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(54) Title: IMPROVED YEAST PERFORMANCE USING COMPATIBLE SOLUTES/OSMOPROTECTANTS

(57) Abstract

The present invention provides a method of increasing the performance of yeast, the method comprising exposing the yeast to at least one compatible solute/osmoprotectant for a period of time sufficient to result in an intracellular concentration of the at least one compatible solute/osmoprotectant of at least 70μMoles/gram dry yeast. It is preferred that the exposure is for a period of time sufficient to result in an intracellular concentration of the at least one compatible solute/osmoprotectant of at least 100μMoles/gram dry yeast. The compatible solutes/osmoprotectants is preferably glycerol.

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Improved Yeast Performance Using Compatible Solutes/Osmoprotectants

Many industrial applications of yeast involve exposure to high osmotic pressures, exerted by various ionic and non-ionic chemicals or solutes (eg. salts and sugars). For example, baker's yeast may be exposed to high osmotic pressures in production (eg., during fermentation and drying or freezing) and application (eg., plain and sugar doughs, frozen doughs). In fact, industrial yeasts in general may be subjected to high osmotic pressures in fermentations involving, for example, production of alcohol (potable and non-potable), soy sauce, miso etc. High external osmotic pressures result in removal of water from cells and increased concentration of internal solutes. This can be brought about by high external solute concentration, leading to loss of intracellular water through osmosis, or by removal of water availability as a solvent (eg. by drying or freezing). Low water activity environments provide for high osmotic pressure. For example the environment encountered by baker's yeasts in different concentration sugar (sweet) doughs can vary in water activity from (Aw ~0.98 for doughs with no added sugar, Aw ~0.95 for doughs with 16% w/w sucrose on flour, to Aw ~0.93, 25% w/w sucrose on flour). Typical liquid culture media have Aw's in excess of these dough systems.

The present inventors believe that in the case of bakers' yeast (and probably, also alcohol producing fermentations etc), there is a need to rapidly produce and retain glycerol when cells are introduced to higher osmotic pressure environments such as doughs. This seems to be a pre-requisite for good fermentation and leavening activity. The demand for this phenotype increases, for example, as the dough sugar content is increased, ie. greater amounts of glycerol are needed to be produced and retained in high sugar than plain doughs. Fast yeast response in glycerol synthesis is particularly important in the process of "no time dough" bread making, where there would be a maximum of about 2 hrs from mixing to baking.

It is generally believed that the performance of yeasts in high sugar dough systems is inversely related to invertase activity (1). However, the present inventors have shown that good fermentation power in dough systems can vary even in the same yeast strain with very similar invertase levels. Investigation of the cause of variation has led to the identification of

glycerol synthesis and internal concentration/ retention by yeasts as being linked very closely with the degree of activity. This suggested that although invertase activity may have some bearing on sugar dough performance, it is not the sole factor responsible for good fermentation in such doughs. Nevertheless it is probable that a low invertase level will offer advantages to yeast cells in high sucrose-containing systems provided they have other, more crucial, osmotic response factors.

Mechanistically it appears that on inoculation into high sugar doughs a yeast must produce, as fast as possible, and retain a certain level of glycerol as a compatible solute, to achieve osmotic stability with the outside environment. The concentration necessary will be determined by the osmotic pressure of the external environment. More glycerol will need to be made and retained in high sugar than plain doughs. The rate at which this occurs determines, in part, the length of lag before yeast achieves maximum gassing rate in doughs. This is seen not just in freshly made yeast, but also stored yeast where there appears to be a correlation between retention of glycerol synthetic capacity and leavening activity in sugar doughs. The significance of lower invertase seems to be that it can "buy time" for the yeast whilst it equilibrates through the glycerol response to high osmotic pressure. The lower invertase activity predisposes to less rapid accumulation of free glucose and fructose from sucrose and hence a lesser osmotic stress is imparted to the yeast before it has time to induce its glycerol production and retention system and thereby be osmotically equilibrated. Thus for higher invertase strains there may be a too rapid hydrolysis of sucrose to "free" glucose and fructose, leading to a rapid increase in external osmotic pressure resulting in a greater requirement for osmotic equilibration. This could be manifested as an extended lag time before significant leavening occurs, and/ or a reduced maximum gassing rate because of excessive requirement for glycerol synthesis - glycerol synthesis occurs as a branching of the glycolytic pathway and as such represents removal of sugar from gas-producing ethanolic fermentation. It should be understood that the nature of metabolism in high sugar doughs is more complex than this simple "model" suggests. Even with good glycerol production and retention, and low invertase, yeast are still somewhat inhibited in high sugar doughs.

Observations regarding various commercial yeasts lead the present inventors to conclude that commercial baker's yeast should have, at least, "as made" invertase activity below a critical level (defined as 15 units of activity, where one unit refers to one μMol glucose released from sucrose per minute per mg protein at pH4.9 and 30°C), in combination with good glycerol production, that results in equal to >0.8 mMol glycerol/ gram equivalent dry yeast after 90 min in a 25% sugar dough system (with >0.7mMol glycerol/ gram equivalent dry yeast retained intracellularly), in order to be good high sugar dough yeast.

Many workers have published on the role of polyols, and in particular, glycerol, as compatible solutes in osmoregulation of yeasts and other organisms in hyperosmotic environments (for example, see 3- 8). It is known that truly salt- and osmo-tolerant yeast (eg., *Debaryomyces hansenii*, *Zygosaccharomyces rouxii, Torulaspora delbruckii*) produce and retain copious amounts of glycerol and other compatible polyols in response to high osmotic pressures, whereas *Saccharomyces cerevisiae* baker's yeast, which are not considered truly osmotolerant strains, show increased glycerol production but also leak much of this from the cell (5-8). Mutant *Saccharomyces cerevisiae* strains deficient for glycerol synthesis are sensitive to hyperosmotic conditions and this sensitivity can be relieved by introduction of functional glycerol synthesis genes (9). suggesting strongly that glycerol production is critical to osmotolerance.

The present inventors have found that the performance of yeast for high osmotic processes can be improved by the addition of compatible solutes such as glycerol to yeast biomass. As will be understood by those experienced in this area there are a number of factors in assessing the performance of yeast. These include, for example, fermentative activity, leavening activity, time taken to proof doughs and retention of activity during storage. Improvement in any one these criteria would be viewed as an increase in performance.

Accordingly, in a first aspect, the present invention consists in a method of increasing the performance of yeast the method comprising exposing the yeast to at least one compatible solute/osmoprotectant for a period of time sufficient to result in an intracellular concentration of the at least one compatible solute/osmoprotectant of at least 70μMoles/gram dry yeast.

In a preferred embodiment of the present invention the yeast is exposed to the at least one compatible solute/osmoprotectant for a period of time sufficient to resnlt in an intracellular concentration of the at least one compatible solute/osmoprotectant of at least 100μMoles/gram dry yeast.

