

Production of the whole wheat flour-containing inulin bread using an inulin-utilizing yeast *Kluyveromyces marxianus*

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(Accepted Dec. 26, 2025)

要旨：私たちは、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)。このパンではイヌリンを発酵用炭水化物として使用しているため、本来イヌリンに期待される食物繊維としての機能は期待しにくい。そこで、本研究では、食物繊維を豊富に含むイヌリンパンの製造を目的として全粒粉を添加した。パンの製造には市販のホームベーカリーを用いた。使用した小麦粉全量に対して全粒粉の含量を 25%と 50%とで試したが、本研究では最終的に 25%を採用した。一方、25%全粒粉を含むパンの製造の際のグルテン 5 g の添加は、パンの膨らみに大きな変化をもたらさなかった。官能評価では、「25%全粒粉入りイヌリンパン」を「ドライイーストで製造した 25%全粒粉入りのパン」または「全粒粉を含まないイヌリンパン」を比較した。「25%全粒粉入りイヌリンパン」は、比較した他のパンと殆どの比較項目で有意な差を示さなかった。全体的にみて「25%全粒粉入りイヌリンパン」は常食として十分なポテンシャルを有することが示された。

キーワード：イヌリンパン、全粒粉、イヌリン資化性酵母、官能評価

Summary

We developed the “inulin bread” using inulin as the sole fermentable carbohydrate with an inulin-utilizing yeast, *Kluyveromyces marxianus* (Kikuchi H. *et al.*, *Curr. Top. Biotech.*, **14**: 13-18, 2023). Because *K. marxianus* utilized inulin as the fermentable carbohydrate, it is difficult to expect the function of inulin as a dietary fiber. Therefore, in this study, whole wheat flour was added to produce the inulin bread rich in dietary fiber. Breads were produced using a commercially available bread maker. At first, the whole wheat flour-containing breads were produced using wheat flour containing 25% or 50% whole wheat flour, but finally 25% was adopted in this study. On the other hand, the addition of 5 g of gluten did not significantly change the rise of the whole wheat flour-containing bread. In the sensory evaluation, the “inulin bread containing 25% whole wheat flour” was compared with the “typical bread containing 25% whole wheat flour bread made with dry yeast” or the “inulin bread without whole wheat flour.” The sensory evaluation showed that the “inulin bread containing 25% whole wheat flour” did not show significant differences in most of the comparison items compared to other two breads. These data suggested that the “inulin bread containing 25% whole wheat flour” has a sufficient potential to be a staple food.

Key words: inulin bread, whole wheat flour, inulin-utilizing yeast, sensory evaluation

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Introduction

Kluyveromyces marxianus, which is known as an inulin/lactose-utilizing yeast, was isolated from traditional alcoholic fermented milk¹⁾, and has also been used as a baker's yeast²⁻⁸⁾. We developed the “authentic milk bread” by fermenting milk using *K. marxianus* as a baker's yeast⁶⁻⁸⁾ instead of ordinary baker's yeast *Saccharomyces cerevisiae*. Furthermore, we successfully developed a new type of bread (called as the “inulin bread”), which was produced using inulin as a fermentable carbohydrate with *K. marxianus*⁹⁾. The inulin bread is expected to contribute to expanding the uses of various inulin-containing crops such as Jerusalem artichoke tubers. Unfortunately, it is difficult to expect the function of inulin as a dietary fiber in the inulin bread because *K. marxianus* digested inulin as the fermentable carbohydrate. Therefore, in this study, we made the inulin bread rich in dietary fiber by addition of whole wheat flour in order to improve the quality of the inulin bread. In addition, gluten was added to improve the rise of bread. Finally, in the sensory evaluation, the quality of the “inulin bread containing whole wheat flour” was compared with those of the “typical bread⁶⁾ containing whole wheat flour bread made with dry yeast” or the “inulin bread without whole wheat flour.”

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*

A loaf of the inulin bread was made as follows. *K. marxianus* cells (5 g, wet weight) were suspended in 210 mL or 250 mL tap water, and mixed with commercially available bread flour

containing 0.33% ash and 11.5% crude protein (Nisshin Seifun, Tokyo, Japan), whole wheat flour (Amazon com, Inc., Seattle, WA, USA), 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). The amounts of bread flour and whole wheat flour were indicated as follows: bread flour 280 g and whole wheat flour 0 g (without whole wheat flour), bread flour 210 g and whole wheat flour 70 g (whole wheat flour 25%), and bread flour 140 g and whole wheat flour 140 g (whole wheat flour 50%). Five grams of gluten (Glico Nutrition Co., Ltd., Osaka, Japan) was added if necessary. 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.

