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Comparison of Proteolytic Activity between Commercial and Homemade Shio-koji

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Abstract

Shio-koji is a fermented seasoning containing enzymes derived from rice koji and is particularly known for its protease-mediated protein degradation activity. In this study, three commercially available shio-koji products from different manufacturers and a homemade shio-koji were examined to compare the effects of differences in enzyme activity on food texture, as well as to evaluate their potential as teaching materials for science education. First, as basic information, the acidic protease activity, pH, and salt concentration of each shio-koji were measured, and differences in enzyme activity among the products were confirmed. Next, as a preliminary model experiment prior to using meat samples, protein degradation was observed using gelatin jelly, and differences in the progression of dissolution under varying temperature conditions were visually confirmed. Subsequently, texture measurements were conducted using pork samples treated with shio-koji and untreated samples. The results indicated that differences in meat hardness could occur depending on the presence or absence of shio-koji treatment, the type of shio-koji used, and the immersion temperature conditions. In addition, weight loss rates were calculated, and the relationship between the release of water and lipids during heating and meat texture was examined. Furthermore, measurements of internal meat temperature during heating revealed differences in the temperature increase process and final temperatures among the experimental conditions, providing supporting evidence for the weight loss rate results. Overall, these findings suggest that a stepwise experimental approach using shio-koji is effective as an educational material that promotes inquiry-based learning by helping students understand enzyme activity and the importance of controlling experimental conditions through the use of familiar fermented foods.

Keywords: *Shio-koji, Fermented food, Enzymatic activity, Inquiry-based learning*

1. Introduction

Fermented foods are deeply rooted in Japanese food culture and are commonly used in everyday home cooking. Shio-koji is a traditional Japanese fermented seasoning produced by fermenting rice koji with salt and water, and it is known to contain various enzymes such as amylases and proteases. In particular, proteases degrade proteins and are believed to tenderize meat and fish; therefore, shio-koji has been used empirically as a seasoning for pre-treatment in cooking. Examining such effects, which are based on everyday culinary experience, through scientific methods and clarifying their mechanisms is important from the perspective of life science education, as it connects daily life with scientific understanding.

On the other hand, commercially available shio-koji products often lack clear information regarding their manufacturing processes or whether heat treatment has been applied, and thus differences in enzyme activity among products may exist [1]. Although heat treatment is expected to cause enzyme inactivation, few studies have compared these differences among commercial products. Therefore, in this study, homemade shio-koji produced without heat treatment was used alongside commercial products, and its properties under non-heated conditions were employed as a reference for evaluation.

In Japanese high school home economics education, practical lessons using fruits such as pineapple, which contain proteolytic enzymes, are introduced through the preparation of gelatin jelly. However, because the purpose of these lessons is jelly preparation, the fruits are heat-treated, resulting in enzyme inactivation [2]. In this context, we considered that applying shio-koji, which also contains proteases, to gelatin jelly could allow students to visually observe protein degradation and thus serve as an effective teaching material in science education. Learning activities that begin with familiar food materials and cooking experiences and involve control of experimental conditions as well as organization and interpretation of data are also meaningful from the perspective of STEM education.

In this study, three commercially available shio-koji products from different manufacturers, homemade shio-koji, and untreated samples were used to examine stepwise the effects of differences in enzyme activity on food texture. After measuring acidic protease activity, pH, and salt concentration as basic parameters, a model experiment using gelatin jelly was conducted. Subsequently, pork samples were used to measure breaking strength and calculate weight loss rates, and the relationships between these parameters and internal meat temperature during heating were also examined.

2. Materials and Methods

2.1 Shio-koji Samples

The shio-koji samples used in this study consisted of three commercially available products manufactured by different companies, which were designated as B, C, and D. In addition, a homemade shio-koji was prepared and designated as E.

The homemade shio-koji was prepared using 170 g of rice koji (Koujiya Satomura), 60 g of salt (Hakatanoshio), and 230 mL of water (Suntory Iga Natural Water). These ingredients were thoroughly mixed until homogeneous in a new container and then transferred to an airtight container. After preparation, the mixture was fermented for seven days in an incubator set at 25°C, during which it was stirred once daily to ensure uniformity. Following fermentation, the sample was stored at 4°C and used as the homemade shio-koji sample (E) in subsequent experiments

2.2 pH, Salt Concentration, and Acidic Protease Activity

The pH of shio-koji samples B, C, D, and E was measured using a pH meter (ATAGO PAL-pH). The salt concentration of the commercial shio-koji samples was calculated based on the values indicated on the product labels, while that of the homemade shio-koji was calculated from the proportions of the raw materials used.