The compatible solutes/osmoprotectants is preferably selected from the group consisting of glycerol, trehalose, sucrose, maltose, glucose, fructose, mannose, ammonium salts, amino compounds, and combinations thereof. The most preferred compatible solute/osmoprotectant is glycerol. Where the solute is glycerol it is preferred that the yeast is exposed to the glycerol for a sufficient period of time to result in an intracellular concentration of glycerol of at least 100μMoles/gram dry yeast equivalents.

In a further preferred embodiment of the present invention the yeast is exposed to glycerol at a concentration of at least 0.1M and preferably at a concentration of at least 0.2M. In the production of a frozen product it is preferred that the concentration of glycerol is at least 0.4M.

In yet a further preferred embodiment of the present invention the yeast is exposed to the compatible solute for at least 24 hours.

In a further preferred embodiment of the present invention the yeast is in the form of yeast having greater than 18% dry yeast material.

In a second aspect the present invention consists in a yeast product produced by the method of the present invention, preferably a cream yeast or compressed yeast product.

In a third aspect the present invention consists in a frozen dough product including a yeast prepared by the method of the present invention.

In a further aspect the present invention consists in a cream yeast product characterised in that the yeast has an internal glycerol concentration of at least 100μMoles/gram dry yeast equivalents.

In yet another aspect the present invention consists in compressed yeast product characterised in that the yeast has an internal glycerol concentration of at least 100μMoles/gram dry yeast equivalents.

In order that the nature of the present invention may be more clearly understood, preferred forms thereof will now be described with reference to the following examples and Figures in which:-

Figures 1 and 2 show uptake of glycerol by yeast stored at 4°C (●, yeast with no added glycerol: ■, ▲, ▼ yeast plus 0.2M glycerol added). Significant benefit from glycerol addition is not observed until at least 24 hr

contact time, therefore optimal application would be by stirring into cream at chilled temperatures and mixing gently for preferably >24hr.

Figure 3 shows CO_2 gas -producing activity and stability (retention of activity with storage time) of yeast cream in 16% sugar doughs (O, yeast plus 0.2M glycerol; ●, yeast with no added glycerol). Benefit is observable in sugar dough application of cream yeast where the level of glycerol proposed gives marked improvement in stability of product.

Figure 4 gives the activity and stability of compressed yeast made from glycerol-treated or non-glycerol-treated cream (O, yeast plus 0.2M glycerol; ●, yeast with no added glycerol). Benefit is observable in sugar dough application of compressed yeast made from glycerol-treated cream where the level of glycerol proposed gives marked improvement in stability of product.

Figure 5 shows plain dough activity and stability of yeast cream in plain doughs (O, yeast plus 0.2M glycerol; ●, yeast with no added glycerol). Benefit is observable in plain dough application of cream yeast where the level of glycerol proposed gives improvement in activity and some improvement in stability of product.

Figure 6 shows plain dough activity and stability of compressed yeast made from glycerol -treated or non-glycerol-treated cream yeast (O, yeast plus 0.2M glycerol; ●, yeast with no added glycerol). Benefit is observable in plain dough application of compressed yeast made from glycerol-treated cream where the level of glycerol proposed gives marked improvement in stability of product.

Figure 7 shows proof times for 10% sugar frozen doughs that have been stored at -21°C for up to 8 weeks. (A, Yeast at 4°C for one day; B, Yeast at 4°C for three days; C Yeast at 4°C for seven days; D, Yeast at 4°C for twenty one days). (●, doughs made with yeast containing no added glycerol; ■, doughs made with yeast to which glycerol was added at 0.2M; ▲, doughs made with yeast to which glycerol was added at 0.3M).

Figure 8 gives proof times for 10% sugar frozen doughs that have been stored at -21°C for up to 8 weeks. (A, Yeast at 4°C for one day; B, Yeast at 4°C for three days; C Yeast at 4°C for seven days; D, Yeast at 4°C for twenty one days). (●, doughs made with yeast containing no added glycerol; ■, doughs made with yeast to which glycerol was added at 0.4M).

EXAMPLES

Materials and Methods

Glycerol (food grade) was obtained from Henkel Australia. Bakers' flour was purchased from Defiance mills (Sydney, Australia); sugar from CSR (Sydney, Australia); Shortening was DPS2; Bread improver was Bakerine Special for activity testing and Bakedoh for Bake testing - both obtained from Mauri Integrated Ingredients (Sydney, Australia). Salt was Analar. Industrially produced yeast was obtained from Burns Philp & Co. Ltd. factories.

Method of Glycerol Incorporation into yeast:

1. Addition to Cream (suspension of yeast at <23% w/w, usually <20%w/w).

Cream yeast is typically about 20% w/w (ie. 100g cream = 20 g dry yeast material and approx. 80 ml aqueous. Of the 80 ml fluid, up to approximately 60% is intracellular). Glycerol is premixed by stirring/ agitating with a portion of the cream yeast and this mixture is then added to the main body of cream to create an homogeneous mixture. Cream is stirred/ agitated at <10°C, and preferably <4°C for > 1 hour and preferably >24 h. Cream can then be used as cream yeast product, or converted to other forms of industrial yeast preparations eg. crumble, compressed, frozen.

2. Addition to Crumbled or Compressed Yeast (yeast solids >23% w/w, usually >27%).

Crumble or compress is usually made by filtering cream yeast to concentrate yeast cells to consistency of the order of 27-35% w/w (ie. 100g compressed = 27-35 g dry yeast material and approx. 65-73 ml fluid. Up to 75% of this fluid is intracellular). Such yeast preparations can be taken and glycerol mixed in, preferably whilst maintaining a cool temperature of the yeast (<10°C, and preferably <4°C). Mixing time is as rapid as possible whilst achieving as homogenous mixing as possible. Yeast can then be

stored (<10°C, and preferably <4°C) as crumble to allow glycerol to equilibrate across membranes prior to making into blocks, or use as crumble in its own right.

Methods for non -frozen doughs and activity tests

Compressed yeast

All activity tests were carried out using an SJA Fermentograph set at 30°C using the dough formulations shown in Table 1. Dry ingredients were weighed into a Farinograph mixing bowl and blended for one minute. Compressed yeast was suspended in water then added to the dry ingredients, together with the salt solution. The complete dough was mixed for three minutes, reaching a final dough temperature of 30°C.

The finished dough was transferred to a standard Fermentograph tin then put into the SJA apparatus. Tests were carried out for two hours knocking the dough down after the first hour.

TABLE 1
Fermentograph Formulations Used in Activity Tests
(Compressed Yeast)

Ingredients	**Plain dough**	**16% sugar dough**	**25% sugar dough**
Flour	280g	250g	250g
Sugar	-	40g	62.5g
Bread Improver*	1.5g	1.3g	1.3g
Water	100ml	91ml	92ml
Salt Solution (9.5% w/v)	57ml	26ml	26ml
Yeast (@ 30% solids)	5g	10g	10g

* Mauri Foods Bakerine Special

Cream yeast

All activity tests were carried out using an SJA Fermentograph set at 30°C using the dough formulations shown in Table 2. Dry ingredients were weighed into a Farinograph mixing bowl and blended for one minute. Cream yeast was suspended in water then added to the dry ingredients, followed by the salt solution. The complete dough was mixed for three minutes, reaching a final dough temperature of 30°C.