3. Bread making using commercial dry yeast

In parallel, bread (called as the “typical breads”⁶⁾) containing 25% whole wheat flour was made using commercial dry yeast (Nisshin Seifun, Tokyo, Japan) for comparison of quality. The dry yeast (2.4 g), 210 mL of tap water, and mixed with 210 g of bread flour, 70 g of whole wheat 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.

4. Measurement of the 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).

5. Sensory evaluation

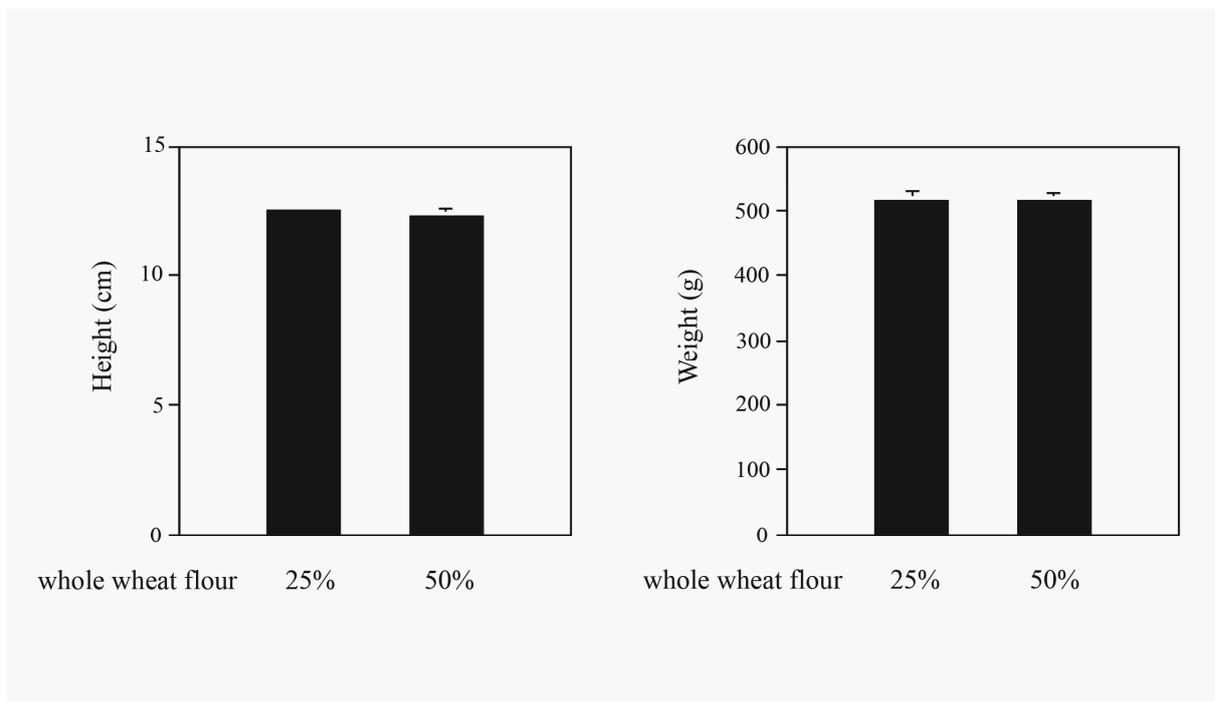
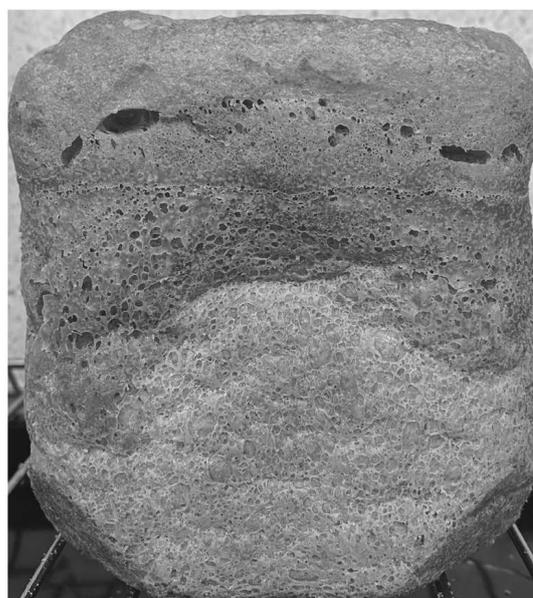


Fig. 1. The height and weight of the inulin bread containing whole wheat flour. The amounts of bread flour and whole wheat flour were indicated as follows: bread flour 210 g and whole wheat flour 70 g (25% whole wheat flour), and bread flour 140 g and whole wheat flour 140 g (50% whole wheat flour). The height of the breads was measured with a ruler, and 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.

