

# Characterization of New Baker's Yeast Strains And Their Leavening Ability in Bread Dough

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## ABSTRACT

Two new Baker's yeast strains obtained from hybridization of commercial baker's yeast parent strains, *Saccharomyces cerevisiae* IFRPD 6080 and IFRPD 6081 with Japanese mating strains were investigated. The hybrids, IFRPD 6171 from parent strain 6080 and IFRPD 6173 from parent strain 6081, were evaluated for their characteristics, namely growth, total carbohydrate and trehalose contents, sugar fermentation ability, cryoresistance at -20 °C and their leavening ability in bread doughs. Both hybrids had good growth and higher maltose and sucrose fermentation abilities than their parents. Carbon dioxide productions in non-sugar, low-sugar and high-sugar dough were also markedly improved. In addition, hybrid 6171 possessed cryoresistance or freeze-tolerant ability in low-sugar dough and could be used for frozen dough preparation. Quality evaluation of white and sweet breads made from hybrids also showed better quality than their commercial parent strains. Thus, the hybrid 6171 was suitable for both white (low-sugar) and sweet breads (high-sugar) whereas hybrid 6173 was particularly good for sweet bread. Moreover, hybrid 6173 was also high in maltose fermentation ability and produced very high carbon dioxide in non-sugar dough, which indicated favorable characteristic to be used in non-sugar bread such as French bread.

**Key words:** *Saccharomyces cerevisiae*, cryoresistance, leavening ability, baker's yeast, bread dough

## INTRODUCTION

Frozen-dough technology for bread making has been extensively developed in the past decade. Beside good dough leavening property, ability of yeast cells to retain fermentation ability at low temperature or under frozen storage is one of the most requirements for baking industry. Baker's yeast strains with freeze-tolerant ability have been isolated and screened from natural sources (Oda *et al.*, 1986; Hino *et al.*, 1987; Hahn and Kawai, 1990; Almeida and Pais, 1996) and by genetically manipulated procedure (Nakagawa and Ouchi, 1994; Kyogoku and Ouchi, 1995;

Shima *et al.*, 1999). Yeast strains other than *Saccharomyces cerevisiae*, namely *Kluyveromyces thermotolerans* FRI 501 (Hino *et al.*, 1987) and *Torulaspora delbrueckii* (Hahn and Kawai, 1990; Oda and Tonomura, 1993; Almeida and Pais, 1996) have also been reported to have freeze-thaw resistance for frozen dough preparation. *Torulaspora* species have been reported to be more cryoresistant and have been potentially applicable in the baking industry (Oda *et al.*, 1986; Oda and Tonomura, 1993).

Hybridization study based on protoplast fusion by respiratory-deficient mutants have been successfully used in the isolation of the hybrids of

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*Saccharomyces cerevisiae* (Spencer *et al.*, 1985). They reported that some of segregants obtained from fusion hybrids between *Saccharomyces diastaticus* (industrial baker yeast strain of *Saccharomyces cerevisiae*) and *Saccharomyces (Zygosaccharomyces) rouxii* had much greater dough-raising capability in simulation bread-dough and sweet dough than either of the original hybrid or a commercial baker yeast. Improvement method of baker's yeast by rare-mating technique was also reported (Oda and Ouchi, 1990a; Wongkhalaung *et al.*, 2004). Nevertheless, the strains constructed by Oda and Ouchi (1990a) did not concurrently retain both freeze-tolerant characteristic and high leavening ability. Hybridization of *Saccharomyces cerevisiae* commercial baker's yeast with cryoresistant mater strain could produce the hybrid with improved leavening ability in low-sugar and high-sugar bread doughs as well as freeze-tolerant ability (Wongkhalaung *et al.*, 2004).

This study intended to investigate and compare the characteristics of the new hybrids with their commercial parent strains. Growth yield, sugar fermentation ability, carbohydrate and trehalose contents, invertase activity, cryoresistance and leavening property in non-sugar, low-sugar and high-sugar bread dough, as well as bread-making quality were evaluated.

## MATERIALS AND METHODS

### Yeast strains

Two commercial baker's yeast strains, *Saccharomyces cerevisiae* No. IFRPD 6080 and IFRPD 6181, from IFRPD Culture Collection and two selected hybrid strains, IFRPD 6171 obtained from parent strain IFRPD 6080; and IFRPD 6173 from parent strain IFRPD 6081 were used in this study. Hybrid IFRPD 6171 and hybrid IFRPD 6173, formerly strain 6080xs4D s27 and strain 6081xs34D s12, respectively, were produced from hybridization using rare-mating technique with Japanese mater strains (Wongkhalaung *et al.*,

2004).

### Culture media

YPD (yeast extract 1 %, polypeptone 2 %, glucose 2 %), solidified with 2 % agar if desired, was used as growth and maintenance medium. Cultivation of yeast cells were carried out in 5-litre flask with baffles containing 1-litre YNB sucrose medium (sucrose 2 %, sodium succinate 1 %, yeast extract 0.5 %, yeast nitrogen base without amino acid 0.67%) at 30 °C with rotation speed of 170 rpm for 40-48 hr. Yeast cells were harvested by centrifugation, washed twice and made up to 200 ml water. This yeast suspension, containing about 4-5 % dry matter (w/v), was used to determine leavening ability in terms of carbon dioxide production in normal dough and frozen dough.

