New Freeze-Tolerant Yeast for Frozen Dough Preparations

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ABSTRACT

Activated bakers' yeast after initial fermentation was more susceptible to freeze damage than nonactivated yeast. In this study, new freeze-tolerant yeasts were isolated from banana peel and identified as *Kluyveromyces thermotolerans* and *Saccharomyces cerevisiae*. These yeasts retained their fermentative ability and bread leavening activity even after one to six weeks of frozen storage in fully prefermented dough. Frozen dough made with these yeasts gave good quality bread similar to that made from unfrozen dough using bakers' yeast.

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Frozen bread dough methods have been extensively adopted in the baking industry and in in-store bakeries. A great deal of research has been carried out on the effect of various ingredients and procedures on the quality of frozen dough, yeast survival ratio in frozen dough (Godkin and Cathcart 1949, Kline and Sugihara 1968), gassing power, and relative proof times after thawing (Meyer et al 1956, Tanaka and Miyatake 1975, Marston 1978, Hsu et al 1979a, Davis 1981, Bruinsma and Giesenschlag 1984). Moreover, the quality of frozen dough was found to be affected by ingredient levels in the breadmaking formulation (McPherson and Lamb 1948, Zaehringer et al 1951, Meyer et al 1956, Lorenz and Bechtel 1965, Sugihara and Kline 1968, Marston 1978), the type of yeast used (Zaehringer et al 1951, Merritt 1960, Kline and Sugihara 1968), and the rates of freezing and thawing of bread dough (Bamford 1975, Hsu et al 1979b, Tanaka et al 1980).

The most important problem encountered in frozen dough processing is how to maintain the viability and gassing power of frozen yeast. Major improvements in the quality of frozen bread dough were achieved by maintaining the yeast stability during frozen storage. In the past, emphasis was placed on minimizing the fermentation activity prior to molding and freezing, either by reducing the time of handling or reducing the dough temperature (Zaehringer et al 1951, McPherson and Lamb 1948, Godkin and Cathcart 1949, Merritt 1960). Study along these lines has been emphasized by Merritt (1960), who suggested that frozen yeast in dough reaches a state of incipient growth and became easily damaged by freezing with the progression of fermentation.

Breads subjected to little or no fermentation before freezing were found not to develop an adequate fermentation flavor during the final proofing period. Improvement can be obtained to a certain extent by changing the amounts of sugar, salt, shortening, and oxidizing agents; however, if the yeast activity is not maintained during frozen storage, the thawed dough will not rise. Fermentation of the dough before freezing plays a more significant role in the stability of the dough than any other single factor.

The yeast manufacturing companies in Japan have recently entered the market for the new frozen dough products, which enables the baking industry to supply a variety of bread products daily. The characteristics of the commercial freeze-tolerant yeasts used in these frozen dough products are not yet totally satisfactory. The present study deals with the detailed characteristics of four new strains of freeze-tolerant yeasts.

MATERIALS AND METHODS

Yeast Strains

Of the five yeasts examined, one yeast (FRI 802) was selected

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from the culture collection of this laboratory, two yeasts (FRI 413 and FRI 501) were isolated by the authors from banana peel, one yeast (commercial freeze-tolerant yeast, CFY), which consisted of a mixture of *Saccharomyces cerevisiae* and *S. rosei*, was provided by Sankyo Co. Ltd. (Japan), and the last yeast was a commercial compressed yeast (CY) provided by the Japan Yeast Industrial Association.

These yeasts were grown for three days at 30° C on a slant of yeast-peptone-glucose (YPG) agar consisting of (on a per liter basis): glucose, 40 g; yeast extract, 10 g; polypeptone, 5 g; KH_2PO_4, 5 g; $MgSO_4 \cdot 7H_2O$, 3 g; and 15 g of agar. The medium composition for preculture was as follows (per liter): molasses (containing 300 g of sugar as glucose per liter), 166 ml; $CO(NH_2)_2$, 2.8 g; $(NH_4)_2SO_4$, 1.0 g; KH_2PO_4, 0.4 g; and $MgSO_4 \cdot 7H_2O$, 0.3 g. Preculture was carried out in a 500-ml shake flask (200 ml liquid volume) at a shaking speed of 140 revolutions/min (10 cm amplitude) under aerobic conditions. A 3-L jar fermenter (diameter 14.3 cm, Iwasiya Co., Japan) with a six-vaned disk impeller was used for the production of a large amount of the yeasts. The initial medium contained 1,200 ml of water and 0.3 g of $MgSO_4 \cdot 7H_2O$. The pH and aeration rate were regulated at 5.2 and 2 v/v·m. The agitation speed and temperature were adjusted to 700 rpm and 30° C throughout the experiment. Adequate supplies of nutrients were provided for 16 hr at intervals of 30 min. The composition of the nutrients supplied for the initial 12-hr period was as follows (per liter): $CO(NH_2)_2$, 10.7 g; $Na_2HPO_4 \cdot 12H_2O$, 3.9 g; 333 ml of molasses as specified previously and 666 ml of water. In addition, a quantity of urea was removed from the above nutrients to enable the yeast to mature for the last 4-hr period. The quantity of nutrients supplied was determined by calculating the rA, where A is the weight in grams of yeast present at any given time, and r is the hourly growth rate requirement expected in grams of sugar per weight in grams of yeast (70% mb) per hour. The value of r was set at 0.16 g sugar/g wet yeast · hr (wet yeast: 70% mb) for 12 hr and subsequently at 0.04 for 4 hr. The yeasts were collected by centrifugation, washed repeatedly with water, and the suspension was dehydrated through a filter paper (No. 4A, Toyoroshi Co., Japan). The moisture content of the dehydrated yeasts was measured at 105° C for 5 hr, and the quantity of yeast was weighed in terms of 70% mb.

