



Studies on endogenous enzymes in pearl millet

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Abstract

*[Objective] Pearl millet (*Pennisetum glaucum*) is a gluten-free grain that can be cultivated reliably even in climates subject to frequent climate change. Its high nutritional value, including iron and calcium, compared to wheat flour makes it a valuable grain resource. However, little research has been done on its cooking properties. Therefore, this study focused on the free sugar content of pearl millet and examined the behavior of endogenous enzymes during heating. This study is expected to contribute to understanding food science and the study of sustainable food resources. [Method] To examine the free sugar content of pearl millet flour, 50 ml of distilled water was added to 5.0 g of pearl millet powder and exposed to temperatures ranging from 40 to 80°C in 10°C increments for 30 minutes. 80% ethanol was then added, followed by shaking and extraction, and suction filtration. The resulting residue was then re-added to 80% ethanol, followed by shaking and extraction, followed by suction filtration. The filtrates were combined, concentrated under reduced pressure, and diluted with distilled water to produce a pearl millet extract. Extracts were prepared in the same manner for unheated samples. The resulting extract was analyzed for glucose, sucrose, fructose, and maltose contents using an F kit (J.K. International Co., Ltd.). [Results] Unheated, the maltose content was high, while the glucose and fructose contents were low. Heating reduced the maltose content and increased the glucose and fructose contents. In particular, exposure to temperatures between 60°C and 70°C reduced the maltose content and increased the glucose and fructose contents. Therefore, it is believed that maltase, α -glucosidase, and sucrase were active in pearl millet. Elucidating enzyme activity will affect the final product and lead to an understanding and learning of food science through food properties. Furthermore, because pearl millet is gluten-free, it offers potential as a new grain resource when considering future food resources.*

Keywords: Pearl millet, Enzyme activity, Free equivalents

1. Introduction

Pearl millet (*Pennisetum glaucum* (L)) (Fig.1. A,B) is a cereal belonging to the genus *Pennisetum* in the Poaceae family. It is resistant to drought and high temperatures and can grow in low-nutrient soils, making cultivation possible even under extreme climatic conditions [1][2]. Consequently, it can be harvested in regions where other cereals struggle to grow, serving as a valuable food source in Africa and India [3]. Additionally, it is gluten-free [4] and has higher nutritional value than wheat flour, containing more iron and calcium. In cooking, it is a versatile ingredient, primarily used as whole grain, cracked grain, ground grain, dough, or as a grain similar to rice. Thus, its ability to withstand harsh conditions of heat and drought while ensuring stable yields, combined with its high nutritional value and versatility, positions it as a food resource with significant potential for the future. However, its distinctive bitterness [5] suggests room for improvement in terms of palatability for it to be recognized as a staple food for daily consumption. Furthermore, while pearl millet has traditionally been consumed in parts of Africa and Asia, few studies have examined its cooking characteristics and palatability from a culinary science perspective. Pearl millet generally suffers from low recognition, and despite its excellent characteristics, research lags behind that of other crops. As climate change progresses, raising concerns about the future stability of food production, the importance of crops tolerant to harsh environmental conditions like drought and high temperatures is increasing. Therefore, pearl millet's drought and heat tolerance makes it highly likely to gain attention as a new crop for the future of our planet. Studying such crops is significant not only for elucidating their food science value but also for providing a perspective that connects sustainable food resources and environmental issues with science education. Specifically, scientifically understanding ingredient properties and material changes during cooking processes can offer an opportunity to connect learning in biology and chemistry—fields covered in science education—to global challenges.

Furthermore, since pearl millet belongs to the grass family, it was considered that milled pearl millet might contain glucose, sucrose, maltose, and fructose. Monosaccharides like glucose and fructose are



generally perceived as sweeter than disaccharides like sucrose and maltose when consumed. Therefore, determining the free sugar content in pearl millet, which has a strong bitterness, becomes crucial for future culinary applications. This suggests the importance of reducing sugars as a flavor indicator.

