to Kazuhiro Ishikawa, Fumiaki Yano, Yohei Mochizuki, Ayako Watanabe, and Yasuhide Matsumura of JA Shizuoka Keizaien Co., Ltd.; Yoshikazu Uehara and Reiko Nishime of Mitsu Chemicals Singapore R&D Center, Pte. Ltd.; Makoto Morohashi and Hidenobu Tousaki of Mitsu Chemicals, Inc.; Yoshiaki Sudo and Yoshiyuki Akama of Suzuyo & Co., Ltd.; Rie Yoshizawa of Suzuyo Singapore Pte. Ltd.; Toshikatsu Hara of Shizuoka VF Co., Ltd.; Norio Azuma of LCCT F&B Solutions Pte. Ltd.; Wataru Fukuda and Toshiko Takeda of Shizuoka Prefectural Government; Tetsuichi Okada, Tatsuya Kimura, Satoshi Hatanaka, and Kaori Chisaki of Waterfront Vitalization and Environment Research Foundation, for their considerable efforts in helping us conduct this research.

This study was conducted as part of the Ministry of Land, Infrastructure, Transport, and Tourism, Japan, the “Agricultural Products Export Promotion Project in cooperation with producers.”

Disclosure statement

No potential conflict of interest was reported by the author(s).
Ethical approval
All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Data availability statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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