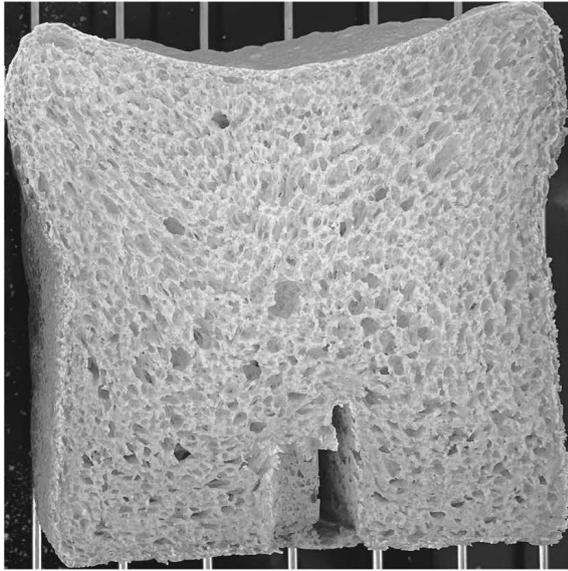


25% whole wheat flour

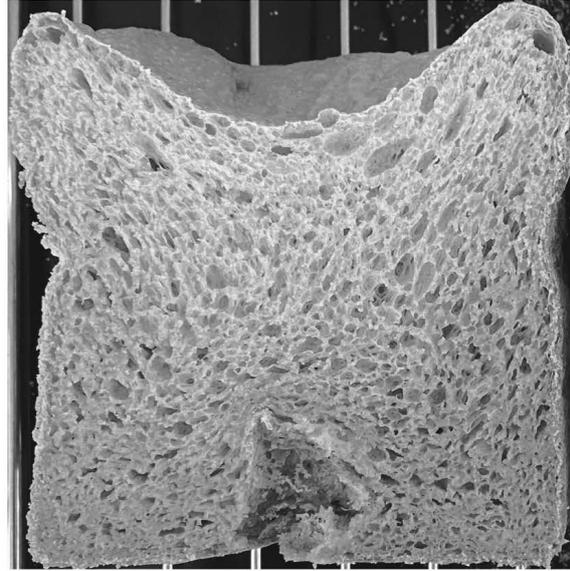


50% whole wheat flour

Fig. 2. Side views of the inulin bread containing whole wheat flour.



25% whole wheat flour



50% whole wheat flour

Fig. 3. Cross-sections of the inulin bread containing whole wheat flour.