Preparation of Bread

The same flour was used for all the dough preparations. It was a commercial bread flour containing 12.4% (13.5% mb) protein with a water absorption of 63% measured by farinograph. For straight doughs, the formulation was as follows: flour, 100 g (13.5% mb); sucrose, 5 g; salt, 2 g; shortening, 4 g; yeast, 2 g (70% mb); and an optimum amount of water. The dough formulation did not include oxidizing agents or additives to protect yeast from freeze injury, in order to compare the characteristics of various yeasts. Each dough was mixed to optimum development in a pin mixer (National Mfg. Co., Lincoln, NE). Fermentation was carried out in a cabinet maintained at 30° C and 90–95% rh for 150 min with punching at 90 min and 135 min. At the end of the fermentation, the dough was sheeted through rolls set at ¼ and ⅛ in. and molded with a dough molder (National). The dough was then panned and proofed at 38° C and 90–95% rh for 55 min. Bread was baked at 215° C for 20 min. Loaf weight and volume were measured immediately after baking. Loaf volume was determined by rapeseed displacement, and sensory evaluation scores were obtained on the following day. The quality rating used in this study included a subjective palatability score as well as a rating based on the objective volume measurement. The latter factor was included because the loaf volume might be an index of the lightness of the bread. The samples were rated on the basis of a maximum score of 100 points apportioned into the following quality characteristics: external appearance, 30 (volume, 10; color of crust, 8; symmetry of form, 5; crust, 4; evenness, 3), and internal appearance, 70 (grain, 15; color, 10; texture, 15; aroma, 10; taste, 20).

Frozen Dough Method

The formulation and processing of frozen dough were the same as those for the straight dough except for the amount of salt (1.5 g), and periods of fermentation (0–180 min). After the end of fermentation, the sheeted dough was wrapped in a polyethylene case and frozen in a laboratory freezer without air flow (model PF-20, Tabai Mfg. Co. Ltd., Japan) at −20° C for seven days. The wrapped frozen dough was transferred from the freezer to a fermentation cabinet, maintained at 30° C and 90–95% rh, and held for 90 min. The thawed dough was transferred to a bowl, turned over, and placed in the fermentation cabinet for 30 min. The dough was sheeted and molded as described above for bread preparation. Then, the dough was panned and proofed at 38° C and 90–95% rh for 70–90 min. Loaves were baked as described above for bread preparation.

Gas Production Measurements

A Gasograph 12B (D&S Instrument Ltd., Pullman, WA) was used in all the gas production measurements (Rubenthaler et al 1980).

Frozen dough system. The formulation for the frozen dough used in the gas production measurement was as follows: flour, 20 g; sucrose, 1 g; yeast, 0.4 g; and an optimum amount of water. Each dough was mixed to optimum development in a small stainless steel bowl with a Teflon-coated rod, placed in the reaction vessel of the Gasograph 12B, and incubated in a water bath for 0–180 min at 30° C. After the end of the prefermentation period, each dough was punched with a spatula to remove gas and then stored for one to six weeks at −20° C. The frozen dough in the reaction vessel was transferred to the water bath of the Gasograph, maintained for 10 min at 30° C, and the rubber stopper in the reaction vessel was pressed to the release pressure before the gassing experiment. The total amount of CO_2 gas released was recorded for 120 min at 30° C without shaking.

Sweet dough system. The formulation for dough containing a high concentration of sugar was as follows: flour, 40 g; sugar, 12 g; yeast, 1.2 g; and an optimum amount of water. Each dough was mixed to optimum development as described above for the frozen dough system. The dough was placed in the reaction vessel of the Gasograph and incubated for 5 min at 30° C, then the rubber stopper in the reaction vessel was pressed. The total amount of CO_2 gas released was recorded for 90 min without shaking.

