Current Topics in
Biotechnology
Vol. 11, 2020

Original Article

Production and evaluation of the authentic milk bread using a lactose-utilizing yeast, *Kluyveromyces marxianus*

Aya Shibata[1,#], Kaori Harata[1,#], Yuko Moriguchi[1], Risako Kanegawa[1], Ayaka Nagahira[1], Misaki Shigematsu[1], Yuri Takamura[1], Haruno Watanabe[1], Takefumi Sagara[1], Harishkumar Madhyastha[2] and Hidehiko Kikuchi[1,*]

[1]Department of Food and Nutrition, Shokei University Junior College, 2-6-78 Kuhonji, Chuo-ku, Kumamoto 862-8678, Japan; [2]Department of Applied Physiology, Faculty of Medicine, University of Miyazaki, 5200, Kihara, Kiyotake, Miyazaki 889-1692, Japan.

ABSTRACT

Since *Saccharomyces cerevisiae* (generally used as baker's yeast) does not express β-galactosidase, it cannot utilize lactose in milk. On the other hand, *Kluyveromyces marxianus* that has β-galactosidase can hydrolyze lactose into glucose and galactose. In this paper, we produce the "authentic" milk bread that is prepared without addition of sucrose and water, using *K. marxianus* as the only baker's yeast and performed evaluation of the authentic milk bread in comparison to bread made using the commercial dry yeast (the typical bread). Various evaluation tests (measurements of height, weight, L*, a*, b*, ΔE, hardness and cohesiveness, and sensory evaluation) revealed that the quality of our authentic milk bread comprehensively was not inferior as compared with the typical bread.

KEYWORDS: baker's yeast, bread, *Kluyveromyces marxianus*, lactose, milk.

INTRODUCTION

Bread has been eaten as one of the staple foods in the world. Although there are many kinds of bread, they are usually produced using flour, water, sugar (carbon sources for yeast), salt, fat and yeast. As is well known, *Saccharomyces cerevisiae* is the main yeast used in making breads, and is also called as baker's yeast. In general, *S. cerevisiae* uses sucrose and/or maltose as carbon sources in bread making. However, *S. cerevisiae* cannot metabolize lactose because it expresses no β-galactosidase (also called as lactase, EC 3.2.1.23) that catalyzes hydrolysis of lactose into glucose and galactose [1]. Therefore, lactose cannot be used as a carbon source in the process of bread preparation.

Lactose-utilizing yeasts *Kluyveromyces* spp. (*e.g.* *K. lactis* and *K. marxianus*) were found from traditional alcoholic fermented milk such as airag in Mongolia [2]. They produce β-galactosidase which is the most important enzyme for the utilization of lactose. In the dairy industry, β-galactosidase from *Kluyveromyces* spp. is used for the production of low-lactose-containing dairy products such as lactose-hydrolyzed milk for lactose intolerant patients [3].

Interestingly, several researchers reported that bread could be produced using *K. marxianus* instead of *S. cerevisiae.* Two strains of *K. marxianus* were used for making of bread containing lactose or whey [4]. While these two strains showed superior proofing activity compared to commercial baker's yeast strains, no difference in bread flavor was shown. Dimitrellou *et al*.

*Corresponding author: masakari@shokei-gakuen.ac.jp
#These authors contributed equally to this work.

carried out bread making using thermally dried *K. marxianus* as baker's yeast [5]. In addition, *K. marxianus* was used for bread making in combination with other microorganisms such as *S. cerevisiae* or *Lactobacillus* spp. [6, 7].

In this paper, we produce the "authentic" milk bread without addition of sugar (sucrose) and water using *K. marxianus* as the only baker's yeast and perform evaluation of the bread.

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, 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.

Bread making using *K. marxianus*

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 (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 bread were independently baked.

Bread making using commercial dry yeast

In parallel, breads for comparison of quality (called as "the typical breads", described below) were made using commercial dry yeast (Nisshin Seifun, Tokyo, Japan). Dry yeast (2.4 g) and 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) were used. Bread making was carried out using a bread maker HBK-101 in the normal mode according to the attached manual. Three pieces of the typical bread were independently baked, and used as standards for evaluation of the authentic milk bread as described below.

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, and incubated at room temperature for 20 h in plastic bag. The height and weight of the breads were measured with a ruler and a scale, respectively. Images of the side view 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).

Evaluation of color of the breads

L*, a* and b* values of the breads (surface and cross section) were measured using a colorimetric method (model CR-300, Konica Minoruta, Tokyo, Japan). Total color differences (ΔE) were calculated from these L*, a* and b* values.