### Mass cultivation in molasses

Each yeast strain was precultured with 2 × 100-ml YNB medium in Sakaguchi flasks for about 45 hr at 30 °C. Cells were harvested and suspended in 500 ml reversed osmosis (RO) water in 1-litre glass cylinder immersed in water bath at 30 °C. Molasses with 20% sugar content and C:N:P ratio of 100:4:0.4 was used as substrate. Cultivation was done by fed-batch culture with incremental feeding of molasses by microtube pump at predetermined rate with an aeration of 2 liter per minute during the cultivation period. After 12 hr cultivation, cells were then harvested by centrifuge and washed twice before suspended in RO water with final volume of 300 ml. This suspension was used for determination of sugar fermentation ability, total carbohydrate and trehalose content, invertase activity as well as bread-making test.

### Yeast characterization

#### Sugar fermentation ability

Ability of parent and hybrid strains to ferment maltose and sucrose were tested with Brom Cresol Purple or BCP method (Y. Nakatomi,

unpublished data). For non-sugar dough or maltose fermentation, substrate containing maltose as sole carbon source was used (maltose 2.4%; yeast extract 0.36 %; polypeptone 0.6% and BCP 0.4% in ethanol, 5 ml/600 ml medium). For high-sugar dough or sucrose fermentation test, maltose was replaced with 60% sucrose and 0.2% of NaCl was also added. One ml of substrate was mixed with 200 µl of yeast suspension (containing less than 10 mg/ml dry matter) and incubated in water bath at 30 °C for 1 hr. After making up to 3ml-volume, intensity of yellow color developed against the color of control substrate (purple) was expressed as plus/minus (yellow/purple).

#### **Total carbohydrate and trehalose determination**

Total carbohydrate and trehalose contents of yeast were determined by the method of McCready *et al.* (1974) and Trevelyan and Harrison (1956), respectively.

#### **Invertase activity**

A 0.5 ml yeast suspension (containing less than 5 mg/ml dry matter) was mixed with 0.5 ml of 6.5 % sucrose in acetate buffer, pH 4.5, in an ice bath. Enzyme activity was carried out in water bath at 30 °C for 3 min. Reaction was terminated by cooling in ice and 3 ml dinitrosalicylic acid reagent (DNS) added. The mixture was brought to boiling for 5 min, adjusted volume to 25 ml and OD measured at 525 nm. The method was determined according to Oda and Ouchi (1990b). One unit of invertase is defined as µg of glucose released at 30 °C per minute per one mg of yeast (dry basis) under the experimental conditions.

#### **CO<sub>2</sub> production measurement of bread dough and frozen dough**

Measurement of CO<sub>2</sub> production from dough was performed using Automatic Measuring System for Dough Testing as described by Hino *et al.* (1988). Each portion of the tested dough; non-sugar dough (20 g wheat flour, 0.4 g compressed yeast, 0.4 g salt and 12 g water); low-

sugar dough (20 g wheat flour, 0.4 g compressed yeast, 1 g sugar, 0.4 g salt and 12.5 g water); and high-sugar dough (30 g wheat flour, 0.6 g compressed yeast, 6 g sugar, 0.1 g salt and 10 g water), was prepared according to the standard method of Japan Yeast Industry Association (1995). The amount of total gas production and/or gas retention in the dough at 30 °C was automatically measured by Fermograph (Atto Co. Ltd., Japan) and was recorded every 10 minutes for total period of 5 hr.

Frozen dough of low-sugar bread dough was also prepared and kept at -20 °C for 1 and 2 weeks after 60-min prefermentation at 30 °C before Fermograph measurement.

#### **Bread-making process and bread quality evaluation**

Bread making was performed to evaluate yeast quality in white bread (low-sugar dough) and sweet bread (high-sugar dough). Each dough was prepared with commercial bread-making flour containing 12 % protein. The standard method of Japan Yeast Industry Association (1995), a modified AACC (American Association of Cereal Chemists, 1983) method, was used and formulations presented in Table 1.

Loaf weight and volume were measured immediately within 1 hr after baking. Loaf volume was measured with 3D Image Analyzer (SELNAC-VM, ASTEX Co., Ltd.). Specific volume was calculated from the ratio of volume/weight. Bread was cooled to 27±1 °C for 1 hr after baking then placed in polyethylene bag and stored at 27±1 °C for 18-24 hr before sensory evaluation. According to Evaluation Method of Bakers' Yeast by Japan Yeast Industry Association (1995), bread was evaluated for both the external characteristics, maximum 50 points (specific volume, 30; crust color, 10; symmetry of form, 5; crust character, 5) and the internal characteristics, maximum 50 points (grain, 10; crumb color, 5; texture, 5; aroma, 15; taste, 15).

