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Freezing Temperature and Freezing Injury of Rough Rice, and Quality of Rough Rice Stored at Temperatures between –50°C and 25°C for Four Years

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Abstract. *A differential scanning calorimeter was used to determine freezing temperature of rough rice. Rice grains with a moisture content of 22.1% froze at about –35°C. Rice grains with a moisture content of less than 20.8% did not freeze at a temperature of –55°C. Incidence of freezing injury of rice grains was determined by germination rate because grains that suffered from freezing injury did not germinate. Rice grains with a moisture content of less than 17.8% germinated after being stored at –80°C for 11 days. Thus, no grain with a moisture content of less than 17.8% froze at a temperature of –80°C.*

Effects of temperatures between –50°C and 25°C on physiological properties and quality characteristics of rough rice during four-year storage were investigated. Low temperature maintained vitality of rice, minimized physiological activities, starch deterioration and rancidity in rice, and consequently preserved rice quality. Eating quality of rice stored at temperatures less than 5°C for four years was the same as that of newly harvested rice. These results indicate that preservation of rough rice quality for several years is possible by storing rice at an average temperature below 5°C during storage. A new on-farm rice storage technique at temperatures below ice point by using natural fresh chilly air in winter has been in practical use in Hokkaido, the northernmost island of Japan, in recent years. Twenty-six grain elevators have been constructed in Hokkaido since 1996. The storage capacity of rough rice in 2004 was 115,000 t.

Keywords. rice storage, super-low-temperature storage, freezing temperature, freezing injury, quality, taste, Long-term rice storage

Introduction

The temperature and moisture content of rice grains greatly affect the quality of rice during storage. In Japan, therefore, the moisture content of brown rice during storage is maintained at a level of 16%. There are two commercial brown rice storage systems in Japan: an environment-temperature storage system, in which the temperature during storage is not controlled, and a low-temperature storage system, in which the temperature is maintained below 15°C during storage. The low-temperature storage system minimizes insect activities and mold growth, and fumigants are therefore not required during storage. However, this system requires electricity to cool the stored brown rice. The total capacity of brown rice storage structures equipped with cooling units in Japan is 6.6 million t, which is about 70% of rice consumption in Japan.

A previous basic study (Kawamura et al., 1997) revealed that the quality of rice stored at a temperature below ice point is comparable to that of newly harvested rice. We (Kawamura et al., 2000) reported that a temperature below ice point minimizes physiological activities in rice and hence minimizes the deterioration of rice quality. Rice storage at a temperature below ice point was named “super-low-temperature storage” by Kawamura et al. (1997, 1999) due to the fact that the storage temperature was much lower than that of low-temperature storage. It was clearly shown that rice storage at a temperature below ice point can preserve rice quality, but freezing temperature and freezing injury of rice were not clear. A basic study was therefore conducted to determine the freezing temperature and the incidence of freezing injury of rice and to determine the effects of temperatures below ice point on the physiological properties and quality of rice during four-year storage.

Methods

Freezing Temperature

The rice samples used to determine freezing temperature were Kirara397 and Hoshinoyume varieties, which are commercial Japonica non-waxy varieties and were produced in Hokkaido, Japan. Rough rice samples were collected at six moisture content (m.c.) levels during the drying process after harvest: 26.5%, 24.2%, 23.1%, 22.1%, 20.8% and 19.5% m.c. Moisture content was determined by the standard method of the Japanese Society of Agricultural Machinery (JSAM): about 10 g of a whole-grain rice sample was placed in a forced-air oven at 135°C for 24 h, and the moisture content was calculated on a wet basis.

A differential scanning calorimeter (DSC 3100 S, Mac Science Co., Tokyo, Japan) was used to measure the freezing temperature of rough rice. The cooling rate was controlled at 2°C/min and the temperature was decreased to –55°C. One grain of rough rice was used for each scan, and five to six grains at each moisture content level were used to measure the freezing temperature.

Freezing Injury

The rice used to determine freezing injury was Kirara397 variety. Rough rice samples were collected at seven moisture content levels during the drying process: 26.5%, 23.1%, 20.8%, 19.5%, 17.8%, 16.8% and 15.7% m.c. Freezing injury of grain seed such as rice is usually determined by germination rate because grains that suffer from freezing injury do not germinate.

