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Fermentation characteristics of yeasts isolated from apple and kiwi

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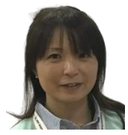

Abstract

This study investigated the fermentation capacity of two natural yeast strains, *Metschnikowia pulcherrima* and *Candida apicola*, isolated from apples and fermented kiwi syrup, respectively. Natural yeasts are increasingly valued for imparting complexity, unique aroma, and distinctive flavor to alcoholic beverages and baked goods. However, their fermentation performance is often weaker and less stable than that of commercial dry yeast. To evaluate optimal conditions, each strain was cultured on agar and inoculated into liquid media containing yeast extract, peptone, and glucose. Bread dough was then fermented with each strain under varying temperatures, and sugar types and concentrations. Fermentation efficiency was assessed by measuring dough height. Statistical analysis was performed using *t*-tests and one-way ANOVA on triplicate data following Fisher's three principles. Results showed that *M. pulcherrima* performed best at 25 °C with 5.0% glucose, while *C. apicola* showed optimal fermentation at 35 °C with 5.0% sucrose. These findings indicate that tailoring fermentation conditions to specific natural yeast strains can significantly enhance the efficiency of alcohol and bread production, offering a promising alternative to commercial yeast in both artisanal and industrial applications.

Keywords: Bread production, Candida apicola, Fermentation temperature, Fermentation, *Metschnikowia pulcherrima*, Sugar type.

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Contents

Contribution of this paper to the literature
This study contributes to existing literature by investigating the fermentation characteristics of two natural yeasts. It provides new insights into the effects of temperature, sugar type, and sugar concentration on the fermentation capacity of these yeasts, thereby deepening our understanding of bread making and sake brewing.

1. Introduction

Alcohol production is primarily carried out through fermentation using *Saccharomyces cerevisiae*, although other natural yeasts are also commonly utilized. For instance, *Torulaspora delbrueckii* is used in beer production [1]. Additionally, *Metschnikowia pulcherrima* is applied in wine production [2], Schizosaccharomyces pombe in rum production, and *Kluyveromyces marxianus* in the fermentation of whey from cheese production to produce vodka and gin [3]. In recent years, bread production with natural yeast has garnered considerable interest [4]. Natural yeast is a type of fungus that thrives under diverse environmental conditions. Unlike bread made with commercial dry yeast, bread produced using natural yeast has a complex taste, as different yeasts impart unique aromas and flavors [5]. Currently, natural yeast bread made with strains isolated from dried grapes [6], sudachi petals, and fruits and flowers collected in Tokachi Region, Hokkaido, Japan, is commercially available [7]. Moreover, bread has been successfully produced using *Lachancea fermentati*, *Lachancea kluyveri*, and *Torulaspora* species isolated from rose cultivars grown in Fukuyama City, Hiroshima, Japan [8]. Saccharomyces rouxii, Saccharomyces bisporus, and Saccharomyces exigus have been isolated from grape juice, pineapple juice, and rice obtained from local markets in Savar, Bangladesh [9]. In bread production, yeast fermentation generates carbon dioxide, which causes wheat gluten to expand, and differences in yeast fermentation capacity directly affect bread quality. Commercial yeast has a strong fermentation capacity, resulting in a softer texture. In contrast, natural yeast is less stable, ferments slowly, and generally shows weaker activity [10]. Therefore, when producing bread with natural yeast, it is essential to adjust the sugar type and concentration as well as temperature to stabilize fermentation and improve efficiency. For example, bread can be successfully produced with *Hanseniaspora meyeri* when monosaccharides such as glucose and fructose are provided at fermentation temperatures of 25–28°C [11]. *Kluyveromyces delphensis* ferments dextrose and sucrose but not fructose or lactose, as demonstrated by sugar-specific fermentation tests [12]. Furthermore, *S. cerevisiae* has an optimum growth temperature of 25–30°C and a fermentation temperature of 45°C; it can utilize glucose, fructose, and sucrose but not in the presence of maltose and amylose [13]. Its ethanol production capacity is maximized at a sugar concentration of 125 g/L rather than 100 g/L. However, ethanol productivity decreases at concentrations above 150 g/L [14], suggesting that both sugar type and concentration substantially influence the fermentation efficiency of yeast. As different natural yeasts respond differently during fermentation, appropriate conditions should be selected for each yeast to ensure successful bread production. In this study, I focused on *Metschnikowia pulcherrima* [15], isolated from fermented apple syrup, and *Candida apicola* [16], isolated from fermented kiwi syrup. *Metschnikowia pulcherrima* has previously been isolated from the spontaneous fermentation of Tannat and Marselan grape musts in Concordia (Entre Rios, Argentina) [17], Cabernet Sauvignon wine in Ningxia, China [18], and Marastina wine [19]. *Candida apicola* has been isolated from naturally fermented Alorena green table olives [20], wine yeasts that ferment grapes with high sugar content [21], and cachaca (sugarcane wine) [22, 23]. Here, the effects of fermentation temperature as well as the sugar type and concentration were assessed to determine the optimal fermentation conditions required by each species in bread production. The results of this study may have potential applications in the production of alcoholic beverages.