## RESULTS AND DISCUSSION

### Growth yields in molasses

Growth yields, expressed as dry matter of yeast (% w/v) of parent strain 6080 (4.42 %) and hybrid strain 6171 (4.41 %) in molasses by fed-batch culture for 12 hr were compatible as shown in Table 2. The yields were slightly lower than those previously cultivated by batch culture for 48 hr in YNB medium (4.58 and 4.68 %, respectively). Similarly, for parent 6081 and hybrid 6173, growth yields were 4.46 and 4.21 %, respectively, in molasses compared to 4.95 and 4.40 % in YNB medium (Wongkhalaung *et al.*, 2004).

### Yeast characterization

#### Total carbohydrate and trehalose determination

Since parent strains 6080 and 6081 are commercial baker's yeast for high sugar bread

dough, they contain relatively high carbohydrate contents. Carbohydrates content of all strains grown in molasses were in the range of 30 to 32 % (Table 2) which were lower than those (34 – 39 %) grown in YNB medium (Wongkhalaung *et al.*, 2004). Trehalose contents were 6.24, 6.74, 6.0 and 5.7 % for strains 6080, 6171, 6081 and 6173, respectively (Table 2) which were also slightly decreased from those grown in YNB medium. This was probably due to rather high feeding rate of molasses during fed-batch cultivation, which induced budding of cells resulting in low accumulation of trehalose. Trehalose has been reported by many investigators to have certain roles in heat and desiccation resistance and cryoresistance in frozen bread dough method (Hino *et al.*, 1990; Meric *et al.*, 1995). Diploid strain of *S. cerevisiae* with >5 % trehalose was shown to be more tolerant to heat and freeze-thaw stress than strains that produced <4 % trehalose (Attfield *et al.*, 1992). Resistance to dehydration

**Table 1** Straight dough formula for bread making.

Ingredient	Amount (parts)	
	White bread dough (low-sugar)	Sweet bread dough (high-sugar)
Bread-making flour (12 % protein)	100	100
Compressed yeast*	2	4
Sugar	5	25
Salt	2	0.7
Shortening	5	6
Water**	65-68	56-58

\* Compressed yeast (66.7% moisture content) was replaced with yeast suspension calculated to have equivalent amount of yeast. \*\*Water was adjusted accordingly.

**Table 2** Growth yields (dry matter, w/v) and some characteristics of parent and hybrid Baker's yeast strains cultivated in molasses.

Strain	Yield (%, w/v)	Fermentation*		Trehalose (%)	Carbohydrate (%)	Invertase (unit/mg)
		Maltose	Sucrose			
Parent 6080	4.42	+	+++	6.24	31.22	210
Hybrid 6171	4.41	++	++++	6.74	32.19	98
Parent 6081	4.46	+	+++	6.0	30.60	227
Hybrid 6173	4.21	+++	++++	5.7	31.64	163

\* high fermentation ability was indicated by the number of +.

of *S. cerevisiae* containing high trehalose content was also increased if high level of intracellular trehalose accumulated in stationary-phase cells or cells incubated in the absence of nitrogen source (Gadd *et al.*, 1987). Among all strains, IFRPD 6171 contained the highest trehalose content (6.74 %).

Due to the fact that trehalose contents in all strains were still higher than 6 %, it was likely to have inevitable effect on cryoresistance of yeast strains. Moreover, some investigators believed that other factors also played an important role in freeze tolerance since the highest trehalose content in the yeast cells did not always correspond to the highest cryoresistance (Almeida and Pais, 1996).

#### **Invertase activity**

Invertase activities of hybrid strain 6171 (98 U/mg) reduced markedly compared to their parent strain IFRPD 6080 (210 U/mg). Hybrid strain 6173 also had lower invertase activity (163 U/mg) than the parent IFRPD 6081 (227 U/mg) as shown in Table 2. Since baker's yeast is highly sensitive to high osmotic pressure created by sugar and/or salt in bread dough, yeast with low invertase helps to prevent adverse effect of high osmotic pressure (Oura *et al.*, 1982). Thus, lower invertase activity of these new strains was considered beneficial for leavening ability in low- and high-sugar bread doughs. This property was most likely to be inherited from mating strains as in the case of freeze-tolerant ability.

#### **CO<sub>2</sub> production measurement in bread doughs**

Leavening ability for both hybrid and parent strains could be observed by measurement of CO<sub>2</sub> production in non-sugar, low-sugar and high-sugar bread dough by Fermograph which appears as Figures 1, 2. Both parent strains, IFRPD 6080 and 6081, are originally commercial active dried yeast, which are mainly used for high sugar bread dough. Thus, the ability to ferment maltose is relatively low and not appropriate for non-sugar dough.

