

## Selection of Yeasts for Breadmaking by the Frozen-Dough Method

YUJI ODA,\* KAZUO UNO, AND SHIGENORI OHTA

*Tokyo Research Laboratory, Kyowa Hakko Co., Ltd., Machida-shi, Tokyo 194, Japan*

Received 16 May 1986/Accepted 8 July 1986

**Eleven yeast strains suitable for frozen dough were selected from over 300 *Saccharomyces* strains. All of these were identified as *Saccharomyces cerevisiae* from morphological, cultural, and physiological characteristics. The selected yeast cells accumulated a higher amount of trehalose than did commercial bakers' yeast cells.**

Frozen dough has been paid increasing attention in the baking industry (2). Well-prepared frozen dough, which is brought into the proofing and baking system as required, can yield acceptable bread after freezer storage for several weeks (8). Bread-leavening yeast is an important ingredient which affects the quality of frozen dough (2). Since fermentation of dough before freezing is detrimental to yeast cell viability (6), freezing injury has been prevented by either omitting fermentation or performing short fermentation under lower temperatures (8). The bread produced just after thawing frozen dough without fermentation lacks the delicious taste and flavor which are expected by most consumers (10). However, maturation of thawed dough diminishes the advantage of frozen dough, namely, that oven-fresh baked goods can be readily available (5). Therefore, the most essential property of a yeast strain suitable for use in frozen dough is a high leavening ability, comparable to that of commercial bakers' yeast, both in nonfrozen dough and in frozen dough which has been fermented for an adequate period before freezing. This paper reports the selection and identification of these yeast strains for breadmaking by the frozen-dough method.

All yeast strains tested, *Saccharomyces* spp., were a part of the collection of Kyowa Hakko Co., Ltd., Tokyo, Japan. The organisms were stocked and maintained in yeast extract-malt extract agar slants (12). Culture of the organisms was carried out at 28°C unless otherwise stated. Yeast cells were grown on 10 ml of the seed medium, containing 1% yeast extract, 2% peptone, and 2% glucose, in a test tube (20 by 200 mm) for 24 h by shaking reciprocally (240 rpm) and were used as an inoculum for preculture. Preculture was performed for 24 h on 250 ml of a medium containing 10% sugar (as molasses), 1% yeast extract, 0.1% urea, and 0.1% KH<sub>2</sub>PO<sub>4</sub>. The broth of preculture was transferred to 1,000 ml of a main culture medium containing 2% sugar (as molasses), 0.05% urea, and 0.01% KH<sub>2</sub>PO<sub>4</sub>. Both the preculture and the main culture were performed in 2-liter Erlenmeyer flasks on a rotary shaker (180 rpm). After 16 h, the cells were harvested, washed twice with distilled water, and pressed. The moisture of the compressed yeast cells was about 70% (wt/wt).

The leavening ability of the yeast cells was determined as the volume of evolved gas from two types of dough (7). The ingredients of sweet bread dough were 200 g of flour, 60 g of sucrose, 1.0 g of salt, 6.0 g of compressed yeast cells, and 52 ml of water, and those of white bread dough were 200 g of flour, 10 g of sucrose, 6.0 g of salt, 4.0 g of compressed yeast cells, and 65 ml of water. All ingredients were kept at 30°C

and mixed by a Shinagawa Mixer 5DMR (Shinagawa Industry Co., Ltd., Tokyo, Japan) to the optimum development. The dough was removed from the mixer and put through a first fermentation for 3 h at 30°C. At the end of the first fermentation, the dough was punched and divided into two 30-g pieces. One was immediately put through a second fermentation for 2 h at 30°C. The other was molded to a 5-mm thickness by hand, frozen at -20°C, wrapped with polyethylene film, and stored at -20°C. After 7 days, the frozen dough was thawed in a proofing cabinet at 30°C for 1 h and put through a second fermentation for 2 h at 30°C.

Yeast cells were identified by the method of van der Walt and Yarrow (11).

Trehalose was extracted from yeast cells with 0.5 M trichloroacetic acid and determined by anthrone (9).

