Note

Possibility for nutritional improvement of the "authentic milk bread" by increasing the amount of milk added

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要旨：私たちの研究室では、通常のパン酵母（*Saccharomyces cerevisiae*）の代わりに乳糖資化性酵母（*Kluyveromyces marxianus*）を用いて、牛乳由来以外の糖や水を加えずに製造する新しいタイプのパン「真正牛乳パン」を開発した（Shibata A. *et al*., *Curr*. *Top*. *Biotech*., **11**: 31-36, 2020）。本研究では、「真正牛乳パン」のさらなる改良を目指して、添加する牛乳の量を増やす試みを行なった。最初に開発した「真正牛乳パン」では 250 mL の牛乳を使用していたが、今回は牛乳 300 mL（予備実験の結果により決定）を使った「真正牛乳パン」を製造して比較した。両者に外観、高さや官能評価での有意な差は認められなかったことから、牛乳の添加量を 50 mL 増やすことにより、品質を損なうことなくタンパク質等の栄養素が豊富になった分、300 mL 牛乳を使用したパンに栄養学的見地からのアドバンテージがあると思われる。今後も様々な観点からの改良を続けていく予定である。

キーワード：真正牛乳パン、乳糖資化性酵母、改良、官能評価

Summary

Recently, we used a lactose-utilizing yeast (*Kluyveromyces marxianus*) instead of a normal baker's yeast (*Saccharomyces cerevisiae*) to produce the "authentic milk bread" without adding sugar and water (Shibata A. *et al*., *Curr*. *Top*. *Biotech*., **11**: 31-36, 2020). In this study, to improve the "authentic milk bread", we increased the amount of milk added. The original "authentic milk bread" was made using 250 mL milk. We made the "authentic milk bread" using 300 mL milk (determined by preliminary experiments) this time, and we compared it with the bread using 250 mL milk. There was almost no difference in the height and the appearance (the side views and the cross-sections) of these two types of breads. The sensory evaluation also revealed that there was statistically no significant difference in the inspection items tested between the bread using 250 mL milk and the bread using 300 mL milk. These results suggested that the nutritional properties might be improved by increased protein derived from 50 mL of additional milk in the bread without spoiling sensory qualities of the breads. To improve the "authentic milk bread", further research will probably be necessary in the future.

Key words: authentic milk bread, *Kluyveromyces marxianus*, improvement, lactose-utilizing yeast, sensory evaluation

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Introduction

Kluyveromyces spp., typical lactose-utilizing yeasts, were found from traditional alcoholic fermented milk[1]. *Kluyveromyces* spp. are used as sources of β-galactosidase[2] which is available for producing low-lactose-containing dairy products. *K. marxianus* belonging to *Kluyveromyces* spp. has also been used as a baker's yeast instead of *Saccharomyces cerevisiae*[3)-6)]. Recently, we developed the "authentic milk bread" that is made without addition of sucrose and water using a lactose-utilizing yeast *K. marxianus* as the only baker's yeast[7]. Various evaluation tests also revealed that the "authentic milk bread" has no inferiority as compared with the typical bread made with *S. cerevisiae* on the whole. The "authentic milk bread" also has the benefit of the presence of the essential amino acids. The insufficient amino acids in wheat (e.g. lysine) can be efficiently taken in by consuming the "authentic milk bread" containing abundant milk proteins. In this paper, to improve the "authentic milk bread", we increased the amount of milk added when the "authentic milk bread" was made.

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 lactose, 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*

K. marxianus cells (5 g, wet weight) were suspended in 250 mL or 300 mL of milk (Kumamoto Dairy, Kumamoto, Japan), and mixed with 280 g of commercially available bread flour (Nisshin Seifun, Tokyo, Japan), 4 g of salt (The Salt Industry Center of Japan, Tokyo, Japan) and 7 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 pieces of the breads were independently baked.

3. 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 of the breads were taken using a digital camera (Canon, Tokyo, Japan). After slicing the breads, images of the cross-sections of the breads were copied using a duplicator (Ricoh, Tokyo, Japan).

4. Sensory evaluation

To assess quality of the breads, a sensory evaluation test was carried out. The breads produced using 300 mL milk were compared with the breads produced using 250 mL milk. After removing crust, these two types of breads were divided into cubes (20 mm x 20 mm x 20 mm) using a bread cutter. Twenty-four of volunteer tasters 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.