Acidic protease activity was measured as follows: 10 g of shio-koji was mixed with 50 mL of 10 mM acetate buffer containing 0.5% NaCl and incubated at 4°C overnight. The mixture was then filtered, and the filtrate was diluted twofold with distilled water. The acidic protease activity of the diluted sample was determined using an acidic protease activity assay kit (Peptide Institute Inc.) according to the manufacturer's instructions. Enzyme activity was expressed as units per gram of shio-koji (U/g koji). One unit (U) was defined as the amount of enzyme that catalyzes the degradation of 1 μmol of substrate per minute under the specified conditions.

2.3 Gelatin Jelly Dissolution Observation

Shio-koji samples B, C, D, and E, powdered gelatin (Morinaga Cook Gelatin), water, and a powdered red food coloring (beni-koji pigment) were used.

Gelatin jelly was prepared at a gelatin concentration of 2% (w/v). Water was heated in a pot, and powdered gelatin and the food coloring were added and stirred until completely dissolved. The solution was poured into cups, allowed to set, and cooled at 4°C to solidify the jelly. After solidification, 7 g of shio-koji was evenly spread on the surface of each gelatin jelly. The samples were then stored at either 25°C or 4°C. The degree of surface dissolution of the gelatin jelly was visually observed at 30-minute intervals for up to 120-minute.

2.4 Pork Meat Texture Measurement

Pork loin was used as the meat sample. After removing visible fat, the meat was cut into rectangular blocks measuring 2 cm × 2 cm × 1.5 cm. Shio-koji samples, including three commercially available products (B, C, and D) and a homemade product (E), were evenly applied to the entire surface of the meat. Samples without shio-koji treatment were used as untreated controls (A).

After shio-koji application, the samples were stored at either 25°C or 4°C for up to 120-minute. The samples were then heated in an oven preheated to 180°C for 8 minutes. After heating, the samples were allowed to stand at room temperature before texture measurement.

Fracture force was measured using a creep meter (Yamaden, RE-33005C). Measurements were performed in the vertical direction at a test speed of 1 mm/s, with a strain rate of 80% and a maximum load of 200 N, using a wedge-shaped plunger with a width of 30 mm.

2.5 Weight Loss Rate

Pork samples were prepared and treated with shio-koji in the same manner as described in Section 4. The weight of each sample was measured before and after heating. The weight loss rate was calculated by dividing the decrease in weight caused by heating by the initial weight of the meat sample and expressing the value as a percentage.

2.6 Changes in Core Temperature during Heating

Untreated pork samples (A) were used for this measurement. Samples that had been kept at 25°C for 30 min and those kept at 4°C were prepared. A small incision was made at the center of each sample using scissors, and a temperature sensor was inserted into the incision. Internal temperature was measured using a compact thermologger (Anritsu Keiki, AM-8000K) equipped with a temperature probe (Anritsu Keiki, ST-23K-050-GW1-ANP). The samples were heated in an oven preheated to 180°C for 8 min, and changes in internal temperature were continuously recorded during heating.

3. Results and Discussion

3.1 pH, Salt Concentration, and Acidic Protease Activity

Table 1. pH, Salt Concentration, and Acidic Protease Activity of Shio-koji Samples

	pH	Salt concentration (%)	Acidic protease activity (U/g koji)
B	5.84	13.0	1026.28^{b}
C	5.21	10.6	748.79^{a}
D	4.79	9.2	1427.16^{c}
E	4.46	13.0	1516.85^{c}