2. Materials and Methods

Metschnikowia pulcherrima (NRBC0863) was purchased from the NITE Biological Resource Center, and *C. apicola* was isolated from kiwi syrup (accession number LC878464, DNA Data Bank of Japan). To prepare each yeast solution, yeast monocultured on agar medium was inoculated into a liquid medium containing 0.25% yeast extract, 0.50% peptone, and 0.10% glucose (for *M. pulcherrima*) or 5.0% glucose (for *C. apicola*). The inoculum was incubated at 30 °C for approximately 1 week. After incubation, the medium was removed using centrifugation (890 × *g*, 5 min, 25 °C), and the yeast was weighed. Subsequently, the yeast was suspended in a 1% glucose solution to obtain a yeast solution with a final concentration of 0.78%–3.9%.

2.1. Examination of the Optimal Fermentation Temperature for Each Yeast

Yeast solution (3.0 g) and strong flour (3.0 g; Tomizawa Shouten Inc., Tokyo, Japan) were mixed in a test tube (1.5 cm φ × 18 cm) and placed in a hot water bath adjusted to 20–35 °C in a low-temperature cooker (Anova; Axia International Co., Ltd., Tokyo, Japan). The increase in dough height was measured every hour for 5 hours and calculated as the increase in dough height per 0.1 g of yeast.

2.2. Investigation of the Optimal Sugar Type for Each Yeast

Yeast solution (3.0 g), strong flour (3.0 g), and sugar (0.15 g; glucose, sucrose, starch, fructose, and maltose) were mixed in a test tube (1.5 cm φ × 18 cm) and placed in a hot water bath at 25°C (*M. pulcherrima*) and 35°C (*C. apicola*) for 12 hours, and the fermentation capacity was calculated as the increase in dough height per 0.1 g of yeast.

2.3. Examination of the Effect of Sugar Concentration

Yeast solution (3.0 g), strong flour (3.0 g), and sugar (0.03–0.15 g) were mixed in a test tube (1.5 φ × 18 cm) and placed in a hot water bath at 25°C (*M. pulcherrima*) and 35°C (*C. apicola*) for 12 hours. The fermentation capacity was calculated as the increase in dough height per 0.1 g of yeast.

2.4. Statistical Analysis

Data were obtained in triplicate using the same sample based on Fisher's three principles. The mean differences between groups were assessed using *t*-tests, and those among three groups were assessed using a one-way analysis

of variance. All statistical analyses were performed using Microsoft Excel (Microsoft Corporation, Redmond, WA). The significance level was set at $p < 0.05$.

3. Results

3.1. Examination of the Optimal Fermentation Temperature for Each Yeast

Figure 1 shows the effect of different temperatures on *M. pulcherrima* fermentation. At 35°C, no change was observed in dough height after 1 hour; however, the dough height increased to 1.3 cm after 5 hours ($p < 0.05$). At 30°C, the height increased to 1.6 cm after 5 hours ($p < 0.05$). At 25°C, no change was observed in dough height after 2 hours; however, the dough height increased to 1.7 cm after 5 hours ($p < 0.05$). At 20°C, no change was observed in dough height after 1 hour, but the height slightly increased to 0.16 cm after 2 hours ($p > 0.05$). Thereafter, no changes were observed up to 5 hours.