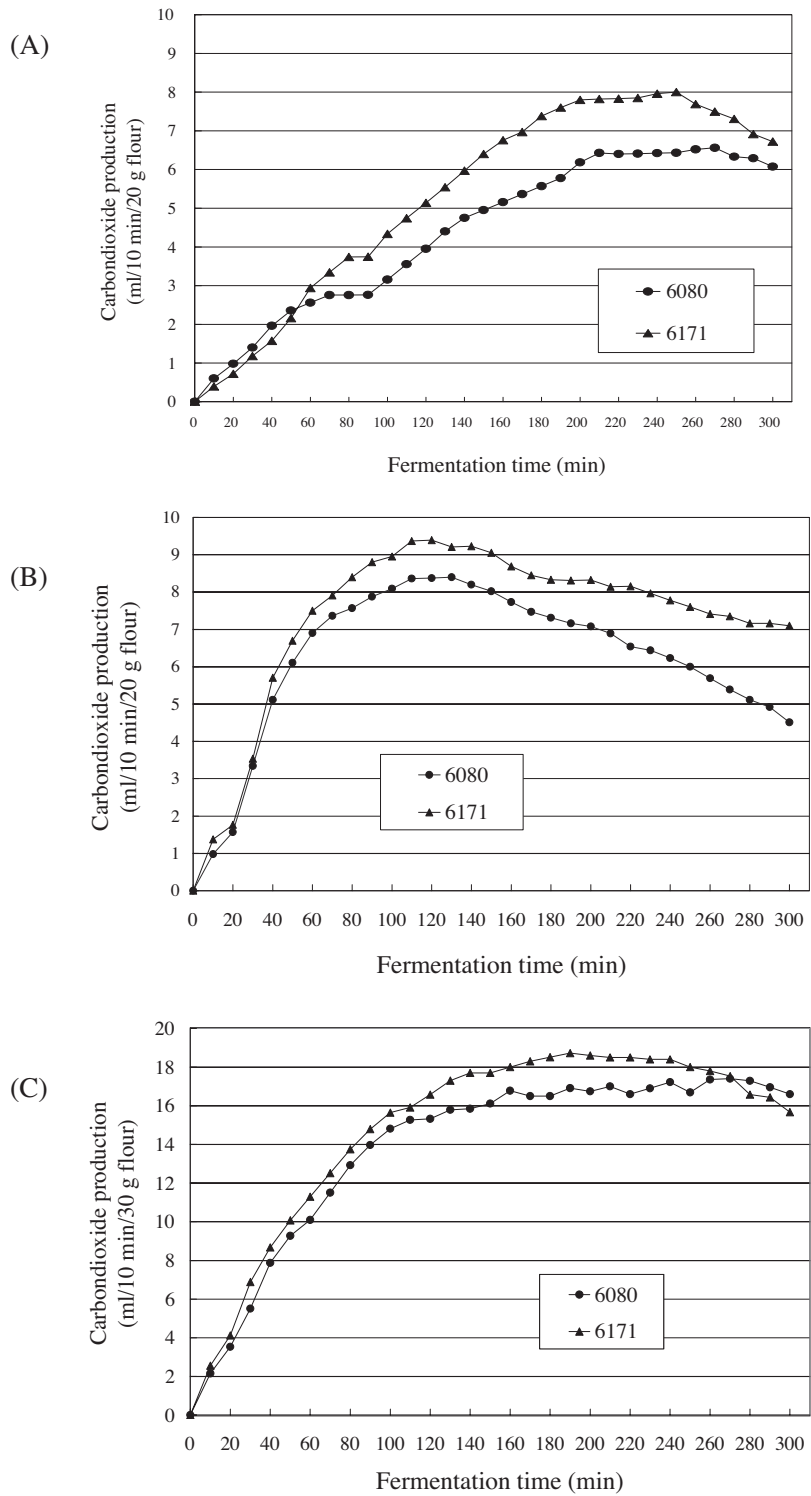
The hybrids showed better maltose fermentation ability than their parents (Table 2). Maltose is the main fermentable sugar present in the flour for non-sugar bread dough. Yeast with high maltose fermentation ability that can liberate maltose from damaged starch in the flour is desirable for non-sugar dough preparation. It was quite understandable that high maltose fermentation ability was been achieved from the mater strain.

Strain 6171 from parent 6080 was better than the parent in terms of total CO<sub>2</sub> production for all types of dough, with considerable increase in non-sugar and low-sugar dough (Figures 1, 3). Parent strain 6081 exhibited higher total carbon dioxide production than parent 6080 in all types of bread dough (Figure 3). Likewise, its hybrid strain 6173 showed improved leavening ability than the parent 6081, especially in non-sugar and high-sugar dough as shown in Figures 2 and 3.