The finished dough was transferred to a standard Fermentograph tin then put into the SJA apparatus. Tests were carried out for two hours knocking the dough down after the first hour.

TABLE 2
Fermentograph Formulations Used in Activity Tests
(Cream Yeast)

Ingredients	Plain dough	16% sugar dough
Flour	280g	250g
Sugar	-	40g
Bread Improver*	1.5g	1.3g
Water	98.0ml	96.0ml
Salt Solution (9.5% w/v)	57ml	26ml
Yeast Cream	7.50ml	15ml

* Mauri Foods Bakerine Special

EXAMPLE. 1. Compressed yeast in plain dough

1 Kg compressed yeast (ca. 28%) solids was mixed for 3 min at 4°C using a Hobart paddle mixer to create a crumble texture. Glycerol was added to a final concentration of 0.2 Molar (based on total water content of yeast) and

mixing continued for 3 min. Yeast was then packed densely into plastic tubs and stored at 4°C. Control yeast that had been crumbled but not had any glycerol added was also stored.

Table 3

Days stored at 4°C	Control (zero added glycerol)		Test (0.2 Molar glycerol)	
	Plain	16% Sugar	Plain	16% Sugar
Fresh (as made)	1360	1285	1370 (+1%)	1415 (+10%)
5	1245	1165	1295 (+4%)	1420 (+22%)
15	1115	845	1135 (+2%)	1300 (+54%)
21	915	795	1170 (+28%)	1185 (+49%)

EXAMPLE. 2. Compressed yeast in sugar dough

Glycerol added or not as above.

Table 4

Days stored at 4°C	Control (zero added glycerol)		Test (0.2 Molar glycerol)	
	16% Sugar	25% Sugar	16% Sugar	25% Sugar
Fresh (as made)	1825	1175	1815 (-1%)	1185 (+1%)
6	1685	990	1810 (+7%)	1245 (+26%)
14	1525	930	1740 (+14%)	1170 (+26%)
21	1455	800	1625 (+12%)	985 (+23%)

Frozen Dough Tests

Example 3. Compressed yeast in frozen dough

Glycerol additions to yeast

Glycerol was added to cream yeast as described previously and compressed yeast samples prepared. These were stored refrigerated for up to 21 days and at intervals yeast was used for preparing frozen doughs.

Frozen Dough Testing

The dough composition used is set out in Table 5. Mixing time was determined by Farinograph and water absorption by Extensograph. Ingredients were weighed into a bucket, other components (except water) added so as to not come into contact with each other, mixed for 1 minute and then water added. The dough was mixed until fully developed. Final dough temperatures were 20 +/-2°C. Seven 520g dough pieces were produced and moulded (six inch Mono Moulder), one being tested immediately for proof time (time to rise to 120 mm) and the other six were blast frozen at -40°C until the core temperature was ~ -5°C. Frozen doughs were then stored at -21°C for 1, 4 and 8 weeks: two doughs were defrosted (at 4°C for 16 hours prior to proof testing) and tested at each time.

Table 5 - frozen dough compositions

Component	Plain dough		10% sugar dough	
	Percentage	Grams	Percentage	Grams
Flour	100	2500	100	2500
Salt	2	50	2	50
Improver	1	25	1	25
Shortening	2	50	2	50
Sugar	-	-	10	250
Water	Variable		Variable	
Yeast (comp.)	5	125	5	125

Figures 7 and 8 show that addition of glycerol to yeast cream followed by a sufficient period for glycerol to enter yeast cells and subsequent preparation of compressed yeast significantly improved stability of 10% sugar dough over eight weeks storage at -21°C. When the yeast was made up to 0.4 M glycerol, the proof times of the resulting frozen 10% sugar doughs increased only slightly from 1 week to 8 weeks storage at -21°C. Untreated yeast suffered an increase in proof time due to initial freezing and especially 0.4 M glycerol additions significantly reduced the loss on freezing.

Benefits were related to the amount of glycerol added in the range 0.2 to 0.4 M (total water basis). The 0.2 M and 0.3 M treatments gave similar results for doughs made after 1, 7 and 14 days storage of the yeast at 4°C, but after 21 days storage the 0.3 M addition gave significantly better results than the 0.2 M addition. 0.4 M glycerol addition resulted in significantly better frozen 10% sugar doughs than the 0.2 M or 0.3 M treatments.

The performance of the treated material relative to the control became greater as storage time of the yeast (as either cream or compressed at 4°C) prior to dough production progressed: the longer untreated yeast was kept at 4°C prior to frozen 10% sugar dough production, the worse the keeping of the activity (at -21°C) became - this occurred to a lesser extent for the 0.2 and 0.3 M treated yeast and did not occur significantly for the 0.4 M glycerol treated yeast.

As will be recognised by those skilled in the art from the above discussion the approach taken by the present inventors to increase yeast performance is to supply exogenous osmoprotectants etc., so that yeast biomass is given a "head start' in subsequent osmotically stressing applications. By mixing yeast with an osmoprotectant/ compatible solute such as glycerol and allowing sufficient time for uptake of that osmoprotectant/ compatible solute into yeast cells, subsequently yeast do not have to synthesise/ retain as much endogenous glycerol in order to osmotically equilibrate with high osmotic pressures exerted by sugar doughs etc. Therefore, glycerol-treated yeast achieve a crucial level of osmotic equilibration earlier than non-glycerol-treated yeast cells. Accordingly, in the case of glycerol, a natural osmoprotectant made by many yeast strains, it is important to allow added glycerol to soak into (or be taken up by) yeast cells so that they are pre-loaded with a significant concentration of

protectant prior to industrial application. Because the basal content of osmoprotectant in yeast product is not normally high enough to cope with immediate osmostress when mixed into a sugar dough, yeast has to make glycerol and so sits in a lag phase, during which little gas production occurs, until it achieves a critical concentration that provides osmotic stability. Only then does gas production become significant. Hence addition of glycerol with enough time to allow this to equilibrate across the yeast membrane, should give the yeast a "head start" by reducing the amount of intrinsic glycerol manufacture to reach the critical concentration.