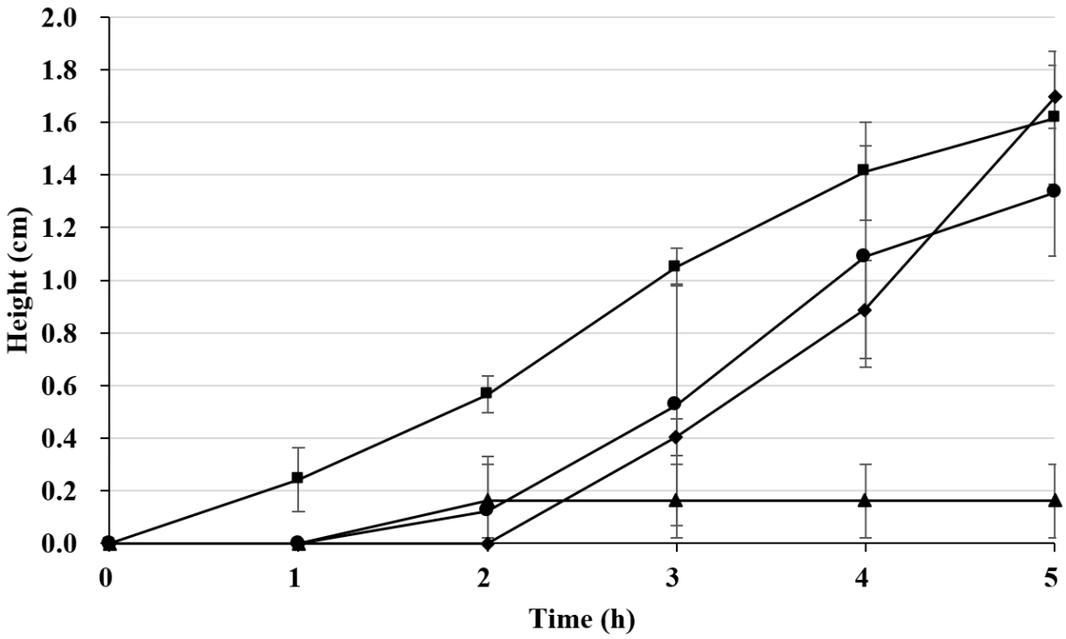

Figure 1. Effect of temperature on Metschnikowia pulcherrima fermentation. ● Indicates 35°C, ■ indicates 30°C, ◆ indicates 25°C, and ▲ indicates 20°C.

Note: The fermentation capacity was calculated as the increase in dough height per 0.10 g of yeast per hour; the results are shown as an integrated value. Measurements were obtained in triplicate. The error bars indicate the standard deviation.

Figure 2 shows the effect of temperature on *C. apicola* fermentation. At 35°C, no change was observed in dough height after 1 hour; however, the dough height increased to 2.0 cm after 5 hours ($p < 0.05$). At 30°C, the height increased to 1.9 cm after 5 hours ($p < 0.05$). At 25°C, no change was observed in dough height up to 4 hours, and the height slightly increased to 0.49 cm after 5 hours ($p > 0.05$). At 20°C, no change was observed up to 2 hours; nevertheless, the height slightly increased to 0.25 cm after 3 hours ($p > 0.05$) and remained unchanged for up to 5 hours ($p > 0.05$).

