International Conference NEW PERSPECTIVES in SCIENCE EDUCATION

Comparison of D-Glucose Concentration Between Terrace Rice and Lowland Rice.

[1] Yuki Otsubo
[2] Ayumu Yamazaki
[3] Kenichi Goto

[1] Toyo University, Japan
[2] Toyo University, Japan
[3] Toyo University, Japan

Abstract

In recent years, rice consumption in Japan has declined significantly due to the diversification of dietary habits and a weakening recognition of rice's cultural and nutritional value. This trend poses challenges not only to agricultural sustainability but also to the transmission of food culture. Terrace rice (tanada-mai), cultivated in mountainous regions, is often described as having a "sweeter" taste compared to lowland rice, yet the scientific basis for this perception remains unclear [1]. Previous studies suggest that terrace rice tends to have higher sugar content [2], but the mechanisms underlying this difference have not been fully elucidated. During the soaking process prior to cooking, endogenous enzymes such as α-glucosidase convert starch into disaccharides and ultimately into D-glucose. However, the effects of temperature on these enzymatic reactions are poorly understood. This study analyses changes in D-glucose concentration during the soaking process of Niigata-grown Koshi Hikari rice [3], comparing terrace and lowland rice from the 2023 (old crop) and 2024 (new crop) harvests. By focusing on enzymatic activity under different temperature conditions, the research aims to clarify the biochemical basis for the sweetness of terrace rice. The findings are expected to contribute to STEM education by providing scientifically grounded insights into rice biochemistry, while also promoting a deeper appreciation of rice as a culturally significant food. Through the integration of food science and cultural studies, this research encourages future learners to rediscover the value of rice and supports the development of educational materials that bridge tradition and inquiry.

Keywords: *Terrace rice, STEM education*

1.Background and Objective

Terraced rice, grown in hillside terraces, is generally considered to have a stronger sweetness than rice grown on flat land (lowland rice). However, scientific evidence supporting this sensory evaluation remains limited. Previous studies in Japan have reported that terraced rice tends to have higher sugar content than lowland rice, but most of these studies have focused only on quantitative differences, and the physiological and biochemical mechanisms underlying these differences are not well understood.

Rice palatability is influenced by factors such as amylose content, protein content, and moisture content, and sugar production during soaking and cooking is also thought to contribute to perceived sweetness. During soaking, rice starch is partially degraded to produce low-molecular-weight sugars. This process involves endogenous enzymes in rice, particularly α-glucosidase, which hydrolyzes starch and oligosaccharides into smaller sugars, including glucose.

Growth conditions differ between terraced fields and flat fields in terms of altitude, temperature variation, water temperature, and sunlight exposure. These environmental factors may influence starch structure and endogenous enzyme activity in rice grains. However, few studies have examined differences in sugar production and enzyme activity during the soaking process between terraced and lowland rice. As a result, the scientific basis for the higher sweetness of terraced rice remains unclear.

From a STEAM education perspective, this research provides an interdisciplinary learning opportunity that integrates Science (biochemistry of starch degradation and enzyme activity), Technology (measurement of glucose concentration and enzyme activity), Engineering (experimental design and control of soaking temperature and time), Mathematics (data analysis and comparison of quantitative results), and Art (sensory evaluation and cultural appreciation of rice quality). By linking traditional agricultural practices with modern scientific analysis, this study demonstrates how everyday foods can be used as effective educational materials to foster problem-solving skills and scientific inquiry.

Based on this background, the aim of this study was to investigate sugar production during soaking in terraced and lowland rice and to examine its relationship with endogenous enzyme activity. Through this approach, the study seeks not only to improve understanding of the palatability of terraced rice but also to illustrate the value of STEAM-based learning in exploring the relationship between cultivation environment, food quality, and biochemical processes.

2. Methods

2.1 Experimental Samples

Six types of rice samples, consisting of flatland-grown and terrace-grown rice from three harvest years (2023, 2024, and 2025), were prepared for this study. Each sample was subjected to soaking experiments under two temperature conditions, 40 °C and 60 °C, to examine the effects of temperature on sugar generation during soaking.