It will be apparent to those skilled in the art that a similar result to that obtained by the addition of compatible solutes may be achieved by altering or treating the yeast such that the yeast produce or retain greater internal levels of glycerol. For example strains of yeast may be engineered or selected (by recombinant technology or breeding/ fusion) for improved glycerol production and retention at, or in excess of, say, >0.4 mMol glycerol/ gram dry yeast within 30 min in a high sugar dough system, preferably with as made invertase levels being below 10 units of activity. One such approach might be to boost the level of glycerol-3-phosphate dehydrogenase in yeasts as this is induced by salt and sugar stress and forms part of the high osmotic glycerol response (10,11). Another approach is to boost the levels of substrates intracellularly available for glycerol synthetic enzymes. Yet another way to increase glycerol production in yeast is to induce this process by treatment with salts or other osmolytes, which is a well known phenomenon and is already used in the industry (12,13). As glycerol is "normally" leaked from cells, unless under osmotic stress, any manipulation (genetic or physiological/osmolyte exposure) would also benefit from having cells modified to retain glycerol in non-osmotic stress conditions, e.g. manipulation of glycerol channel/porter proteins.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

REFERENCES

1. Loiez, A., Clement, P. & Colavizza, D. (Le Saffre Patent Application). Document number AU A 15180/92 . Priority date 1991.

2. Mager, W.H. & Varela, J.C.S. 1993. Osmostress response of the yeast *Saccharomyces*. *Molecular Microbiology*, 10, 253-258.

3. Hocking, A.D. 1986. Effects of water activity and culture age on the glycerol accumulation patterns of five fungi. *Journal of General Microbiology*, 132, 269-275.

4. Brown, A.D. 1974. Microbial water relations: features of the intracellular composition of sugar-tolerant yeasts. *Journal of Bacteriology*, 118, 769-777.

5. Tokuoka, K., Ishitani, T. & Chung, W-C. 1992. Accumulation of polyols and sugars in some sugar tolerant yeasts. *Journal of General and Applied Microbiology*, 38, 35-46.

6. Brown, A.D. & Edgley, M. 1980. Osmoregulation in Yeast, in *Genetic Engineering of Osmoregulation, Impact on Plant Productivity for Food, Chemicals, and Energy*. (Eds. D.W. Rains, R.C. Valentine & A. Hollaender). Plenum Press New York and London.

7. Larsson, C., Morales, C., Gustafsson, L. & Adler, L. 1990, Osmoregulation of the salt tolerant yeast *Debaryomyces hansenii* grown in a chemostat at different salinities. *Journal of Bacteriology*, 172, 1769-1774.

8. Blomberg, A. & Adler, L. 1989. Roles of glycerol and glycerol-3-phosphate dehydrogenase (NAD+) in acquired osmotolerance of Saccharomyces cerevisiae. *Journal of Bacteriology*, 171, 1087-1092.

9. Larsson, K., Ansell, R., Eriksson, P. & Adler, L. 1993. A gene encoding *sn*-glycerol 3-phosphate dehydrogenase (NAD+) complements an osmosensitive mutant of *Saccharomyces cerevisiae*. *Molecular Microbiology*, 10, 1101-1111.

10. Albertyn, J., Hohmann, S. & Prior, B.A. 1994. Characterisation of the osmotic-stress response in *Saccharomyces cerevisiae*: osmotic stress and glucose repression regulate glycerol-3-phosphate dehydrogenase independently. *Current Genetics*, 25, 12-18.

11. Albertyn, J., Hohmann, S., Thevelein, J.M. & Prior, B.A. 1994. *GPD1*, which encodes glycerol-3-phosphate dehydrogenase, is essential for growth under osmotic stress in *Saccharomyces cerevisiae*, and its expression is regulated by the high-osmolarity glycerol response pathway. *Molecular and Cellular Biology*, 14, 4135-4144.

12. Blomberg, A. & Adler, L. 1989. Roles of glycerol and glycerol-3-phosphate dehydrogenase (NAD+) in acquired osmotolerance of *Saccharomyces cerevisiae*. *Journal of Bacteriology*, 171, 241-248.

13. Chen, S.L. & Gutmanis, F. (Universal Foods Corp.). USA Patent No. 4, 420, 563. Filed 1982.

Claims:-

1. A method of increasing the performance of yeast the method comprising exposing the yeast to at least one compatible solute/osmoprotectant for a period of time sufficient to result in an intracellular concentration of the at least one compatible solute/osmoprotectant of at least 70μMoles/gram dry yeast.

2. A method as claimed in claim 1 in which the yeast is exposed to the at least one compatible solute/osmoprotectant for a period of time sufficient to result in an intracellular concentration of the at least one compatible solute/osmoprotectant of at least 100μMoles/gram dry yeast.

3. A method as claimed in claim 1 or claim 2 in which the compatible solutes/osmoprotectants is selected from the group consisting of glycerol, trehalose, sucrose, maltose, glucose, fructose, mannose, ammonium salts, amino compounds, and combinations thereof.

4. A method as claimed in any one of claims 1 to 3 in which the compatible solutes/osmoprotectants is glycerol.

5. A method as claimed in claim 4 in which the yeast is exposed to the glycerol for a sufficient period of time to result in an intracellular concentration of glycerol of at least 100μMoles/gram dry yeast equivalents.

6. A method as claimed in claim 4 or claim 5 in which the yeast is exposed to glycerol at a concentration of at least 0.1M.

7. A method as claimed in claim 6 in which the yeast is exposed to glycerol at a concentration of at least 0.2M.

8. A method as claimed in claim 6 or claim 7 in which the yeast is exposed to glycerol at a concentration of at least 0.4M.

9. A method as claimed in any one of claims 1 to 8 in which the yeast is exposed to the compatible solute for at least 24 hours.

10. A method as claimed in any one of claims 1 to 9 in which the yeast is in the form of yeast having greater than 18% dry yeast material.

11. A yeast product produced by the method as claimed in any one of claims 1 to 10.

12. A frozen dough product including a yeast prepared by the method as claimed in any one of claims 1 to 10.

13. A cream yeast product characterised in that the yeast has an internal glycerol concentration of at least 70, preferably at least 100 µMoles/gram dry yeast equivalents.

14. A compressed yeast product characterised in that the yeast has an internal glycerol concentration of at least 70, preferably at least 100 µMoles/gram dry yeast equivalents.