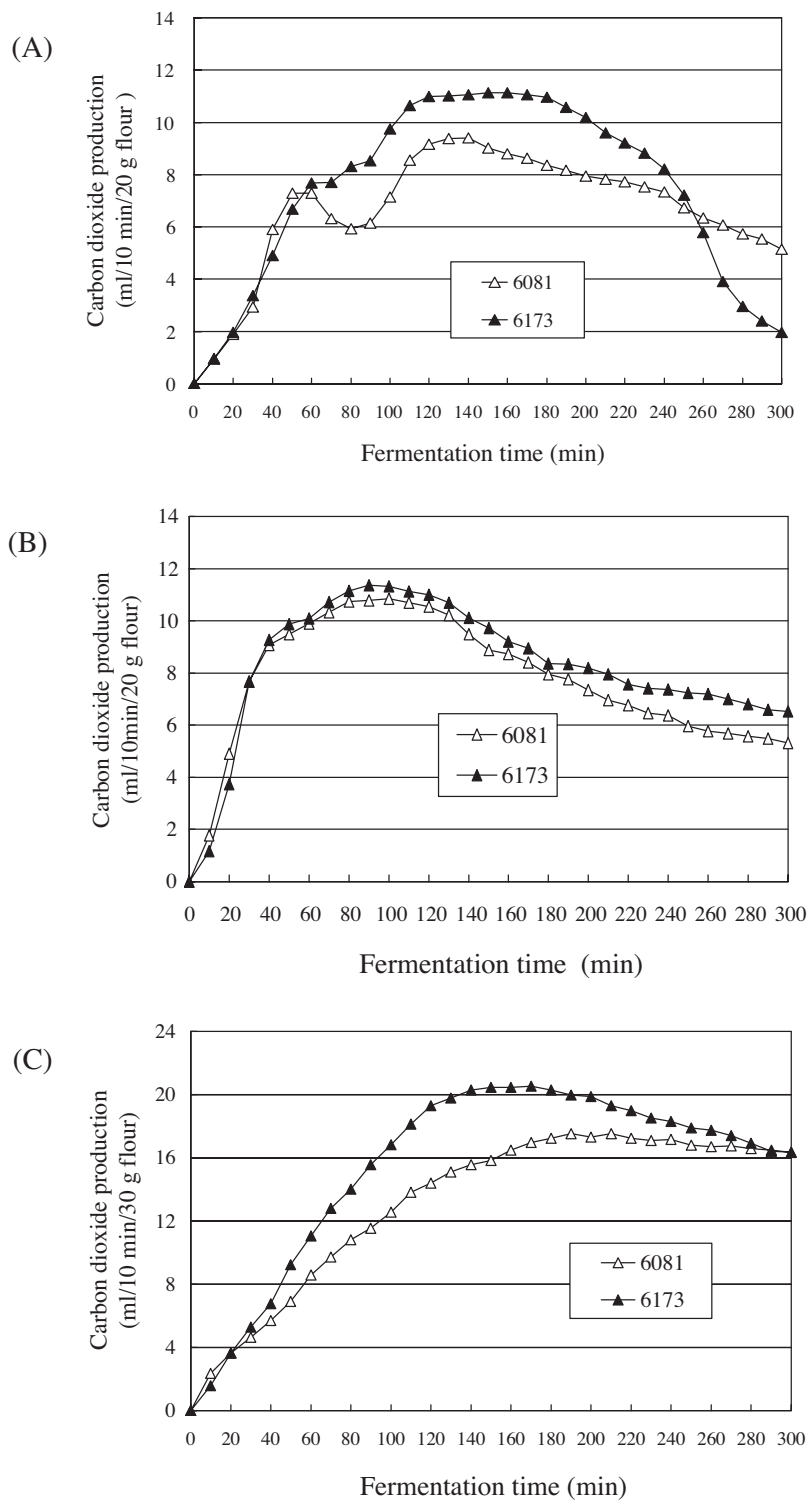
#### **Cryoresistance in frozen dough**

Traditional *Saccharomyces cerevisiae* baker's yeast strain is generally susceptible to freeze damage during prolonged storage of prefermented dough and renders it to have less potential for frozen-dough method (Hsu *et al.*, 1979). Cryoresistance ability including freeze-thaw stability is affected by physiological property of the cells and trehalose is considered as an important factor (Gadd *et al.*, 1987). Cryoresistance was evaluated by measurement of CO<sub>2</sub> production in low-sugar bread dough, after frozen-storage for 1 and 2 weeks at -20 °C.

