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Original Article

Production and evaluation of a unique type of bread using inulin as the only carbohydrate source for bread fermentation with the inulinase-producing yeast, *Kluyveromyces marxianus*

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ABSTRACT

Bread is consumed as one of the major staple foods all over the world. Similarly, Jerusalem artichoke is cultivated all over the world, and its tubers containing a large amount of inulin are eaten raw, heated or pickled. However, the effective utilization of the tubers of Jerusalem artichoke and inulin is still in the development stage. In this study we developed a unique type of bread 'the inulin bread' that is made using inulin as an only carbohydrate for bread fermentation with an inulin-utilizing yeast *Kluyveromyces marxianus*, but not the common baker's yeast *Saccharomyces cerevisiae*. Our findings in this paper suggest that the use of *K. marxianus* cultured in the presence of inulin and 3 h of pre-fermentation is remarkably effective to make the inulin bread. Evaluation of the various physical properties (measurements of height, weight, hardness and cohesiveness) and sensory evaluation revealed that the quality of the inulin bread is not inferior when compared with the common white bread. We expect that the inulin bread developed by us can not only contribute to the effective utilization of tubers of Jerusalem artichoke which is rich in inulin but also will open up new possibilities of bread making in the future.

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KEYWORDS: bread, inulin, Jerusalem artichoke, *Kluyveromyces marxianus*.

INTRODUCTION

Jerusalem artichoke is cultivated all over the world. The tubers of Jerusalem artichoke are eaten raw, heated or pickled. Notably, inulin is considered as a dietary fiber that helps in reducing the risk of type 2 diabetes and a prebiotic because it is not digested in the human intestine and utilized by intestinal bacteria [1, 2]. However, the understanding of effective utilization of the tubers of Jerusalem artichoke and inulin remains poor.

We have produced the 'authentic' milk bread that is made without addition of sugar (sucrose) and water using a lactose-utilizing yeast *Kluyveromyces marxianus* as the only baker's yeast and evaluated its quality [3]. *Kluyveromyces* spp., *e.g. K. lactis* and *K. marxianus*, are found in traditional alcohol-fermented milk such as airag in Mongolia [4]. Interestingly, *K. marxianus* can produce inulinase, which catalyzes hydrolysis of inulin, and can utilize inulin for growth [5]. Using inulinase-producing *K. marxianus*, Struyf *et al.* have eliminated fructan from wheat whole meal and produced the low fermentable oligo-, di, monosaccharides and polyol (FODMAP) whole wheat bread [6]. However, no bread has been made using inulin as a carbohydrate source for fermentation.

In this study, we developed a unique type of bread 'the inulin bread' that is made using inulin as an only carbohydrate source for bread fermentation with *K. marxianus* and evaluated its quality.

MATERIALS AND METHODS

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 lactose or inulin, 5 g/L of peptone, 3 g/L of yeast extract and 3 g/L of malt extract. Cells were washed by tap water twice, and used for bread making.

Inulin bread making using *K. marxianus*

K. marxianus cells (5 g, wet weight) were suspended in 190 mL tap water, and mixed with 280 g of commercially available bread flour (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), 7 g of unsalted butter (Megmilk Snow Brand, Cso., Ltd., Tokyo, Japan) and inulin (Tokyo Chemical Industry, Tokyo, Japan). Bread making was performed using a bread maker HBK-101 (MK Seiko Co., Ltd., Nagano, Japan) in the normal mode or the natural yeast mode (3 h of pre-fermentation plus normal mode) according to an attached protocol. Three loaves of the inulin bread were independently baked.

Bread making using commercial dry yeast

To evaluate the quality of the inulin bread, bread for comparison (called as "the typical bread") were made using commercial dry yeast (Nisshin Seifun, Tokyo, Japan) as described in our previous report [3]. In brief, bread making was carried out using a bread maker HBK-101 in the normal mode according to an attached manual using dry yeast (2.4 g), 190 mL of tap water, 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). The typical bread was baked, and used as a standard for evaluation of the inulin bread as described in 'Sensory evaluation of the inulin bread' section.

Measurement of height and weight, and evaluation of appearance of the inulin bread

After baking, the inulin bread was cooled down to room temperature for 1 h, and incubated for 20 h in a plastic bag. The height and weight of the inulin bread were measured with a ruler and a scale, respectively. Images of the side view of the inulin bread were taken using a digital camera (Canon, Tokyo, Japan). After slicing the inulin bread, images of the cross-sections of the inulin bread were also taken using a digital camera.

Evaluation of the physical properties of the inulin bread

The inulin bread was divided into rectangles (40 mm x 40 mm x 20 mm) using a slicer (model E16, Ritter, Grobenzell, Germany) and a bread cutter (model EK700, Black and Decker, MD, USA). The physical properties (hardness and cohesiveness) of the inulin bread were measured using a creep meter (model TPU, Yamaden, Tokyo, Japan) with these pieces of the inulin bread. The measurement conditions were as follows: plunger, circular form (8 mm in diameter); measurement strain rate, 50%; compression speed, 5 mm/s.