Evaluation of physical properties of the breads

The breads were divided into rectangles (40 mm × 40 mm × 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 breads were measured using a creep meter (model TPU, Yamaden, Tokyo, Japan) using these pieces of the breads. The measurement conditions were as follows: plunger, circular form (8 mm in diameter); measurement strain rate, 50%; compression speed, 5 mm/s.

Sensory evaluation

To assess quality of the breads, a sensory evaluation test was carried out. The breads produced using *K. marxianus* (called as "the authentic milk bread") were compared with the breads produced using commercial dry yeast (called as "the typical bread"). After removing crust, these two types of breads were divided into cubes (20 mm × 20 mm × 20 mm) using a bread cutter. Twenty-three 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.

Statistical analysis

Data (height, weight, L*, a*, b*, hardness and cohesiveness) are presented as averages of measured values from three each of these two kinds of breads (the authentic milk breads and the typical breads). Data of sensory evaluation are presented as averages of the evaluation scores (n = 23). Statistical differences between the typical breads and the authentic milk breads were calculated with Student's t-test.

RESULTS AND DISCUSSION

General properties of the breads

We prepared the authentic milk bread that was made from flour, milk, salt and fat (without addition of sugar and water) using *K. marxianus*, and evaluated them from various perspectives as compared with the typical bread as follows. The height and weight of the authentic milk bread were almost the same as that of the typical bread (Figure 1). In addition, there was almost no difference in the appearance of the side view and the cross-sections of these two types of breads (Figure 2). Non-utilization of sucrose and water showed little effect on the bread baking. In other words, the lactose and moisture alone contained in milk would be enough to raise the bread. As a side note, about 12.5 g of lactose is contained in 250 mL of milk because milk contains ~5% of lactose in general [8]. The comparison of L*, a*, b* and ΔE values between the typical bread and the authentic milk bread is shown in Table 1. In both the side view and the cross-sections of these two types of breads, there was no significant difference in the four values tested. Moreover, hardness and cohesiveness of the authentic milk bread were not significantly different from those of the typical bread (Figure 3). These results showed that *K. marxianus* could produce morphologically good-looking bread under the condition mentioned above.

Sensory evaluation of the breads

To evaluate the quality of the authentic milk bread, sensory evaluation was carried out. Figure 4 shows the results of sensory evaluation. Surprisingly, sensory evaluation on the seven inspection items (appearance, color, fragrance, moist feeling, chewy texture, taste and overall quality) of the authentic milk bread has no significant difference with respect to that of the typical bread. On the other hand, the bread made from only flour and milk (without salt and fat) was remarkably inferior to the authentic

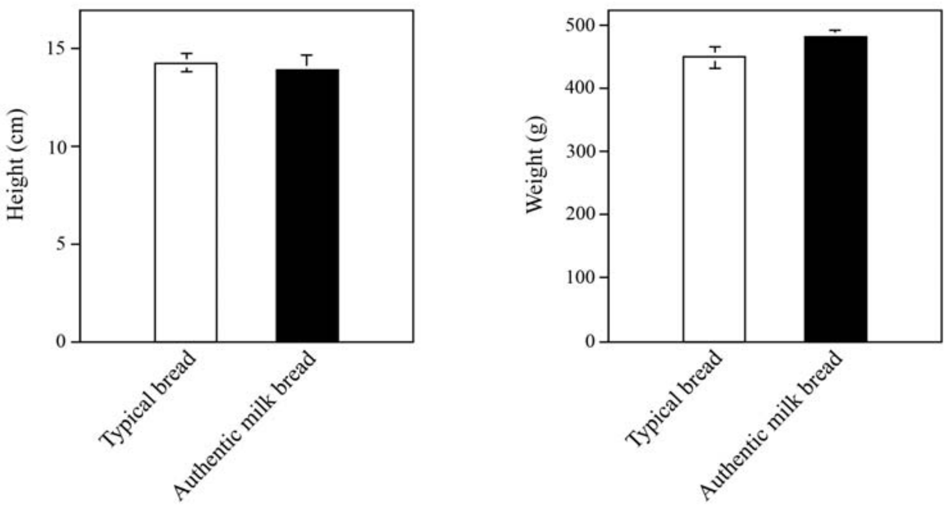

Figure 1. The height and weight of the typical bread and the authentic milk bread. After baking, the breads were cooled down to room temperature for 1 h, and incubated at room temperature for 20 h in plastic bags. The height and weight of the breads were measured with a ruler and a scale, respectively. 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.