Regarding the increase in reducing sugars during cooking, it is known that reactions involving enzymes within rice grains are influenced by soaking time and heating temperature in rice. Therefore, it is reasonable to assume that enzymatic reactions also play a role in pearl millet. Quantifying the levels of glucose, fructose, sucrose, and maltose in pearl millet after heating can help determine which enzymes are active at specific temperature ranges. Furthermore, differences in enzyme reactions and heating temperatures during the cooking process represent an important learning topic in science education. Specifically, the function of enzymes, changes in reaction rates due to temperature conditions, and optimal activity temperatures are covered in secondary school science. However, within science education, these topics are often addressed only through abstract explanations of principles and sequences based primarily on words and sentences, making them difficult to grasp. Conversely, everyday cooking operations like heating and soaking grains are familiar phenomena where enzyme reactions and chemical changes actually occur. Quantitatively capturing these processes is considered an effective teaching tool for fostering scientific thinking skills. Therefore, presenting the effects of enzyme activity in cooking as numerical data or graphs visualizes invisible reactions, aiding understanding of the scientific inquiry process from hypothesis formulation to verification and analysis.

Therefore, this experiment focuses on the free sugar content of pearl millet to examine the behavior of sugar production by endogenous enzymes during heating.



Fig.1. Pearl Millet
A: During cultivation, B: Grain, C: Powder

2. Experimental Methods

2.1. Samples

Pearl millet used was commercially available Indian pearl millet powder (Atta Pearl Millet (Bajra Flour) SARTAJ; hereafter referred to as pearl millet). It was stored in a polyethylene bag at 4°C in a refrigerator until use (Fig.1. C).

2.2. Observation Of Pearl Millet By Scanning Electron Microscopy

Pearl millet powder was observed without fixation or dehydration. It was dispersed on a sample stage coated with conductive tape and examined for morphology using a scanning electron microscope (JSM-IT210, JEOL Ltd.) at an acceleration voltage of 10.0 kV and a magnification of 1,000× [21.1] (10.0 kV, 1000×). For comparison, wheat flour (low-gluten flour, Tokuhoshi brand, Masuda Seifun Co, Ltd.) and rice flour (bread-type rice flour, Mizuhokichara brand, Kumamoto Seifun Co, Ltd.) were used.



2.3. Measurement Of Free Sugars

Add 30 ml of 80% ethanol to 5.0 g of pearl millet powder and shake at 130 rpm for 1 hour using a shaker (AS ONE Corporation Double-Action Lab Shaker SRR-2). The resulting extract was filtered using 0.45 μm filter paper (ADVANTEC Qualitative Filter Paper 70 mm), separating it into residue and supernatant. An additional 30 ml of 80% ethanol was added to the residue and shaken at 130 rpm for 1 hour using the shaker. The resulting extract was filtered using 0.45 μm filter paper. The filtrate was combined with the previous filtrate to form the free sugar extract. Ethanol was evaporated from this extract using an evaporator (EYELA Rotary Vacuum Evaporator N-1000), and the volume was adjusted to 25 ml with distilled water. To investigate the behavior of sugar production by endogenous enzymes during heating, 5.0 g of pearl millet powder was mixed with 50 ml of distilled water. The mixture was allowed to soak at 40°C for 30 minutes to ensure complete immersion of the powder in the distilled water. Subsequently, 200 ml of 80% ethanol was added, and the mixture was shaken at 140 rpm for 1 hour using a shaker. The resulting extract was filtered using 0.45 μm filter paper, separating it into residue and supernatant. An additional 30 ml of 80% ethanol was added to the residue. The mixture was shaken at 130 rpm for 1 hour using a shaker, filtered using filter paper, and the supernatant was combined with the previous supernatant to form the sugar extract. Ethanol was evaporated from this using an evaporator, and the volume was adjusted to 50 ml using distilled water (Fig.2). The same procedure was performed for samples maintained at 50, 60, 70, and 80°C.