Maltose liquid fermentation. Maltose fermentative ability was measured using a modification of the liquid fermentation media described by Atkin et al (1945). The medium composition for liquid fermentation was as follows (g/20 ml): yeast, 0.4; maltose, 0.1; glucose, 0.006; and 3 ml of 1/15 M phosphate buffer (pH 5.6), 2 ml of nutrient solution. Each 1 L of nutrient solution contained 57 g of urea, 29 g of diammonium sulfate, 23 g of magnesium sulfate, 46 mg of thiamine hydrochloride, 46 mg of pyridoxine hydrochloride, and 460 mg of niacin. A 20-ml sample of this medium was incubated in the reaction vessel of the Gasograph 12B for 5 min at 30° C; then 0.4 g of yeast was placed in the solution, and the rubber stopper in the reaction vessel was pressed. The total amount of CO_2 gas released was recorded for 90 min without shaking.

Zymotachygraph Studies of Sponge Dough Fermentation

The dough formulation used for the Zymotachygraph studies was as follows: flour, 200 g; salt, 4 g; yeast, 4 g; and an optimum amount of water. Each dough was mixed to optimum development in the pin mixer. The dough was placed in an airtight chamber of the Zymotachygraph (Syst. M. Chopin, France) at 30° C. After 12 min, the exhaust cock of the chamber was closed. The rate of CO_2 gas produced and the degree of gas retention in the dough mass were recorded for 5 hr.

Survival Ratio of Yeast

The counts of surviving yeasts were determined as follows: yeasts were grown on slants of YPG medium for three days at 30° C. The cell suspension from the slant was inoculated to 100 ml of YPG broth in a Sakaguchi flask. After 24 hr of shaking culture at 30° C, 2 ml of the culture was transferred to the same medium. After 6 hr of cultivation, 10 ml of logarithmic phase cells were

collected by centrifugation. The cells were washed with a 0.5% sodium chloride solution, and the suspension was found to have a volume of 100 ml. Further dilution of the suspension was made to give yeast counts that ranged between 50 and 200 colonies per plate. YPG agar with the pH adjusted to 5.0 was used for the plate counts. Yeast counts were obtained after 72 hr of incubation at 30° C.

RESULTS AND DISCUSSION

The results of baking tests of nonfrozen bread dough produced with the previously described formulation are shown in Table I. The quality of breads made with three strains of FRI yeasts was roughly comparable to that made with a commercial yeast. Quality scores of the breads made with the FRI 501 and FRI 413 yeasts were not inferior to the control except that slightly lower loaf volume and denser texture were observed.

The quality of bread prepared from frozen dough with ordinary bakers' yeast was usually lower than that of the fresh control for all the parameters (Table II). For example, the grain of bread from frozen dough was coarser and less uniform than that of bread prepared from fresh dough. The odor was flat and yeasty, and the eating quality was cottony. Moreover, when frozen dough was baked without first molding and rounding, a misshapen coarse grain and rugged crust top resulted, as described by Meyer et al (1956). Based on the report of Sternberg (1973) on gluten development in the dough-making process, we considered that the gluten structure that was damaged by freezing and thawing, recovered during the molding and rounding operations after thawing. Although breads prepared from frozen dough made with three FRI yeasts exhibited a rugged crust top without the make-up

TABLE I
Quality of Straight-Dough Bread Produced with Five Different Yeasts

Strain[a]	Loaf Volume (ml)	Specific Volume	Bread Quality (points)
FRI 802	720	4.8	86.5
FRI 501	675	4.5	84.9
FRI 413	660	4.3	83.5
CFY	700	4.7	84.2
CY	740	5.1	88.3

[a]CFY = Commercial freeze-tolerant yeast, CY = commercial yeast.

TABLE II
Quality of Bread from Frozen Dough Stored at −20°C for Seven Days

Strain[a]	Proof Time (min)	Loaf Volume (ml)	Specific Volume	Bread Quality (points)
FRI 802[b]	90	655	4.4	80.8
FRI 501[c]	70	680	4.6	84.2
FRI 413[c]	90	640	4.3	80.2
CFY[b]	90	680	4.7	81.8
CY[b]	90	475	3.1	66.9

[a]CFY = Commercial freeze-tolerant yeast, CY = commercial yeast.
[b]Frozen dough fermented for 90 min prior to freezing.
[c]Frozen dough fermented for 120 min prior to freezing.