A. 2023 harvest, Niigata Prefecture, Niigata-grown Koshihikari, no-wash rice, flatland-grown rice (hereafter referred to as 23-P)

B. 2023 harvest, Uonuma region, Niigata Prefecture, Koshihikari, no-wash rice, terrace-grown rice (hereafter referred to as 23-T)

C. 2024 harvest, Niigata Prefecture, Niigata-grown Koshihikari, no-wash rice, flatland-grown rice (hereafter referred to as 24-P)

D. 2024 harvest, Uonuma region, Niigata Prefecture, Koshihikari, no-wash rice, terrace-grown rice (hereafter referred to as 24-T)

E. 2025 harvest, Niigata Prefecture, Niigata-grown Koshihikari, no-wash rice, flatland-grown rice (hereafter referred to as 25-P)

F. 2025 harvest, Uonuma region, Niigata Prefecture, Koshihikari, no-wash rice, terrace-grown rice (hereafter referred to as 25-T)

The following reagent was used in this study:

G. Liquid D-glucose E-Kit (J.K. International Co., Ltd.).

In this study, this method was used to measure the D-glucose concentration in Experiment 1.

Principle：D-glucose + ATP — HK → Glucose-6-phosphate + ADP

G6P + NAD^+ — G6P-DH → 6-Phosphogluconate + NADH + H^+

This assay is based on enzymatic reactions catalyzed by hexokinase (HK) and Glucose-6-phosphate dehydrogenase (G6P-DH). In the presence of hexokinase and ATP, D-Glucose is phosphorylated to D-glucose-6-phosphate (G6P) with the concomitant formation of adenosine diphosphate (ADP). Subsequently, in the presence of Glucose-6-phosphate dehydrogenase, D-glucose-6-phosphate is oxidized to 6-phosphogluconate, accompanied by the reduction of NAD^+ to NADH.

H. Saccharifying Enzyme Activity Fractionation Assay Kit (Kikkoman Biochemifa Company).

In this study, this method was used to measure α-glucosidase activity in Experiment 2.

Principle：4-Nitrophenyl α-D-glucoside (PNPG), a substrate for measuring α-glucosidase activity, is hydrolyzed to produce p-nitrophenol (PNP). The reaction is terminated by the addition of sodium carbonate. Simultaneously, the pH of the reaction mixture shifts to the alkaline range, resulting in maximal color development of PNP. The absorbance of PNP is then measured at a wavelength of 400 nm.

2.2 Experimental Procedure

2.2.1 Experiment 1: Measurement of D-Glucose Concentration

1. Rice samples were sieved (1.00 mm – 2.36 mm) to standardize grain size.
2. Water baths (12 pt buckets) were prepared using a low-temperature water cooker and set to the desired soaking temperatures (40 °C and 60 °C).
3. For each rice sample, 20 g of rice and 24 mL of distilled water were placed in a 50 mL beaker. The beakers were positioned on a raised platform inside the water bath, and the rice was soaked for 70 minutes.

4. During soaking, the solution was stirred in a circular motion with a glass rod 15 times every 10 minutes, and 100 μL of soaking solution was collected each time, for a total of seven samplings over 70 minutes.
5. To 100 μL of the soaking solution, 2000 μL of Reagent 1 (NAD^+ and buffer) was added and mixed thoroughly. The mixture was transferred to a cuvette and incubated at 25°C for exactly 3 minutes.
6. Absorbance (A_1) was measured at 340 nm using a spectrophotometer.
7. Subsequently, 500 μL of Reagent 2 (HK and G6P-DH) was added to the mixture, mixed thoroughly, and incubated at 25°C for exactly 15 minutes.
8. Absorbance (A_2) was measured at 340 nm.
9. D-glucose concentration (g/mL) was calculated from the measured absorbance values (A_1, A_2) according to the manufacturer's instructions:
   D-glucose concentration (g/mL)=($A_2 - A_1$)×blank correction (0.052)×0.744
10. Steps 1–9 were repeated three times for each sample, and the mean value was calculated.

