Addition of more lactose and butter remarkably improves the quality of the authentic milk bread

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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」（以下、「オリジナル真正牛乳パン」と呼ぶ）を開発した（Shibata A. *et al*., *Curr. Top. Biotech*., **11**: 31-36, 2020）。本研究では、「オリジナル真正牛乳パン」のさらなる改良を目指して、乳糖 5 g 及び無塩バター20 g を添加した「改良型真正牛乳パン」を製造した。「オリジナル真正牛乳パン」は製造時に無塩バター7 g を使用し、乳糖は加えていない（勿論牛乳には含まれる）。「改良型真正牛乳パン」と「オリジナル真正牛乳パン」を比較したところ、「改良型真正牛乳パン」は高さが 3.2 cm「オリジナル真正牛乳パン」を上回るとともに、官能評価では「香り」「しっとり感」「味」及び「総合評価」の項目で有意に高い評価を得たことから、乳糖とバターの増量が「オリジナル真正牛乳パン」の品質改良をもたらすことが示された。

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” (hereinafter, this is called “original authentic milk bread”) without adding sugar and water (Shibata A. *et al*., *Curr. Top. Biotech*., **11**: 31-36, 2020). Although the “original authentic milk bread” was made adding no lactose (of course, lactose is contained in milk) and 7 g of unsalted butter, we added 5 g of lactose and 20 g of unsalted butter in order to improve the “original authentic milk bread” in this study. The height of the “improved authentic milk bread” was 3.2 cm higher than that of the “original authentic milk bread”. In addition, the sensory evaluation revealed that the “improved authentic milk bread” is significantly superior to the “original authentic milk bread” in some inspection items (fragrance, moist feeling, taste and overall quality). These results revealed that addition of more lactose and butter would improve the quality of the “original authentic milk bread”.

Key words: authentic milk bread, *Kluyveromyces marxianus*, improvement, lactose-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)–5)]. Recently, we developed the "authentic milk bread" (hereinafter, this is called "original authentic milk bread"), which was made without addition of sucrose and water using a lactose-utilizing yeast *K. marxianus* as a baker's yeast[6)] instead of *Saccharomyces cerevisiae*. Next, in order to improve the nutritional properties of the "original authentic milk bread", we increased the amount of milk added from 250 mL to 300 mL[7)]. Because the milk-rich "original authentic milk bread" 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 milk-rich "original authentic milk bread". In this paper, we aimed to improve the overall quality of the "original authentic milk bread" by addition of more lactose and butter. The "improved authentic milk bread" rose very well, the height of it was higher than that of the "original authentic milk bread". Sensory evaluation test revealed that the quality of the "improved authentic milk bread" is significantly better than that of the "original authentic milk bread".

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 "improved authentic milk bread" was made as follows. *K. marxianus* cells (5 g, wet weight) were suspended in 250 mL of milk (Kumamoto Dairy, Kumamoto, Japan), and mixed with 280 g of commercially available bread flour with a protein content 12.6 grams per 100 grams (Nisshin Seifun, Tokyo, Japan), 5 g of lactose (LOHAStyle 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). In contrast, the "original authentic milk bread" was made as follows. *K. marxianus* cells (5 g, wet weight) were suspended in 250 mL of milk, and mixed with 280 g of commercially available bread flour, 4 g of salt and 7 g of unsalted butter. 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. In contrast, the "original authentic milk bread" was made using no lactose and 7 g of unsalted butter as described previously[6)]. The making methods of these two types of bread are the same except for the addition of lactose and butter usage. Three pieces of the bread 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 and cross-sections of the breads were taken using a digital camera (Canon, Tokyo, Japan).

4. Sensory evaluation

To assess quality of the "improved authentic milk bread", a sensory evaluation test was carried out as described previously[6)]. Twenty-five 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.