Figure 1

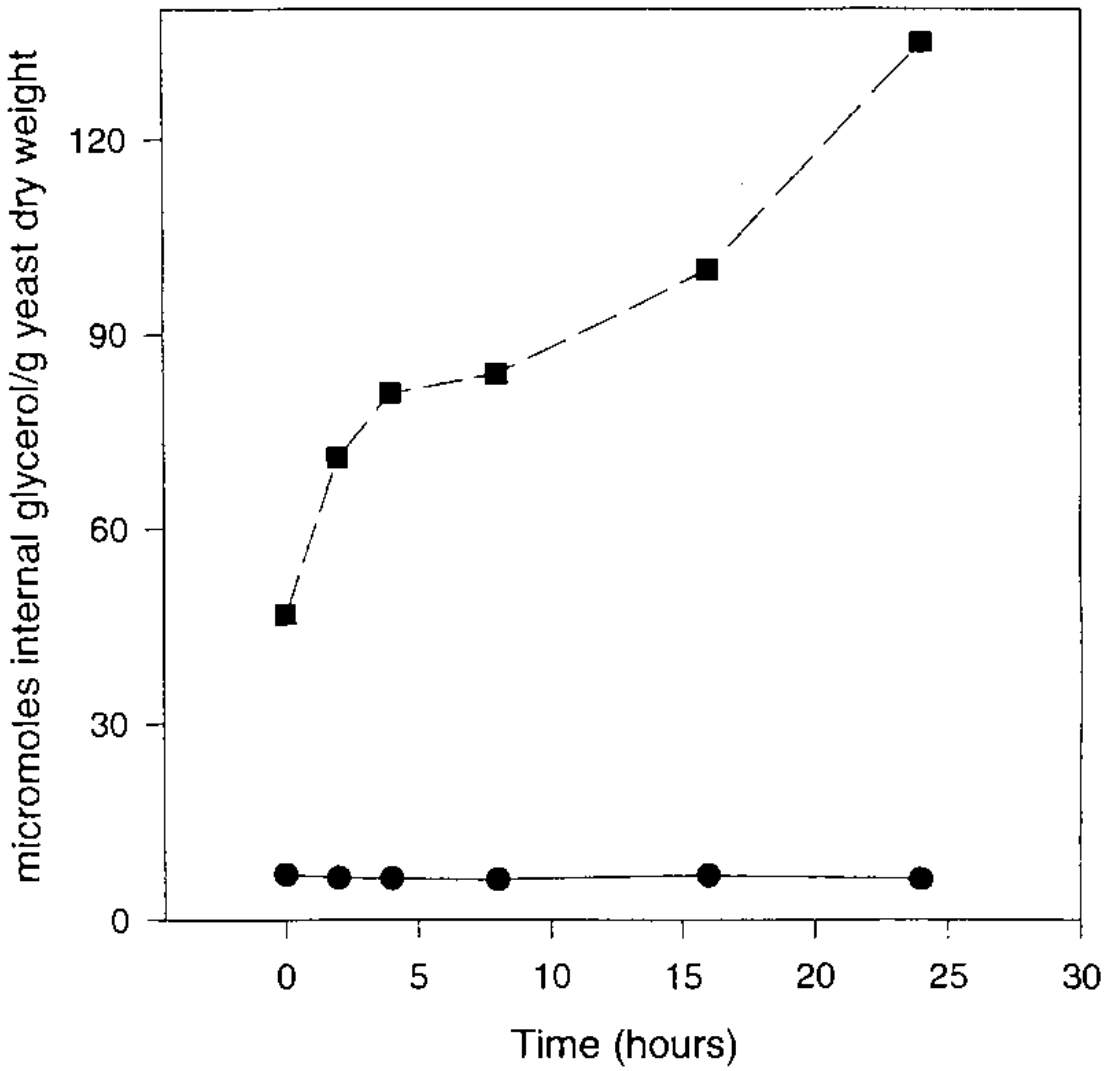

micromoles internal glycerol/g yeast dry weight
120
90
60
30
0
0
5
10
15
20
25
30
Time (hours)

Figure 2

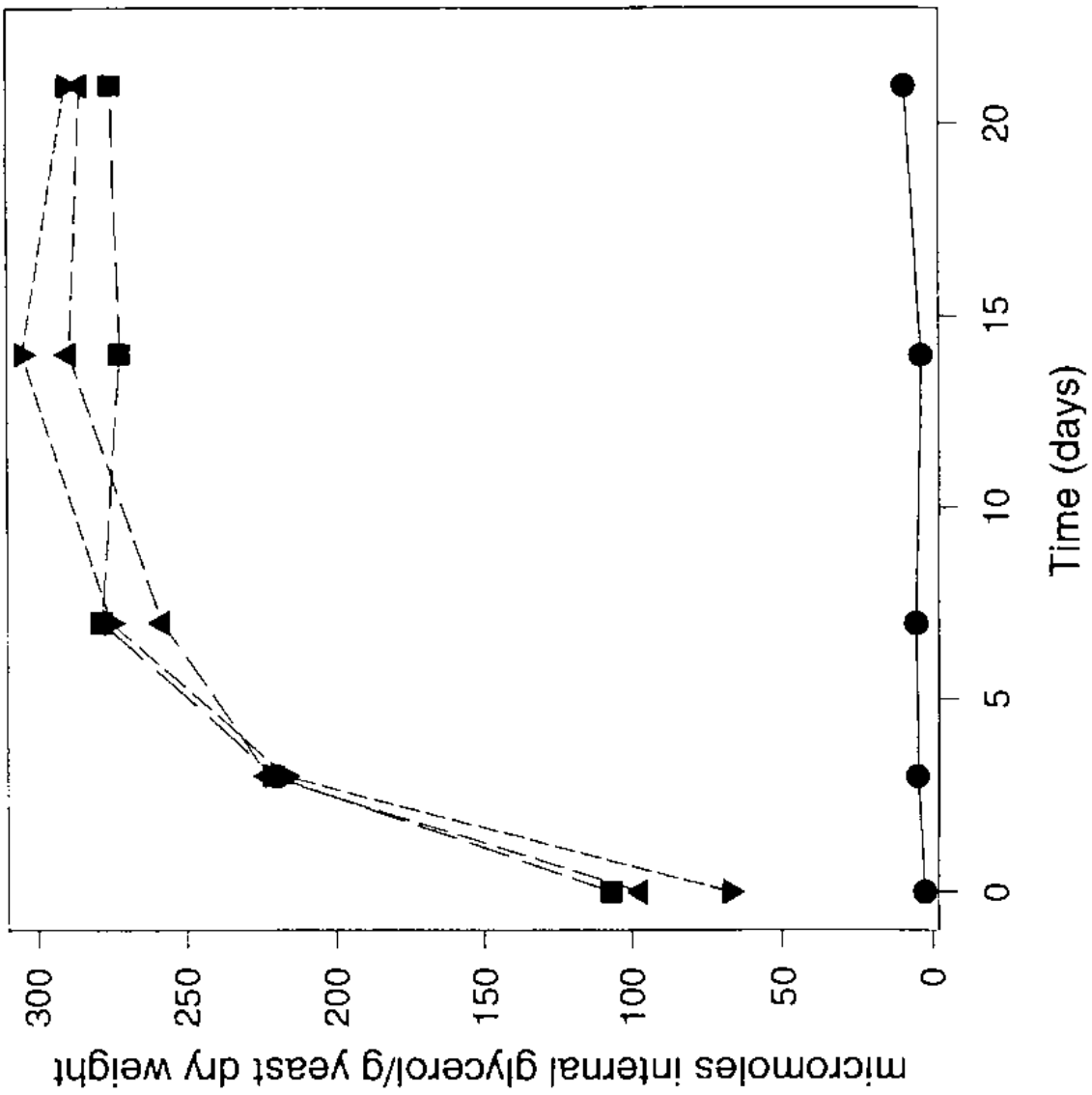

micromoles internal glycerol/g yeast dry weight
300
250
200
150
100
50
0
0
5
10
15
20
Time (days)

