Development of a new type of bread using Jerusalem artichoke tuber as a raw material by fermentation with an inulin-utilizing yeast *Kluyveromyces marxianus*

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(Accepted Jul. 22. 2025)

要旨：私たちの研究室では、通常のパン酵母（*Saccharomyces cerevisiae*）の代わりにイヌリン資化性酵母（*Kluyveromyces marxianus*）を用いて、イヌリンを唯一の発酵用炭水化物として製造する新しいタイプのパン「イヌリンパン」を開発した（Kikuchi H. *et al*., *Curr*. *Top*. *Biotech*., **14**: 13-18, 2023）。本研究では、イヌリンを含有する作物そのものを原材料とした新しいタイプのパンの製造を目指して、キクイモ抽出液を使用した「キクイモパン」を開発・製造した。キクイモ抽出液は、生キクイモに水を添加してジューサーミキサーで破砕した後に、破砕液をガーゼで漉して調製した。パンの製造は市販のホームベーカリーを用いた。パン 1 斤あたりの生キクイモ使用量 62.5 g でパンの高さは 15 cm を超え、1 斤あたりの生キクイモ使用量 167 g までパンの高さは殆ど変わらなかった。官能評価で「キクイモパン」と「ドライイーストで製造した通常のパン」を比較したところ、「味」の項目でキクイモパンは有意に劣勢であったが、その他の項目「外観」「色彩」「香り」「しっとり感」「もちもち感」及び「総合評価」の項目では遜色無い評価であった。キクイモ独特の風味が評価に影響を及ぼした可能性があるが、全体的にみて「キクイモパン」は十分常食に耐えうる品質を有することが示された。

キーワード：キクイモパン、イヌリン、イヌリン資化性酵母、官能評価

Summary

We developed the inulin bread produced by fermentation using an inulin-utilizing yeast (*Kluyveromyces marxianus*) instead of ordinary baker's yeast with inulin as the sole carbohydrate source (Kikuchi H. *et al*., *Curr. Top. Biotech*., **14**: 13-18, 2023). In this study, to produce a new type of bread using inulin-containing crops as raw materials, we developed the “Jerusalem artichoke bread”. Raw Jerusalem artichoke tubers were crushed using a juicer mixer, and filtering the resulting homogenate through gauze. A commercially available home bakery was used to produce bread. The height of the Jerusalem artichoke bread exceeded 15 cm when 62.5 g of raw Jerusalem artichoke tubers were used per loaf, and the height of the bread remained almost the same up to 167 g of raw tubers used per loaf. In a sensory evaluation comparing the Jerusalem artichoke bread with the bread made by commercial dry yeast, the Jerusalem artichoke bread was significantly inferior only in taste, but was almost equal in other evaluation items. These results showed that the Jerusalem artichoke bread is useful as a staple food although the unique flavor of Jerusalem artichoke may affect the overall evaluation.

Key words: Jerusalem artichoke bread, inulin, inulin-utilizing yeast, sensory evaluation

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Introduction

Kluyveromyces marxianus was found from traditional alcoholic fermented milk[1)], and has also been used as a baker's yeast[2~8)]. Recently, we developed the "inulin bread" (hereinafter, this is called the "inulin bread"), which was produced using an inulin-utilizing yeast *K. marxianus* as a baker's yeast[9)] instead of ordinary baker's yeast *Saccharomyces cerevisiae*. In this study, to develop a new type of bread made from inulin-containing crops as raw materials, we produced the "Jerusalem artichoke bread" as a starting point. The Jerusalem artichoke is a highly productive crop with delicious tubers that can be used to make soup, roasted, mashed and so on. Because Jerusalem artichoke tubers contain 10-22 g of inulin /100 g fresh weight[10, 11)], it is thought that Jerusalem artichoke tubes are suitable as a raw material for making bread using *K. marxianus*. To evaluate the quality of the resulting Jerusalem artichoke bread, sensory evaluation test was carried out and compared the Jerusalem artichoke bread and the bread made using commercial dry yeast.