Sensory evaluation of the inulin bread

To assess the quality of the inulin bread, a sensory evaluation test was performed using the typical bread as a standard. The two types of bread were baked, cooled down to room temperature for 1 h, and incubated for 20 h in a plastic bag. After removing the crust, these two types of bread were divided into cubes (20 mm x 20 mm x 20 mm) using a bread cutter. Twenty-six volunteer tasters aged 19-20 evaluated these bread pieces used as samples. They gave the evaluation scores as follows: -2 (bad), -1 (slightly bad), 0 (neither), +1 (slightly good) and +2 (good) for attributes of appearance, color, fragrance, moist feeling, chewy texture, taste and overall quality.

Statistical analysis

Data (height, weight, hardness and cohesiveness) are presented as averages of measured values from three loaves of the inulin bread. Data of sensory evaluation are presented as averages of the evaluation scores (n=26). Statistical differences

between the typical bread and the inulin bread were calculated using Student's *t*-test.

RESULTS AND DISCUSSION

In this study using *K. marxianus*, we developed a novel type of bread namely 'the inulin bread' that was made using flour, inulin (instead of sugar), salt, lactose-free skim milk and fat, and evaluated its quality by various experiments.

First, we investigated the effects of the types of carbohydrates (lactose or inulin) in the yeast growth media and of pre-fermentation on inulin bread making. The height of the bread made using *K. marxianus* grown in the presence of inulin was significantly higher than that of the bread made using *K. marxianus* grown with lactose (Figure 1A). These data demonstrate that inulin is better than lactose as a carbohydrate in the growth media in order to make the bread. Additionally, *S. cerevisiae* strains utilize maltose derived from starch hydrolysis catalyzed by wheat amylase in the dough, resulting in their leavening ability without addition of sugar. However, maltose in dough does not contribute in the fermentation by *K. marxianus* because *K. marxianus* cannot utilize maltose [7]. Therefore, only inulin makes the dough rise in the inulin bread. In addition, even if the bread making is performed using *K. marxianus* grown in the presence of inulin, the height of the bread made with the natural yeast mode (3 h of pre-fermentation plus normal mode) was significantly higher than that of the bread made with the normal mode (Figure 1A). These findings suggest that 3 h of pre-fermentation is remarkably effective to make the inulin bread because hydrolysis of inulin can be progressed during the 3 h of pre-fermentation. In contrast, there is no effect of both the types of carbohydrates (lactose or inulin) in the yeast growth media and pre-fermentation on the weight of the inulin bread (Figure 1B).

Second, we investigated the effects of the quantity of inulin added on inulin bread making. Judging from the height of the bread, 15 g of inulin was significantly more effective than 10 g on inulin bread making (Figure 2). In addition, there was no significant change in the height of the bread using

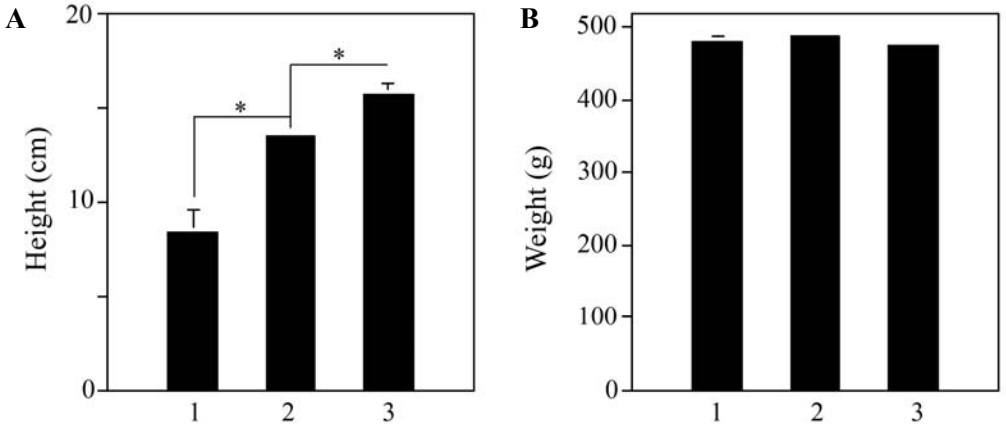

Figure 1. The effects of the types of carbohydrates (lactose or inulin) in the yeast growth media and of pre-fermentation on inulin bread making. *K. marxianus* cells (5 g, wet weight) grown in the presence of lactose or inulin were suspended in 190 mL tap water, and mixed with 280 g of commercially available bread flour, 6 g of lactose-free skim milk, 4 g of salt, 7 g of unsalted butter and 20 g of inulin. Bread making was performed using a bread maker in the normal mode or the natural yeast mode (3 h of pre-fermentation plus normal mode) according to an attached manual. Lanes 1: the inulin bread made in the normal mode using *K. marxianus* grown in the presence of lactose, lanes 2: the inulin bread made in the normal mode using *K. marxianus* grown in the presence of inulin, lanes 3: the inulin bread made in the natural yeast mode (3 h of pre-fermentation plus normal mode) using *K. marxianus* grown in the presence of inulin. Data are presented as averages of measured values from three independent loaves of bread. Error bars indicate standard deviation. Statistical differences were calculated using Student's *t* test. $*p < 0.05$.