Figure 3

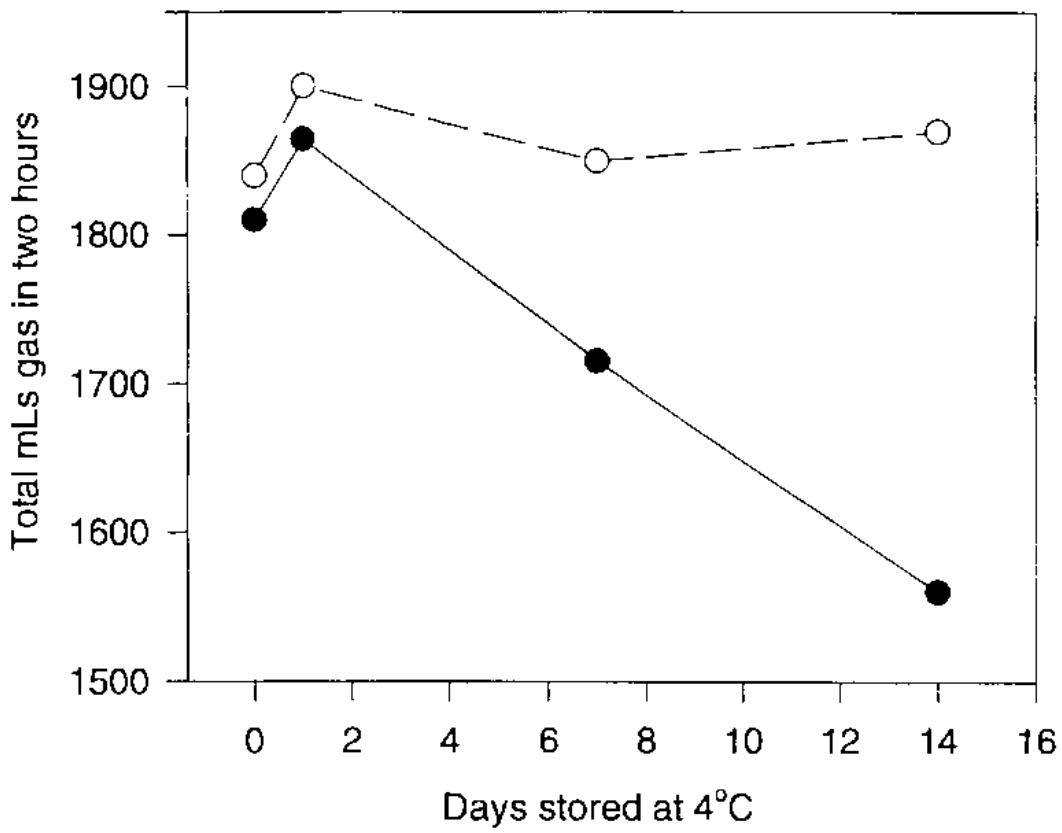

1900
1800
1700
1600
1500
Total mLs gas in two hours
0
2
4
6
8
10
12
14
16
Days stored at 4°C

