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(54) IMPROVEMENTS IN OR RELATING TO YEASTS

(71) We, KONINKLIJKE NEDERLANDSCHE GIST-EN SPIRITUSFABRIEK N.V., a Dutch Body Corporate of 1, Wateringseweg, Delft, The Netherlands, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to new bakers' yeast hybrids which are useful in the manufacture of active dried yeast having a high gas production activity.

Although active dried yeasts are available which are sufficiently active to be used as substitutes for compressed yeasts, their gas production activity is lower than that of the compressed yeasts so that larger quantities of the active dried yeasts than of the compressed yeasts are required to achieve a particular result.

Yeasts of high protein content, which have a high activity in the compressed form, are generally unsuitable for use as they suffer a drastic loss of activity during drying. Active dried yeasts of good stability have been obtainable without undue loss of activity only from yeasts having a relatively low activity in the compressed form. Thus, of the previously available dried yeasts, none has an activity comparable with the best compressed yeasts.

Yeast hybrids have now been bred which possess good activity in the compressed form and can be dried without undue loss of activity, giving active dried yeasts of high activity not only after mixing with dry dough components, e.g. flour, and rehydrating during dough-making but also when rehydrated separately before addition to other dough components.

Accordingly, the invention provides bred yeast hybrids having a protein content of 47 to 60% (%N $\times$ 6.25, as determined by the Kjeldahl method), preferably 49 to 55%, based on the dry matter content which, in compressed form, exhibit an activity of at least 550 when determined by the test procedure

which consists of suspending a quantity of the compressed yeast corresponding to 450 mg. of dry matter in 55 ml. of an aqueous solution containing 2 g. of sodium chloride at a temperature of 28°C., adding to the suspension 100 g. of flour and thereafter mixing the mass for 6 minutes at 28°C., maintaining the dough obtained at 28°C. and measuring the activity as the volume of gas in ml. at 28°C. and 760 mm.Hg evolved in the period from 10 to 175 minutes after the start of mixing of the dough; and which yeast hybrids, after conversion to an active dried form having a dry matter content of at least 85%, preferably 90 to 98%, by drying at a maximum temperature of 50°C., have (a) an activity of at least 510 when determined by the test procedure which consists of mixing a quantity of the active dried yeast corresponding to 450 mg. of dry matter with 100 g. of flour and adding 55 ml. of an aqueous solution containing 2 g. of sodium chloride, and thereafter mixing the mass and measuring the activity in the manner hereinbefore specified, and (b) an activity of at least 385 when measured by the test procedure which consists of maintaining a quantity of the active dried yeast corresponding to 450 mg. of dry matter in contact with 8 ml. of water for 10 minutes at 28°C., mixing the suspension obtained with 100 g. of flour and 47 ml. of an aqueous solution containing 2 g. of sodium chloride, and thereafter mixing the mass and measuring the activity in the manner hereinbefore specified.

In the compressed form (dry matter content about 25—33% by weight), the new hybrids have a high activity. The residual activity of the active dried form amounts to at least 84% of the activity of the compressed yeast when incorporated directly into the dough mixture prior to rehydration, or at least 70% when rehydrated before addition to the other dough components.

Samples of the yeast hybrids exhibiting these properties, designated Ng 2031 and Ng 2103, have been deposited at the "Centraal Bureau voor Schimmelcultures, Yeast Divi-

sion" at Delft, The Netherlands, where they have been given the numbers C.B.S. 6128 and C.B.S. 6131 respectively.

The yeasts according to the invention may be cultured in a manner known for the production of bakers' yeast using a nutrient medium containing sugars such as molasses, nitrogen being added as ammonium salt and/or ammonia, and phosphate. The propagation of the yeast hybrid can be performed under the usual conditions of temperature and pH in several stages with a final stage consisting of growth under aerobic conditions converting the sugars mainly into yeast. The compressed yeast may be prepared, for instance, by centrifugation and washing of the yeast fermentation liquid and filtration. Since filtration is usually carried out on a vacuum filter, it is preferable to dissolve an osmotically highly active substance such as sodium chloride in the yeast suspension before filtering, and then to wash the osmotic liquid with a solution of lower osmotic pressure, such as tap-water, during filtration, as described in Specification No. 763,926.