To measure free sugar content, the prepared sugar extract was analyzed using J.K. International's E-Kit Liquid Maltose/Sucrose/D-Glucose (ENZYTEC Liquid Maltose-Sucrose-D-Glucose E8170), Sucrose/D-Glucose (ENZYTEC Liquid Sucrose/D-Glucose E8180), D-Glucose/D-Fructose E8160) to determine glucose, fructose, sucrose, and maltose levels. The measurement principle was an enzymatic method using hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6P-DH), with the generated NADH measured for absorbance at 340 nm.

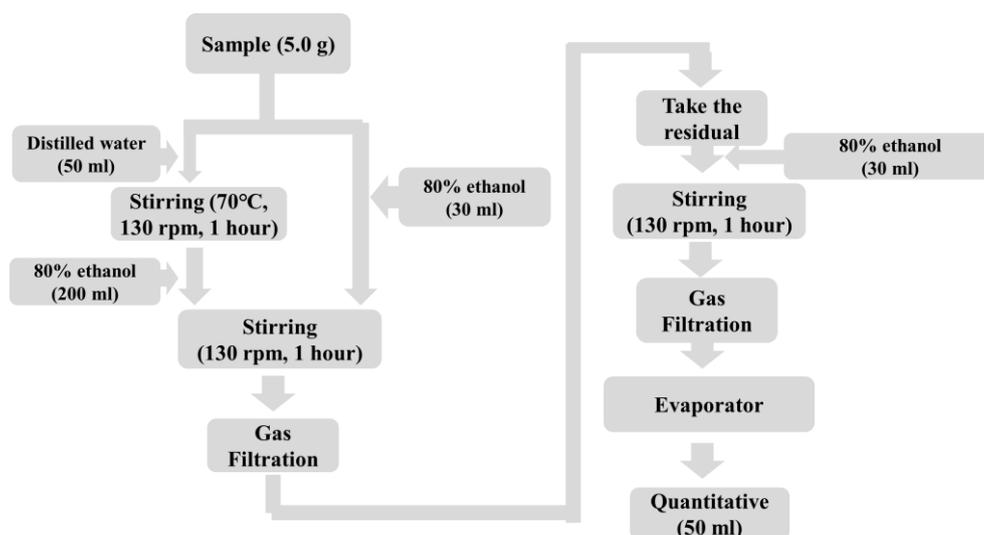


Fig.2. Preparation of sugar extracts

3. Results And Discussion

3.1. Morphological Observation Of Pearl Millet

The morphology of the aforementioned pearl millet, wheat flour, and rice flour was observed using a scanning electron microscope (Fig.3).

Comparing the particle shape and particle size observations, the average particle size tended to be largest in pearl millet powder, followed by wheat flour, and then rice flour. Particularly in pearl millet powder, a large variation in particle size was observed, with a mixture of particles ranging from relatively large to fine, exhibiting diverse sizes and shapes. In contrast, wheat flour and rice flour showed a relatively uniform particle size distribution compared to pearl millet powder, and their particle shapes also exhibited consistent tendencies. These results suggest that differences in grain species lead to variations in particle size distribution and particle shape within the powders. Furthermore, since



the specific grinding method used for the pearl millet powder in this study was not detailed, comparisons based on differences in grinding conditions were not performed. However, it is conceivable that differences in grinding methods could have introduced variability in the particle size distribution within the samples. Additionally, it cannot be ruled out that the powder inherently possesses the characteristic of containing a mixture of large and small particles, independent of grinding conditions. This study did not conduct detailed investigations into particle size distribution measurements or the effects of grinding conditions. Therefore, these points remain unclear at present and are considered topics for future research.