2.2.2 Experiment 2: Measurement of A-Glucosidase Activity

1. Rice samples were sieved (1.00 mm – 2.36 mm) to standardize grain size.
2. Water baths were prepared using a low-temperature cooker and set to the desired soaking temperatures (40 °C and 60 °C).
3. For each rice sample, 20 g of rice and 24 mL of distilled water were placed in a 50 mL beaker, which was then soaked in the water bath for 70 minutes.
4. During soaking, the solution was stirred in a circular motion with a glass rod 15 times every 10 minutes, and 100 μL of soaking solution was collected each time, for a total of seven samplings over 70 minutes.
5. In a separate water bath, 2000 μL of the substrate solution (PNPG) was pre-warmed in small test tubes at 37 °C for approximately 5 minutes.
6. To initiate the reaction, 100 μL of the soaking solution was added to the pre-warmed substrate solution and mixed thoroughly.
7. The reaction mixture was incubated at 37 °C for exactly 10 minutes. The reaction was then stopped by adding 1000 μL of stop solution and mixing thoroughly.
8. The absorbance of the reaction mixture was measured at 400 nm using a spectrophotometer (E2s).
9. α-glucosidase activity (U/mL) was calculated based on the absorbance measurement according to the manufacturer's instructions:
   α-glucosidase activity (U/mL)={ E2s−blank correction }×0.171
10. Steps 1–9 were repeated three times for each sample, and the mean value was calculated.

3. Results

3.1 Measurement of D-Glucose Concentration

Flatland and terrace rice from the 2023, 2024, and 2025 harvests were soaked at 40 °C for 70 minutes. Samples of the soaking solution were collected every 10 minutes, for a total of seven time points per trial, and each measurement was repeated three times. The mean D-glucose concentrations obtained are shown in **Fig. 1.**

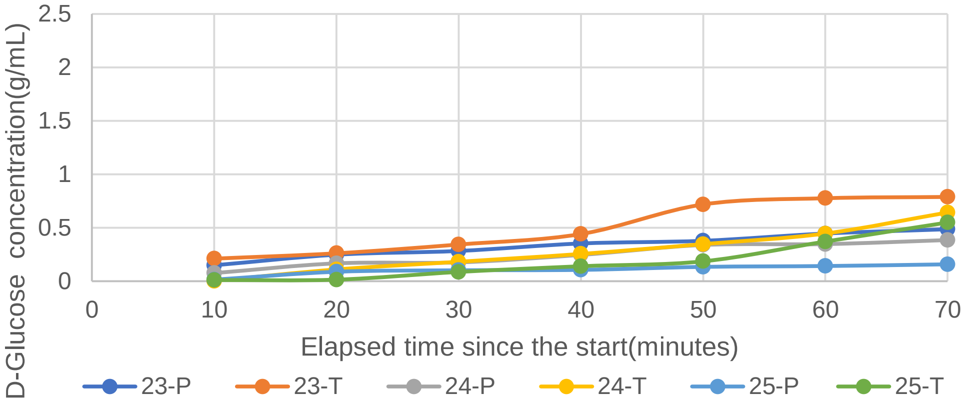

Fig. 1.D-Glucose concentrations in lowland rice and terraced rice at 40℃

Flatland and terrace rice from the 2023, 2024, and 2025 harvests were soaked at 60°C for 70 minutes. Samples of the soaking solution were collected every 10 minutes, for a total of seven time points per trial, and each measurement was repeated three times. The mean D-glucose concentrations obtained are shown in **Fig. 2.**

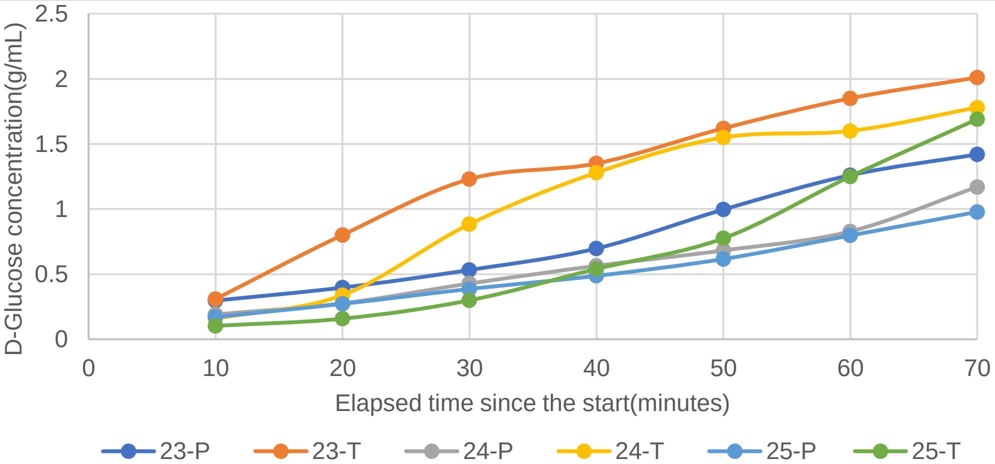

Fig. 2.D-Glucose concentrations in lowland rice and terraced rice at 60℃

Under both 40°C and 60°C conditions, D-glucose concentrations increased with reaction time. At 70 minutes, D-glucose concentrations at 60°C were higher in all samples than those at 40°C.