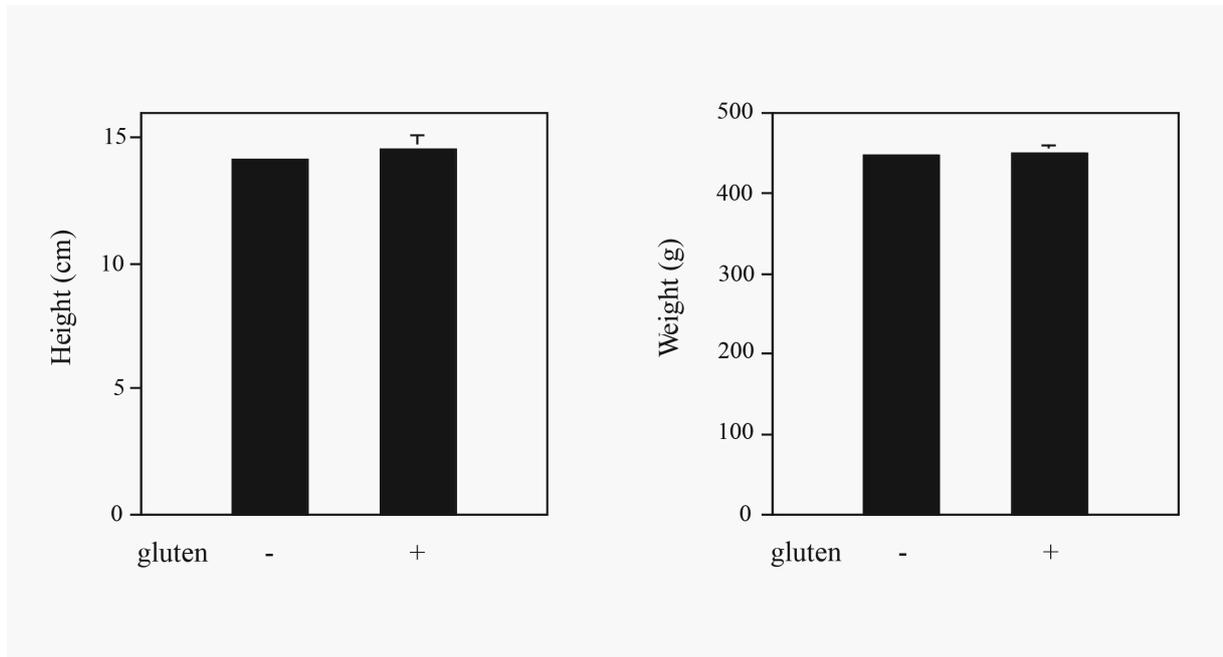


Fig. 4. The effects of addition of 5 g gluten on the height and weight of the inulin bread containing 25% whole wheat flour (bread flour 210 g and whole wheat flour 70 g). The height of the breads was measured with a ruler, and the weight of the breads was measured with a scale. Data are presented as averages of measured values from three independent loaves of the breads.



gluten –

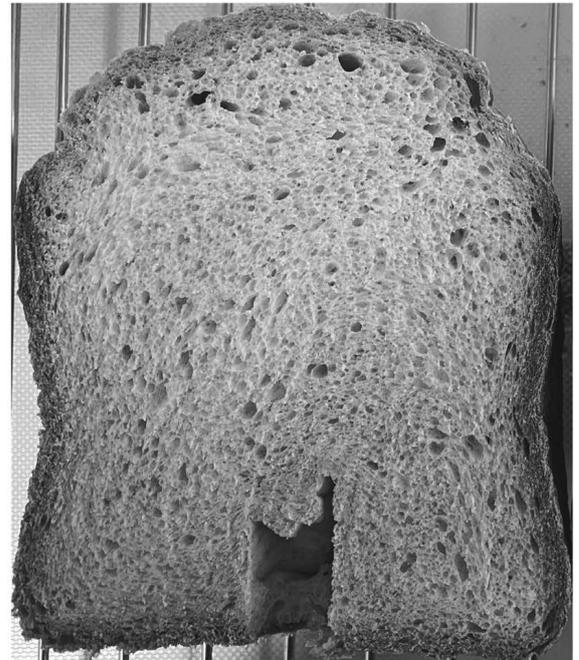


gluten +

Fig. 5. Side views of the inulin bread containing whole wheat flour with or without 5% gluten.



gluten –



gluten +

Fig. 6. Cross-sections of the inulin bread containing whole wheat flour with or without 5% gluten.

Table 1. Sensory evaluation of the inulin bread containing 25% whole wheat flour and the typical bread containing 25% whole wheat flour.

Inspection items	Inulin bread containing 25% whole grain	Typical bread containing 25% whole grain	P value *	Statistical significance
Appearance	2.63 ± 1.02	2.82 ± 0.87	$p > 0.05$	No
Color	2.61 ± 0.97	2.89 ± 0.83	$p > 0.05$	No
Flavor	2.45 ± 1.13	2.92 ± 1.00	$p > 0.05$	No
Moist feeling	2.66 ± 0.97	2.84 ± 1.00	$p > 0.05$	No
Chewy texture	2.50 ± 0.98	2.61 ± 0.95	$p > 0.05$	No
Taste	2.68 ± 1.02	2.87 ± 0.90	$p > 0.05$	No
Overall quality	2.79 ± 1.02	2.87 ± 0.88	$p > 0.05$	No

Thirty-eight volunteer tasters evaluated pieces of the “inulin bread containing 25% whole wheat flour” and the “typical bread containing 25% whole wheat flour”. 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 ($n = 38$). * Statistical differences were calculated using Student’s *t* test.

Table 2. Sensory evaluation of the inulin bread containing 25% whole wheat flour and the inulin bread without whole wheat flour.

Inspection items	Inulin bread containing 25% whole grain	Inulin bread without whole grain	P value *	Statistical significance
Appearance	2.63 ± 1.02	3.00 ± 0.90	$p > 0.05$	No
Color	2.61 ± 0.97	3.16 ± 0.82	$p < 0.05$	Yes
Flavor	2.45 ± 1.13	2.76 ± 0.97	$p > 0.05$	No
Moist feeling	2.66 ± 0.97	2.50 ± 1.16	$p > 0.05$	No
Chewy texture	2.50 ± 0.98	2.82 ± 1.23	$p > 0.05$	No
Taste	2.68 ± 1.02	3.16 ± 1.03	$p > 0.05$	No
Overall quality	2.79 ± 1.02	3.24 ± 0.88	$p > 0.05$	No

Thirty-eight volunteer tasters evaluated pieces of the “inulin bread containing 25% whole wheat flour” and the “inulin bread without whole wheat flour”. 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 ($n = 38$). * Statistical differences were calculated using Student’s *t* test.