Rough rice samples (about 50 g per each sample) were stored in refrigerators controlled at 2°C, –10°C, –20°C, –30°C, –40°C or –80°C for 11 days. The samples were then dried to about 16%

m.c. in a forced-air oven at 40 - 45°C and hulled. Germination rate were determined according to the standard method of the Japan Food Agency (JFA): three hundred sound brown rice grains were soaked in a hydrogen peroxide solution (1% [w/w] concentration) and placed in an incubator at 20°C. The germination rate was calculated by counting the number of grains that germinated within a period of seven days.

Freezing Trace

To observe freezing trace inside a rice grain, about 10 g of Hoshinoyume rice with a moisture content of 24.2% was placed in a refrigerator at a temperature of –40°C. After a one-day cooling period in the refrigerator, some grains form the sample were put in Freon 22 (–150°C) for quick freezing (cooling rate: about 10,000°C/min) and fixing of tissues of the rice grain. Freezing trace inside the rice grain was observed by using a scanning electron microscope (SEM).

Four-year Rice Storage Experiment

The rice used for the four-year rice storage experiment was Hoshinoyume variety. The moisture content of the rough rice sample was 16.2%. About 15 kg of rough rice was placed in each polyethylene container with a screw lid, and the containers were stored in incubators or refrigerators controlled at 25°C, 15°C, 5°C, –5°C, –20°C or –50°C for a period of 48 months, from October 1998 until October 2002.

To understand the physiological properties and quality characteristics of the rice samples, vigor rate, germination rate, free fat acidity, texturogram property and eating quality were investigated. Some grains were periodically taken from the rough rice samples and hulled. Vigor and germination rate were determined according to the standard method of JFA. The vigor rate was calculated by counting the number of grains that germinated within a period of 72 hours, and the germination rate was calculated by counting the number of grains that germinated within a period of 7 days. Grains that had lost their vitality (i.e., dead seeds) did not germinate.

Free fat acidity was determined by the rapid method of the American Association of Cereal Chemists (AACC, method 02-02): free fat acid was extracted from ground brown rice in a benzene solution, and the extracted solution was then titrated with potassium hydroxide solution. Texturogram property (hardness-stickiness ratio) of cooked rice was defined as the ratio of the first positive peak (hardness) divided by the first negative peak (stickiness) of the texture profile measured by a texturometer (Zenken, Tokyo, Japan).

To determine the eating quality of cooked rice, a sensory test was carried out according to the Japanese official rice taste testing method standardized by the JFA. Panelists were selected with gender and age balance and were trained for the test in advance. The number of panel members was 48. The sensory test was a multiple comparison test. The reference sample was Hoshinoyume variety, the same variety as the stored sample and grown by the same rice farmer. The reference sample was freshly harvested rice in the year after the completion of the four-year storage experiment (i.e., 2002 crop year). Panel members continually received two plates in the test and were asked to compare six samples with the reference sample on the basis of five sensory determinations: appearance, aroma, hardness, cohesiveness and overall flavor of the cooked rice. The directions of difference between the reference sample and six compared samples in overall flavor, for instance, were “good” and “bad”, and the degrees of difference were “no difference”, “very slight difference”, “slight difference”, “moderate difference”, “great difference” and “extreme difference”. Numerical scores were assigned to the directions and degrees with “extremely better than the reference” being +5, “no difference to the reference” being zero and “extremely worse than the reference” being –5. The reference sample was always scored zero. The evaluation value ranged within +-5 for appearance, aroma and overall

flavor and +-3 for the other items on a discrete scale. The overall flavor was eating quality of the sample.