5. Statistical analysis

Data (height and weight) are presented as averages of measured values from three each of these two kinds of breads (the bread using 250 mL milk and the bread using 300 mL milk). Data of sensory evaluation are presented as averages of the evaluation scores ($n = 24$). Statistical differences between the bread using 250 mL milk and the bread using 300 mL milk were calculated with Student's *t*-test.

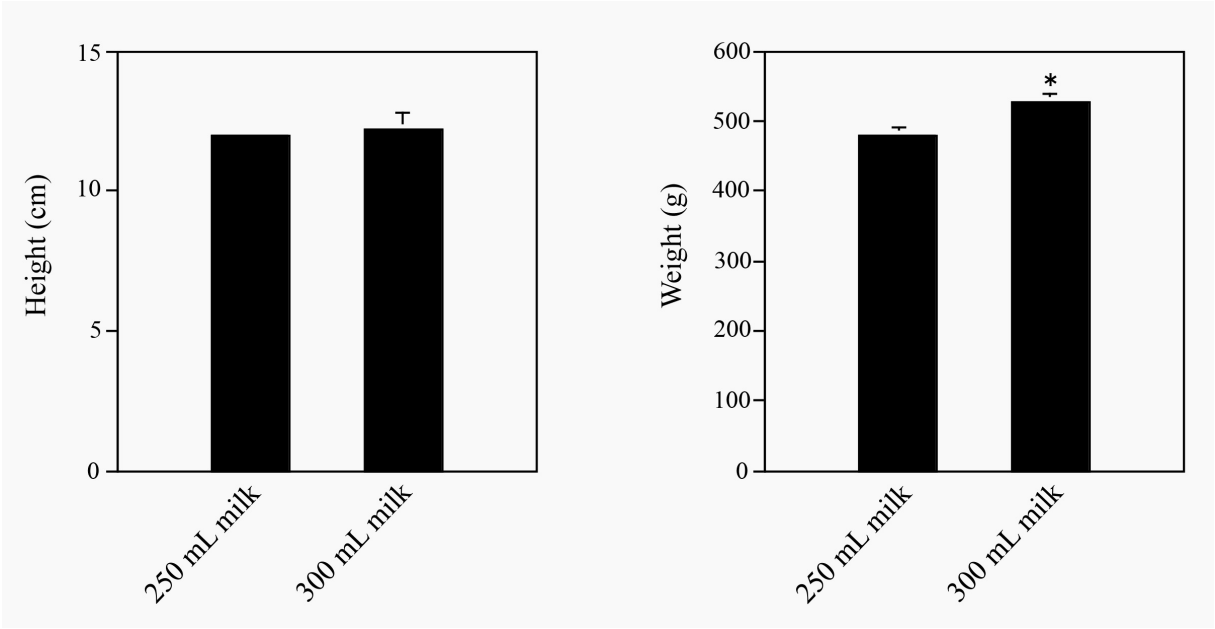

Fig. 1. The height and weight of the bread using 250 mL milk (250 mL milk) and the bread using 300 mL milk (300 mL milk). The height and weight of the breads were measured with a ruler and a scale, respectively. Data are presented as averages of measured values from three independent breads. Error bars indicate standard deviation. Statistical differences were calculated using Student's *t* test. *, $P < 0.05$ compared with the data of the bread using 250 mL milk.