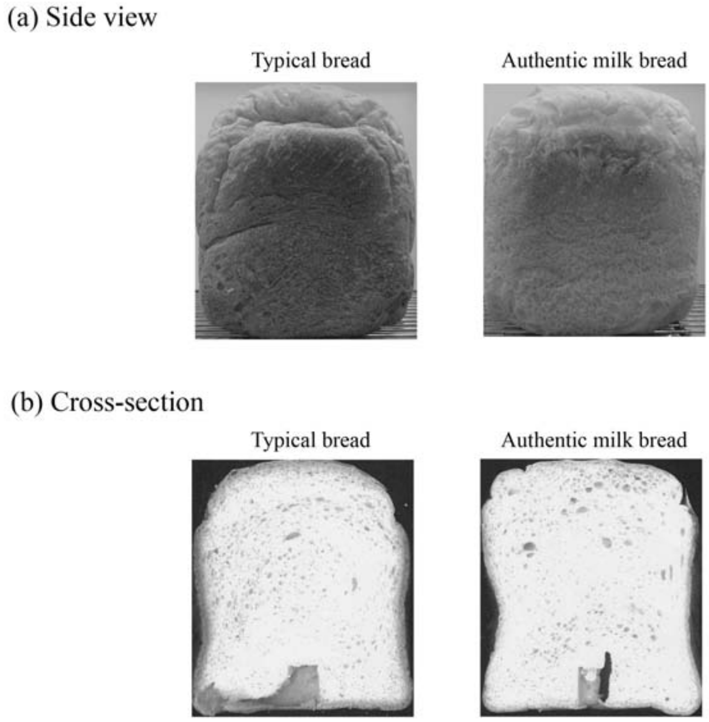

Figure 2. Appearance of the typical bread and the authentic milk bread. (a) Side views of the typical bread and the authentic milk bread, (b) Cross-sections of the typical bread and the authentic milk bread.

Table 1. Differences in characteristics of color (L*, a* and b* values) between the typical bread and the authentic bread.

	Typical bread			**Authentic milk bread**			
	L*	**a***	**b***	**L***	**a***	**b***	**ΔE**
Cross-section	67.49 ± 2.66	-3.54 ± 0.55	12.03 ± 1.19	68.57 ± 3.68	-3.39 ± 0.33	11.03 ± 1.94	1.48
Surface	51.97 ± 3.08	7.50 ± 1.12	20.42 ± 2.31	55.08 ± 6.96	6.79 ± 2.01	21.51 ± 3.76	3.37

Data (L*, a* and b*) are shown as means ± standard deviation (n = 3).

milk bread in eating quality (data not shown). Although *K. lactis* could raise breads as with *K. marxianus*, the bread made using *K. lactis* was inferior to the bread made using *K. marxianus* in fragrance and eating quality (data not shown). These results showed that *K. marxianus* might produce the high-quality milk bread.

CONCLUSION

In this study, we developed the authentic milk bread that was made from flour, milk, salt and fat (without addition of sugar and water) using *K. marxianus* as the only baker's yeast and performed the evaluation tests in comparison to the typical bread. Interestingly, various evaluation tests revealed that the authentic milk bread developed by us has no inferiority as compared with the typical bread on the whole.

On the other hand, 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

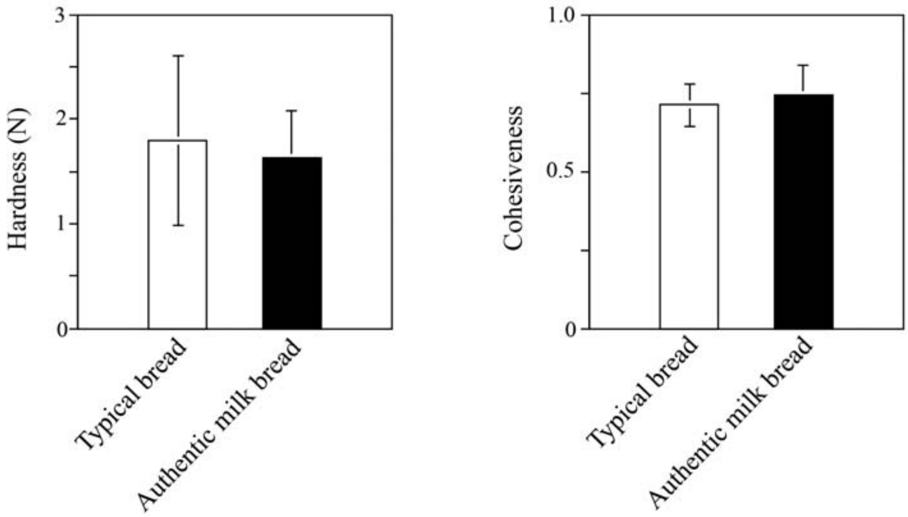

Figure 3. Physical properties of the typical bread and the authentic milk bread. The breads were divided into rectangles (40 mm × 40 mm × 20 mm) using a slicer and a bread cutter. The physical properties (hardness and cohesiveness) of the breads were measured using a creep meter with these rectangular pieces of the breads. 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.