Materials and Methods

1. Yeast

K. marxianus (NBRC 1735) was provided by NITE Biological Resource Center (Kisarazu, Chiba, Japan), and grown at 30 °C in the culture medium containing 10 g/L of inulin, 5 g/L of peptone, 3 g/L of yeast extract and 3 g/L of malt extract. Cells were washed by sterilized water twice, and used for bread making.

2. Bread making using *K. marxianus*

The Jerusalem artichoke bread was made as follows. Raw Jerusalem artichoke tubers from Akita Prefecture were purchased from MOA (Shizuoka, Japan). The Jerusalem artichoke tubers extract was prepared by adding tap water to raw Jerusalem artichokes tubers (50-167 g fresh weight), crushing them in a juicer mixer, and filtering the homogenate through gauze. Finally, the volume of the resulting extract was adjusted to 200 mL with tap water. *K. marxianus* cells (5 g, wet weight) were suspended in 200 mL of the Jerusalem artichoke tubers extract, and mixed with 280 g of commercially available bread flour containing 0.33% ash and 11.5% crude protein (Nisshin Seifun, Tokyo, Japan), 6 g of lactose-free skim milk (Morinaga Milk Industry Co., Ltd., Tokyo, Japan), 4 g of salt (The Salt Industry Center of Japan, Tokyo, Japan) and 20 g of unsalted butter (Megmilk Snow Brand, Co., Ltd., Tokyo, Japan). Bread making was carried out using a bread maker HBK-101 (MK Seiko Co., Ltd., Nagano, Japan) in the natural yeast mode according to the attached manual. Three loaves of the breads were independently baked for each amount of raw Jerusalem artichoke tubers used.

3. Phenol-sulfuric acid method

Total saccharide content of the Jerusalem artichoke tubers extract was determined by phenol-sulfuric acid method according to the standard manual[12)] using inulin as a standard saccharide.

4. Bread making using commercial dry yeast

In parallel, breads (called as the "typical breads") were made using commercial dry yeast (Nisshin Seifun, Tokyo, Japan) for comparison of quality. The dry yeast (2.4 g), 190 mL of tap water, and mixed with 280 g of bread flour, 4 g of salt, 20 g of unsalted butter and 6 g of skim milk (Megmilk Snow Brand), and 20 g of sugar (Dai-Nippon Meiji Sugar Co., Ltd., Tokyo, Japan) were used. Bread making was carried out using a bread maker HBK-101 with the normal mode according to an attached manual.

5. Measurement of height and weight, and evaluation of appearance of the breads

After baking, the breads were cooled down to room temperature for 1 h. The height and weight of the breads were measured with a ruler and a scale, respectively. Images of the side views and cross-sections of the breads were taken using a digital camera (Canon, Tokyo, Japan).