To assess quality of the inulin bread containing 25% whole wheat flour, a sensory evaluation test was carried out as described⁶⁻⁹⁾. Thirty-eight of volunteer tasters (female students aged 19-20) 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.

6. Statistical analysis

Data (the 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 ($n = 38$). Statistical differences were calculated with Student's *t*-test.

Results and Discussion

We have previously reported that the “inulin bread” is produced using purified inulin as the sole carbohydrate source fermented by *K. marxianus*⁹⁾. As is well known, inulin is becoming a focus of attention as an effective dietary fiber. However, it is difficult to expect the function of inulin as a dietary fiber in the inulin bread because *K. marxianus* utilized inulin as the fermentable carbohydrate. On the other hand, as is widely known, whole wheat flour is rich in dietary fiber. Therefore, in this study, we produced the “inulin bread containing whole wheat flour” so as to endow the inulin bread with function of the food containing dietary fiber.

First, we considered the content of whole wheat flour added in the inulin bread. When making the inulin bread containing 50% whole wheat flour, 250 mL of tap water was added to the dough in order to knead well. To set the same condition, 250 mL tap water also used for production of the inulin bread containing 25% whole wheat flour. Figure 1 shows the effects of the amounts of whole wheat flour added on the height and weight of the inulin bread. No differences were observed between the inulin bread containing 25%

whole wheat flour and the inulin bread containing 50% whole wheat flour. Typical appearances of the side view of these two breads are shown in Fig. 2. No difference was observed between the two breads. In contrast, there was an interesting difference in the cross-sections between the inulin bread containing 25% whole wheat flour and the inulin bread containing 50% whole wheat flour (Fig. 3). The top of the inulin bread containing 50% whole wheat flour appeared to be concave compared to that of the inulin bread containing 25% whole wheat flour. This phenomenon may be due to inhibition of bread rise by whole wheat flour. Moreover, texture and taste of the inulin bread containing 50% whole wheat flour also seemed to be inferior to those of the inulin bread containing 25% whole wheat flour. Therefore, the inulin bread containing 25% whole wheat flour was produced in the following experiments.

Second, we examined the effect of addition of gluten on the bread rise. When making the inulin bread containing 25% whole wheat flour, 210 mL of tap water was sufficient for kneading dough well. Unexpectedly, the addition of 5 g gluten had little effect on the height, weight, appearances of the side view and appearances of the cross-section of the bread (Fig. 4-6). However, the top of the bread was properly risen with or without addition of gluten. These results suggested that 250 mL of tap water seemed too much and inappropriate for making the inulin bread containing 25% whole wheat flour using *K. marxianus* (see Fig. 1).

Finally, in order to evaluate the quality of the inulin bread containing 25% whole wheat flour, sensory evaluation was performed using the typical bread containing 25% whole wheat flour and the inulin bread without whole wheat flour as comparison targets. Comparison data of sensory evaluation comparing the inulin bread containing 25% whole wheat flour and the typical bread containing 25% whole wheat flour are shown in Table 1. The evaluation values of the inulin bread containing 25% whole wheat flour were roughly equivalent to those of the typical bread containing 25% whole wheat flour in all

evaluation items. On the other hand, comparison data of sensory evaluation comparing the inulin bread containing 25% whole wheat flour and the inulin bread without whole wheat flour are shown in Table 2. The evaluation values of the inulin bread containing 25% whole wheat flour were significantly inferior only in color, but there was no significant difference between the inulin bread containing 25% whole wheat flour and the inulin bread without whole wheat flour in other evaluation items. As mentioned above, female students aged 19-20 participated in the sensory evaluation as participants. Of course, the results of sensory evaluation may be different if it is performed by different age or sex groups as participants.

In this study, we succeeded in producing the inulin bread containing 25% whole wheat flour using *K. marxianus*. In other words, the inulin bread rich in dietary fiber could be produced without significantly compromising texture, taste and so on. Our data obtained suggested that the “inulin bread containing 25% whole wheat flour” has a sufficient potential to be a staple food.

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. 2024-02), and informed consent was obtained from all participants.

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