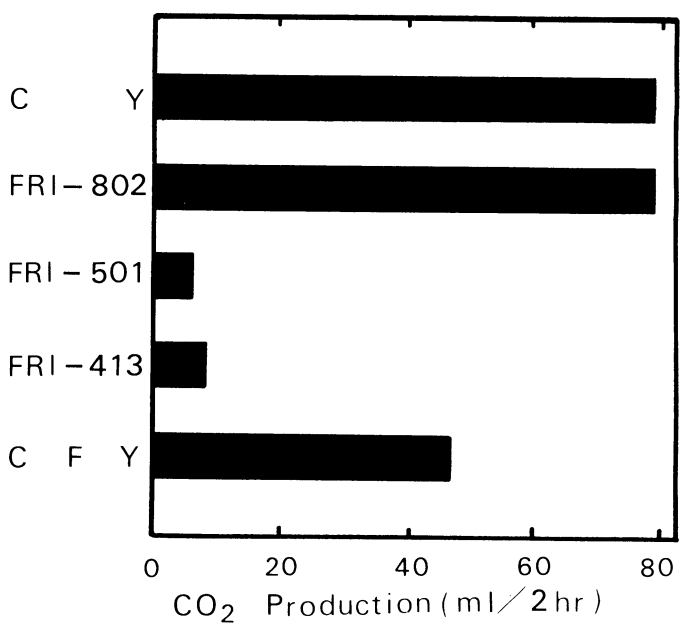

Fig. 1. Maltose fermentative ability of various yeasts measured as CO_2 production for 20 ml of the liquid fermentation media.

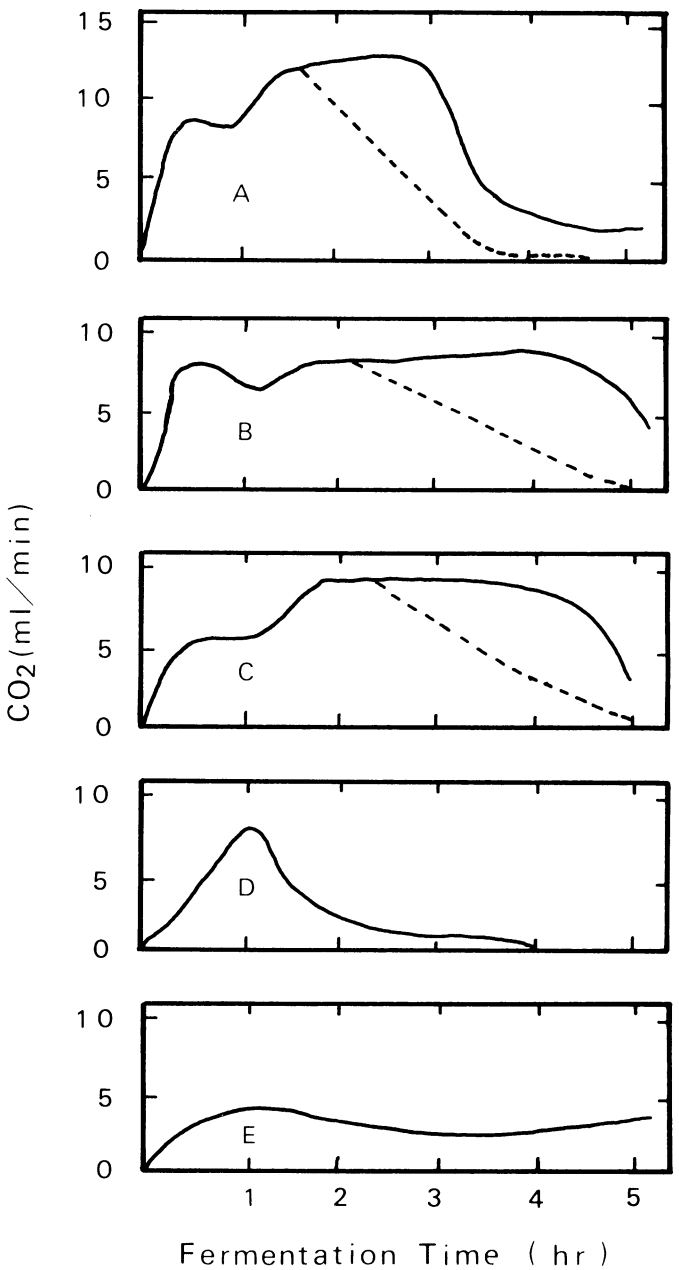

Fig. 2. Zymotachygrams of sponge dough made from various yeasts. **A,** Commercial yeast; **B,** commercial freeze-tolerant yeast; **C,** FRI 802 yeast; **D,** FRI 501 yeast; **E,** FRI 413 yeast. CO_2 production for dough made with 200 g of flour. Broken line shows the degree of gas retention in the dough mass.

steps after thawing, those from frozen doughs with make-up steps after thawing had almost perfect properties (Table II). The volumes and scores of loaves prepared from frozen dough made with different yeasts are presented in Table II. Yeast strain is very important in frozen dough stability. These data show that the utilization of freeze-tolerant yeasts as a substitute for bakers' yeast results in a significant improvement in the loaf volume and quality, even in products prepared from frozen doughs subjected to some fermentation prior to freezing.