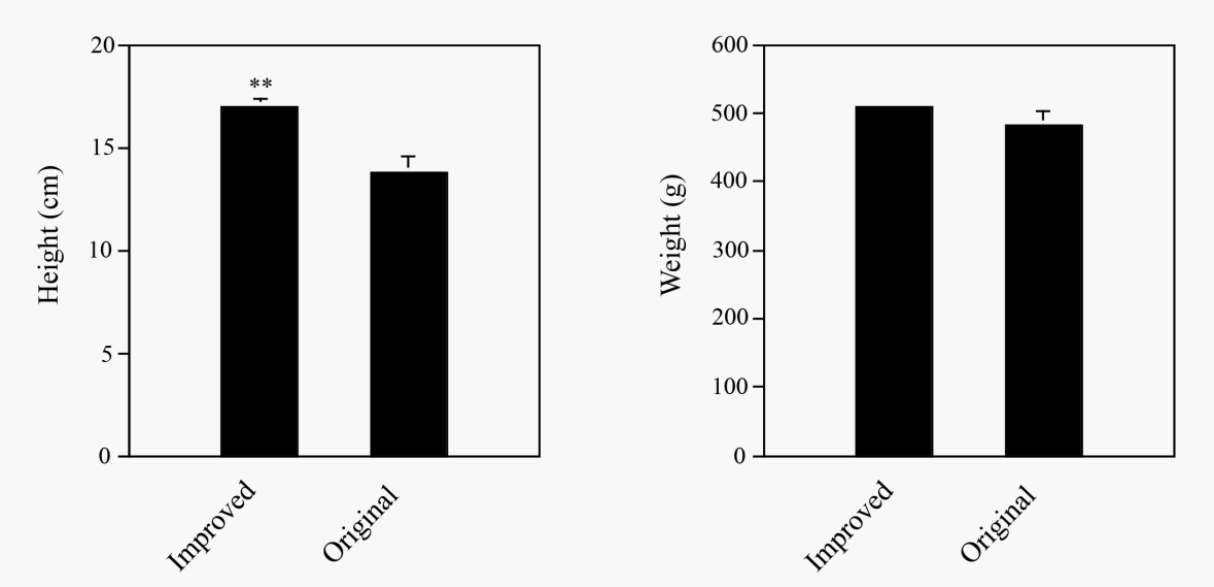

Fig. 1. The height and weight of the "improved authentic milk bread" and the "original authentic milk bread". The height and weight of the breads were measured with a ruler and a scale, respectively. Data are presented as averages of measured values from three independent breads. Error bars indicate standard deviation. Statistical differences were calculated using Student's *t* test. **, $P < 0.01$ compared with the data of the "original authentic milk bread".