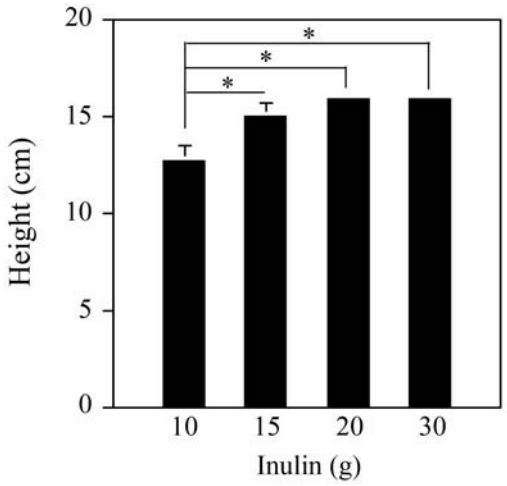

Figure 2. The effects of the quantity of inulin added on inulin bread making. *K. marxianus* cells (5 g, wet weight) grown in the presence of inulin were suspended in 190 mL tap water, and mixed with 280 g of commercially available bread flour, 6 g of lactose-free skim milk, 4 g of salt, 7 g of unsalted butter and 10, 15, 20 or 30 g of inulin. Bread making was performed using a bread maker in the natural yeast mode (3 h of pre-fermentation plus normal mode) according to an attached manual. Data are presented as averages of measured values from three independent loaves of bread. Error bars indicate standard deviation. Statistical differences were calculated using Student's *t* test. $*p < 0.05$.

more than 15 g of inulin. Hereafter, we made inulin bread using 20 g of inulin.

Third, we investigated the general properties of the inulin bread. Figure 3 shows the side view and the cross-section of the inulin bread made using 20 g of inulin (the height of the bread: 15.7 cm). The side view shows that inulin bread turned to golden brown, and the shape and number of air bubbles also seem to be nearly the same as those of the typical bread shown in our previous report [3]. On the other hand, hardness and cohesiveness of the inulin bread were not significantly different from those of the typical bread (Figure 4). These results showed that the inulin bread is the morphologically good-looking bread under the conditions mentioned above.

Finally, to evaluate the quality of the inulin bread, sensory evaluation was carried out. Figure 5 shows the results of sensory evaluation. Notably, sensory evaluation revealed that the seven characteristics of the inulin bread (appearance, color, fragrance, moist feeling, chewy texture, taste and overall quality) inspected not only displayed a high score, but also had no significant difference with respect to those of the typical bread. Although *K. lactis* was able to raise the bread using inulin similar to *K. marxianus*, the inulin bread made using

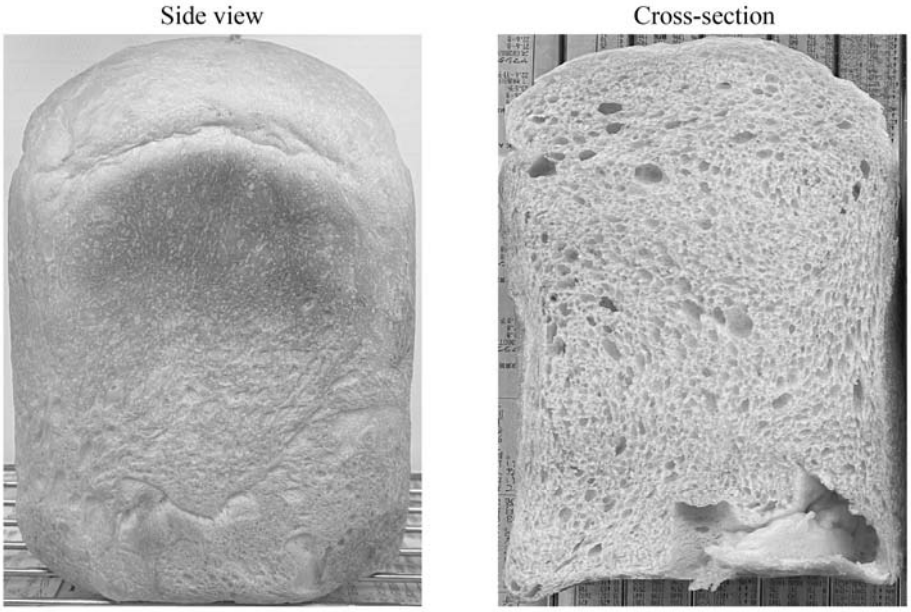

Figure 3. Side view and cross-section of the inulin bread.