As shown in Figure 1, the maltose fermentative ability of the FRI 802 yeast was roughly comparable to that of CY, whereas the maltose fermentative ability of the commercial freeze-tolerant yeast (CFY) was about half that of CY. This trend was also observed in the Zymotachygrams, as shown in Figure 2. For the FRI 802 and CFY yeasts, the type of fermentation curve was similar to that of CY and the degree of gas retention in the dough was approximately the same as that of CY for 5 hr. The fermentative ability of the FRI 802 yeast was always inferior to that of CY, but gas production of the dough made with the FRI 802 yeast in the latter phase of fermentation was prolonged. This behavior may be ascribed to the freeze tolerance of the CFY and FRI 802 yeasts as described later.

TABLE III
Effect of Fermentation Before Freezing on Bread Quality from Frozen Dough

Strain[a]	Time[b] (min)	Volume (ml)	Bread Quality (points)
FRI 802	0	680	80.0
	30	690	80.3
	60	620	75.9
	90	620	74.2
	120	545	68.0
	150	490	62.5
FRI 501	0	540	68.6
	30	580	74.2
	60	620	75.0
	90	675	80.6
	120	680	84.4
	150	670	84.3
FRI 413	0	470	61.5
	30	520	68.5
	60	570	77.5
	90	615	80.1
	120	640	82.1
	150	640	82.3
CFY	0	620	81.3
	30	615	81.3
	60	620	80.6
	90	580	75.0
	120	565	63.8
	150	350	49.0
CY	0	630	80.6
	30	565	74.1
	60	550	68.9
	90	445	53.8
	120	370	53.1
	150	370	53.1

[a]CFY = Commercial freeze-tolerant yeast, CY = commercial yeast.
[b]Prefermentation time.

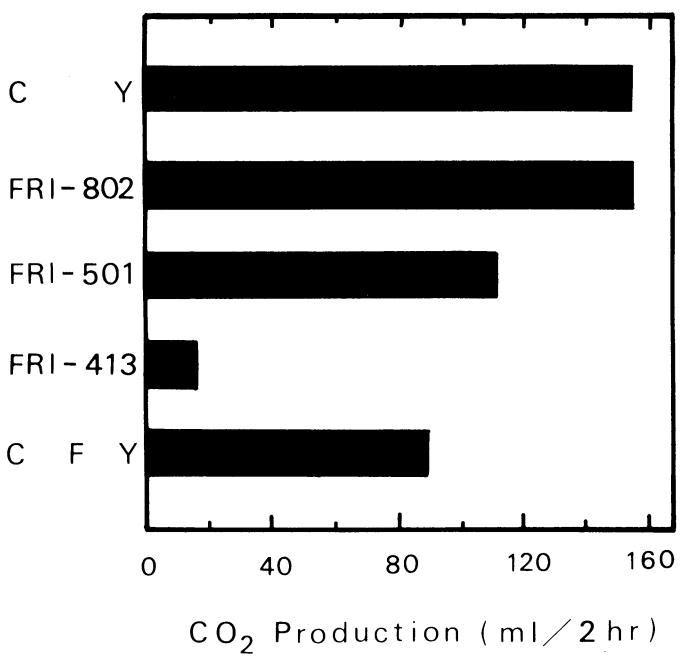

Fig. 3. Sweet dough fermentative ability of various yeasts measured as CO_2 production for dough made with 40 g of flour.