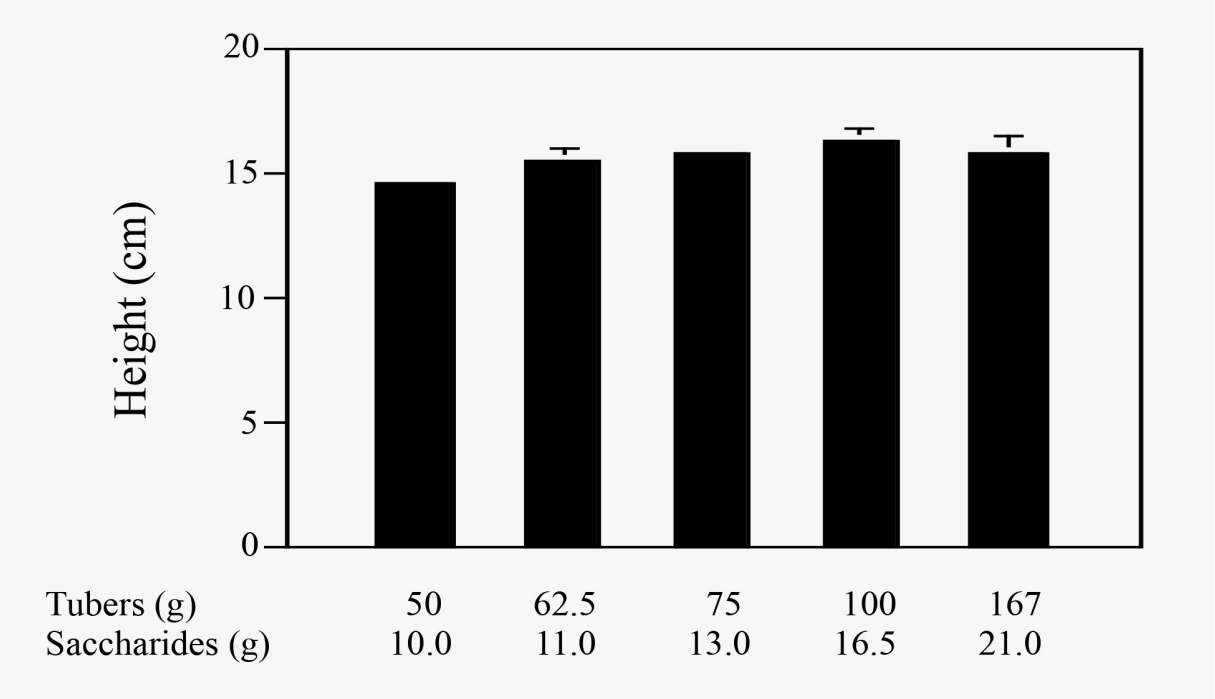

Fig. 1. The height of the Jerusalem artichoke bread. The height of the breads was measured with a ruler. Data are presented as averages of measured values from three independent loaves of the breads. Tubers (g): The Jerusalem artichoke bread were made using various weights of raw tubers as shown in the figure. Saccharides (g): The amounts of total saccharide contained in 200 mL of the Jerusalem artichoke tubers extract used in bread making were determined by phenol-sulfuric acid method.