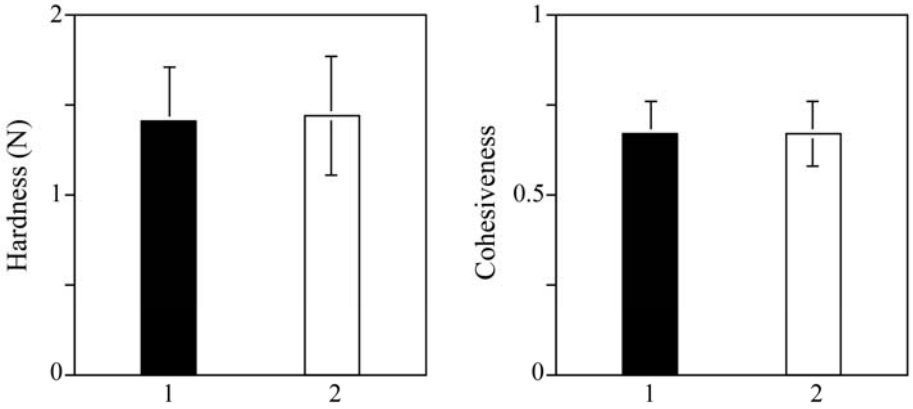

Figure 4. Physical properties of the inulin bread. The physical properties (hardness and cohesiveness) of the inulin bread (lanes 1) and the typical bread (lanes 2) were measured using a creep meter. Data are presented as averages of measured values from three independent loaves of bread. Error bars indicate standard deviation. Statistical differences were calculated using Student's *t* test.

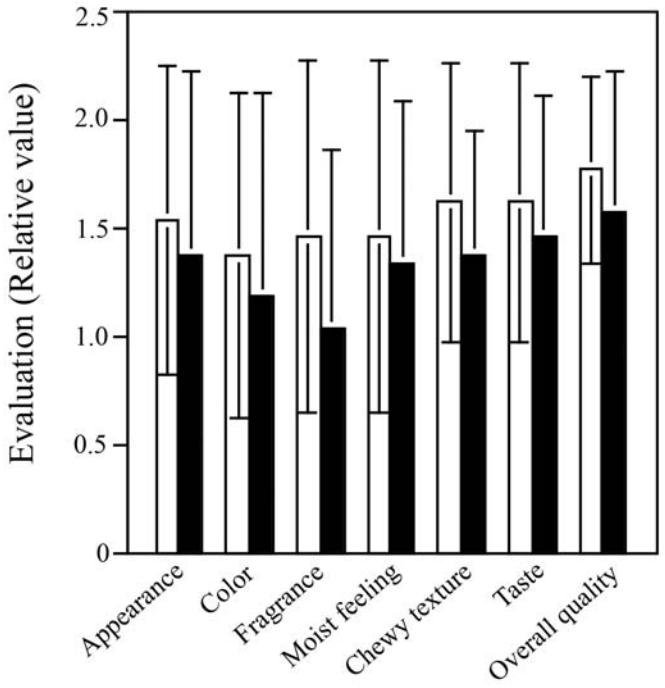

Figure 5. Sensory evaluation of the typical bread and the inulin bread. The pieces of the typical bread (open bars) and the inulin bread (closed bars) used as samples were evaluated by twenty-six volunteer tasters. They gave the evaluation scores as follows: -2 (bad), -1 (slightly bad), 0 (neither), +1 (slightly good) and +2 (good) for attributes of appearance, color, fragrance, moist feeling, chewy texture, taste and overall quality. Data are presented as averages of the evaluation scores (n=26), and error bars indicate standard deviation. Statistical differences were calculated using Student's *t* test.

K. lactis was inferior to that made with *K. marxianus* in terms of its ability to raise the bread (data not shown).

These results revealed that *K. marxianus* can produce high-quality inulin bread. We expect that the inulin bread can not only contribute to the

effective utilization of Jerusalem artichoke but also will open up new possibilities of bread making in the future.

CONCLUSION

In this study, we developed a novel type of bread called 'the inulin bread' using *K. marxianus*. Interestingly, various evaluation tests revealed that the inulin bread developed by us is not inferior to the white bread made using commercial dry yeast namely 'the typical bread'. Moreover, the inulin bread can also contribute to the effective utilization of tubers of Jerusalem artichoke. Of course, the inulin bread has tremendous potential, and additional research is needed to find out how the overall quality of the inulin bread can be improved further.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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