Eleven strains, MA233, MA249, MA251, MA257, MA325, MA335, MA340, MA378, MA379, MA380, and MA381, were selected from over 300 *Saccharomyces* strains and compared with the seven yeasts, CY1 to CY7, which are used for the production of commercial compressed yeast (Table 1). The selected strains had slightly lower leavening activity in both the first and second fermentations without freezing, whereas they had higher activity in the second fermentation after freezing than did the commercial strains. The freeze-thaw resistance of the selected yeast strains was shown clearly in the white bread dough but not in the sweet bread dough, since the high amount of sugar in the formula prevents the organisms from receiving freezing injury (6). Damaging effects of freezing on yeast cells in frozen dough increase in proportion to the fermentation period, since the freezing of activated yeast cells with fermentation products was shown to be detrimental to yeast viability (6). With the eleven strains, the observation of higher activity in the second fermentation after freezing might have been based on a lower extent in the first fermentation. However, this was not the case. To make the extent of fermentation before freezing equal, dough was frozen when the amount of evolved gas reached 150 ml in the first fermentation. Residual activity for 2 h after freeze-thaw was 36.5 ml with MA233 and 17.0 ml with CY1, and these differences were also found between the selected strains and commercial strains (data not shown). It was evident that the eleven yeast strains were more resistant to freezing injury in the fermented dough than were the commercial yeasts.

Morphological and cultural properties were similar among the eleven strains. After 3 days at 25°C, most cells were globose or subglobose, were 3.8 to 5.2 μm by 4.3 to 6.5 μm in size, and reproduced by multilateral budding in malt extract. Neither ring nor film was found after monitoring incubation at 20°C for 1 month. On malt agar, the streak culture was butyrous, slightly brownish, slightly raised and

\* Corresponding author.

TABLE 1. Leavening ability and trehalose content of selected and commercial yeast strains

Strain	Leavening ability (ml of CO <sub>2</sub> /30 g of dough)						Trehalose content (mg/g of dry wt)
	Sweet bread dough			White bread dough			
	First fermentation (3 h)	Second fermentation (2 h)		First fermentation (3 h)	Second fermentation (2 h)		
Without freezing		With freezing	Without freezing		With freezing		
MA233	75.0	83.0	80.5	130.5	85.0	44.5	37.7
MA249	59.0	70.5	72.0	117.5	86.0	44.0	43.1
MA251	60.0	64.0	75.5	116.5	79.5	39.5	37.8
MA257	67.5	78.0	84.0	117.0	83.0	32.0	34.2
MA325	56.5	71.5	74.0	121.0	79.0	38.0	27.2
MA335	60.5	75.0	77.0	125.0	83.0	31.0	35.7
MA340	56.0	79.0	82.0	119.0	81.5	29.5	36.0
MA378	56.0	65.0	66.0	117.0	84.0	39.5	41.2
MA379	56.5	65.5	67.0	119.0	79.0	40.0	40.4
MA380	50.5	65.5	61.0	113.0	83.5	41.0	38.7
MA381	50.5	64.0	66.0	111.0	77.5	32.5	35.7
CY1	90.0	90.0	89.0	156.0	85.5	20.5	10.5
CY2	78.5	83.5	81.0	153.5	85.5	19.5	10.8
CY3	73.0	86.0	81.0	152.5	86.0	21.0	10.3
CY4	75.0	87.5	85.0	149.0	85.0	22.5	10.2
CY5	78.5	90.0	87.5	153.0	87.0	14.5	10.7
CY6	57.5	64.0	62.0	146.5	86.0	18.5	13.7
CY7	92.0	95.0	92.0	148.5	82.0	18.0	12.6

smooth with light striations, and dull. Pseudohyphae were formed on Dalmau plates of either corn meal agar or potato glucose agar. Vegetative cells were transformed directly into asci containing one to four globose ascospores in acetate agar. Each strain fermented glucose, galactose, and sucrose but not lactose. Maltose was fermented in five strains, MA233, MA325, MA335, MA340, and MA378. All strains assimilated glucose, galactose, sucrose, maltose, trehalose, and raffinose but not cellobiose, lactose, soluble starch, D-xylose, L-arabinose, D-ribose, L-rhamnose, erythritol, ribitol, succinic acid, citric acid, or inositol. Mannitol was assimilated in MA249 and MA378. Each strain utilized ammonium sulfate as a nitrogen source but not potassium nitrate or ethylamine hydrochloride. The following physiological characteristics were common among the selected strains: vitamin requirement, biotin (essentially) and pantothenic acid (stimulatively); weak to positive growth on 50% (wt/wt) glucose-yeast extract agar; growth at 37°C; no growth in the presence of 100 ppm (100 µg/g) of cycloheximide. There were no essential or consistent differences among the characteristics of the selected strains as described above, and all were identified as *S. cerevisiae*.