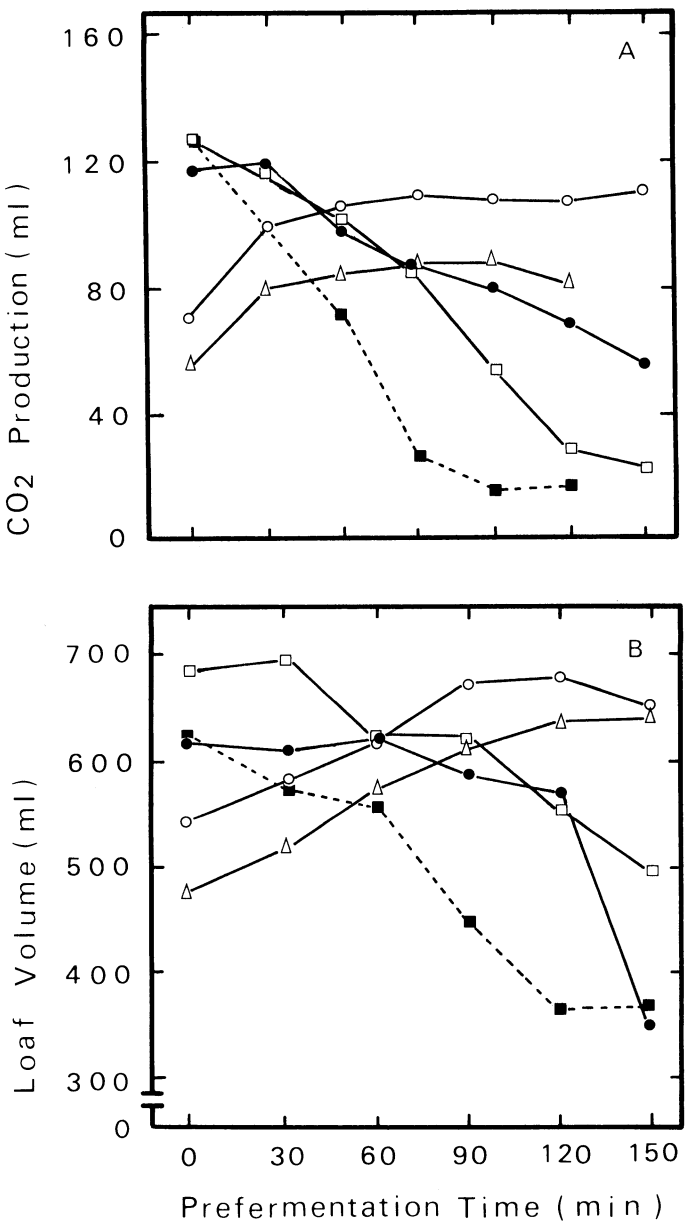

Fig. 4. Effect of fermentation before freezing on fermentative ability of frozen dough stored at −20° C for seven days. **A,** CO_2 production for frozen dough made with 20 g of flour. **B,** Loaf volume for frozen dough made with 100 g of flour. ■ = Commercial yeast, ● = commercial freeze-tolerant yeast, □ = FRI 802 yeast, o = FRI 501 yeast, and △ = FRI 413 yeast.

Because the FRI 501 yeast was found to exhibit a negative maltose fermentative ability, as shown in Figure 1, and only an early phase of liquid fermentation (Fig. 2), the release of CO_2 gas from FRI 501 in the maltose fermentation test was attributed to the presence of a trace amount of glucose in the medium. The time required to reach the maximum level of fermentative ability was longer, but the maximum level in the early phase was approximately the same as that of CY. The fermentative ability of the FRI 413 yeast was rather low throughout the test period, as shown in Figure 2.

Figure 3 indicates the fermentative ability of these yeasts in sweet dough systems. FRI 802, FRI 501, and CFY yeasts had the ability to ferment the sweet dough, whereas FRI 413 yeast lacked this ability. These results suggest that 1) FRI 802 and CFY yeasts can be used both in lean and rich dough formulations. 2) Although FRI 501 yeast cannot be used in the lean doughs, it has an applicability in rich dough preparations, such as sponge-dough systems in which 5–10% sugar is added for the production of sweet goods in Japan and in straight-dough systems. 3) The applicability of FRI 413 yeast in sponge-dough systems is low, but the yeast may be used in straight-dough systems.

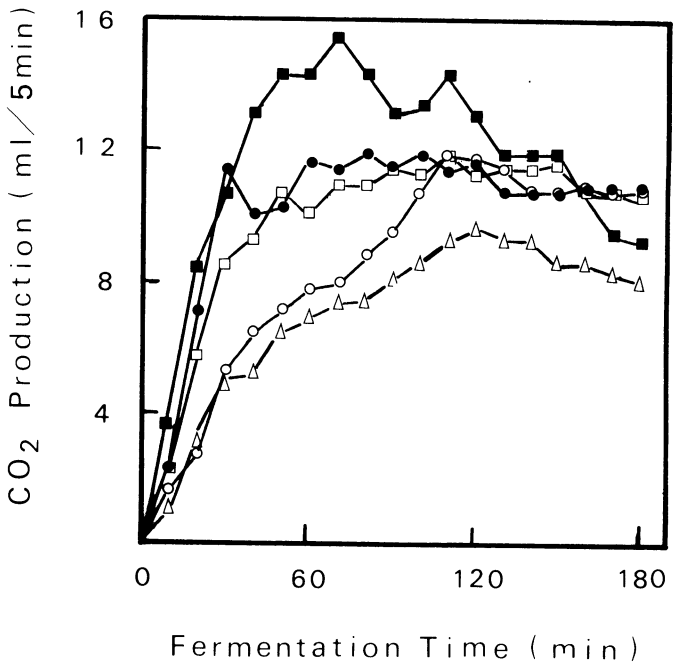

Fig. 5. Fermentation curve of bread dough using various yeasts. ■ = Commercial yeast, • = commercial freeze-tolerant yeast, □ = FRI 802 yeast, o = FRI 501 yeast, and △ = FRI 413 yeast. CO_2 production for dough made with 20 g of flour.