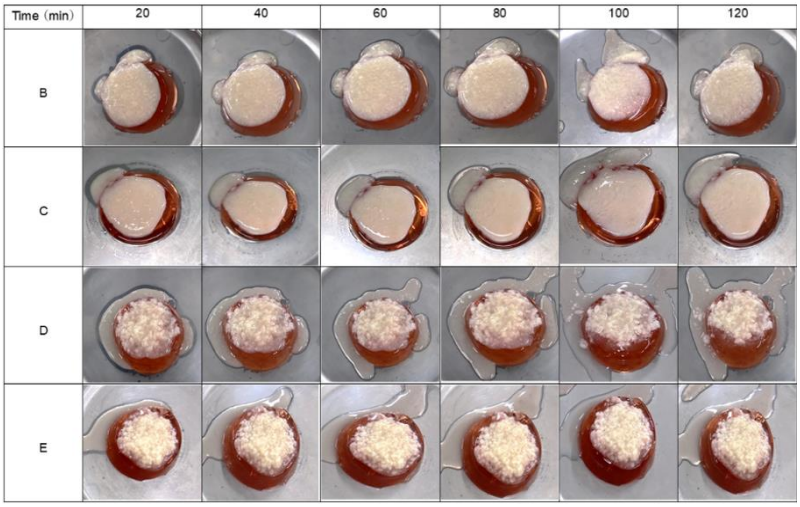
Values with different letters indicate a significant differences ($p < 0.05$).

Table 1 shows the pH, salt concentration, and acidic protease activity of each shio-koji sample. Acidic protease activity was detected in all shio-koji samples. Among the commercially available products, sample C exhibited the lowest acidic protease activity, which was significantly lower than that of the other samples. Sample B showed higher activity than sample C; however, its activity was significantly lower than that of samples D and E. In contrast, samples D and E exhibited the highest acidic protease activity, and no significant difference was observed between these two samples.
Regarding salt concentration, it has been suggested that enzyme activity may be inhibited under high-salt conditions. However, in this study, sample E, which had a salt concentration of 13%, exhibited high acidic protease activity. This result suggests that acidic protease activity may not be markedly suppressed at salt concentrations of approximately 13%. In addition, because only acidic protease activity was evaluated in this study, it is possible that samples C and B may possess relatively higher activities of neutral or alkaline proteases.
The pH values of the shio-koji samples ranged from 4.46 to 5.84, and the salt concentrations ranged from 9.2% to 13.0%. These values were within the typical ranges for shio-koji [3]. The pH of shio-koji is weakly acidic, which provides favorable conditions for the growth of molds and yeasts involved in fermentation, while simultaneously inhibiting the growth of many bacteria and actinomycetes. Such pH conditions are known to selectively regulate the microbial community associated with shio-koji, thereby contributing to stable fermentation processes and ensuring product safety.

3.2 Gelatin Jelly Dissolution Observation

Table 2. Appearance of gelatin jelly coated with various shio-koji samples at 25°C

Table 3. Appearance of gelatin jelly coated with various shio-koji samples at 4°C



Tables 2 and 3 show the changes in the appearance of gelatin jelly coated with shio-koji under 25 °C and 4 °C conditions. Under the 25 °C condition, collapse and dissolution of the jelly surface were observed over time, and differences were noted in both the onset and progression of dissolution depending on the type of shio-koji used.For sample D, gelatin dissolution was observed only at 20 min, indicating the earliest onset among the samples; however, subsequent progression was relatively slow, and with time its degree of dissolution was exceeded by the other samples. In contrast, sample E showed a rapid progression of dissolution between 80 and 100 min, and at 120 min the degree of dissolution followed the order B, C, E, and D, from greatest to least.

In contrast, under the 4 °C condition, no marked collapse or dissolution of the jelly surface was observed for any of the shio-koji samples. These results indicate that the rate of gelatin degradation differed depending on the type of shio-koji. In addition, the level of acidic protease activity did not necessarily correspond to the degree of dissolution of the gelatin jelly, suggesting the involvement of enzymes other than acidic protease.Furthermore, comparison of the grain texture of the shio-koji samples showed that samples B and C had smaller grains, whereas samples D and E had larger grains. Because the samples that exhibited faster dissolution of the gelatin jelly tended to have smaller grains, the differences in dissolution behavior of the gelatin jelly may have been influenced by variations in grain texture among the shio-koji samples.

Moreover, degradation progressed under the 25 °C condition but showed little progression under the 4 °C condition, reconfirming that gelatin degradation is a temperature-dependent enzymatic reaction. In addition, differences in dissolution morphology may also be associated with variations in grain texture among the shio-koji samples.