Although morphological, cultural, and physiological characteristics of the selected yeast strains coincided with the standard description of *S. cerevisiae* (13), the trehalose content in the cells of these strains was found to be distinguishable from that in the commercial bakers' yeasts (Table 1). The trehalose content in the cells of the eleven strains was threefold higher than that in the commercial yeasts. Intracellular trehalose stabilizes membrane lipids under anhydrobiotic conditions and protects many organisms from dehydration (3). Since a high trehalose content improves the stability of bakers' and wine yeasts in storage, these yeast cells are cultured so as to accumulate the highest amount of trehalose for commercial production (1, 4). However, the commercial compressed cells of CY1, which had a much higher trehalose content (about 150 mg/g), were as susceptible to freezing injury as the cells cultured in the present experiment. In fermented dough, accumulated trehalose in

the cells of commercial bakers' yeast is unable to protect yeast cells from freezing injury. The selected yeast strains possessed a common ability to accumulate a higher trehalose content than did the commercial yeast cells under experimental culture conditions. This property seems to be related to the higher viability of these yeast cells in frozen dough.

We thank Shigemi Tokunaga for her technical assistance.

#### LITERATURE CITED

- Bekers, M. J., B. E. Damberg, I. J. Krauze, E. J. Ventina, and J. G. Kontakevich. 1981. Factors of wine yeast resistance at their dehydration, p. 117-121. In G. G. Stewart and I. Russel (ed.), Current developments in yeast research. Pergamon Press, Inc., Toronto.
- Bruinsma, B. L., and J. Giesenschlag. 1984. Frozen dough performance: compressed yeast-instant dry yeast. *Baker's Dig.* 58(6):6-11.
- Crowe, J. H., L. M. Crowe, and D. Champman. 1984. Preservation of membranes in anhydrobiotic organisms: the role of trehalose. *Science* 223:701-703.
- Grba, S., E. Oura, and H. Suomalainen. 1979. Formation of trehalose and glycogen in growing baker's yeast. *Finn. Chem. Lett.* 1979:61-64.
- Himmelstein, A. 1984. Enzyme treatment of flour. Will it help frozen and retarded doughs? *Baker's Dig.* 58(5):8-12.
- Hsu, K. H., R. C. Hoseney, and P. A. Seib. 1979. Frozen dough. I. Factors affecting stability of yeasted doughs. *Cereal Chem.* 56:419-424.
- Japan Yeast Industry Association. 1975. Experimental methods for baker's yeast, p. 1-2. Japan Yeast Industry Association, Tokyo.
- Marston, P. E. 1978. Frozen dough for breadmaking. *Baker's Dig.* 52(5):18-37.
- Panek, A. D., A. L. Sampaio, G. C. Bratz, S. J. Baker, and J. R. Mattoon. 1979. Genetic and metabolic control of trehalose and glycogen synthesis. New relationships between energy reserves, catabolite repression and maltose utilization. *Cell. Mol. Biol.* 25:334-354.
- Richard-Molard, D., M. C. Nago, and R. Drapron. 1979. Influence of the breadmaking method on French bread flavor. *Baker's Dig.* 53(3):34-38.

11. **van der Walt, J. P., and D. Yarrow.** 1984. Methods for the isolation, maintenance, classification and identification of yeasts, p. 45–104. *In* N. J. W. Kreger-van Rij (ed.), *The yeasts: a taxonomic study*, 3rd ed. Elsevier Science Publishers B. V., Amsterdam.
12. **Wickerham, L. J.** 1951. *Taxonomy of yeast*. U.S. Department of Agriculture technical bulletin no. 1029. U.S. Department of Agriculture, Washington, D.C.
13. **Yarrow, D.** 1984. Genus 22. *Saccharomyces* Meyer ex Reess, p. 379–395. *In* N. J. W. Kreger-van Rij (ed.), *The yeasts: a taxonomic study*, 3rd ed. Elsevier Science Publishers B. V., Amsterdam.