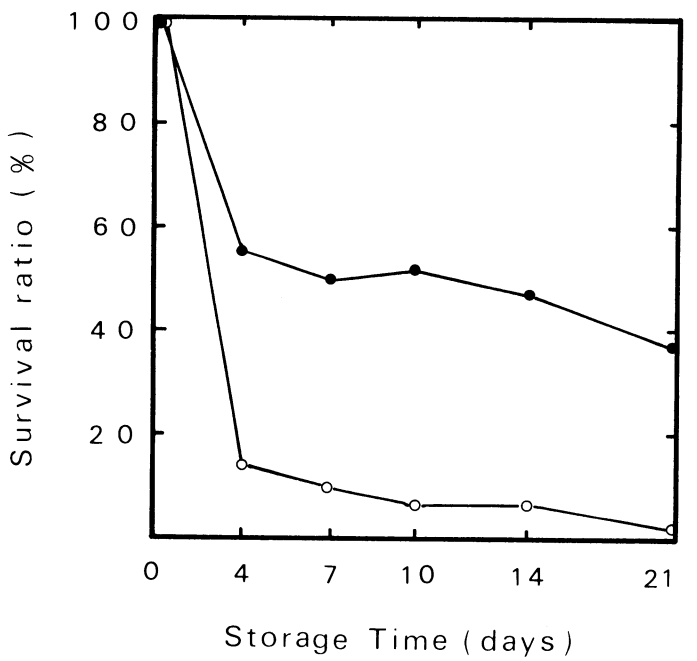

Fig. 6. Survival ratio of the logarithmic phase cells of commercial and FRI 501 yeasts stored at −20°C. o = Commercial yeast and • = FRI 501 yeast.

The results of CO_2 production of thawed doughs and the loaf volume of bread prepared from frozen doughs that were fermented for some time with the various yeasts before freezing, are shown in Figure 4. Commercial yeast showed a very low stability during frozen storage, even with 30 min of prefermentation of the straight dough. The baked products from frozen dough made with CY, even without prefermentation, were of poor quality (Table III).

The stability of freeze-tolerant yeasts can thus be classified into two types based on these experiments (Fig. 4 and Table III). In the first type, frozen dough made with the CFY and FRI 802 yeasts, the CO_2 production of the thawed dough decreased with the increase in the period of fermentation prior to freezing (Fig. 4A). The loaf volume of bread made with these yeasts slightly decreased up to 90–120 min of prefermentation, but decreased markedly beyond 120 min (Fig. 4B). CFY used in this experiment consisted of a mixture of the two strains as described above. When the frozen dough was made with *S. rosei* only, the pattern of CO_2 production of the thawed dough was different from that with CFY and similar to that with FRI 501 and FRI 413, as reported by Tanaka (1982) and Saito et al (1982).

On the other hand, the CO_2 production, loaf volume, and bread quality from frozen dough made with the FRI 413 and FRI 501 yeasts increased slightly with the increase of the period of fermentation prior to freezing. We considered that the differentiation into two types may result from the pattern of CO_2 production from dough during the prefermentation period and the degree of freeze tolerance.

The rate of CO_2 production per 5 min from unfrozen bread dough prepared from 20 g of wheat flour was recorded by the Gasograph for 3 hr, and the results are shown in Figure 5. Commercial yeast, CFY, and FRI 802 yeast gave the maximum gassing rate in a short time. After 60 min, the CO_2 production for CY decreased slightly, but the CO_2 production for CFY and FRI 802 did not. It took a longer time for the FRI 501 and FRI 413 yeasts to give the maximum gassing rate, and the volume of released gas was only 65–75% that of the CY at the end of 3 hr. If the biological activity or physiological state of the yeasts is proportional to the mass of CO_2 produced, the mass of fermentation products that affect yeasts during freezing would also be increased with increasing fermentation. Tanaka et al (1976) reported that the coexistence of flour and ethanol is harmful to activated yeast during frozen storage. The rate of CO_2 production and the maximum level of CO_2 produced were higher for CY than for the freeze-tolerant yeasts. These findings indicate that higher levels of ethanol and other fermentation products from yeast may result in the inhibition of fermentation of CY after 60 min and also cause a higher degree of damage to the CY during frozen storage. The ordinary commercial yeast was very sensitive to freezing injury when fermentation prior to freezing was allowed. For this reason, even if the frozen doughs were made with CY by other improved methods, there would still be a limit to the yeast viability. The maximum level of CO_2 production for the CFY and FRI 802 yeast was lower, although the time to reach the maximum level was roughly comparable to that of CY. These yeasts would need a longer prefermentation period to produce the same amount of metabolites as CY does to cause the same degree of freezing injury. Previous reports indicated that it may be preferable to use dry yeasts or yeasts maintained at 2°C for a long time for the preparation of frozen dough, presumably because of the longer lag period after incorporation into the dough (Merritt 1960, Zaehringer et al 1951, Kline and Sugihara 1968, Reed 1966). The use of the FRI 802 yeast could afford the production of frozen dough products of good quality within a short period of