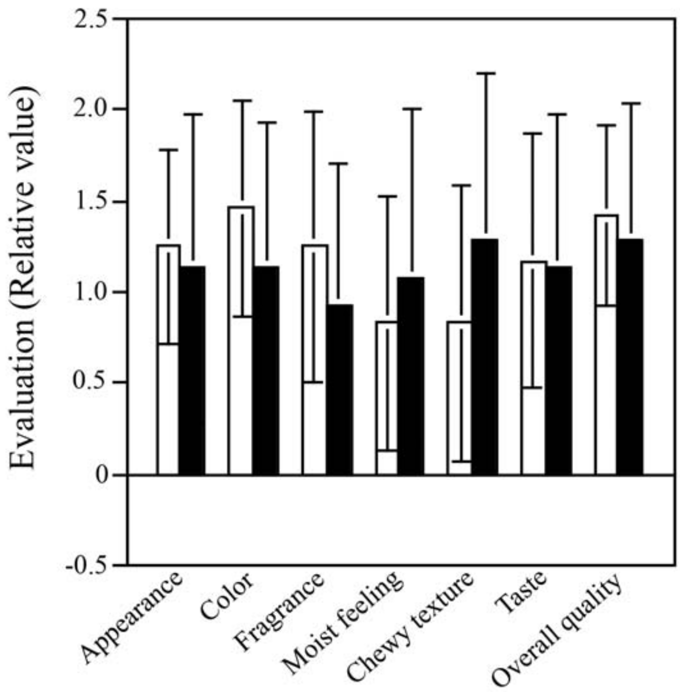

Figure 4. Sensory evaluation of the typical bread and the authentic milk bread. Twenty-three volunteer tasters evaluated pieces of the typical bread (open bars) and the authentic milk bread (closed bars) samples. They gave the evaluation scores as follows: -2 (bad), -1 (slightly bad), 0 (neither), +1 (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 = 23), and error bars indicate standard deviation. Statistical differences were calculated using Student's *t* test.

consuming the authentic milk bread containing milk proteins. If milk was used as one of the ingredients of breads in order to improve nutritional property of the breads, almost all of the lactose remains in the breads by using *S. cerevisiae* instead of *K. marxianus*. In this case, it may be difficult for lactose-intolerant patients to intake the breads containing lactose. In addition, this bread would be higher in energy than the authentic milk bread because of the residual lactose. In contrast, these problems can be probably resolved by the authentic milk bread because certain lactose would be probably consumed by *K. marxianus*. Moreover, the authentic milk bread may contribute effective utilization of the surplus milk. Of course, the quality (e.g. texture and taste) of our authentic milk bread can be improved further through additional research. We would like to promote future development of the authentic milk breads.

ACKNOWLEDGEMENTS

We thank R. Madhyastha for editorial reading of the manuscript.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

REFERENCES

1. Kikuchi, H. 2018, J. Assoc. Food Sci. Edu. Japan, 9, 9-12.
2. Miyamoto, T. 2015, Sci. Rep. Fac. Agric. Okayama Univ., 104, 35-47.
3. Hronska, H., Grosova, Z. and Rosenberg, M. 2009, J. Food Nutr. Res., 48, 87-91.
4. Caballero, R., Olguin, P., Cruz-Guerrero, A., Gallardo, F., Garcia-Garibay, M. and Gomez-Ruiz, L. 1995, Food Res. Int., 28, 37-41.
5. Dimitrellou, D., Kandylis, P., Kourkoutas, Y., Koutinas, A. A. and Kanellaki, M. 2009, Food Chem., 115, 691-696.
6. Plessas, S., Bekatorou, A., Gallanagh, J., Nigam, P. and Koutinas, A. A. 2008, Food Chem., 107, 883-889.
7. Plessas, S., Fisher, A., Koureta, K., Psarianos, C., Nigam, P. and Koutinas, A. A. 2008, Food Chem., 106, 985-990.
8. Jenkins, T. C. and McGuire, M. A. 2006, J. Dairy Sci., 89, 1320-1310.