Results

Freezing Temperature

Figure 1 shows freezing temperatures of rough rice grains with various levels of moisture content. Rice of 26.5% m.c. froze at temperatures in the range of –13°C to –25°C. The moisture content of each sample was measured using 10 g of rough rice. There were about 350 grains in 10 g of rough rice. Although each grain had various moisture contents, the average moisture content value of 350 grains (i.e., 26.5%) was used for analysis. The freezing temperature was measured using one grain for each scan. The freezing temperatures of rice of 26.5% m.c. were therefore distributed in the range of –13°C to –25°C because of the distribution of moisture contents of the grains. Rice of 22.1% m.c. froze at about –35°C. Rice samples of 20.8% m.c. and 19.5% m.c. did not freeze even at a temperature of –55°C, which was the minimum temperature of the DSC used in this study.

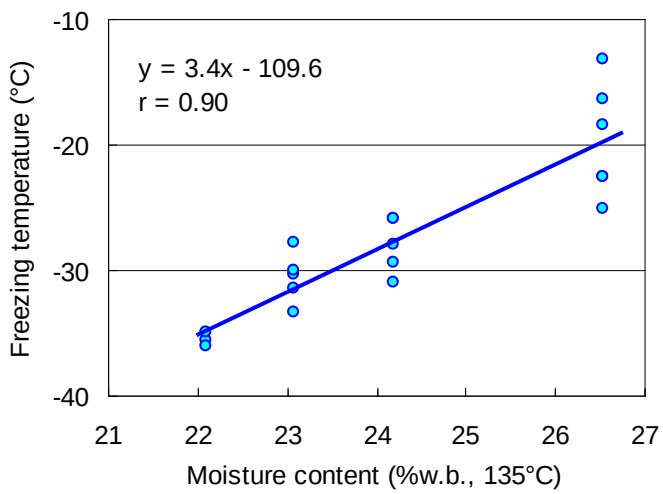

Figure 1. Freezing temperatures of rough rice grains with various levels of moisture content.

Freezing Injury

Figure 2 shows the germination rates (incidence of freezing injury) of rice grains with seven levels of moisture content after being cooled at six temperature levels for 11 days. Freezing injury did not occur in any of the rice grains stored at 2.5°C because the germination rate of these rice grains was almost 100%, while freezing injury occurred in all of the rice grains of 26.5% m.c. stored at temperatures below –19.1°C because the germination rates were 0%. There was no freezing injury in 64% of the rice grains of 23.1% m.c. stored at –19.1°C. Rice grains with moisture contents of less than 17.8% germinated after being stored at –80°C. Thus, no grain with a moisture content of less than 17.8% froze even at a temperature of –80°C.

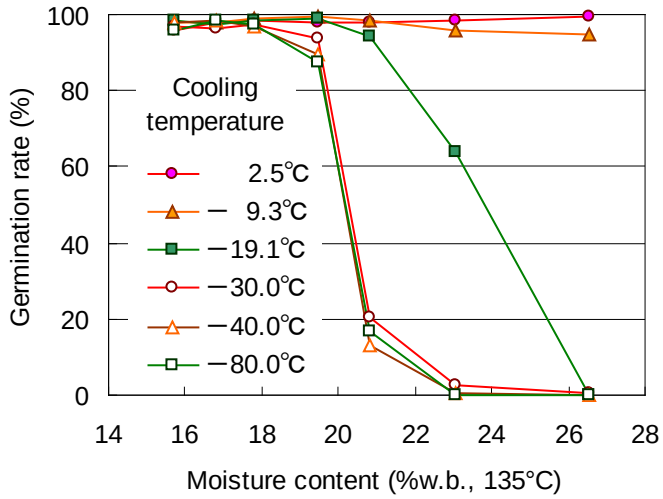

Figure 2. Germination rates (incidence of freezing injury) of rice grains with various levels of moisture content after 11-day cooling treatments.

Freezing Trace

A cross section of a rice grain of 24.2% m.c. cooled at –40°C observed by using a SEM is shown in Figure 3. Rough surface of cross section indicates freezing trace after sublimation of ice crystal in rice grain. Freezing traces were observed on the surface of the cross section. The embryo and a small section in the endosperm were observed as rough surface indicating freezing trace (Figures 3 and 4). Rice grains whose embryos suffered from freezing did not germinate.

