Improving the Freeze Tolerance of Bakers’ Yeast by Loading with Trehalose

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We examined the freeze tolerance of bakers’ yeast loaded with exogenous trehalose. Freeze-tolerant and freeze-sensitive compressed bakers’ yeast samples were soaked at several temperatures in 0.5 M and 1.0 M trehalose and analyzed. The intracellular trehalose contents in both types of bakers’ yeast increased with increasing soaking period. The initial trehalose-accumulation rate increased with increasing exogenous trehalose concentration and soaking temperature. The maximum trehalose content was almost identical (200–250 mg/g of dry cells) irrespective of the soaking temperature and the type of bakers’ yeast, but depended on the exogenous trehalose concentration. The leavening ability of both types of bakers’ yeast loaded with trehalose was almost identical to that of the respective original cells, irrespective of the soaking conditions. The freeze-tolerant ratio (FTR) of both types of bakers’ yeast increased with increasing intracellular trehalose content. However, FTR decreased during over-soaking after the maximum amount of trehalose had accumulated. FTR of the freeze-sensitive bakers’ yeast was more efficiently improved than that of the freeze-tolerant type.

Key words: bakers’ yeast; trehalose; freeze-tolerance; leavening ability

The process for manufacturing bread from frozen dough is widely utilized in the baking industry. It enables not only providing fresh bread to consumers, but also simplifies the manufacturing process for the bread to reduce working hours doing the night and early morning. Bakers’ yeast, however, is usually susceptible to freeze injury during the storage of frozen dough, so that the yeast cells can’t retain their original leavening ability after freezing and thawing.1) Several freeze-tolerant types of bakers’ yeast have therefore been developed and are now used.2-4)

The freeze-tolerance mechanism for bakers’ yeast has been studied to improve the freeze tolerance.4-7) We have reported that the intracellular trehalose content and lipid composition of bakers’ yeast were factors affecting its freeze tolerance.8-11) Trehalose (α-D-glucopyranosyl-α-D-glucopyranoside) is a non-reducing disaccharide, and has been reported to play the roles of a reserved carbohydrate and a stress protectant of the cytoplasmic membranes against dryness, freezing and heat stress.9) Several attempts by gene technology have been made to accumulate trehalose in yeast cells.12,13) No report, however, has been published on a rapid and convenient method to load trehalose into bakers’ yeast, although bakers’ yeast loaded with exogenous glycerol has been reported to improve the leavening ability of sweet dough.14)

Saccharomyces cerevisiae possesses trehalose activity but can also absorb trehalose without prior hydrolysis to glucose. Two types of trehalose transport activities have been reported in S. cerevisiae: high-affinity transport (Km = 4 mM), and low-affinity transport (Km > 100 mM) that could involve a facilitated diffusion process.15) While the high-affinity transport activity was induced by α-methylglucoside but not by trehalose, the low-affinity uptake was constitutively expressed in S. cerevisiae.16-18) Therefore, bakers’ yeast soaked in a high concentration of trehalose may accumulate trehalose in the cells by the latter mechanism. We describe here the loading of bakers’ yeast with exogenous trehalose, resulting in high freeze tolerance in dough.

Materials and Methods

Materials. Trehalose was purchased from Hayashibara Co. (Okayama, Japan) and from Ajinomoto Co. (Tokyo, Japan). Trehalose was purchased from Sigma (St. Louis, MO, USA; from porcine kidney, 1.4 units/mg of solid). Freeze-sensitive and freeze-tolerant types of compressed bakers’ yeast were obtained from one of the Japanese bakers’ yeast companies, stored at 4°C, and used within 3 d. All other chemicals were of analytical reagent grade.

Loading bakers’ yeast with trehalose. Compressed bakers’ yeast cells were suspended in 0.5 M and 1.0 M trehalose to give a cell density of 3 g of dry cells per 100 ml, and incubated at various temperatures. After harvesting the cells at 4°C by centrifugation, the cells were washed twice with 0.5 M KCl and then with

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distilled water.