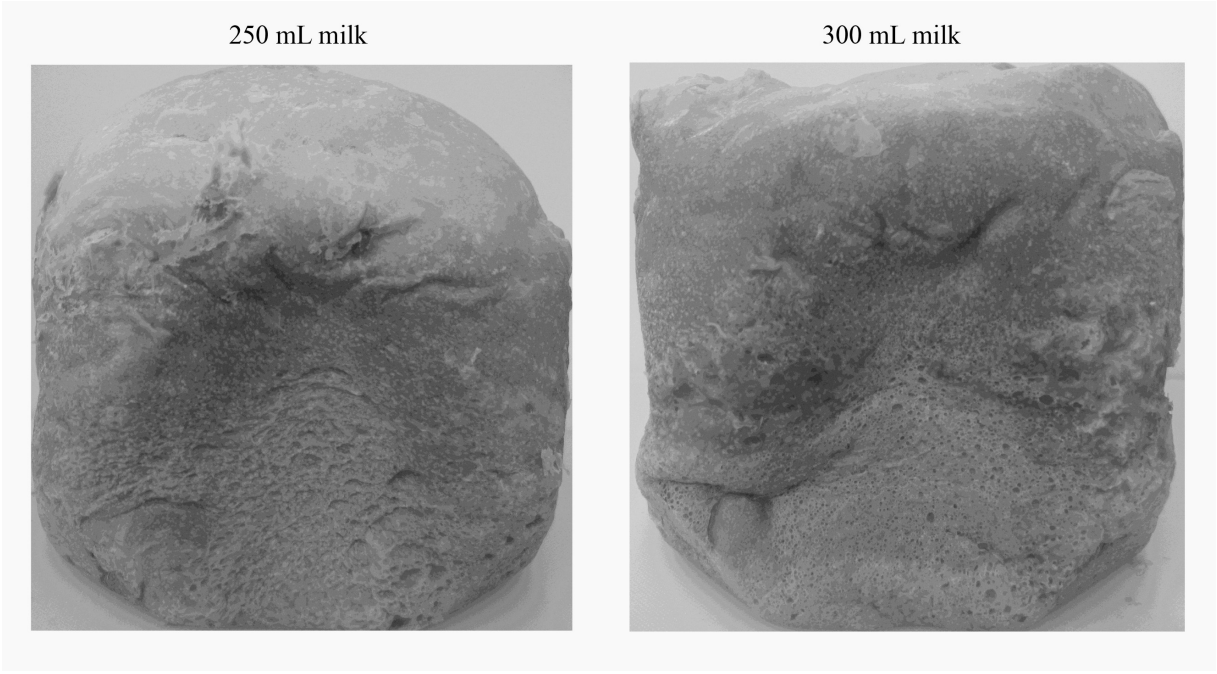

Fig. 2. Side views of the bread using 250 mL milk (250 mL milk) and the bread using 300 mL milk (300 mL milk).

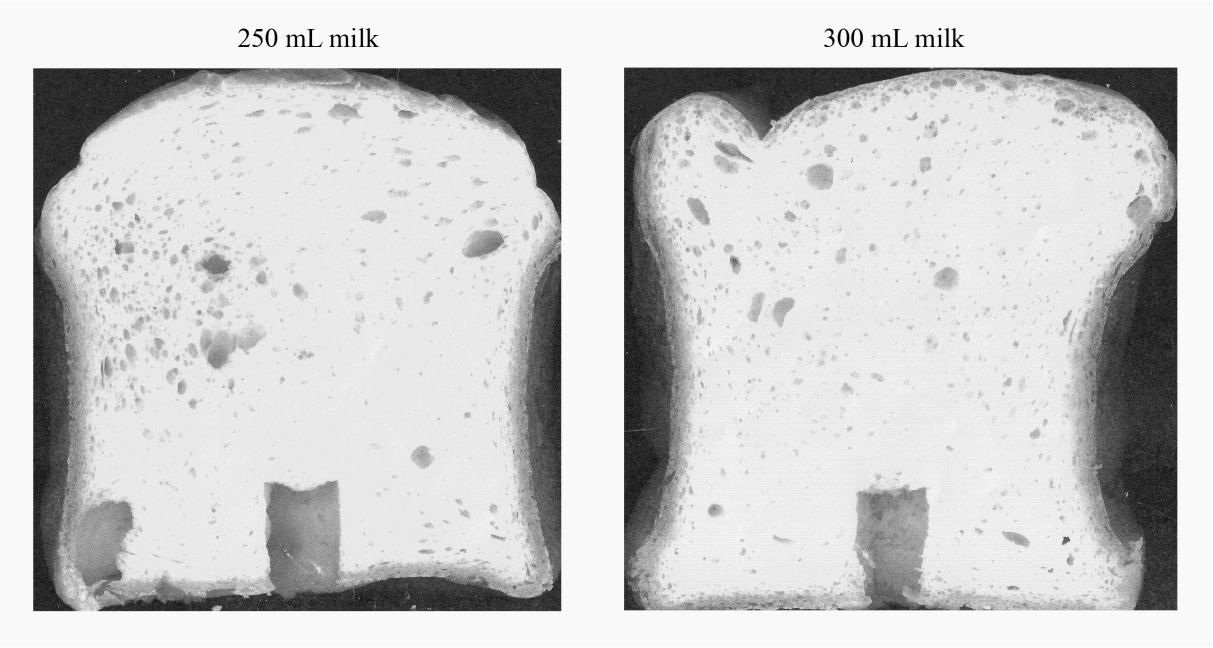

Fig. 3. Cross-sections of the bread using 250 mL milk (250 mL milk) and the bread using 300 mL milk (300 mL milk).