3.3 Pork Meat Texture Measurement

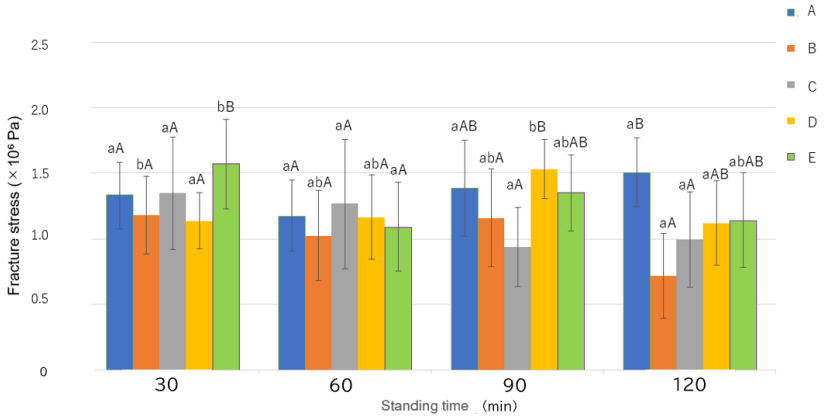


Fig. 1. Fracture stress of roasted pork treated with shio-koji at 25 °C

[a,b] Different small letters indicate significant differences between standing times within the same sample ($p < 0.05$).
[A,B] Different uppercase letters indicate significant differences between samples at same standing time ($p < 0.05$).

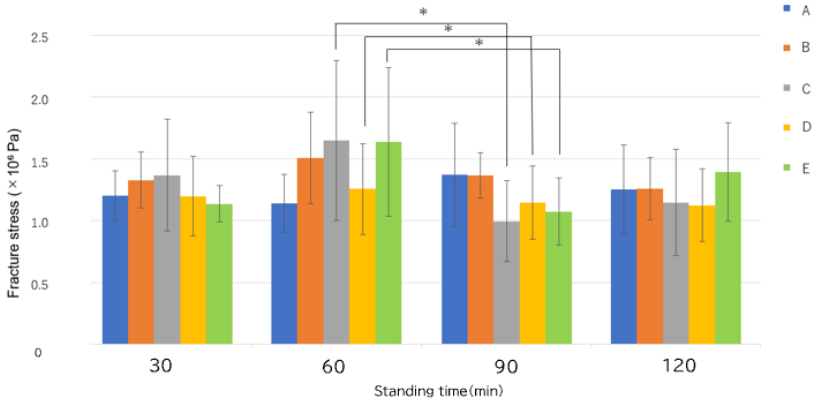


Fig. 2. Fracture stress of roasted pork treated with shio-koji at 4 °C
* Statistically significant differences ($p < 0.05$).

Figure 1 shows the time course of hracture stress after cooking pork treated with shio-koji at 25 °C. In the untreated sample (A), hracture stress tended to increase with longer standing time. In contrast, in the shio-koji–treated samples (B–E), hracture stress after 120 min of standing was lower than that of the untreated sample, indicating that shio-koji treatment resulted in meat tenderization. In addition, differences in hracture stress were observed among the different types of shio-koji even at the same standing time. Sample B showed lower hracture stress than the untreated sample at all standing times, indicating a continuous tenderizing effect. Furthermore, the order of meat tenderness after 120min of standing at 25 °C was consistent with the order of gelatin jelly dissolution observed after 120 min at 25 °C described in the previous section.

Figure 2 shows the time-dependent changes in fracture stress of pork after cooking following the application of shio-koji at 4 °C. Under the 4 °C condition, overall changes in fracture stress were smaller than those observed at 25 °C. In samples C, D, and E, a decrease in fracture stress was observed after 60 min of standing; however, the fracture stress values at 30 min and 120 min were almost identical, and no consistent change in hardness with increasing standing time was observed. Thus, the softening observed at 60min and the temporary increase in fracture stress at 90 min under low-
temperature conditions were considered to be transient fluctuations rather than representative of an overall trend. In addition, no significant differences among shio-koji types were observed at the same standing time.

At 25 °C, the decrease in fracture stress observed in shio-koji–treated samples suggests that proteases in shio-koji were active under room-temperature conditions and contributed to meat tenderization through the degradation of myofibrillar and connective tissue proteins [4]. In contrast, the increase in fracture stress observed in untreated samples was presumably due to moisture evaporation from the surface and protein contraction, resulting in progressive hardening. These results indicate that the presence or absence of shio-koji treatment markedly affected changes in meat texture. Moreover, the trend in fracture stress after 120 min of standing at 25 °C was consistent with the results of the gelatin gel model experiment, indicating that protease-mediated protein degradation was the dominant factor in both tests.