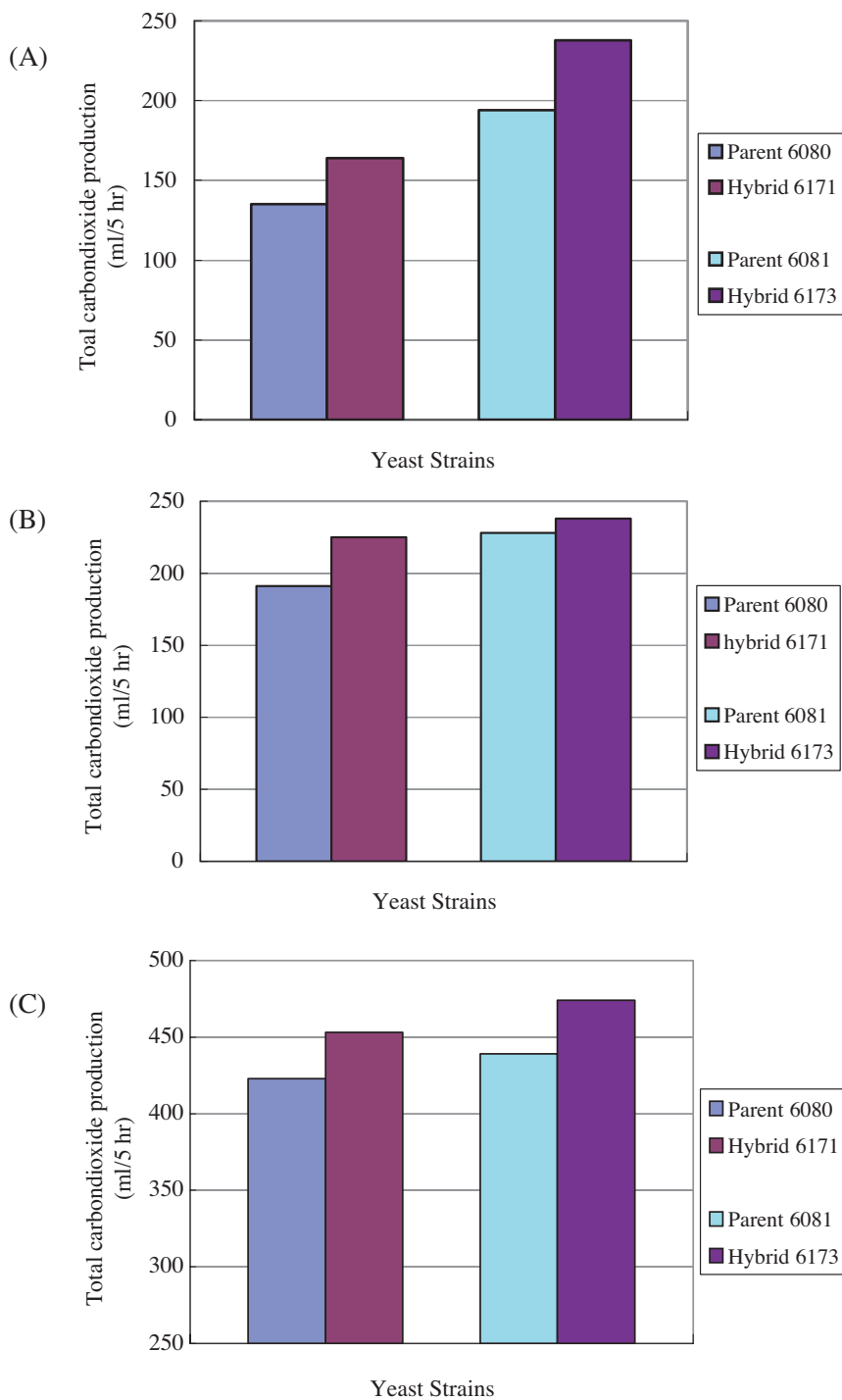
Hybrid 6171 from parent 6080 maintained better fermentation ability than the parent with a slight decrease in CO<sub>2</sub> production after 1-week storage (Figure 4). Total gas production was about 20 % lower than the non-frozen dough after 2 week-storage but still much higher than that of the parent 6080. Thus, strain 6171 was considered to possess freeze-tolerant ability in low-sugar (5%) bread dough when prefermented for 60 min before freezing up to 1 week storage at -20 °C.



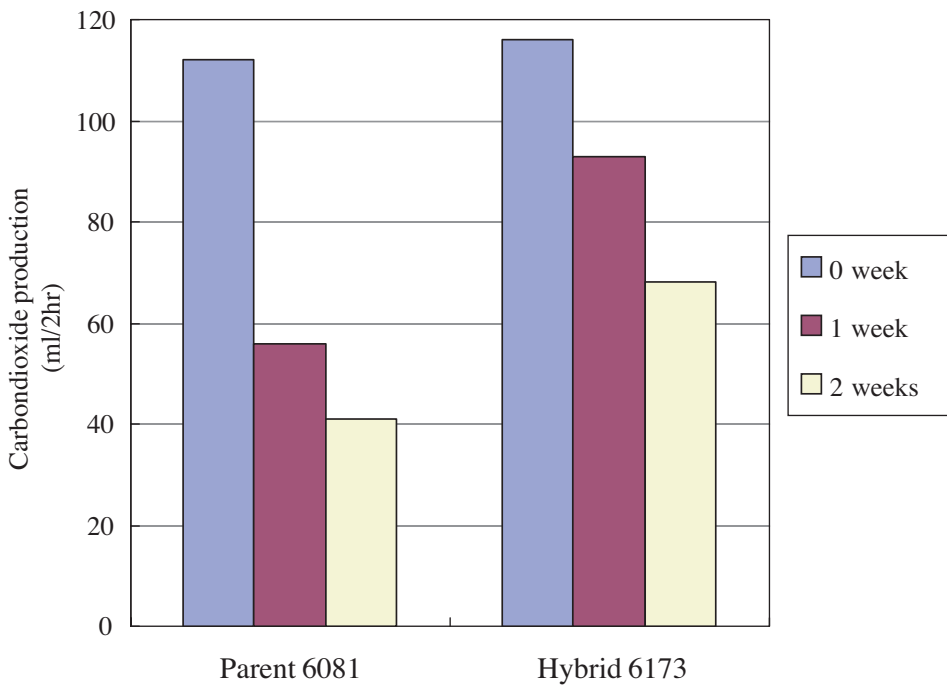
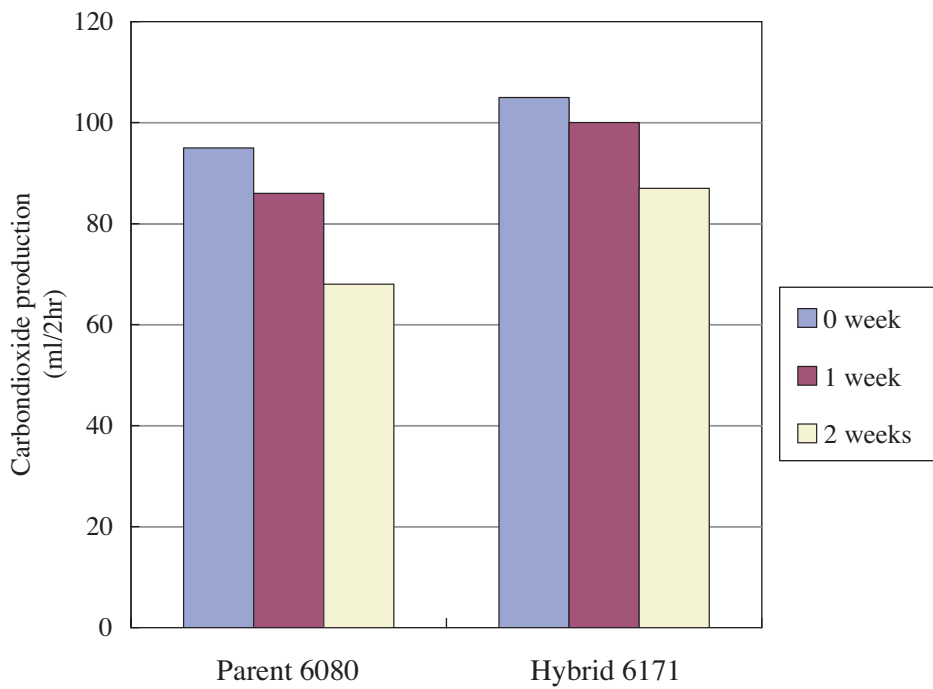
**Figure 1** Carbon dioxide production pattern of non-sugar (A), low-sugar (B) and high-sugar (C) bread dough of hybrid strain 6171 and commercial parent strain 6080.