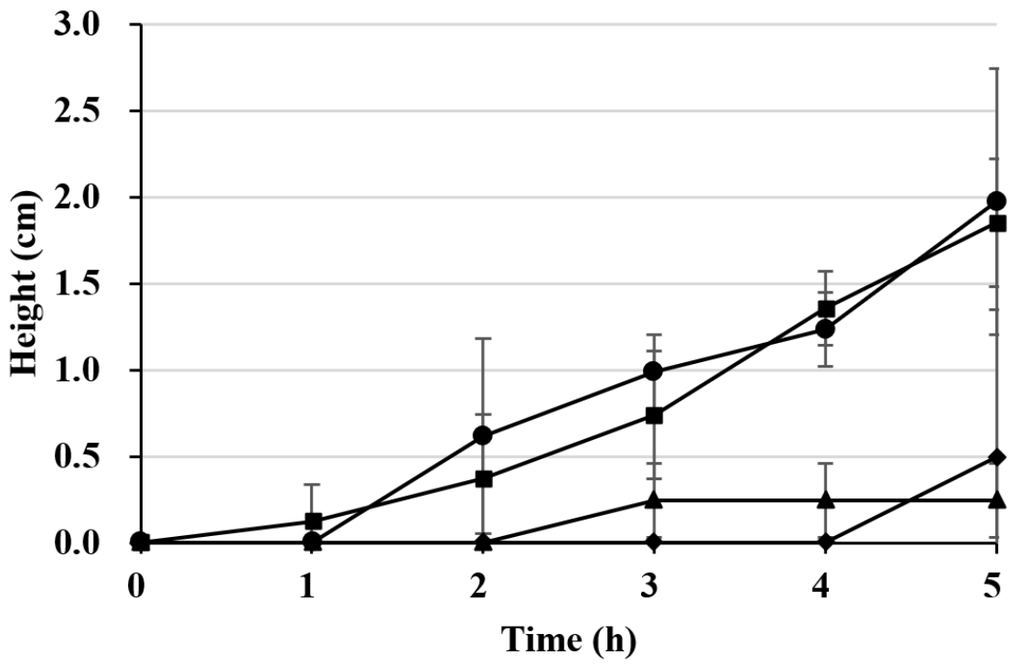

Figure 2. Effect of temperature on Candida apicola fermentation. ● Indicates 35 °C, ■ Indicates 30 °C, ◆ Indicates 25 °C, and ▲ Indicates 20 °C.

Note: The fermentation capacity was calculated as the increase in dough height per 0.10 g of yeast per hour; the results are shown as integrated value. Measurements were obtained in triplicate. The error bars indicate the standard deviation.

3.2. Investigation of the Optimal Sugar Type for Each Yeast

Figure 3 shows the effects of various sugars on *M. pulcherrima* fermentation. The addition of glucose increased the dough height to 8.1 cm after 12 h ($p < 0.05$). The addition of sucrose did not alter the dough height until 3 h, but it increased to 0.17 cm after 4 h ($p < 0.05$). Thereafter, no change was observed in dough height until 6 h ($p > 0.05$), followed by an increase to 0.34 cm after 7 h ($p < 0.05$). No further change was observed until 12 h ($p > 0.05$). With starch addition, the height increased to 3.2 cm after 8 h ($p < 0.05$), then slightly increased to 3.3 cm after 12 h ($p > 0.05$). With fructose addition, the height increased to 6.7 cm after 11 h ($p < 0.05$), but slightly decreased after 12 h ($p > 0.05$). With maltose addition, the height increased to 2.5 cm after 7 h ($p < 0.05$); however, no change was observed after 8 h ($p > 0.05$). A slight increase to 2.9 cm was observed at 9 h ($p > 0.05$). No further change was observed until 12 h.