Freeze-tolerant ratio of the yeast. The washed cells were placed on a water-absorbent clay plate for several min to produce a compressed yeast cake, like the commercial products. This yeast cake (2 g) was mixed at 30°C for 20 min with 100 g of flour, 5 g of glucose, 1.5 g of NaCl and 62 ml of distilled water by a dough mixer (ProKM-230, Aikosha Co., Saitama, Japan), and divided into 50-g portions. The dough was pre-fermented at 30°C for 2 h and then stored at -20°C for 7 d. After storage, the dough samples were thawed at 30°C for 10 min, and the leavening ability was determined by measuring the gas volume (A) evolved from each sample at 30°C over 2 h with a Fermonograph AF-1000 (Atto Co., Tokyo, Japan). As a control experiment, pre-fermented dough without being frozen was further fermented at 30°C for 2 h, and the gas volume (B) evolved from the dough was determined under the same conditions. Three replications were made for each dough sample. The freeze-tolerant ratio (FTR) was calculated as A/B × 100.  

Survival ratio of the yeast after freezing and thawing. Commercial bakers’ yeast cells loaded with or without trehalose were suspended in 0.85% NaCl to give an absorbance of 0.2 at 660 nm, before being frozen at -20°C for 7 d. After thawing at 30°C for 1 h, an aliquot of the suspension was spread on YPG plates after appropriate dilution and incubated at 30°C for 48 h. The survival ratio was calculated from the number of living cells before and after freezing.

Intracellular trehalose content. Trehalose was extracted from the yeast cells with 12.5% trichloroacetic acid, and measured either by the anthrone method or by high-performance liquid chromatography (HPLC). Comparable results were obtained by either technique. When the HPLC method was used, the extract was evaporated to dryness under reduced pressure at 40°C, after having removed most TCA in the extract with diethyl ether. The dried sample was dissolved in water and adjusted to pH 6–7 with NaOH. After filtering through a Cosmonice W filter (0.45 μm pore size, Millipore Japan Co., Tokyo, Japan), the filtrate was analyzed by HPLC. The filtrate was also treated with trehalase at 37°C for 1 h in a 0.2 M citric acid-phosphate buffer (pH 5.7) and then analyzed by HPLC. Commercial fructose, glucose, sucrose, maltose and trehalose were each used as standard sugars at a concentration of 0.1%. Analytical conditions: detector, Shodex RID-2A; column, Shodex AXpak WA-624; column temperature, 50°C; eluent, acetonitrile:distilled water = 3:1; flow rate, 1 ml/min; and pressure, 60 kg/cm².

Results and Discussion

Loading commercial compressed bakers’ yeast with exogenous trehalose

To load trehalose into bakers’ yeast cells, freeze-tolerant and freeze-sensitive compressed bakers’ yeast samples were soaked in a 0.5 M or 1 M trehalose solution at 4°C, before being analyzed. As shown in Fig. 1, the intracellular trehalose level in both types of bakers’ yeast increased with increasing soaking period. Since soaking for more than 7 d did not lead to any further increase in the intracellular trehalose content, soaking for 7 d was deemed sufficient to load the maximum level of trehalose into the cells. However, a browning reaction was observed with both types of bakers’ yeast cells soaked for 7 d, so loading for 7 d appears to have been unsuitable for bread making. When the cells were soaked in 0.85% NaCl, the intracellular trehalose content slightly decreased after a prolonged soaking period. One molar trehalose was more effective for loading trehalose into the cells than 0.5 M. The trehalose-accumulation rate and the maximum trehalose content of the cells appeared to depend on the trehalose concentration of the soaking solution. Although the initial trehalose level of the freeze-tolerant bakers’ yeast was lower than that of the freeze-sensitive type, this low level of intracellular trehalose was not a specific feature of other freeze-tolerant bakers’ yeast products. The initial intracellular trehalose contents of six commercial freeze-tolerant bakers’ yeast products (91 ± 41 mg/g of dry cells) were almost identical to those of five freeze-sensitive types (103 ± 51 mg/g of dry cells). When we used compressed

![Fig. 1. Change in the Intracellular Trehalose Content of Compressed Bakers’ Yeast Soaked in Trehalose Solutions.](image-url)
bakers' yeast from other manufacturers, exogenous trehalose could be loaded into the cells to the same extent (200–250 mg of trehalose/g of dry cells) by soaking at 4°C in 1 M trehalose for 7 d. Therefore, the maximum level of trehalose loaded into the yeast cells appeared to be almost constant irrespective of the type of bakers' yeast. When we used 0.01 M trehalose for soaking, the intracellular trehalose level remained low in all bakers' yeast samples used. These results suggest that exogenous trehalose was incorporated by the low-affinity trehalose transport system (a facilitated diffusion process), irrespective of the type of bakers' yeast.