**Figure 2** Carbon dioxide production pattern of non-sugar (A), low-sugar (B) and high-sugar (C) bread dough of hybrid strain 6173 and commercial parent strain 6081.



**Figure 3** Total carbon dioxide production of new hybrids and their parent strains in  
(A) Non-sugar bread dough  
(B) Low-sugar bread dough  
(C) High-sugar bread dough



**Figure 4** Freeze-tolerant abilities upon 1 and 2-week storages at  $-20^{\circ}\text{C}$  after 60-minute prefermentation of low-sugar bread dough.

Hybrid strain 6173 developed from the parent 6081 had lower freeze-tolerant ability than hybrid strain 6171 as demonstrated in Figure 4. Its total CO<sub>2</sub> production was apparently much better than the parent strain 6081 after 1 and 2-week frozen storage. However, the amount of CO<sub>2</sub> production in dough decreased about 25 and 40 %, respectively, after 1 and 2 weeks frozen storage at -20 °C which made it not suitable for frozen dough preparation.

### Bread quality evaluation

Quality of bread made from yeast suspension of strains 6080, 6081, 6171 and 6173 cultivated in molasses, were evaluated and shown as Table 3. The pictures of white bread and sweet bread prepared from all strains are demonstrated in Figure 5. The hybrid strain 6171 showed improved leavening ability with distinctively higher scores in both white bread (low-sugar) and sweet bread (high sugar) with the score of 84 (B Rank) and 84.2 (B Rank), respectively, whereas

the commercial parent strain 6080 got only C rank for both white bread (74) and sweet bread (78.2). Quality ranking at B level is considered as good-quality bread while C level is normal.

Similar results were obtained with strain 6173 which exhibited higher scores than the parent 6081. The scores of 78 (C) for white bread and 81.5 (B) for sweet bread were obtained compared to 71.2 (C) and 70.5 (C), respectively, for parent strain 6081.

It was evident that the new hybrid strains have high potential for industrial application as a result of their better breads quality compared to their parent strains.

Interestingly, though hybrid strain 6173 proved to be highly promising for leavening ability in all types of bread dough in terms of total gas production, when applied in bread making, quality rankings of both white and sweet breads were slightly lower than those of the hybrid strain 6171.

It could be concluded that hybrid 6171 was excellent for both white (low-sugar) and sweet

**Table 3** Quality evaluation of white bread and sweet bread made from hybrids (6171 and 6173) and strains (6080 and 6081)

	White bread			
	Parent 6080	Hybrid 6171	Parent 6081	Hybrid 6173
Loaf volume (ml)	695	745	660	680
Loaf weight (g)	151	149	151	150
Specific volume*	4.6	5.0	4.4	4.5
Score of specific vol.	16	20	14	15
Quality score**	74	<b>84</b>	71.2	<b>78</b>
	Sweet bread			
	Parent 6080	Hybrid 6171	Parent 6081	Hybrid 6173
Loaf volume (ml)	805	880	715	850
Loaf weight (g)	163	165	166	167
Specific volume*	4.9	5.3	4.3	5.1
Score of specific vol.	19	23	13	21
Quality score**	78.2	<b>84.2</b>	70.5	<b>81.5</b>

Each value is the mean of two samples.