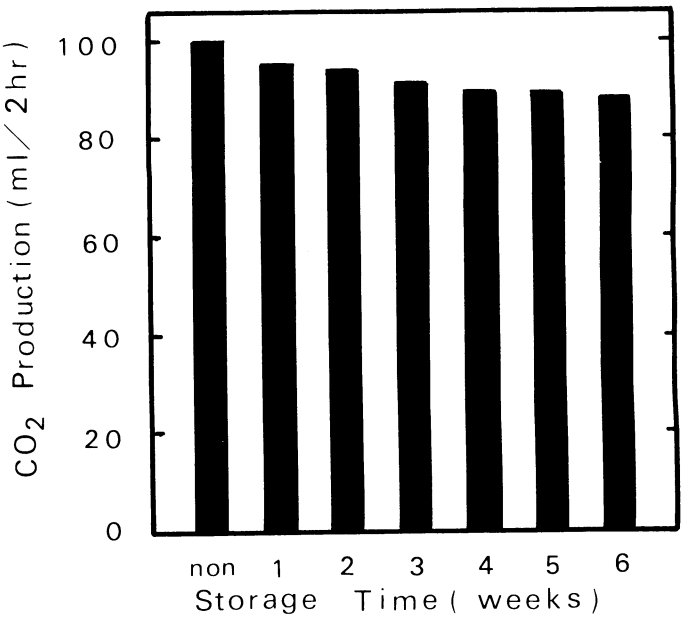

Fig. 7. Fermentative ability of frozen dough made with FRI 501 yeast and stored at −20° C for 1–6 weeks. CO_2 production for dough made with 20 g of flour.

prefermentation, because the maximum level of CO_2 production was lower. It thus appears that because of the prolonged prefermentation period, the yeast became sensitive to freezing. In the case of CFY, the freeze-sensitive *S. cerevisiae* yeast in the CFY underwent freezing injury after a short period of prefermentation, whereas addition of the freeze-tolerant *S. rosei* yeast enabled it to compensate for the loss after thawing. These yeasts, therefore, enabled good quality breads to be produced from frozen dough after a prefermentation of up to 30–60 min (Table III).

The maximum level of CO_2 production for the FRI 501 and FRI 413 yeasts was roughly comparable to that for the CFY and FRI 802 yeast, but the time for reaching that level was longer than in the case of CFY and FRI 802 yeast. It is likely that the amount of fermentation products would be lower than with the CFY and FRI 802 yeast during the prefermentation period, and these yeasts would be affected after thawing. The frozen dough prefermented with FRI 501 was mature, and the bread made from this frozen dough had a very pleasant flavor. The above observations were corroborated by the high survival rate of the activated yeast after freezing as shown in Figure 6. FRI 501 yeast was tolerant to freezing in the activated state. The rate of freezing injury of activated CY was very high compared to that of the FRI 501 yeast. If the fermentation products were to coexist in the freezing condition, as is the case in the frozen dough, the freezing injury would be more pronounced. Kline and Sugihara (1968) postulated that the reducing substance released from dead cells during frozen storage are related to the decreased gas retention associated with dough weakening. Wolt and D'Appolonia (1984) reported an increase in the content of sulfhydryl compounds in the dough during frozen storage; these compounds have a harmful effect on the rheological and baking properties of frozen dough. Only a small amount of fermentation products was accumulated in the dough when the FRI 501 yeast was used for a fermentation period of 180 min, and the activated cells were tolerant to freezing. Commercial yeast upon prefermentation for 90 min sustained destructive injury, whereas the FRI 501 yeast was not affected by freezing when the prefermentation lasted 180 min. Consequently, frozen dough made with full prefermentation may display good rheological properties, and vigorous yeast cells may ferment the thawing dough.

The CO_2 production and loaf volume from frozen and thawed dough made with the FRI 501 yeast with 90 min of fermentation prior to freezing and stored for six weeks at −20° C are indicated in Figure 7. CO_2 production hardly decreased during frozen storage for six weeks.

The three strains of FRI yeasts were identified mainly by the method described by Lodder and Kreger-van Rij (1952), Kreger-van Rij (1984), and Wickerham (1951). FRI 802 and FRI 413 were identified as *S. cerevisiae*, and FRI 501 as *Kluyveromyces thermotolerans*.

Based on the results of this study, it was confirmed that the freeze-tolerant yeasts showed different characteristics in their fermentative ability, gassing rate before and after freezing, and the degree of CO_2 retention in the dough mass after thawing. Similarly, their suitability for rich or lean dough formulations also varied. There are some limitations in the use of these freeze-tolerant yeasts, as the dough must be prepared under optimum conditions. Bread dough that has been well prepared with optimum prefermentation time before freezing can now be expected to yield acceptable bread after several weeks of proper storage. The characteristics of these freeze-tolerant yeasts, however, may change if the culture conditions such as aeration, feed rate of sugar, and other available nutrients change during the production of yeast. More detailed studies on the culture conditions should be carried out for the effective utilization of these yeasts.

S. cerevisiae is the most highly utilized species of yeasts for baking; however, some other species of yeasts can also be used (Sanderson 1985). We anticipate that frozen doughs may be prepared from other yeasts with better qualities isolated from various natural sources in the future.

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