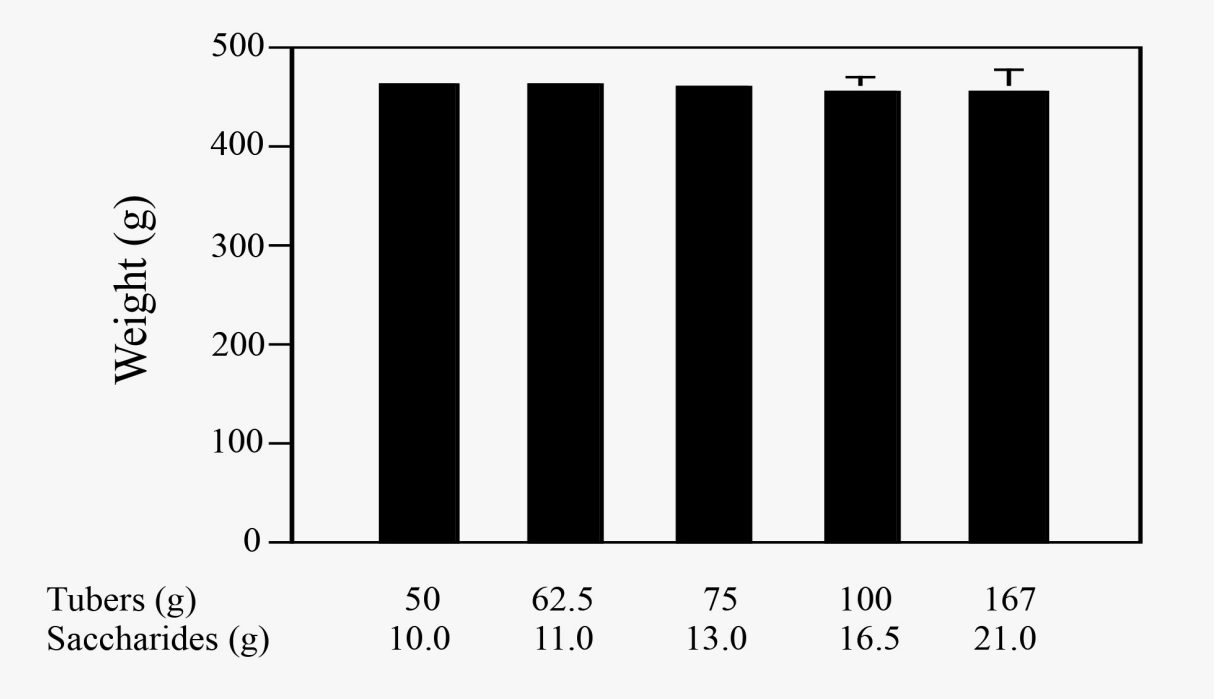

Fig. 2. The weight of the Jerusalem artichoke bread. The weight of the breads was measured with a scale. Data are presented as averages of measured values from three independent loaves of the breads. Tubers (g): The Jerusalem artichoke bread were made using various weights of raw tubers as shown in the figure. Saccharides (g): The amounts of total saccharide contained in 200 mL of the Jerusalem artichoke tubers extract used in bread making were determined by phenol-sulfuric acid method.

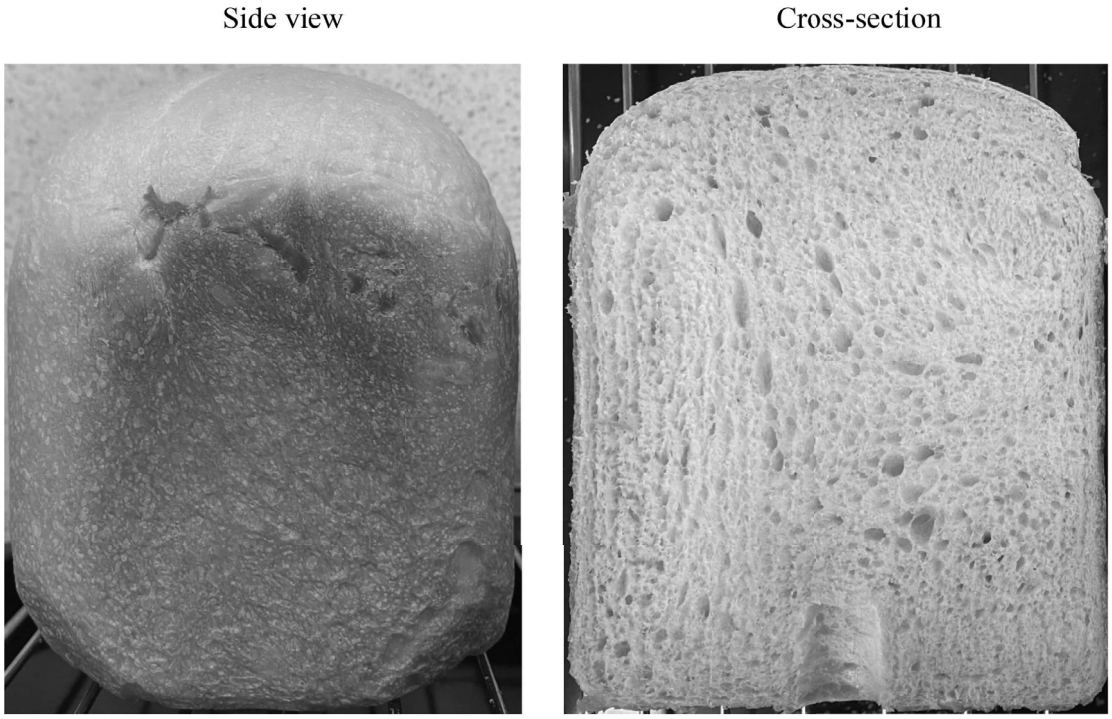

Fig. 3. Side view and cross-section of the Jerusalem artichoke bread using 167 g of raw tubers.