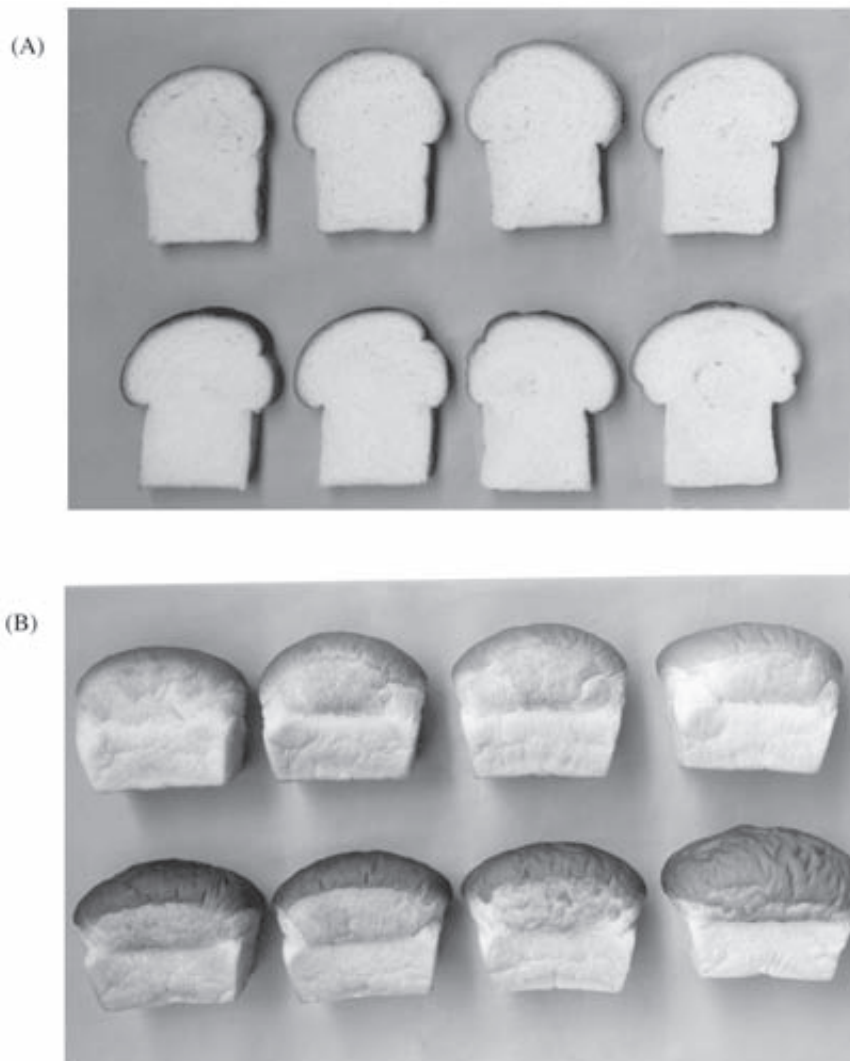
\* Specific volume is ratio of loaf volume per weight. The value  $\geq 6$  is very good (30 points), 5.4-5.9 is good (24-29 points), 4.8-5.3 is normal (18-23 points), 4.2-4.7 is poor (12-17 points), and 4.0- 4.1 is very poor (10-11 points).

\*\* Quality score is obtained from external appearance (50) and internal characteristic (50). The score  $>90.1$  is very good, 80.1-90 is good, 70.1-80 is normal, 60.1-70 is poor and  $<60$  is very poor.

(high-sugar) breads whereas hybrid 6173 was appropriate for sweet bread. However, due to the fact that hybrid 6173 was high in maltose fermentation ability and produced very high carbon dioxide in non-sugar dough, it appeared to be suitable for non-sugar bread dough such as French bread as well.

## CONCLUSION

Growth in molasses medium by fed-batch culture showed comparable yields of two new hybrids with the parents. Leavening property and fermentation ability in maltose and sucrose were much better in the hybrid strains. Carbon dioxide productions in non-sugar, low-sugar and



**Figure 5** (A) Slices of white bread (top row) and sweet bread (bottom row) made from strains 6080; 6081; 6171 and 6173 (from left).  
(B) Loaves of white bread (top row) and sweet bread (bottom row) made from strains 6080; 6081; 6171 and 6173 (from left).

high-sugar dough were also markedly improved. Cryoresistance determination of the hybrids after frozen-storage for 1 and 2 weeks at -20°C revealed more freeze-tolerant ability than the parent strains. Freeze-tolerant ability was remarkable in strain 6171 and was qualified for frozen dough application.

Hybrid 6171 was suitable for both white (low-sugar) and sweet (high-sugar) breads whereas hybrid 6173 was good for sweet bread. In addition, hybrid 6173 also possessed good maltose fermentation activity and produced high carbon dioxide in non-sugar dough.

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