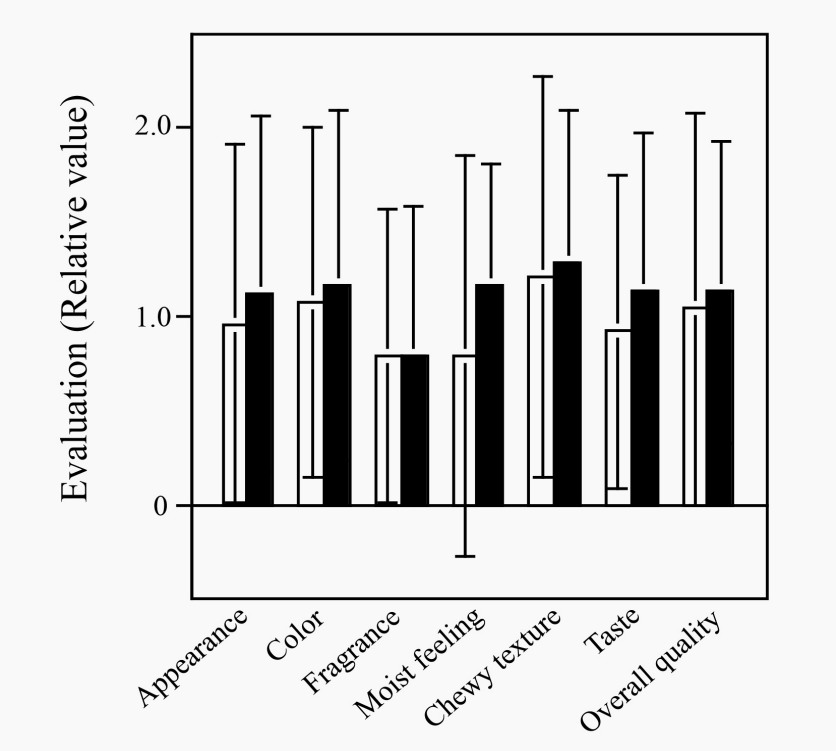

Fig. 4. Sensory evaluation of the bread using 250 mL milk and the bread using 300 mL milk. Twenty-four volunteer tasters evaluated pieces of the bread using 250 mL milk (open bars) and the bread using 300 mL milk (closed bars) samples. They gave the evaluation scores as follows: -2 (bad), -1 (slightly bad), 0 (neither), +1 (slightly good) and +2 (good) for attributes of appearance, color, fragrance, moist feeling, chewy texture, taste and overall quality. Data are presented as averages of the evaluation scores (n = 24), and error bars indicate standard deviation. Statistical differences were calculated using Student's t test.

Results and Discussion

Although we have developed the "authentic milk breads"[7], we think that the quality (e.g. texture and taste) of them can be improved further through additional research. In this paper, as part of improvement of the "authentic milk breads", we made them by changing the amount of milk added. While we originally made the "authentic milk breads" using 250 mL milk[7], we made them using 300 mL, 325 mL and 350 mL milk this time. However, addition of 325 mL or 350 mL milk caused poor bread volume (data not shown). Therefore, we made the bread using 300 mL milk, and compared it with the bread using 250 mL milk. The height of the bread using 300 mL milk was also almost the same as that of the bread using 250 mL milk (Fig. 1). In contrast, the bread using 300 mL milk was about 40 g heavier than the bread using 250 mL milk, the difference was statistically significant (Fig. 1). It seemed that the difference in weight of these two types of breads was due to the difference in the amount of milk added. On the other hand, there was almost no difference in the appearance of the side views and the cross-sections of these two types of breads (Fig. 2 and Fig.3). In addition, hardness and cohesiveness of the bread using 250 mL milk were not significantly different from those of the bread using 300 mL milk (data not shown). Next, to evaluate the quality of these two types of breads, sensory evaluation was performed. Figure 4 shows the results of sensory evaluation. There was statistically no significant difference in the inspection items tested between the bread using 250 mL milk and the bread using 300 mL milk. However, it seemed that there might be some kind of difference in moist feeling of these two types of breads. After all, these data revealed that there is almost no difference in sensory quality between the bread using 250 mL milk and the bread using 300 mL milk. In addition, the bread using 300 mL milk should contain milk proteins more than the bread using 250 mL milk. As a side note, about 1.6 g of protein may be contained in 50 mL of milk because milk contains approximately 3.22% of protein in general[8].

These results obtained in this paper suggested that the nutritional properties might be improved by increasing protein in the bread without spoiling qualities of the breads. We hope that we can continue to improve the "authentic milk bread" in the future.

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