The compressed yeast, after dividing it into particles, e.g. by extrusion, may be dried in not more than 120 minutes, preferably less than 20 minutes, to a dry matter content of at least 85% by weight by means of a drying gas (preferably air) flow in such a manner that the yeast particles are held within a temperature range of from 20° to 50°C. It is advantageous to add a swelling and/or wetting agent to the compressed yeast before drying. Suitable swelling agents are cellulose derivatives, such as methyl cellulose, carboxymethyl cellulose and hydroxypropyl cellulose. suitable wetting agents are compounds with a hydrophilic and a lipophilic group, such as esters of saturated or unsaturated fatty acids and suitable alcohols, for instance sorbitan fatty acid esters, e.g. sorbitan monolaurate, sorbitan monostearate, sorbitan monopalmitate and sorbitan mono-oleate; glycerol fatty acid esters, e.g. glycerol monostearate, glycerol monopalmitate and glycerol distearate; mono- and diglycerides of edible fatty acids modified with lower organic acids such as acetic acid, lactic acid, citric acid, tartaric acid and diacetyl tartaric acid, such as glyceryl stearate lactate; propylene glycol fatty acid esters, e.g. propylene glycol monostearate; or mixtures of two or more of the abovementioned compounds.

The morphology of the new hybrids in Difco (a Trade Mark) malt extract, on Difco malt agar and in slide cultures on corn meal was determined according to the methods described by Lodder and Kreger-van Rij in "The Yeasts" (1952), Chapter II, North Holland Publishing Company, Amsterdam. After 3 days of growth at 25°C. in the malt extract the cells of the new hybrids Ng 2031 and Ng 2103 are spherical to ovoid, single or in pairs or in short chains or groups. After 1 month at

25°C. a sediment and a ring are formed, but no pellicle or islets. After 3 days of growth at 25°C. on malt agar the cells are spherical to ovoid, single, in pairs or in short chains. The streak culture after one month is cream to yellowish-brown in colour, soft, smooth and flat. In the slide cultures, the new hybrids show a more or less well-developed pseudomycelium.

Sporulation was studied by incubating a loopful of a 2 days-old culture grown on malt agar at 25°C., the inoculum being first washed with sterile 0.3% sodium chloride solution, on the following medium: sodium acetate (0.4% (weight/volume) and agar 1.5% (weight/volume) in tap water, the pH of the medium being adjusted to 6.8 ± 0.2 before autoclaving for 20 min. at 120°C. After 3 to 5 days at 25°C., the strains Ng 2031 and Ng 2103 formed about 25—50% sporulating cells and about 2 to 20% four-spore asci. Addition of potassium chloride (1% weight/volume) to the medium had no effect on sporulation.

Carbohydrate fermentation was examined using the method described by Lodder and Kreger-van Rij, *op. cit.* It was found that in addition to glucose, galactose, saccharose and maltose, the new hybrids fermented the trisaccharide melezitose within 2 days. Trehalose was fermented within 3 weeks by strain Ng 2031 but not by strain Ng 2103. α -Methylglucoside was quickly fermented by strain Ng 2031 (within 1 to 2 days), especially in the presence of trace amounts of glucose, and within one week by Ng 2103.

The requirements of the new hybrids for growth factors were determined according to the method of E. J. Miller, J. Inst. Brewing (1960) 66, 235. Limited growth was observed in the strains when the vitamins B₁ and B₆ or inositol were omitted from the basic growth medium. Addition of uracil to the same basic medium did not show any significant inhibitory effect.

The following Examples further illustrate the invention. The letters B², B³ and B⁴ refer to the three tests for measuring the activity of yeasts described below:

TEST PROCEDURE

In all three tests B², B³ and B⁴ a dough is prepared from an amount of yeast corresponding to 450 mg. of dry matter, 100 g. of flour, and 55 ml. of water containing 2 g. of NaCl, the water being at a temperature of 28°C. The dough obtained is placed on a water-bath adjusted to a temperature of 28°C., and the amount of gas produced is determined during the interval from 10 minutes to 175 minutes after the start of mixing the dough. The volume of gas produced is given in ml. at 28°C. and 760 mm.Hg.