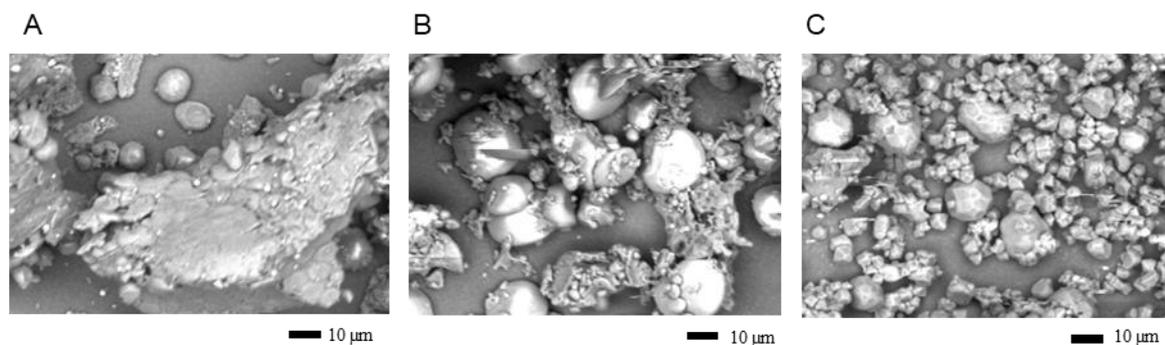


Fig.3. Scanning Electron Microscope Images of Pearl Millet, Wheat Flour, and Rice Flour
A: Pearl Millet, B: Wheat Flour, C: Rice Flour

3.2. Free Sugar Content

Fig.4 shows the glucose, fructose, maltose, and sucrose contents in pearl millet powder samples heated at different temperatures. Maltose content began increasing from the unheated (20°C) sample, peaked at 50°C, and decreased most significantly at 70°C. In contrast, based on the glucose production activity of pearl millet shown in Fig.4, the glucose content increased between 50°C and 70°C. Thus, the sugar composition clearly changed in stages depending on the heating temperature. This suggests that starch was broken down by α -amylase at 40°C, increasing maltose production. Furthermore, around 60°C, α -amylase activity subsided, and maltase or α -glucosidase, which break down maltose, likely became active. It is known that rice α -amylase II-4 exhibits high activity around 37°C, while α -amylase I shows high activity around 70°C. [6] In rice, the presence of α -glucosidase, which decomposes maltose and oligosaccharides to produce glucose, has been confirmed, with an optimal activity temperature around 60°C [7].

Next, examining the sucrose content shown in Fig.4, heating reduced it from 30 mg/100 g in the untreated sample decreased to 10 mg/100 g after heating. This decrease is consistent with previous studies indicating the presence of sucrose in unheated pearl millet [8]. In contrast, the fructose production activity of pearl millet, shown in Fig.4, indicated that fructose content increased from 40°C, peaking at 70°C, similar to glucose. Therefore, it is considered that sucrose decomposed it into glucose and fructose.

Comparing the behavior of monosaccharides and disaccharides, the temperature range where disaccharides like maltose and sucrose decreased coincided with the temperature range where glucose and fructose increased. This allowed simultaneous observation of the quantitative changes in each sugar type during the heating-induced decomposition process. While α -amylase and α -glucosidase, which break down starch and maltose, are similar to those in rice [9], sucrase, which is thought to be present in pearl millet in this study, is not commonly observed in other cereals or tubers.