D-glucose concentrations increased gradually in all samples, reaching approximately 0.2-0.9 g/mL after 70 min at 40℃. In particular, terraced rice harvested in 2023 (23-T) showed higher values than the other samples, with a marked increase observed after 50 min. Among the production years, sugar production tended to follow the order: 2023 > 2024 > 2025. Within the same production year, terraced rice (T) tended to exhibit higher D-glucose concentrations than lowland rice (P).

Sugar production was markedly enhanced at 60°C compared with 40°C, and D-glucose concentrations reached approximately 1.0-2.1 g/mL after 70 min at 60℃.Terraced rice harvested in 2023 (23-T) showed the highest D-glucose concentrations, with a rapid increase observed at the early reaction stage (20-30 min). While terraced rice harvested in 2024 (24-T) and lowland rice harvested in 2024 (24-P) also exhibited relatively high values, rice harvested in 2025 tended to show lower D-glucose concentrations overall. Differences between cultivation conditions became more pronounced at 60°C, with terraced rice exhibiting consistently higher D-glucose production than lowland rice.

3.2 Measurement of A-Glucosidase Activity

Lowland rice and terraced rice harvested in the 2023–2025 harvest years were soaked at 40°C for 70 minutes. α-glucosidase activity was measured every 10 minutes (seven time points) with three replicates, and the mean values are presented in **Fig. 3**.

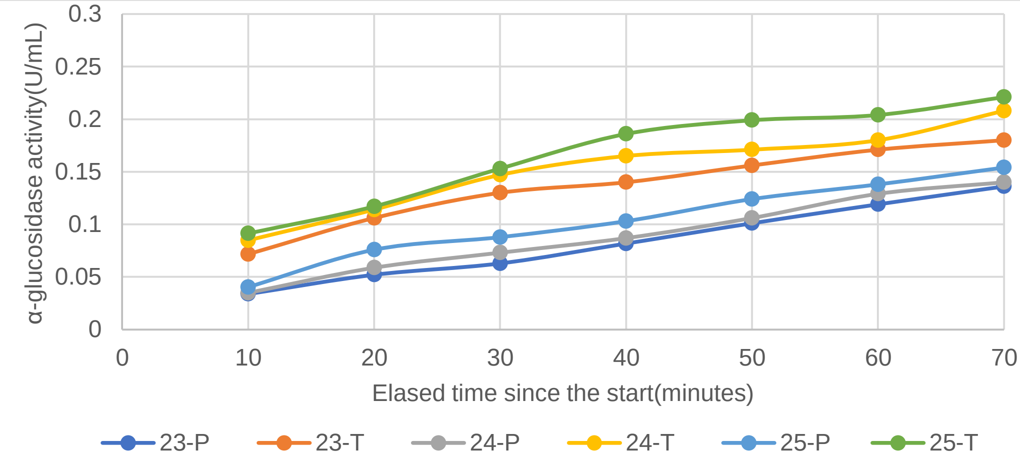

Fig. 3. α-Glucosidase activity in lowland rice and terraced rice at 40℃

Lowland rice and terraced rice harvested in the 2023–2025 harvest years were soaked at 60 °C for 70 minutes. α-glucosidase activity was measured every 10 minutes (seven time points) with three replicates, and the mean values are presented in **Fig. 4**.