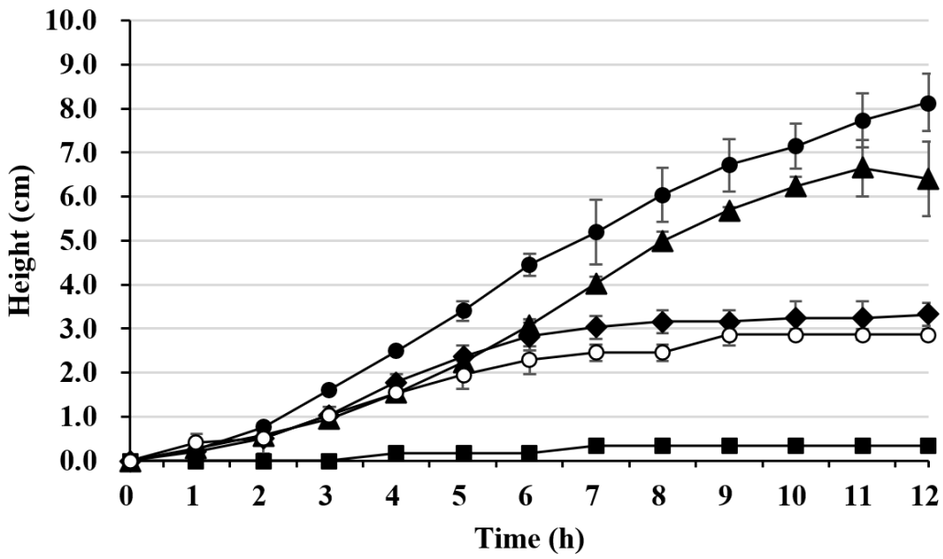

Figure 3. Effect of various sugars on Metschnikowia pulcherrima fermentation. ● Indicates glucose, ■ Indicates sucrose, ◆ Indicates starch, ▲ Indicates fructose, and ○ Indicates maltose.

Note: The fermentation capacity was calculated as the increase in dough height per 0.10 g of yeast per hour; the results are shown as integrated value. Measurements were obtained in triplicate. The error bars indicate the standard deviation.

Figure 4 shows the effects of various sugars on *C. apicola* fermentation. The addition of glucose increased the dough height to 4.9 cm after 10 h ($p < 0.05$); however, no significant change was observed after 11 h ($p > 0.05$), followed by a slight increase to 5.2 cm after 12 h ($p > 0.05$). Sucrose increased the height slightly to 1.6 cm after 2 h ($p > 0.05$); the height remained unchanged until 3 h ($p > 0.05$), but increased to 7.7 cm after 12 h ($p < 0.05$). With starch addition, the height increased to 0.25 cm after 2 h ($p < 0.05$), remained unchanged until 6 h ($p > 0.05$), and then increased to 0.81 cm after 8 h ($p < 0.05$). Thereafter, it remained unchanged until 11 h ($p > 0.05$) and increased to 1.3 cm after 12 h ($p < 0.05$). With fructose addition, no change was observed until 4 h ($p > 0.05$), and the height increased to 1.7 cm after 9 h ($p < 0.05$). Thereafter, no change was observed until 11 h ($p > 0.05$). Subsequently, the height increased to 2.2 cm after 12 h ($p < 0.05$). With maltose addition, the height increased to 0.50 cm after 2 h ($p < 0.05$), but did not change until 6 h ($p > 0.05$). Thereafter, it increased slightly to 1.2 cm after 9 h ($p > 0.05$), remained unchanged until 10 h ($p > 0.05$), and increased to 2.0 cm after 12 h ($p < 0.05$).

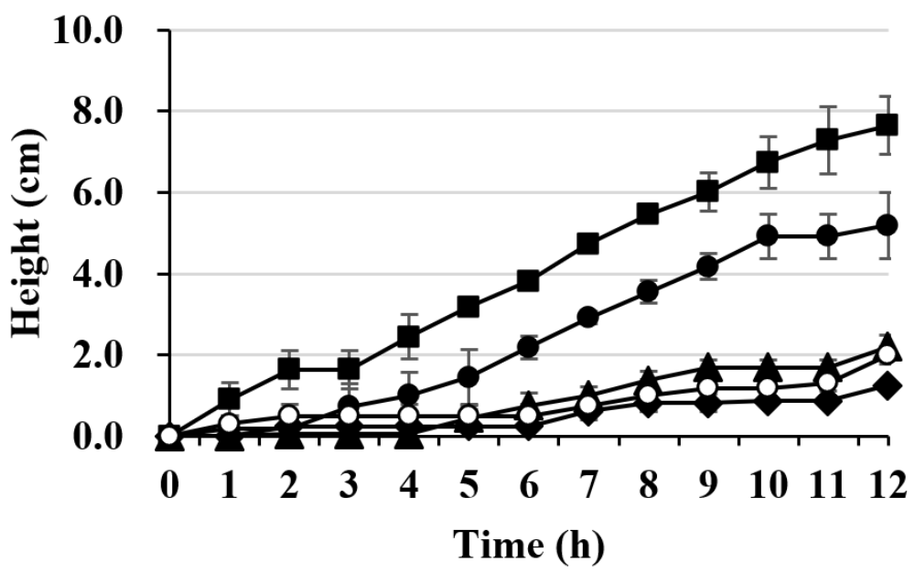

Figure 4. Effect of various sugars on Candida apicola fermentation. ● Indicates glucose, ■ Indicates sucrose, ◆ Indicates starch, ▲ Indicates fructose, and ○ Indicates maltose.