In contrast, under the 4 °C condition, no pronounced decrease in fracture stress was observed, confirming that protease activity in shio-koji is highly temperature-dependent and that its tenderizing effect on meat is limited under low-temperature conditions.

3.4 Weight Loss Rate

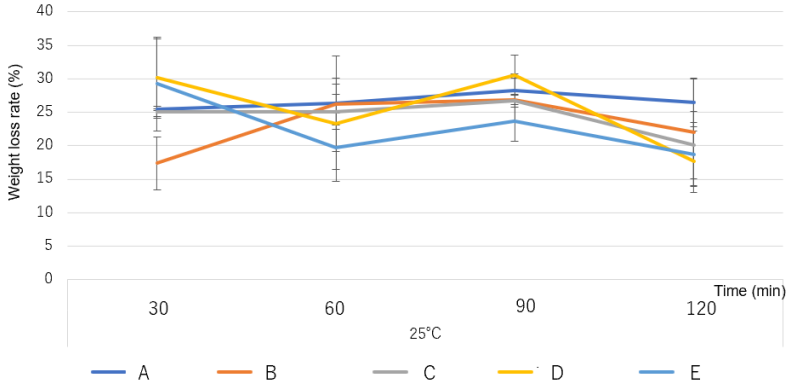


Fig. 3. Weight loss rate of pork samples treated with various shio-koji before and after cooking at 25 °C

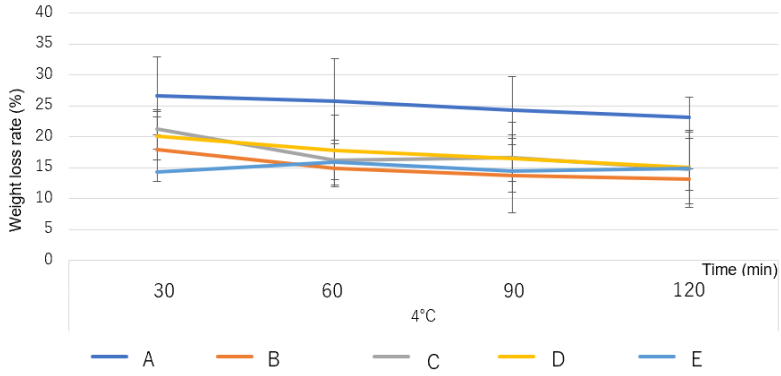


Fig. 4. Weight loss rate of pork samples treated with various shio-koji before and after cooking at 4 °C

Figure 3 shows the weight loss rate of pork samples held at 25 °C and then cooked at 180 °C for 8 min. Under the 25 °C condition, significant differences in weight loss rate were observed. After 120 min, significant differences were found between sample A and samples D and E, with the untreated sample exhibiting significantly greater hardness.

Figure 4 shows the post-cooking weight loss rate of pork samples held at 4 °C. Under the 4 °C condition, the weight loss rate ranged approximately from 15% to 25%, which was overall lower than that observed under the 25 °C condition. After 120 min, significant differences were observed between the untreated sample (A) and the shio-koji–treated samples, with the untreated sample showing significantly greater hardness.

Because the weight loss rate under the 25 °C condition did not change monotonically with increasing standing time, it is suggested that changes in weight during cooking are influenced not only by moisture evaporation but also by interactions between shio-koji components and muscle proteins. In particular, the tendency for the weight loss rate to decrease after 120 min of standing suggests that

sufficient penetration of salt and enzymatic components may have altered and stabilized the structure of myofibrillar proteins, thereby improving water-holding capacity during heating. In other words, during the process of tissue hardening, water-holding capacity may not have been fully expressed, resulting in increased weight loss.

In contrast, under the 4 °C condition, the overall reduction in weight loss rate compared with the 25 °C condition can be attributed to suppressed enzymatic activity and limited moisture migration within the muscle under low-temperature conditions. In shio-koji–treated samples, penetration of salt and components such as amino acids may have stabilized the myofibrillar protein structure, leading to improved water-holding capacity during cooking. The highest weight loss rate observed in untreated samples indicates that, even under low-temperature conditions, the absence of pretreatment results in poor water-holding capacity. In the present shio-koji treatment, in addition to salt addition, pH may have been altered due to the production of organic acids and amino acids[5]. However, previous studies have reported that salt concentration exerts a more dominant effect on meat water-holding capacity than pH[6]. In light of this, the low weight loss rates observed in the high-salt samples B and E after 120 min of standing under the 4 °C condition are considered to strongly reflect the effect of salt. Moreover, although sample B exhibited a pH close to the isoelectric point, it still showed a low weight loss rate, which is consistent with findings from previous studies.