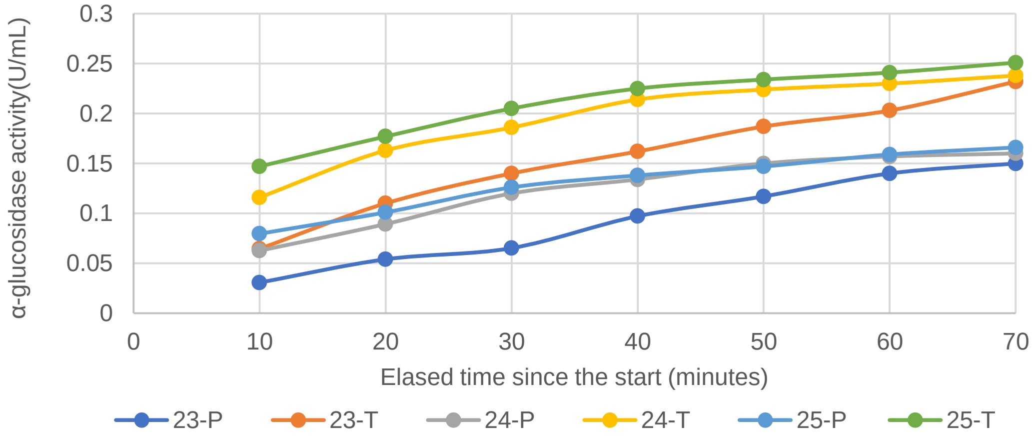

Fig. 4. α-glucosidase activity in lowland rice and terraced rice at 60℃

Under both 40°C and 60°C conditions, α-glucosidase activity increased over time. At 70 minutes, α-glucosidase activity at 60°C were higher in all samples than those at 40°C. α-glucosidase activity increased gradually in all samples, reaching approximately 0.14–0.23 U/mL after 70 min at 40℃. Terraced rice (T) tended to exhibit higher α-glucosidase activity than lowland rice (P). Among the production years, terraced rice harvested in 2024 and 2025 (24-T and 25-T) showed relatively higher values. Rice harvested in 2023 tended to exhibit slightly lower α-glucose concentrations overall.

α-glucosidase activity was markedly enhanced compared with that at 40°C, reaching approximately 0.15–0.25 U/mL after 70 min at 60℃. Terraced rice harvested in 2025 (25-T) and 2024 (24-T) showed high α-glucosidase activity and maintained higher values from the early reaction stage. For all production years, terraced rice (T) consistently exhibited higher α-glucosidase activity than lowland rice (P). The effects of both production year and cultivation condition became more pronounced at 60°C.

Statistical analysis was performed using two-way ANOVA with cultivation type (terraced vs. lowland) and production year (2023–2025) as fixed factors for each temperature condition. Post-hoc comparisons were conducted using Tukey's HSD test (α = 0.05). All data are presented as mean ± standard deviation of three independent measurements.

Under both 40°C and 60°C conditions, D-glucose and α-glucose concentrations increased with reaction time in all samples. Higher sugar concentrations were consistently observed at 60°C than at 40°C, indicating that elevated temperature enhanced starch degradation.

At 40°C, sugar production increased gradually, whereas at 60°C, both D-glucose and α-glucose showed a more pronounced and rapid increase, particularly during the early to mid reaction stages. D-glucose concentrations reached approximately 0.2–0.9 g/mL at 40 °C and 1.0–2.1 g/mL at 60°C, while α-glucose concentrations reached approximately 0.14–0.23 U/mL at 40°C and 0.15–0.25 U/mL at 60°C after 70 min.

Terraced rice (T) generally exhibited higher D-glucose and α-glucose concentrations than lowland rice (P) under most conditions. This difference was more pronounced at 60°C, suggesting that terraced rice was more susceptible to enzymatic starch degradation or possessed different substrate properties.

Differences in sugar production were observed among production years. Rice harvested in 2023 and 2024 tended to show higher D-glucose concentrations, whereas rice harvested in 2025 generally exhibited lower D-glucose values. In contrast, α-glucose concentrations were relatively higher in terraced rice harvested in 2024 and 2025, indicating that year-to-year effects differed between sugar forms. α-glucose concentrations increased in parallel with increases in D-glucose concentrations, although the absolute values of α-glucose were lower. The similar time-course profiles of both sugars indicate that starch degradation proceeded consistently throughout the reaction period.

4. Discussion

In this study, the effects of cultivation environment (flatland rice and terraced paddy rice), production year (2023–2025), and reaction temperature (40°C and 60°C) on the saccharification characteristics of rice starch were evaluated based on the time-course changes in D-glucose and α-glucose concentrations. The experimental design and analytical process of this study not only contribute to fundamental knowledge in food science but also can be positioned as a practical model of inquiry-based learning within STEAM education.