Note: The fermentation capacity is calculated as the increase in dough height per 0.10 g of yeast per hour; the results are shown as integrated value. Measurements were obtained in triplicate. The error bars indicate the standard deviation.

3.3. Examination of the Effect of Sugar Concentration

Figure 5 shows the effect of glucose concentration on *M. pulcherrima* fermentation. When 1.0% glucose was added, no change was observed in dough height after 1 hour, but the dough height increased to 6.9 cm after 11 hours ($p < 0.05$) and remained unchanged until 12 hours ($p > 0.05$). At a concentration of 2.0%, no change was observed in dough height after 1 hour; however, the height increased to 7.3 cm after 12 hours ($p < 0.05$). At a concentration of 3.0%, no change was observed after 1 hour ($p > 0.05$), but after 12 hours, the height increased to 8.7 cm ($p < 0.05$). At a concentration of 4.0%, the height slightly increased to 0.12 cm after 3 hours ($p > 0.05$), followed by an increase to 8.7 cm after 12 hours ($p < 0.05$). Following the addition of 5.0% glucose, the height increased to 10 cm after 12 hours ($p < 0.05$).

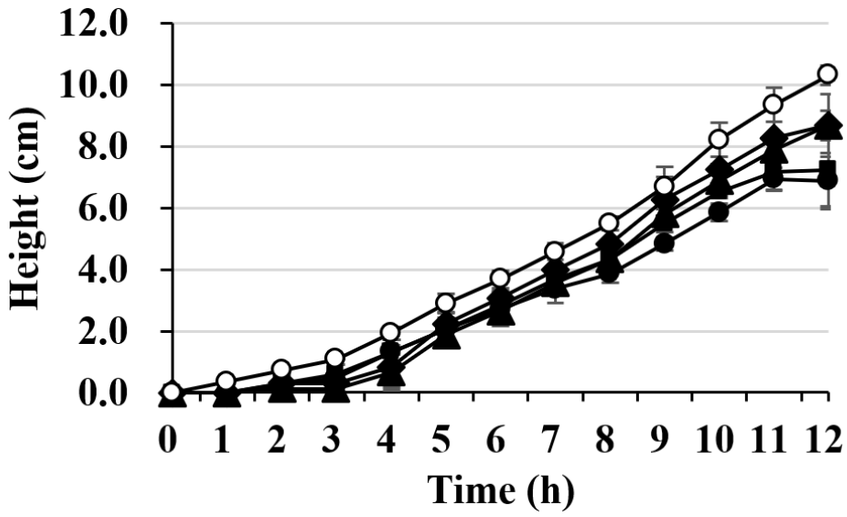

Figure 5. Effect of glucose concentration on Metschnikowia pulcherrima fermentation. ● Indicates 1.0%, ■ Indicates 2.0%, ◆ Indicates 3.0%, ▲ Indicates 4.0%, and ○ Indicates 5.0%.

Note: The fermentation capacity was calculated as the increase in dough height per 0.10 g of yeast per hour; the results are shown as integrated value. Measurements were obtained in triplicate. The error bars indicate the standard deviation.

Figure 6 shows the effect of sugar concentration on *C. apicola* fermentation. When sucrose was added at a concentration of 1.0%, no change in dough height was observed after 12 hours. Furthermore, at 2.0%, no change was observed in dough height up to 2 hours, but it increased slightly to 0.57 cm after 3 hours ($p > 0.05$) and remained unchanged after 12 hours ($p < 0.05$). At 3.0%, no change was observed after 1 hour, but the height increased slightly to 0.71 cm after 3 hours ($p > 0.05$) and remained unchanged until 12 hours ($p > 0.05$). Similarly, no change was observed in dough height at 4.0% until 1 hour; however, after an increase in dough height to 0.71 cm after 2 hours ($p < 0.05$), no further change was observed until 4 hours ($p > 0.05$). After 10 hours, the height increased to 6.0 cm ($p < 0.05$), and no change was observed after 12 hours ($p > 0.05$). At 5.0%, the height increased to 3.3 cm after 3 hours ($p < 0.05$), but no change was observed after 4 hours ($p > 0.05$). Thereafter, it increased to 11 cm after 12 hours ($p < 0.05$).