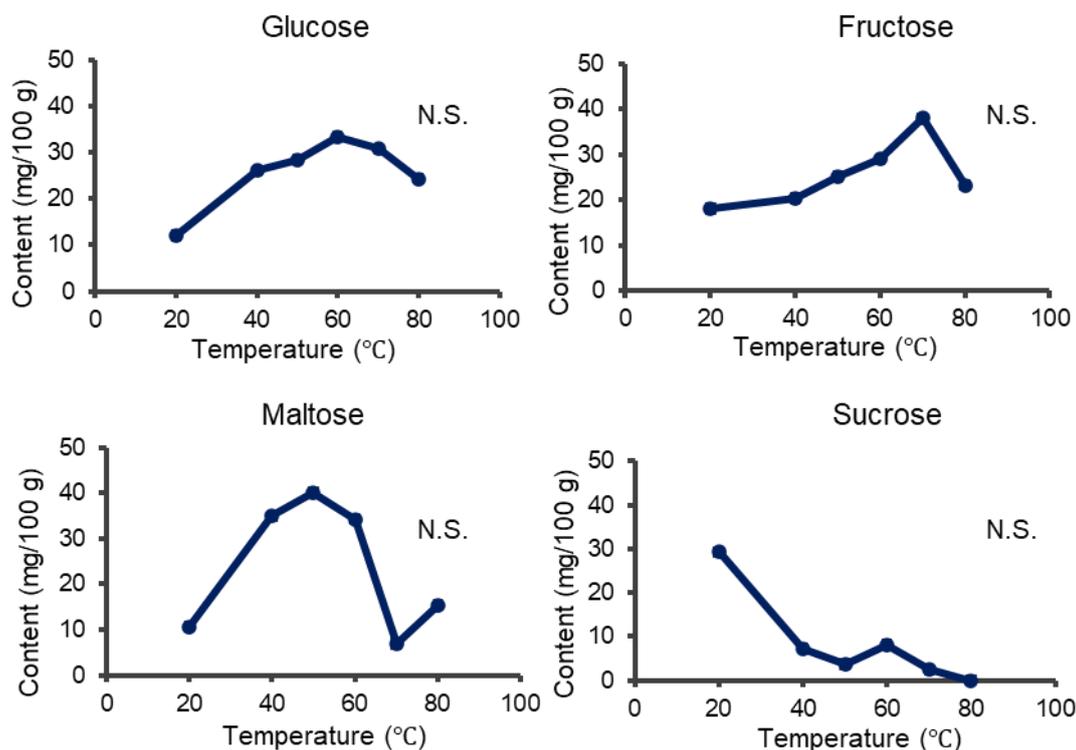


Fig.4. Free sugar content

4. Summary

This study aimed to clarify changes in free sugar content in pearl millet powder under different heating conditions. We performed histological observation using scanning electron microscopy and sugar quantification via enzymatic methods.

Histological observation confirmed that pearl millet powder has a larger average particle size compared to wheat flour and rice flour and contains a mixture of particles of various shapes and sizes. Free sugar content measurements showed that sucrose was the main component detected in unheated samples. However, as the heating temperature increased, sucrose decreased while glucose and fructose tended to increase. Particularly significant changes were observed in the 60–70°C temperature range, suggesting the involvement of sucrase. Furthermore, maltose was detected in higher amounts at 50°C and decreased at 70°C, indicating that starch was likely broken down into maltose by α -amylase. At 70°C, the maltose production reaction ceased, and the reaction converting maltose to glucose became strongly active. Consequently, maltose decreased and glucose increased between 60°C and 70°C, suggesting the action of maltase or α -glucosidase. These results provide important insights that extend beyond mere evaluation of food properties, contributing to the understanding of sustainable food resources and the advancement of science education addressing environmental issues. Pearl millet is a crop capable of growing in arid regions and high-temperature environments. In today's society facing advancing climate change, it is gaining attention as a potential future staple grain substitute. Studying such grains holds educational value, as it allows for a scientific explanation of “why this crop is necessary” from the perspectives of food supply stability and environmental adaptability. Furthermore, the increase and decrease in free sugar content accompanying temperature changes visually demonstrate enzyme temperature dependence and the mechanisms of material transformation through quantitative data. This expands the potential for developing teaching materials that enable students to understand abstract concepts like enzyme reactions and decomposition/synthesis based on experimental results. Therefore, as interdisciplinary research bridging food science, environmental science, and science education, it provides foundational insights that will contribute to future educational material development and the advancement of STEM education.



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