First, focusing on the effects of temperature conditions, at the 70 min time point, both D-glucose and α-glucose concentrations were significantly higher at 60 °C than at 40 °C for all samples ($\alpha < 0.05$). This result quantitatively demonstrates the food chemistry principle that higher temperatures promote starch gelatinization and enzymatic reactions (Science). At the same time, experimental procedures such as temperature control and maintenance of constant reaction conditions highlight the importance of utilizing experimental equipment and environments (Technology) as well as constructing appropriate experimental systems (Engineering). Furthermore, the visualization of the obtained data and comparison between temperature conditions contribute to the development of quantitative reasoning and data interpretation skills (Mathematics).

Regarding the effect of reaction time, time-dependent increases in D-glucose and α-glucose concentrations were observed under all conditions. In particular, the rapid increase in sugar production during the initial phase of the reaction, followed by a reduced rate of increase in the later phase, provides a clear example for understanding scientific concepts such as enzyme reaction kinetics and substrate limitation through actual data. The analysis of such time-course changes clearly illustrates the inquiry process of hypothesis formulation, verification, and interpretation, making it well suited to problem-solving–based learning in STEAM education.

With respect to differences in cultivation environment, terraced paddy rice tended to exhibit higher sugar production than flatland rice, with the difference being significant especially under the 60°C condition ($p < 0.05$). The unique topographical and climatic characteristics of terraced paddies, including larger diurnal temperature variations and differences in water management, are likely to influence the grain-filling process. These results link agricultural and regional environmental factors with food science properties and strongly reflect the STEAM education perspective of examining the relationship between natural environments and human activities from multiple angles. Using rice, a familiar food material, as a study subject provides high educational value by enabling learners to connect regional agriculture and sustainability with scientific analysis.

In addition, significant differences in sugar production were observed among production years, with rice produced in 2025 showing a suppressed progression of saccharification compared with rice from other years ($p < 0.05$). This finding suggests that high-temperature conditions during the grain-filling period may have affected starch structure, and it connects scientific knowledge in food chemistry with the contemporary social issue of climate change. Such year-to-year comparisons go beyond simple interpretation of experimental results and serve as an important learning element in STEAM education by fostering the ability to scientifically evaluate the impact of climate change on food production.

Furthermore, differences in the generation behavior of D-glucose and α-glucose indicate that D-glucose, as the final degradation product, serves as a more sensitive indicator of the overall progression of the saccharification reaction. The process of examining the appropriateness of indicator selection cultivates a critical perspective on data interpretation and contributes to the development of higher-order thinking skills emphasized in STEAM education.

In conclusion, this study elucidates the saccharification characteristics of rice starch from a food science perspective while simultaneously presenting a learning model that encompasses experimental design, measurement, data analysis, and interpretation as an integrated inquiry process. The framework of this research can be utilized as educational material in STEAM education to foster scientific thinking, quantitative analysis skills, and technological literacy in an integrated manner, and it represents a practical example of inquiry-based learning linked to regional resources and societal challenges.

5. Future Perspectives

From the viewpoint of science education, the measurement of D-glucose concentration in rice provides an effective model for linking chemical analysis with real-world food science. By examining how temperature conditions influence D-glucose production during starch degradation, students can directly explore the relationship between enzymatic reactions, molecular structure, and observable quantitative data.

In future studies, this experimental framework could be expanded to compare different rice varieties or processing conditions, enabling learners to formulate hypotheses and evaluate how biological and chemical factors affect glucose concentration. Such comparisons may deepen students' understanding of carbohydrate metabolism and food chemistry while reinforcing data analysis and statistical reasoning skills.

Furthermore, integrating advanced analytical approaches, such as structural analysis of starch using solid-state NMR, could offer an opportunity to introduce students to modern scientific techniques. This would help learners connect macroscopic measurements, such as glucose concentration, with microscopic and molecular-level structures, fostering interdisciplinary thinking across chemistry, biology, and materials science.

Overall, the D-glucose concentration measurement in rice represents a versatile educational tool that supports inquiry-based learning and promotes new perspectives in science education by bridging everyday materials with advanced scientific concepts.

6. REFERENCES

[1] Kikuchi, Chinana. (2021). "A Study on the Branding Process of Terrace Rice."

[2] Hokuriku National Agricultural Experiment Station. (1997). "A Simple Quantitative Method for Water-Soluble Sugars on the Surface of Milled Rice Using a Digital Refractometer."National Agriculture and Food Research Organization (NARO), Kyushu University Institutional Repository.

[3] Kikuchi, Itsuki. (2023). "Visible Quantification of Glucose in Rice During Cooking: Toward Application as an Educational Material."Undergraduate Thesis.