The tests differ in the method of making the dough.

Test B²: The active dried yeast is mixed with

100 g. of flour in a mixer. The salt solution is added and the components are mixed for 6 minutes at 28°C. into a dough.

5 *Test B²*: The compressed yeast is suspended in the salt solution, 100 g. of flour are added and the components are mixed for 6 minutes at 28°C. into a dough.

10 *Test B⁴*: The active dried yeast is rehydrated for 10 minutes with 8 ml. of water at a temperature of 28°C., and the suspension obtained is mixed with 100 g. of flour and 47 ml. of water containing 2 g. of salt. The components are mixed for 6 minutes at 28°C. into a dough.

15 EXAMPLE I

In this Example, the properties of hybrids Ng 2031 and Ng 2103 of the present invention, are compared with the properties of the known yeast strains Ng 720, Ng 723 and Ng 1777.

20 Compressed yeasts were prepared from these strains in a 100,000 litre vessel under the same conditions of pH, temperature etc. commonly used in this type of propagation under aerobic conditions. At the start of fermenta-

tion 170 kgs. of ammonium phosphate were added. During fermentation, a total of 12,900 kgs. of molasses and 1,380 litres of ammonia (25% NH₃ w/v) were added, giving yeasts that after drying into the active dried form 30 had a protein content of between 52.0 and 52.9% (% N × 6.25) based on dry matter. The cultures were filtered and washed and the compressed yeasts obtained were mixed with 35 2% by weight of sorbitan monostearate in the form of an aqueous suspension.

Active dried yeasts were obtained by drying the compressed yeasts, divided into particles, in a stream of hot air at a starting temperature of 120°C., which temperature was 40 gradually decreased in such a manner that the temperature of the yeast particles themselves did not rise over about 35°C. The over-all drying time was 8 minutes. The active dried yeasts obtained had a dry matter content of 45 93.0 to 93.9% by weight.

The averaged results of several experiments involving the Test Procedures hereinbefore described are given in the following Table.

TABLE I

Yeast	Ng 720	Ng 723	Ng 1777	Ng 2031	Ng 2103
Compressed yeasts					
ml. gas evolved in test B ³	610	554	586	616	610
Active dried yeasts					
Dry matter content (%)	93.3	93.7	93.5	93.8	93.8
Protein content (% N × 6.25) based on dry matter	52.6	52.5	52.0	52.1	52.3
ml. gas evolved in test B ²	461	466	499	560	560
percentage activity B ² /B ³	75.6	84.2	85.2	91.0	91.8
ml. gas evolved in test B ⁴			333	485	482
percentage activity B ⁴ /B ³			56.3	78.8	79.2

Thus yeast Ng 720, which is used in practice as a good quality compressed yeast, exhibits a good activity in the compressed form, but has only poor activity in the dried form when rehydrated according to test B² (rehydration after mixing the yeast with flour for making the dough), as evidenced by the low 50 percentage activity of 75.6% (expressed as a percentage of the gas production compared

with that of the compressed yeast). Therefore, this yeast is not suitable for the production of an active dried yeast of good quality. 55

The yeast Ng 723, which is used in practice for the production of conventional active dried yeast, has a relatively poor activity in the compressed form, so that, although the ratio of the activities observed in tests B² and B³ is higher, the absolute activity according to test B² is 60

low. Therefore, although a better stability to drying and rehydration according to test B³ characterises this yeast, this yeast is also not suitable for the production of an active dried yeast of good quality.

The yeast Ng 1777 has the high activity in the compressed form needed to obtain a high activity in the active dried form, but the active dried form of this yeast is very sensitive to the rehydration method used; thus, in test B² a relatively high activity is observed, whereas in test method B⁴ the activity is low (333), representing only 56.3% of the activity of the compressed yeast observed in test B³.

However, the new yeast hybrids of the present invention possess high activities in the compressed form, show after drying, according to test B², a considerably higher activity than than of the yeast Ng 1777, and have a pronouncedly better activity than that yeast in test B⁴. The new hybrids are thus highly desirable for making active dried yeast.