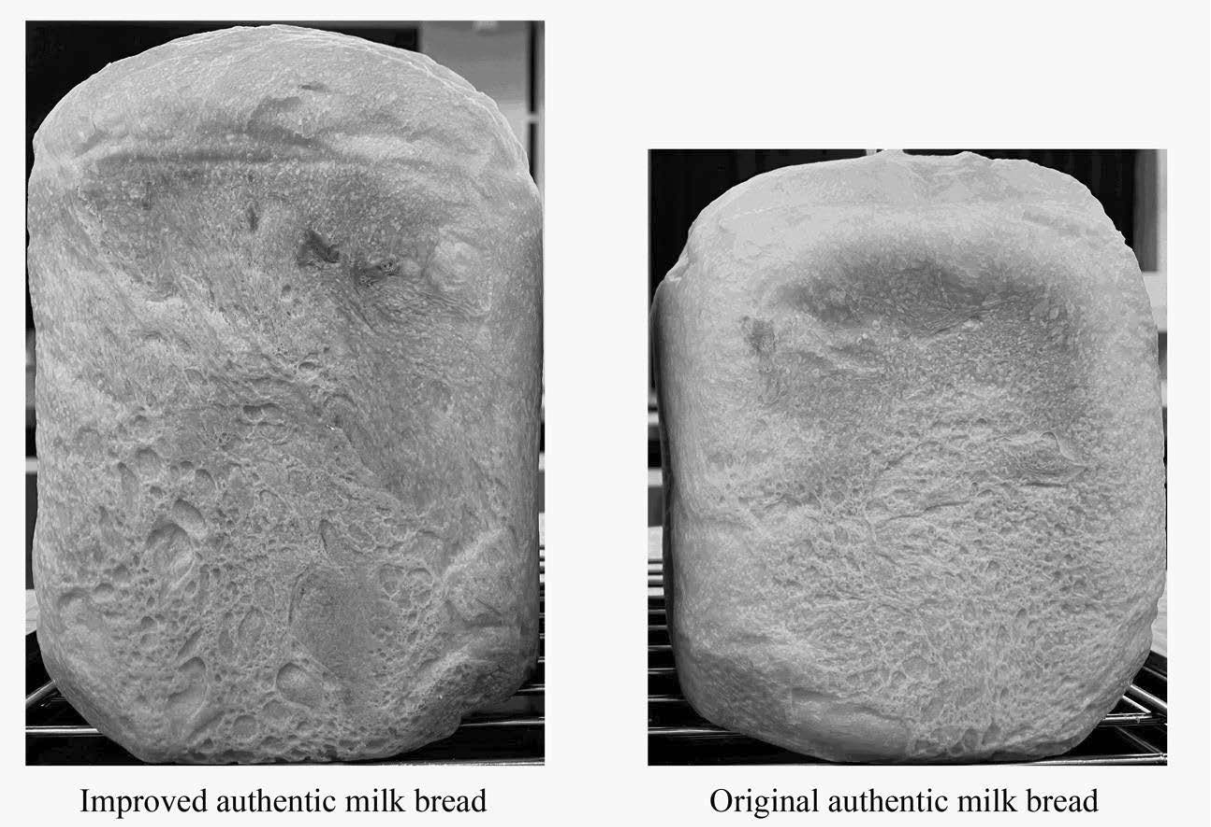

Fig. 2. Side views of the "improved authentic milk bread" and the "original authentic milk bread".

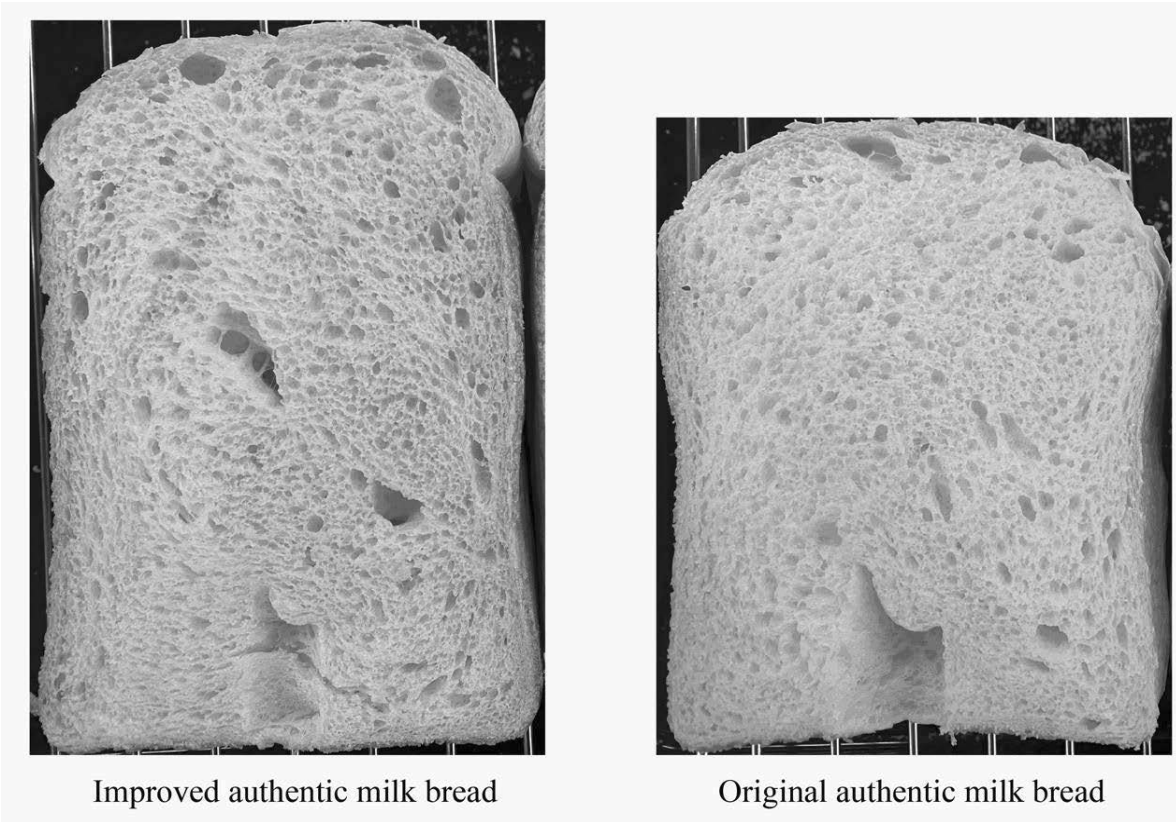

Fig. 3. Cross-sections of the "improved authentic milk bread" and the "original authentic milk bread".