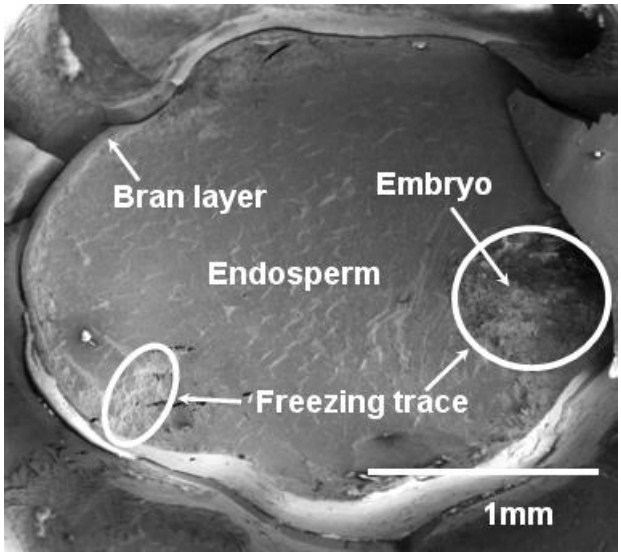

Figure 3. Cross section of a rice grain cooled at –40°C observed by using a scanning electron microscope (moisture content: 24.2%).

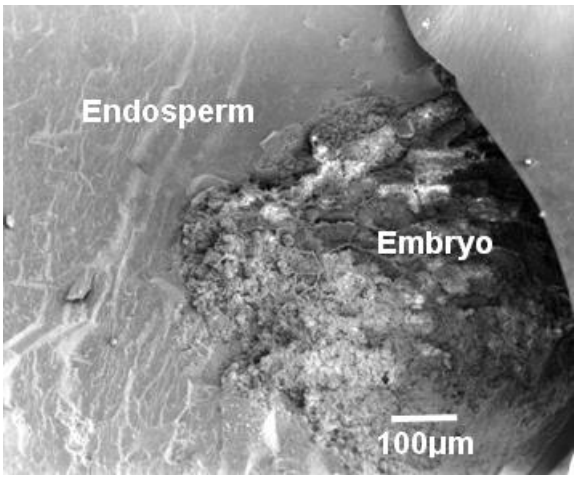

Figure 4. Freezing trace of the embryo of a rice grain cooled at -40°C (moisture content: 24.2%).

Four-year Rice Storage Experiment

Vigor Rate and Germination Rate

Vigor rate and germination rate are indices of physiological properties of rice grains. Vigor rate indicates the extent of dormancy of rice grains, and germination rate indicates the extent of vitality of rice grains. For example, rice grains of low vigor rate and high germination rate mean that the grains do not germinate within 72 hours because of deep dormancy and have the capacity of germination within 7 days because of breaking dormancy. The deeply dormant grains have low physiological activity, hence minimize deterioration of the quality during storage. On the other hand, rice grains of high vigor rate and high germination rate mean light dormancy and high physiological activity, hence accelerate deterioration. Rice grains of low vigor rate and low germination rate do not have any vitality as rice seeds (i.e., dead seeds).

At the beginning of storage (just after harvesting and drying of the rice sample), the vigor rate and germination rate were about 50% and 100%, respectively, indicating deep dormancy and high vitality.

Vigor rate of the rice sample stored at 25°C increased to more than 90% after 2 months of storage and then decreased to 0% after 9 months of storage (Figure 5). Germination rate of the rice sample stored at 25°C decreased to 0% over a period of 12 months of storage.

Vigor rate of the rice sample stored at 15°C increased to more than 90% after 6 months of storage and then decreased to 0% after 36 months of storage (Figure 6). Germination rate of the rice sample stored at 15°C remained at almost 100% during 12 months of storage and then decreased to 0% over a period of 48 months of storage.

These results indicate that the rice samples stored at 25°C and 15°C had lost dormancy from the beginning of storage because of the high temperatures and that respiration and metabolism increased, finally resulting in loss of vitality.

Vigor rate of the rice sample stored at 5°C increased to more than 90% after 12 months of storage and remained at more than 90% during 48 months of storage (Figure 7). Vigor rate of the rice sample stored at –5°C gradually increased during storage and reached more than 90%

after 48 months of storage (Figure 8). Germination rates of the rice samples stored at 5°C and –5°C remained at almost 100% during 48 months of storage.