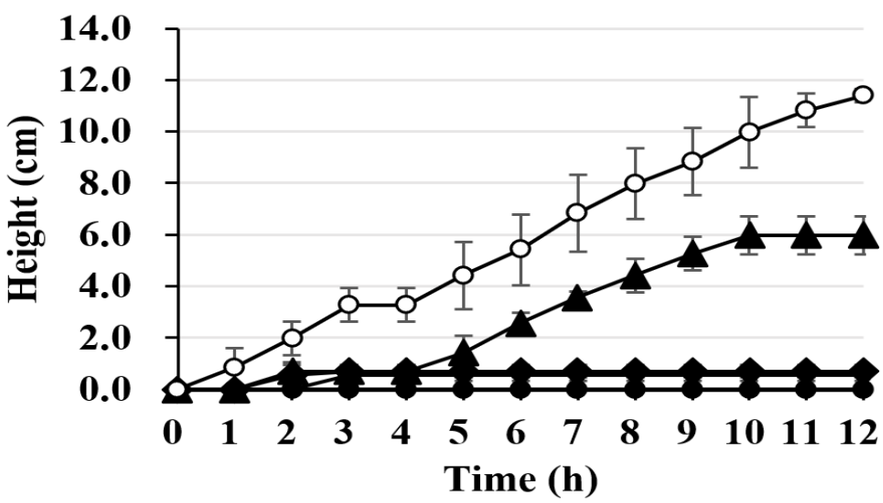

Figure 6. Effect of sucrose concentration on Candida apicola fermentation. ● Indicates 1.0%, ■ Indicates 2.0%, ◆ Indicates 3.0%, ▲ Indicates 4.0%, and ○ Indicates 5.0%.

Note: The fermentation capacity was calculated as the increase in dough height per 0.10 g of yeast per hour; the results are shown as integrated value. Measurements were obtained in triplicate. The error bars indicate the standard deviation.

4. Discussion

4.1. Examination of the Optimal Fermentation Temperature for Each Yeast

In this study, the optimal temperature, sugar type, and concentration required for fermentation using *M. pulcherrima* and *C. apicola* as natural yeasts in bread production were investigated. After 5 hours of fermentation, *M. pulcherrima* and *C. apicola* showed high fermentation capacities at 25°C and 35°C, respectively; however, after 5 hours, they exhibited relatively high fermentation capacities at 30°C and 35°C, respectively. *Metschnikowia pulcherrima*

grows efficiently at temperatures in the range of 15–20°C [2]; whereas, *C. apicola* grows efficiently at 25–37 °C [12], indicating that it does not grow well in low-temperature environments. *Metschnikowia pulcherrima* has been isolated as a dominant fermentation species during the fermentation of Fiano di Avellino grapes at 9 °C in wine production [24], suggesting that it can survive at low temperatures and show high fermentation potential. In addition, *C. apicola* can survive at low temperatures while marginally maintaining its fermentation potential. Detailed reports on the optimal fermentation temperature of *C. apicola* are lacking. As a species similar to *C. apicola*, *C. tropicalis* has been reported to be heat-resistant and capable of producing ethanol from xylose [25]. *Candida* species may have a high fermentation capacity at relatively high temperatures, suggesting that it is difficult for *C. apicola* to ferment substrates at lower temperatures.