Table 1. Sensory evaluation of the "improved authentic milk bread" and the "original authentic milk bread".

Inspection items	Improved authentic milk bread	Original authentic milk bread	Values of p^*	Statistical significance
Appearance	1.60 ± 0.76	1.48 ± 0.77	$p > 0.05$	No
Color	1.56 ± 0.77	1.48 ± 0.82	$p > 0.05$	No
Fragrance	1.68 ± 0.63	1.12 ± 0.88	$p < 0.05$	Yes
Moist feeling	1.44 ± 0.92	0.92 ± 0.86	$p < 0.05$	Yes
Chewy texture	1.40 ± 0.82	1.16 ± 0.62	$p > 0.05$	No
Taste	1.80 ± 0.50	1.20 ± 0.65	$p < 0.05$	Yes
Overall quality	1.84 ± 0.37	1.40 ± 0.58	$p < 0.05$	Yes

* Statistical differences were calculated using Student's *t* test.

Twenty-five volunteer tasters evaluated pieces of the "improved authentic milk bread" and the "original authentic milk bread". 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 ($n = 25$), and error bars indicate standard deviation.

Data of sensory evaluation are presented as averages of the evaluation scores (n = 25). Statistical differences between the "improved authentic milk bread" and the "original authentic milk bread" were calculated with Student's t-test.

Results and Discussion

In our previous study, we developed the "original authentic milk bread" using *K. marxianus*[6]. Sensory evaluation showed that the quality of the "original authentic milk bread" is not inferior as compared to the typical bread made using a commercial dry yeast. We expect that the quality (*e.g.* texture and taste) of the "original authentic milk bread" can be improved further through additional research. Therefore, in this paper, in order to improve the quality of the "original authentic milk bread", we made them by increasing 5 g of lactose and 13 g (total 20 g) of butter. Because 250 mL of milk would contain about 12.5 g of lactose in general[8], about 17.5 g of total lactose would be used for making the "improved authentic milk bread". Increased lactose and butter would improve the rise and taste of the "original authentic milk bread", respectively.

Figure 1 shows the effects of addition of more lactose and butter on the height and weight of the "authentic milk bread". The height of the "improved authentic milk bread" was significantly taller than that of the "original authentic milk breads" by 3.2 cm. Typical appearances of the side view of these two types of bread are shown in Fig. 2. In contrast, the weight of the "improved authentic milk bread" was almost the same as that of the "original authentic milk bread". Next, the cross-sections of these two types of bread are displayed in Fig. 3. There seemed to be some differences between the "improved authentic milk bread" and the "original authentic milk bread" in distribution and size of gas bubbles (Fig. 3).

Finally, in order to evaluate the quality of the "improved authentic milk bread", sensory evaluation was performed using the "improved authentic milk bread" and the "original authentic milk bread. Comparison data for sensory evaluation are shown in Table 1. The significance threshold was set at p = 0.05 in Student's t-test. As expected, these data reveal that "improved authentic milk bread" would be significantly superior to the "original authentic milk bread" in fragrance, moist feeling, taste and overall quality.

In this study, we succeeded in developing the "improved authentic milk bread" by addition of more lactose and butter. In addition, increases of both lactose and butter were necessary for making the "improved authentic milk bread" (data not shown). On the other hand, as shown in our previous report[6], the quality of the "original authentic milk bread" was assessed the same level as that of the bread made using a commercial dry yeast. Therefore, the quality of the "improved authentic milk bread" seems to be very high. Recently, we successfully developed a unique type of bread using inulin as the only carbohydrate source with *K. marxianus*[9]. In the current situation, *K. marxianus* would not be pretty much used for making bread. However, our data shows that *K. marxianus* has the potential to be an excellent baker's yeast. We hope that the establishment of producing dry yeast and breeding of *K. marxianus* can transform it into an excellent baker's yeast next to *S. cerevisiae* in the future.

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