EXAMPLE II

The yeast hybrid Ng 2103 was propagated in a 100,000 litre vessel. 16,200 kgs. of molasses, 1,770 litres of ammonia and 215 kgs. of ammonium phosphate were added. The yeast obtained had a protein content (% N $\times$ 6.25) based on dry matter of 53.5% after drying into an active dried form, and evolved as compressed yeast 661 ml. of gas when determined according to test B³.

After processing into an active dried yeast in the same manner as described in Example I using the same amount of wetting agent, the product obtained had the following properties: dry matter content 93.6%; ml. of gas evolved in test B² 599; and percentage activity B²/B³ 90.6.

Continuing the drying process to a dry matter content of 96.6%, the following results were obtained: ml. of gas evolved in test B² 586, and percentage activity B²/B³ 88.6.

WHAT WE CLAIM IS:—

1. A compressed yeast, other than a naturally occurring yeast, having a protein content of 47 to 60% (% N $\times$ 6.25, as determined by the Kjeldahl method) based on dry matter content, which exhibits an activity of at least 550 when determined by the test procedure consisting of suspending a quantity of the compressed yeast corresponding to 450 mg. of dry matter in 55 ml. of an aqueous solution containing 2 g. of sodium chloride at a temperature of 28°C., adding to the suspension 100 g. of flour and thereafter mixing the mass for 6 minutes at 28°C., maintaining the dough obtained at 28°C. and measuring the activity as the volume of gas in ml. at 28°C. and 760 mm.Hg evolved in the period from 10 to 175 minutes after the start of mixing the dough: and which compressed yeast after conversion by drying at a maximum temperature of 50°C.

to an active dried bakers' yeast having a dry matter content of at least 85% by weight exhibits (a) an activity of at least 510 when determined by the test procedure consisting of mixing a quantity of the active dried yeast corresponding to 450 mg. of dry matter with 100 g. of flour and adding 55 ml. of an aqueous solution containing 2 g. of sodium chloride and thereafter mixing the mass for 6 minutes at 28°C., maintaining the dough obtained at 28°C. and measuring the activity as the volume of gas in ml. at 28°C. and 760 mm.Hg evolved in the period from 10 to 175 minutes after the start of mixing the dough; and (b) an activity of at least 385 when measured by the test procedure consisting of maintaining a quantity of the active dried yeast corresponding to 450 mg. of dry matter in contact with 8 ml. of water for 10 minutes at 28°C., mixing the suspension obtained with 100 g. of flour and 47 ml. of an aqueous solution containing 2 g. of sodium chloride, and thereafter mixing the mass and measuring the activity in the manner hereinbefore specified.

2. A compressed yeast according to claim 1 having a protein content of 49 to 55% based on dry matter content.

3. A compressed yeast according to claim 1 or 2 in which the yeast hybrid is Ng 2031 (C.B.S., Yeast Division, number 6128).

4. A compressed yeast according to claim 1 or 2 in which the yeast hybrid is Ng 2103 (C.B.S., Yeast Division, number 6131).

5. A compressed yeast according to claim 1 when prepared by the method substantially as hereinbefore described with especial reference to Example I or II.

6. An active dried baker's yeast having a protein content of 47 to 60% (% N $\times$ 6.25, as determined by the Kjeldahl method) based on dry matter content, said dried yeast being obtainable by drying at a maximum temperature of 50°C. from a compressed yeast as claimed in claim 1 and having a dry matter content of at least 85% by weight, which exhibits (a) an activity of at least 510 when determined by the test procedure consisting of mixing a quantity of the active dried yeast corresponding to 450 mg. of dry matter with 100 g. of flour and adding 55 ml. of an aqueous solution containing 2 g. of sodium chloride and thereafter mixing the mass for 6 minutes at 28°C., maintaining the dough obtained at 28°C. and measuring the activity as the volume of gas in ml. at 28°C. and 760 mm.Hg evolved in the period from 10 to 175 minutes after the start of mixing the dough; and (b) an activity of at least 385 when measured by the test procedure consisting of maintaining a quantity of the active dried yeast corresponding to 450 mg. of dry matter in contact with 8 ml. of water for 10 minutes at 28°C., mixing the suspension obtained with 100 g. of flour and 47 ml. of an aqueous solution containing 2 g. of sodium chloride, and

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The yeast Ng 1777 has the high activity in the compressed form needed to obtain a high activity in the active dried form, but the active dried form of this yeast is very sensitive to the rehydration method used; thus, in test B² a relatively high activity is observed, whereas in test method B⁴ the activity is low (333), representing only 56.3% of the activity of the compressed yeast observed in test B³.