3.5 Changes in Core Temperature During Heating

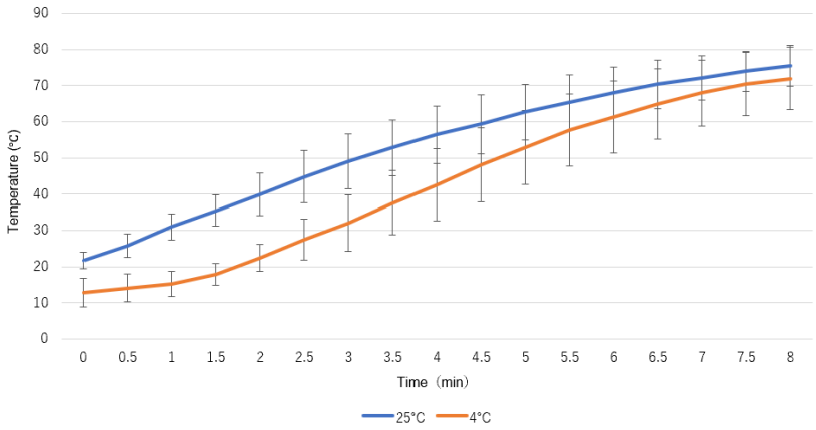


Fig. 5. Changes in the core temperature of pork during heating under 25 °C and 4 °C conditions

Based on the changes in internal temperature shown in Fig. 5, samples held at 4 °C exhibited a more gradual temperature increase during the initial stage of heating and reached a lower final internal temperature compared with those held at 25 °C. This result is consistent with the trend of lower weight loss rates observed under the 4 °C condition (Fig. 4).

In general, the water-holding capacity of meat decreases with increasing heating temperature; thermal denaturation of proteins begins at approximately 40–50 °C, and collagen contraction occurs around 65 °C [4]. In the present study, samples held at 4 °C passed through these temperature ranges more rapidly than those held at 25 °C, and the attainment of higher temperature ranges was further suppressed. As a result, thermal denaturation and contraction of myofibrillar proteins were relatively reduced, limiting moisture exudation and potentially leading to the observed decrease in weight loss rate.

4. Conclusions

In this study, three commercially available shio-koji products, a homemade shio-koji, and an untreated control were used to systematically examine how differences in protease activity among shio-koji samples affect protein degradation behavior and changes in food texture. The results revealed that the type of shio-koji influenced the breaking strength and weight loss rate of both meat and gelatin jelly, demonstrating that each sample exhibited distinct characteristics in texture modification.

Furthermore, based on the breaking strength of pork and the dissolution behavior of gelatin jelly, the most effective condition for achieving softening during cooking was identified as standing samples B and C at 25 °C for 120 min. However, when performing such standing treatments, sufficient consideration should be given to food hygiene.

These findings indicate that differences in protease activity are not directly or uniformly reflected in changes in food texture. Rather, texture modification is a complex phenomenon influenced by multiple interacting factors, including salt concentration, reaction temperature, and standing time. In addition, differences in standing temperature affected enzyme reaction rates, moisture migration, and heat transfer behavior during cooking, thereby influencing myofibrillar protein denaturation, water-holding capacity, and ultimately the final texture and weight loss rate of the food. Furthermore, the results of the gelatin jelly model experiment were generally consistent with the softening tendencies observed in pork samples, suggesting that even a simple model system can qualitatively capture trends in protein degradation occurring in real food matrices.

This study proposes an instructional model in which stepwise experimental activities utilizing familiar fermented foods enable learners to develop an integrated understanding of the relationship between microscopic enzymatic reactions and macroscopic changes in food properties. Furthermore, the experimental framework designed in this study can be structured as a learning sequence that systematically engages students in fundamental processes of scientific inquiry—namely hypothesis formulation, control of variables, observation and measurement, and data interpretation—within contexts grounded in everyday life. Collectively, these findings underscore the practical relevance of this approach to science education, as it deepens conceptual understanding of enzyme action through explicit connections to observable phenomena and contributes to the cultivation of scientific reasoning skills.

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