Vigor rates of the rice samples stored at –20°C and –50°C remained almost the same as those at the beginning of storage, and their germination rates remained at almost 100% during 48 months of storage (Figures 9 and 10). These results indicate that low temperatures such as –20°C and –50°C maintained dormancy, minimized physiological activities and maintained vitality of rice for four years.

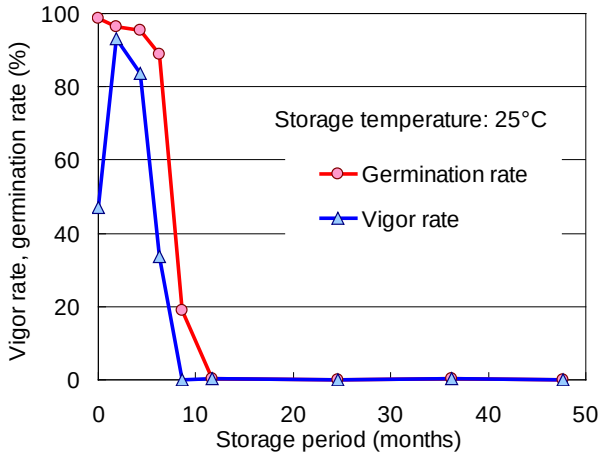

Figure 5. Changes in vigor rate and germination rate during storage at 25°C.

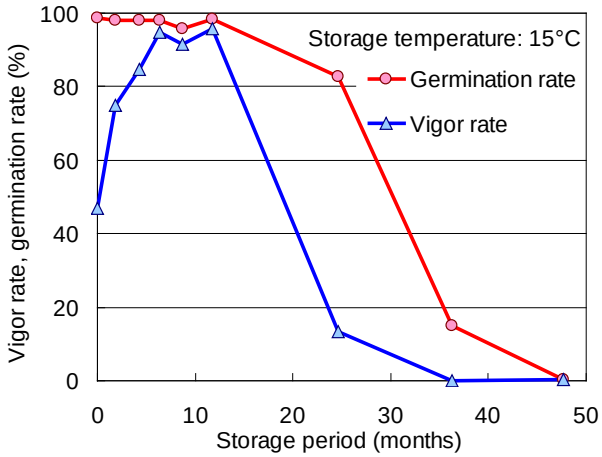

Figure 6. Changes in vigor rate and germination rate during storage at 15°C.

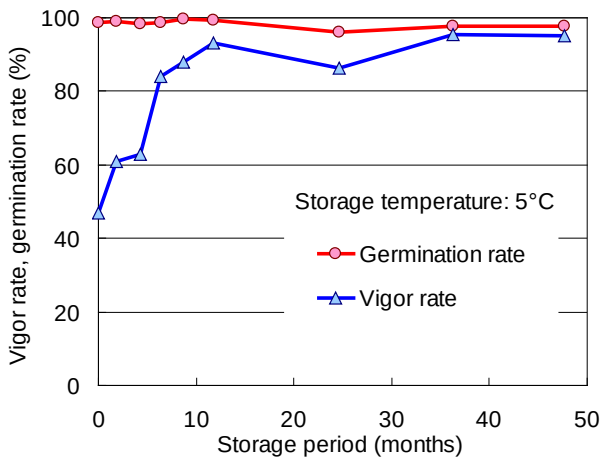

Figure 7. Changes in vigor rate and germination rate during storage at 5°C.

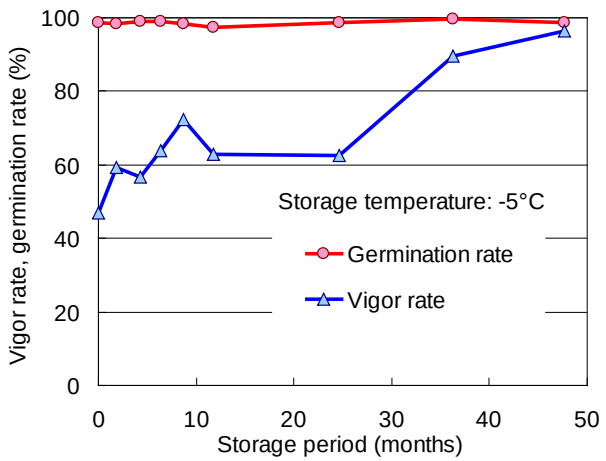

Figure 8. Changes in vigor rate and germination rate during storage at -5°C.