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2. A compressed yeast according to claim 1 having a protein content of 49 to 55% based on dry matter content.

3. A compressed yeast according to claim 1 or 2 in which the yeast hybrid is Ng 2031 (C.B.S., Yeast Division, number 6128).

4. A compressed yeast according to claim 1 or 2 in which the yeast hybrid is Ng 2103 (C.B.S., Yeast Division, number 6131).

5. A compressed yeast according to claim 1 when prepared by the method substantially as hereinbefore described with especial reference to Example I or II.

6. An active dried baker's yeast having a protein content of 47 to 60% (% N $\times$ 6.25, as determined by the Kjeldahl method) based on dry matter content, said dried yeast being obtainable by drying at a maximum temperature of 50°C. from a compressed yeast as claimed in claim 1 and having a dry matter content of at least 85% by weight, which exhibits (a) an activity of at least 510 when determined by the test procedure consisting of mixing a quantity of the active dried yeast corresponding to 450 mg. of dry matter with 100 g. of flour and adding 55 ml. of an aqueous solution containing 2 g. of sodium chloride and thereafter mixing the mass for 6 minutes at 28°C., maintaining the dough obtained at 28°C. and measuring the activity as the volume of gas in ml. at 28°C. and 760 mm.Hg evolved in the period from 10 to 175 minutes after the start of mixing the dough; and (b) an activity of at least 385 when measured by the test procedure consisting of maintaining a quantity of the active dried yeast corresponding to 450 mg. of dry matter in contact with 8 ml. of water for 10 minutes at 28°C., mixing the suspension obtained with 100 g. of flour and 47 ml. of an aqueous solution containing 2 g. of sodium chloride, and



thereafter mixing the mass and measuring the activity in the manner hereinbefore specified.

- 5 7. An active dried yeast according to claim 6 having a protein content of 49 to 55% based on dry matter content.

8. An active dried yeast according to claim 6 or 7 in which the dry matter content is 90 to 98% by weight.

- 10 9. An active dried yeast according to claim 6, 7 or 8 in which the yeast hybrid is Ng 2031 (C.B.S., Yeast Division, number 6128).

10. An active dried yeast according to claim 6, 7 or 8 in which the yeast hybrid is Ng 2103 (C.B.S., Yeast Division, number 6131).

- 15 11. An active dried bakers' yeast as claimed in claim 6 when prepared substantially as described in Example I or II.

- 20 12. A compressed or dried yeast according to any one of claims 1 to 11 which includes a swelling and/or wetting agent.

- 25 13. A process for the preparation of an active dried bakers' yeast as claimed in claim 6 which comprises dividing a fresh compressed yeast as claimed in any one of claims 1 to 5 into particles and drying the particles over a period of not more than 120 minutes, to a dry matter content of at least 85% by weight by means of a drying gas flow in such a manner that the yeast particles are held within
30 the temperature range of from 20° to 50°C.

14. Process according to claim 13 in which the drying gas flow is hot air initially at a maximum temperature of 120°C. and its temperature is gradually decreased as the yeast particles are dried so that the temperature of the yeast particles at any time does not rise above 35°C. 35

15. A process according to claim 13 or 14 in which the yeast particles are dried to a dry matter content of 90 to 98% by weight. 40

16. A process according to claim 13, 14 or 15 in which the yeast particles are subjected to the drying gas flow for a period of less than 20 minutes.

17. A process according to claim 13, 14, 15 or 16 in which the compressed yeast contains a swelling and/or wetting agent. 45

18. A process for preparing an active dried bakers' yeast according to claim 13 substantially as hereinbefore described in Example I or II. 50

19. Active dried yeast as claimed in claim 6 when prepared by the process claimed in any one of claims 13 to 18.

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