Figure 4

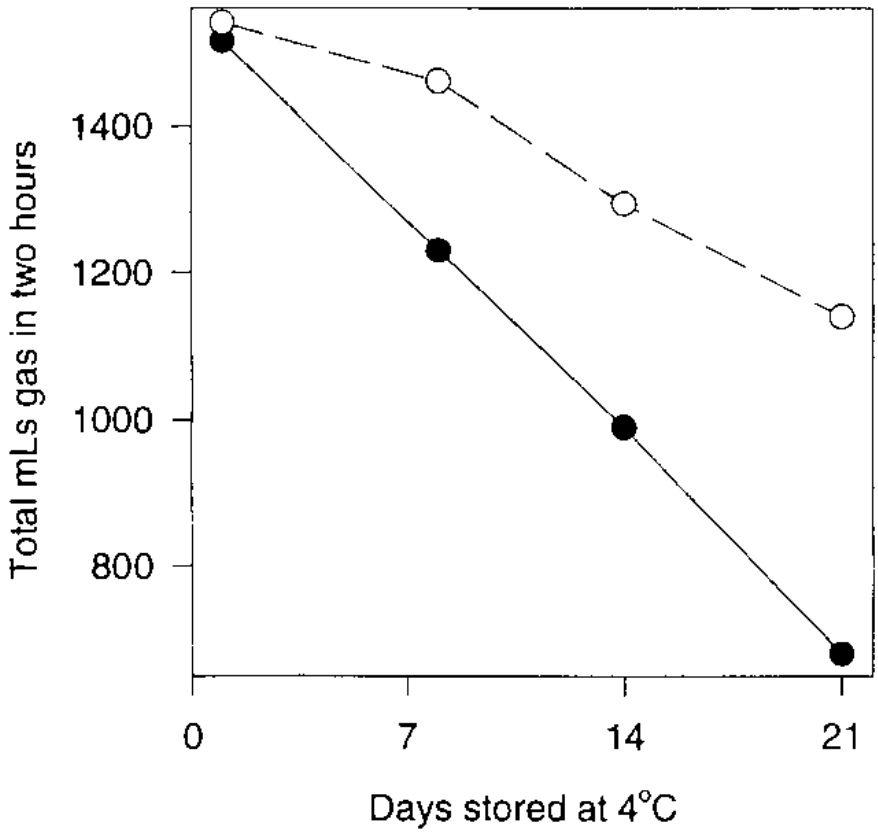

Total mLs gas in two hours
1400
1200
1000
800
0
7
14
21
Days stored at 4°C

Figure 5

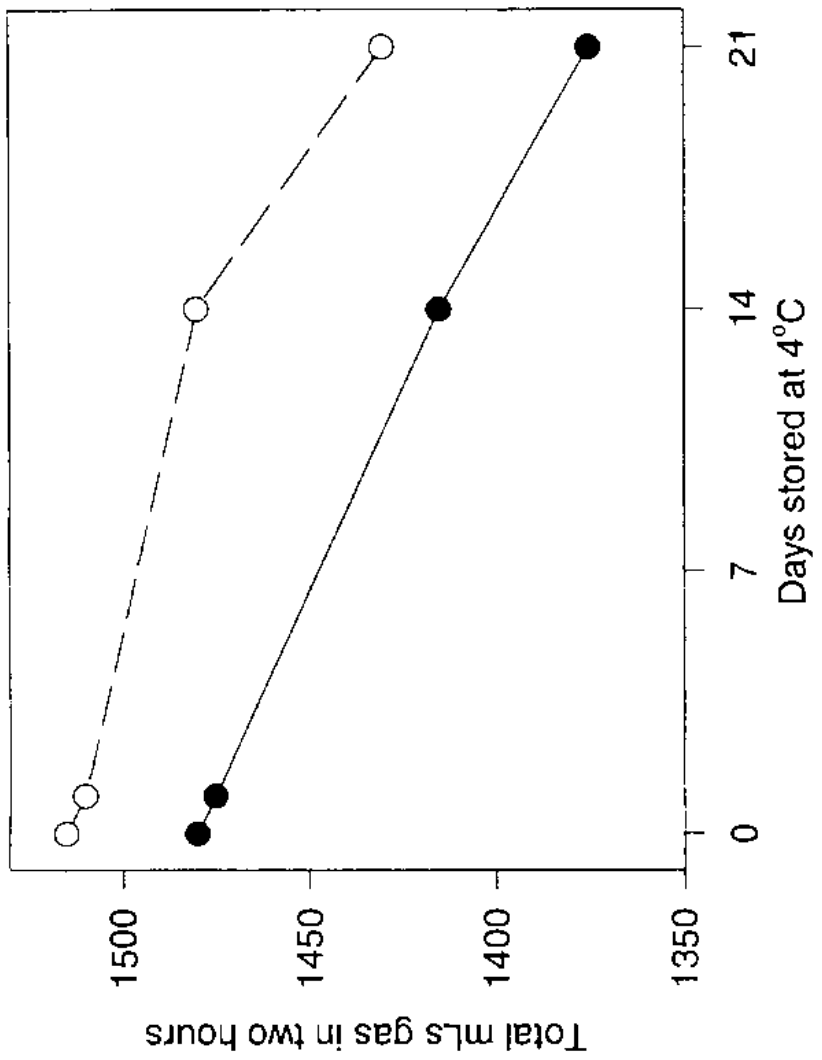

Total mLs gas in two hours
1500
1450
1400
1350
0
7
14
21
Days stored at 4°C

Figure 6

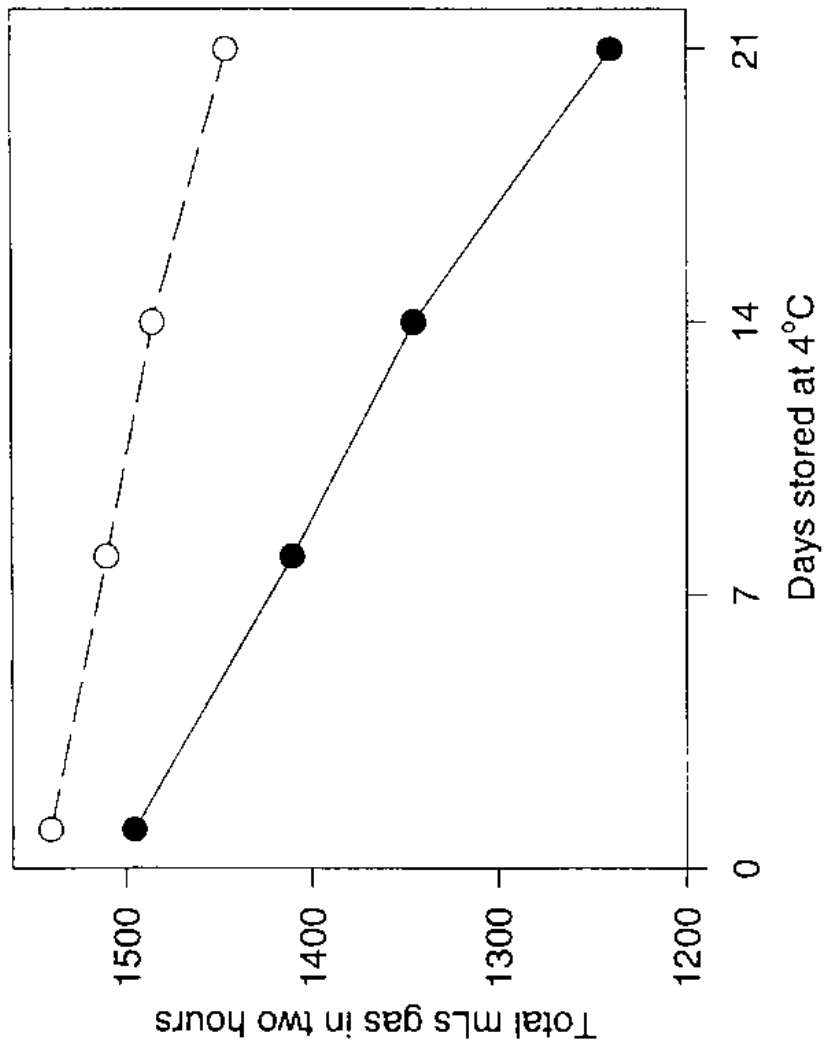

Total mLs gas in two hours
1500
1400
1300
1200
0
7
14
21
Days stored at 4°C

Figure 7

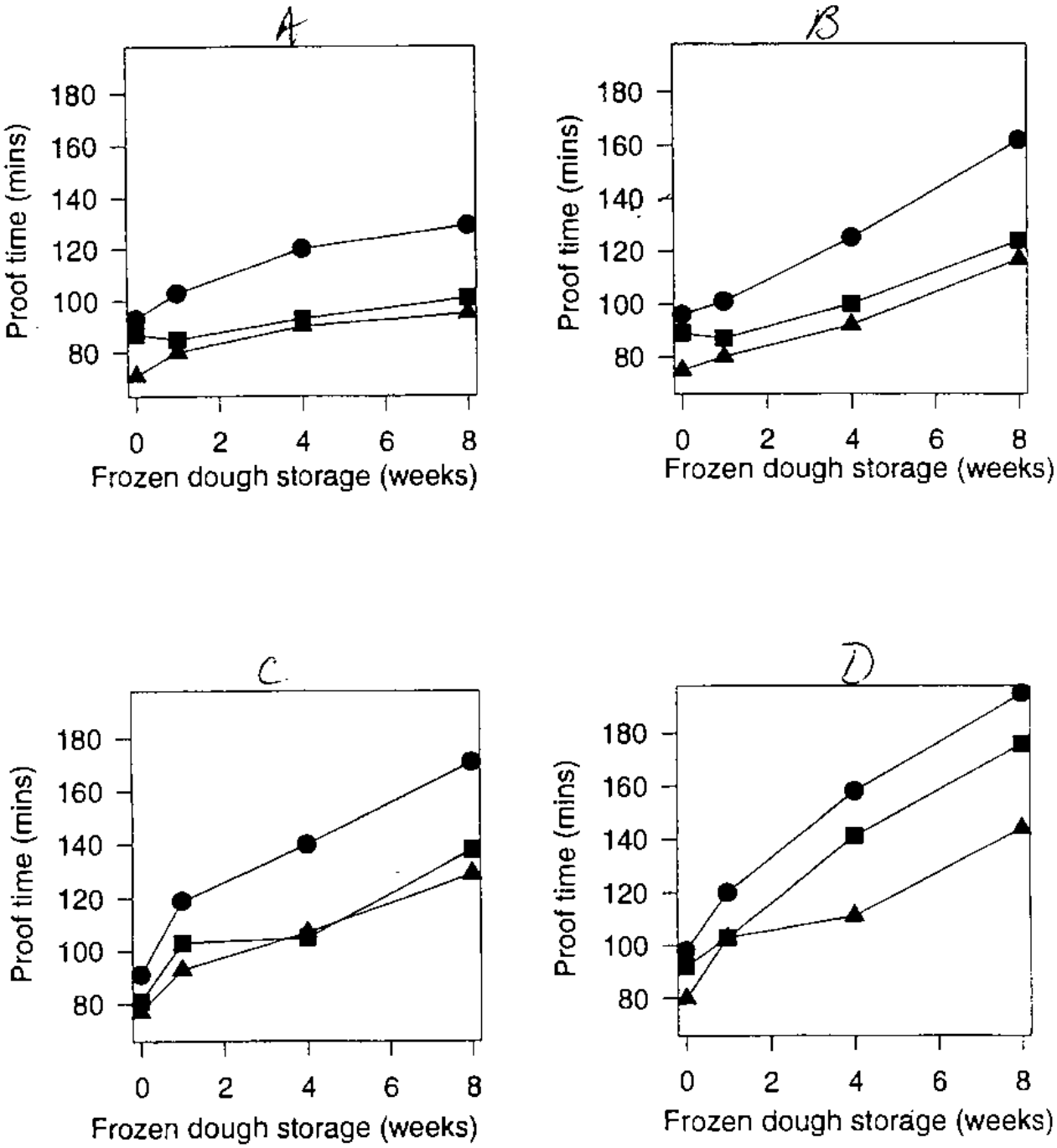
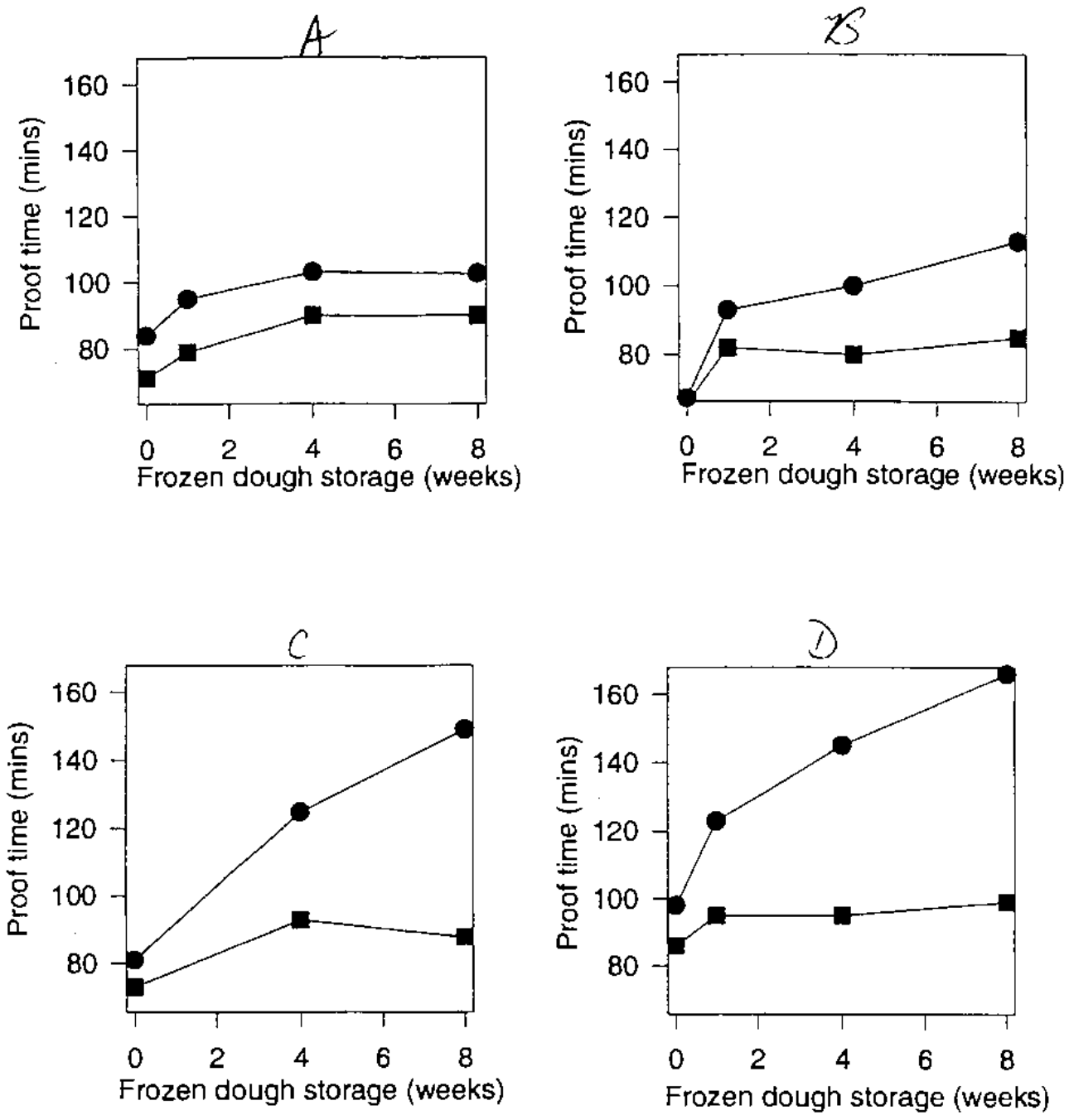

A
B
C
D
Proof time (mins)
180
160
140
120
100
80
0
2
4
6
8
Frozen dough storage (weeks)

Figure 8


A
B
C
D
Proof time (mins)
160
140
120
100
80
0
2
4
6
8
Frozen dough storage (weeks)

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 96/00719

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: C12N 1/16, 1/18; A21D 8/04 // (C12N 1/16, C12R 1:645) (C12N 1/18, C12R 1:865)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: As above

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT : IPC and (OSMOPROTECT: OR GLYCEROL OR TREHALOSE OR SUCROSE OR MALTOSE OR GLUCOSE OR FRUCTOSE OR MANNOSE OR AMMONIUM OR AMINO:)
CASM, FSTA, BIOT : YEAST# and Keywords As Above

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Derwent Abstract Accession No. 89-316351, Class D16, CS,A, 8708359 (BREIEROVA E.) 12 September 1989 Abstract	
A	EP,A, 435606 (ICHINOBE BAKING CO LTD & DAIKIN INDUSTRIES LIMITED) 3 July 1991	

☐ Further documents are listed in the continuation of Box C ☐ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
22 November 1996

Date of mailing of the international search report
29 NOV 1996

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