We also examined the effect of the soaking temperature on loading trehalose into bakers' yeast. As shown in Fig. 2, the trehalose-accumulation rate increased with increasing soaking temperature. The intracellular trehalose was accumulated to its maximum level after soaking at 10°C for 5 d, 20°C for 1 d, and 30°C for 18 h. The maximum level was almost identical, irrespective of the soaking temperature. However, a browning reaction occurred in the bakers' yeast cells soaked at 10°C for 7 d, at 20°C for 3 d, and at 30°C for 1 d. No browning was apparent when the cells were incubated in 0.85% NaCl; therefore, browning may have occurred after the maximum level of trehalose had been accumulated in the cells. It may thus have been that over-soaking caused browning of the yeast cells. Similar results were also obtained when we used compressed bakers' yeast products from other manufacturers. Although the initial intracellular trehalose content of the compressed bakers' yeast samples used were decreased by 5–10% during storage at 4°C for 3 d after purchase, the maximum intracellular trehalose level after loading was almost constant.

**Leavening ability of bakers' yeast loaded with trehalose**

The leavening ability of bakers' yeast loaded with trehalose was analyzed in dough (Fig. 3). The yeast appeared to retain its original leavening ability after loading under various soaking conditions, and a difference in the intracellular trehalose content between the soaked and original bakers' yeast cells did not result in different leavening ability. This finding agrees well with our previous result that the intracellular trehalose content of bakers' yeast was not correlated with its leavening ability. Although the bakers' yeast browned under some soaking conditions as just described, such browning did not reduce the leavening ability.

**Freeze-tolerance of bakers' yeast loaded with trehalose**

The FTRs of freeze-tolerant and freeze-sensitive bakers' yeast samples was observed in the increase in ratio of freeze-tolerance when soaked at 4–10°C for 3–5 d, at 20°C for 1–3 d, and at 30°C for 18 h. While the freeze tolerance of the freeze-sensitive bakers' yeast was markedly improved by soaking, that of the freeze-tolerant type was only slightly improved. Similar results were also obtained when we used other samples of compressed bakers' yeast. The freeze tolerance of yeast is known to depend on several genetic factors besides physiological and environmental ones. Almeida and Pais have reported that some commercial bakers' yeast samples having more than 20% trehalose were freeze sensitive. Therefore, the effect of trehalose on the

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**Fig. 2.** Effect of Soaking Temperature on Loading of Trehalose into Yeast Cells.

Compressed bakers' yeast was soaked in a 1 M trehalose solution at 10°C, 20°C, and 30°C (open symbols). As a control experiment, bakers' yeast was soaked in 0.85% NaCl (closed symbols). The intracellular trehalose was extracted from each bakers' yeast sample with 12.5% trichloroacetic acid at 37°C for 1 h and then analyzed. A, freeze-sensitive bakers' yeast; B, freeze-tolerant bakers' yeast. Symbols: ○, 10°C; •, 20°C; and △, 30°C. Each result is expressed as the mean value of triplicate experiments, for which the standard deviation is shown.
freeze tolerance may depend on the yeast strain. In addition, since trehalose is known to stabilize the cytoplasmic membrane, freeze-tolerant bakers' yeast may already have a cytoplasmic membrane that is resistant to freezing; therefore, its freeze-tolerance may be only slightly improved by loading with trehalose. In fact, we have previously reported that the lipid composition of commercial freeze-tolerant bakers' yeast had high fluidity of the cytoplasmic membrane, and suggested that this high fluidity reflected the freeze tolerance of bakers' yeast.\(^\text{11}\)

We have described the effect of loading trehalose into bakers' yeast. This trehalose loading appeared to be effective for improving the freeze tolerance of bakers' yeast. This is the first report on a rapid and convenient method to improve the freeze tolerance of bakers' yeast.

References

Fig. 4. Correlation between the Intracellular Trehalose Content and Survival Ratio after Freezing.
Freeze-tolerant and freeze-sensitive bakers' yeast samples were soaked in a 1 M trehalose solution at 4–10°C for 0–5 d, at 20°C for 0–3 d, and at 30°C for 0–0.75 d. The intracellular trehalose content was plotted against the survival ratio after freezing at −20°C for 7 d. “r” in the figure is the correlation factor. A, freeze-sensitive bakers' yeast; B, freeze-tolerant bakers' yeast.