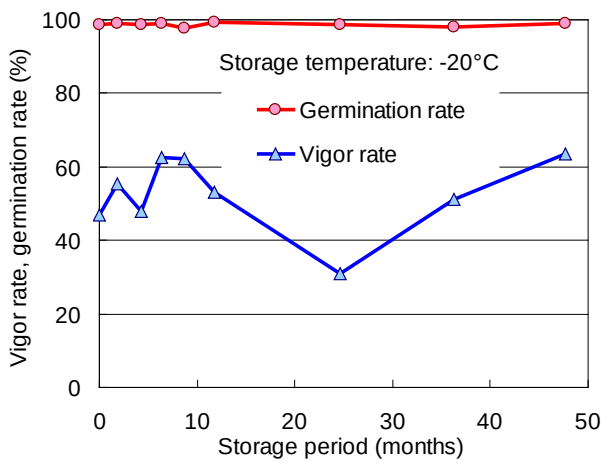

Figure 9. Changes in vigor rate and germination rate during storage at -20°C.

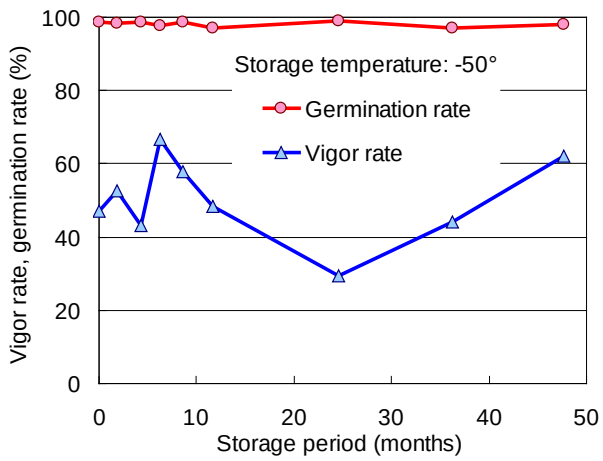

Figure 10. Changes in vigor rate and germination rate during storage at -50°C.

Quality Characteristics

Lipase hydrolyzes fat in rice grains to fatty acid. When the activity level of lipase in rice grains is high, the amount of free fat acidity increases during storage. This increase in the amount of free fat acidity causes deterioration of the quality of rice (rancid rice) during storage. The free fat acidity of rice stored at temperatures below 5°C was almost the same as that at the beginning of storage (Figure 11). However, the free fat acidity of rice stored at 25°C dramatically increased during storage.

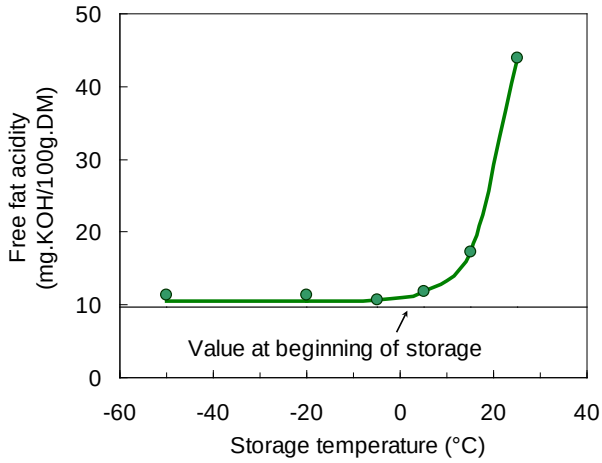

Figure 11. Free fat acidity of rice after 48-month storage.

Generally, the hardness of cooked rice increases and the stickiness of cooked rice decreases during storage. The texturogram property (hardness-stickiness ratio) increases accordingly as rice ages. The changes in the texture of cooked rice are caused by deterioration in rice starch. There was no change in the hardness-thickness ratio of rice stored at temperatures below 5°C (Figure 12).