4.2. Investigation of the Optimal Sugar Type for Each Yeast

In this study, *M. pulcherrima* and *C. apicola* showed high fermentation capacities following the addition of glucose and sucrose, respectively. Glucose, a monosaccharide, is used by yeast in ethanol fermentation. In a previous study, 31 types of *S. cerevisiae* isolates from palm wine were provided with glucose, galactose, fructose, sucrose, maltose, trehalose, and raffinose, either in combination or individually, to ferment a substrate, and glucose was used for fermentation in all tests [26]. Among the aforementioned sugars, glucose, galactose, and fructose are monosaccharides; sucrose, maltose, and trehalose are disaccharides; and raffinose is the only trisaccharide. As these disaccharides and the trisaccharide contain glucose, they can be fermented if the yeast produces enzymes that can break them down into glucose. Reportedly, the strength of sucrase activity varies among different species of *S. cerevisiae* [27]. Hence, the type of sugar available and the fermentation rate may differ among different yeast species. In this study, the fermentation capacity of *M. pulcherrima* for sucrose was weaker than that for maltose and starch (Figure 3). *Metschnikowia pulcherrima* uses glucose and not sucrose in the fermentation of soy whey and produces ethanol as a product [28-30], consistent with the findings of the present study, suggesting that *M. pulcherrima* does not produce sucrase or possesses weak sucrase activity. Alcohol fermentation has been reported to be slow with maltose [31], and as starch is maltose-bound, *M. pulcherrima* may have weak maltase and amylase activities. *Candida apicola* has weak maltase or amylase activity (Figure 4) and shows high fermentation efficiency for sucrose. In fermentation capacity tests by sugar type, *C. apicola* was reportedly able to ferment sucrose but not fructose [12]. Similar results were obtained in this study. Hence, *C. apicola* has high sucrase activity; however, it was also able to ferment the dough with fructose in this study. The glucose and fructose produced from sucrose through sucrase were used for fermentation. However, the slow onset of fermentation of glucose and fructose suggests that sucrose may promote fermentation by *C. apicola*. In addition, as *C. apicola* has been reported to tolerate high ethanol concentrations [21], it is possible that ethanol produced through fermentation promoted the fermentation of glucose and fructose. However, baking bread was the focus of this study, and therefore, the fermentation mechanism of these yeasts in baking is a subject for future research.

4.3. Examination of the Effect of Sugar Concentration

Metschnikowia pulcherrima and *C. apicola* showed the maximum fermentation efficiency at a sugar concentration of 5.0%. During fermentation, the fermentation capacity of *C. apicola* was affected by sugar concentration. In a previous study, fermentation by *S. cerevisiae* increased ethanol concentration with increasing glucose concentrations from 2.0% to 8.0% [32]. Furthermore, as *Starmerella zemplinina* (synonym *Candida zemplinina*) has been reported to grow faster when glucose is added at concentrations of 2.0% and 20% [21], fermentation efficiency may have increased with increasing glucose concentrations up to 20%. In contrast, the fermentation efficiency of *Hanseniaspora guilliermondii* reportedly decreases at sugar concentrations >300 g/L [14]. These results indicate that the effect of sugar concentration on fermentation varies depending on the yeast type. The yeasts investigated in this study showed the highest fermentation efficiency at a 5.0% sugar concentration, and it is highly probable that the efficiency can be increased by increasing the sugar concentration. However, as a considerable increase in sugar concentration significantly affects the flavor of bread, the effect of sugar concentration on flavor must be considered.

This study has some limitations. As both wheat and water are used in bread production, the effects of wheat and the ions in water should be considered. However, these factors were not examined in this study; therefore, their influence on the fermentation capacity of *M. pulcherrima* and *C. apicola* should be investigated in future studies to clarify the conditions suitable for bread production. Moreover, the fermentation capacity of natural yeasts could change depending on the environment. The present study did not focus on this aspect, warranting future studies on the effects of environmental factors on the fermentation capacity of these yeasts.

5. Conclusion

The effects of temperature, sugar type, and concentration on yeast fermentation capacity were investigated in this study. The optimal fermentation temperature was 25°C for *M. pulcherrima* and 35°C for *C. apicola*. The concentration and type of sugar that most enhanced the fermentation efficiency of each yeast were 5.0% glucose for *M. pulcherrima* and 5.0% sucrose for *C. apicola*. Overall, fermentation using *M. pulcherrima* and *C. apicola* under appropriate temperature and sugar concentration conditions could potentially enhance the efficiency of bread production. Given the limited research examining the use of these natural yeasts in food, further studies are required to ensure their safety, effectiveness, and hygienic handling.

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