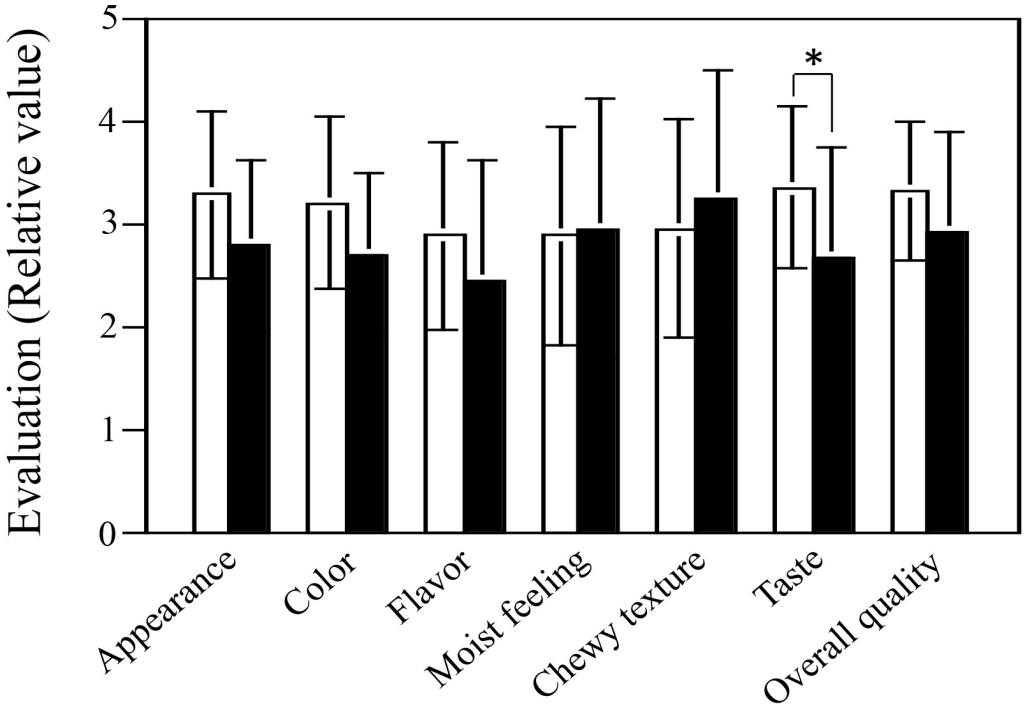

Fig. 4. Sensory evaluation of the Jerusalem artichoke bread and the typical bread. Twenty-eight volunteer tasters evaluated pieces of the typical bread (open bars) and the Jerusalem artichoke bread using 100 g of raw tubers (closed bars). They gave the evaluation scores as follows: 0 (bad), 1 (slightly bad), 2 (neither), 3 (slightly good) and 4 (good) for attributes of appearance, color, flavor, moist feeling, chewy texture, taste and overall quality. Data are presented as averages of the evaluation scores (n = 28), and error bars indicate standard deviation. Statistical differences were calculated using Student's t test. *, $p < 0.05$.

6. Sensory evaluation

To assess quality of the Jerusalem artichoke bread, a sensory evaluation test was carried out as described[6-9]. Twenty-eight of volunteer tasters (female students aged 18-20) evaluated these bread pieces used as samples. They gave the evaluation scores as follows: 0 (bad), 1 (slightly bad), 2 (neither), 3 (slightly good) and 4 (good) for attributes of appearance, color, flavor, moist feeling, chewy texture, taste and overall quality.

7. Statistical analysis

Data (height and weight) are presented as averages of measured values from three each of these two kinds of breads. Data of sensory evaluation are presented as averages of the evaluation scores ($n = 28$). Statistical differences between the Jerusalem artichoke bread and the typical bread were calculated with Student's *t*-test.