The eating quality (overall flavor) of rice stored at temperatures below 5°C was the same as that of the reference sample (newly harvested rice) after 48 months of storage (Figure 13).

These results indicate that low temperature minimized rancidity and starch deterioration in rice and thus preserved rice quality.

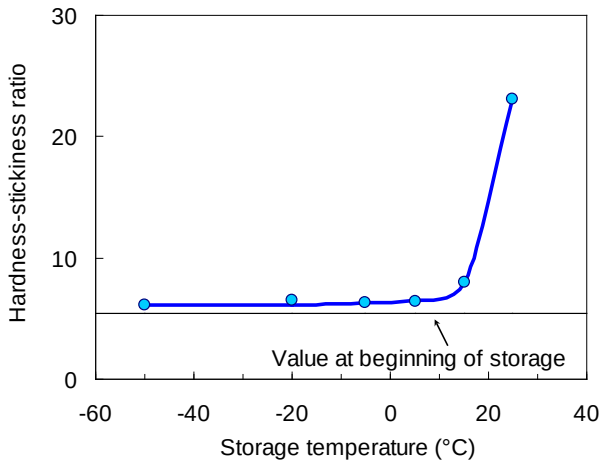

Figure 12. Texturogram property of cooked rice after 48-month storage.

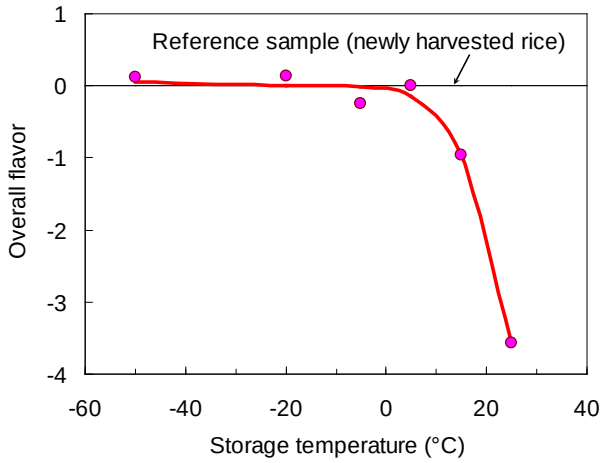

Figure 13. Eating quality (overall flavor) of cooked rice after 48-month storage.

Discussion

In Japan, rough rice is always dried until the moisture content is about 16% after harvesting. In the present study, rough rice with a moisture content of less than 17.8% did no freeze even at a temperature of –80°C. Thus, there is no need for concern about freezing of rice stored in farm silos in winter.

The quality of rough rice stored at temperatures less than 5°C for four years was the same as that of newly harvested rice, indicating that preservation of rough rice quality for several years is possible by storing rice at an average temperature of less than 5°C.

We (Kawamura et al., 2004) have developed a new technique named super-low-temperature storage for storing rough rice at a temperature below ice point using ambient fresh chilly air in winter. When the grain temperature is cooled down below ice point in winter, the average temperature during storage can be easily maintained below 5°C. A combination of rice storage at a temperature below ice point and utilization of chilly fresh air enables the quality of rice to be preserved at a high level without the requirement of a cooling unit or electricity. Thus long-term storage of rice without deterioration of quality can be achieved by using natural cold energy.

The use of the super-low-temperature storage technique has been increasing in cold regions of Japan in recent years. In Hokkaido, the northernmost island of Japan, 26 grain elevators have been constructed since 1996. The storage capacity of rough rice in Hokkaido in 2004 was 115,000 t.

Conclusion

No rice grain with a moisture content of less than 17.8% froze at a temperature of –80°C. Low temperature maintained vitality of rice, minimized physiological activities, starch deterioration and rancidity in rice, and consequently preserved rice quality at a level of newly harvested rice. Preservation of rough rice quality for four years was possible by storing rice at temperatures below 5°C. The average grain temperature in farm silos could be maintained below 5°C by using ambient fresh chilly air in winter.

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