Results and Discussion

In our previous study, we developed the "inulin bread" using purified inulin as the sole carbohydrate source fermented by *K. marxianus*[9]. In this study, as the next step, we developed the "Jerusalem artichoke bread" so as to produce a new type of bread using inulin-containing crops as raw materials. The Jerusalem artichoke is a highly productive crop with delicious and relatively inexpensive tubers which are loaded with inulin (10-22 g of inulin/100 g fresh weight of tubers). In addition, Jerusalem artichoke tuber mostly contains inulin as the carbohydrate for storage. Therefore, we expected that the Jerusalem artichoke tubers are suitable as a raw material for making bread using *K. marxianus.*

As expected, we successfully developed the "Jerusalem artichoke bread". Figure 1 shows the effects of the amounts of tubers used on the height of the Jerusalem artichoke bread. Although bread using 50 g of raw tubers per loaf was slightly (but not significantly) lower, no differences were observed between 62.5 g and 167 g of raw tubers used. On the other hand, rise of the breads distinctly decreased when raw tubers used was less than 50 g per loaf. (data not shown). Based on the saccharide content in 200 mL of the Jerusalem artichoke tubers extract determined by phenol-sulfuric acid method, height of the Jerusalem artichoke breads plateaued using even above 11 g of inulin per loaf. These results generally consistent with our previous data obtained from making bread with purified inulin[9]. Interestingly, *K. marxianus* strains are unable to utilize maltose[13]. In fact, the bread barely rose at all without the Jerusalem artichoke tubers extract. These data showed that the Jerusalem artichoke tubers extract containing abundant inulin certainly contributed to producing the Jerusalem artichoke bread as the most important source of carbohydrates. Figure 2 shows the effects of the amounts of tubers used on the weight of the Jerusalem artichoke bread. No difference was observed between 50 g and 167 g of raw tubers used.

Typical appearances of the side view and cross-section of the Jerusalem artichoke bread using 167 g of tubers are shown in Fig. 3. The side view turned to golden brown, and the shape and number of air bubbles also seen to be nearly the same as those of the inulin bread shown in our previous report[9]. There was almost no difference in the side view and cross-section of the Jerusalem artichoke bread depending on the amounts of tubers used, except that color of the cross-section became slightly darker as the amounts of tubers used increased (data not shown).

Finally, in order to evaluate the quality of the Jerusalem artichoke bread, sensory evaluation was performed using the typical bread and the Jerusalem artichoke bread. The Jerusalem artichoke bread using 100 g of raw tubers was selected as a sample for the sensory evaluation considering the balance between fragrance and color derived from raw Jerusalem artichoke tubers. Comparison data of sensory evaluation are shown in Fig. 4. The Jerusalem artichoke bread was significantly inferior only in taste, but was almost equal in other evaluation items. The Jerusalem artichoke bread showed that color of the crumb of

it was slightly darker than that of the typical bread and it also had a distinctive flavor of Jerusalem artichoke. These characteristics may bring about such somewhat low evaluation. In this study, female students aged 18-20 took part in the sensory evaluation as tasters. The results of sensory evaluation may be different if it is carried out by different age or sex groups as tasters. In addition, it may be possible to create a bread that is more suitable for everyone using less raw tubers.

We succeeded in developing the Jerusalem artichoke bread using *K. marxianus.* Our data reveals that the "inulin bread" can be made from inulin-containing crops as raw materials. We will produce various types of the inulin breads using other inulin-containing crops. On the other hand, in this study, only the extract from raw Jerusalem artichoke tubers was used to making bread, and the residue was discarded. Because the residue contains a lot of nutrients such as dietary fibers, we also would like to consider effective methods to utilize it in the future.

Institutional Review Board Statement

This study conducted in accordance with the ethical guidelines of the Declaration of Helsinki. Sensory evaluation in this study was approved by the Bioethical Committee at the Shokei University/Shokei University Junior College (No. 2024-23), and informed consent was obtained from all tasters.

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