IMPROVEMENT OF BAKER'S YEAST CHARACTERISTICS BY MUTATION AND GROWTH FACTORS

By

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B.Sc. Agric. Sci. (Food Technology), Fac. Agric., Cairo Univ., 2002

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ABSTRACT

In an attempt to improve the quality characteristics of baker's yeast, two methods were used: optimization of the growth conditions and mutation. For this purpose, four strains of *Saccharomyces cerevisiae i.e*. Y-2034, Y-2072, Y-2073, and commercial baker's yeast strain were tested for their quality characteristics required for good bread making. Results showed that, *S. cerevisiae* Y-2034 exhibited higher biomass productivity and yield, maltase activity, trehalose and protein content, fermentation power and storage stability followed by commercial strain compared to the other strains.

Optimization of the environmental conditions of *S. cerevisiae* Y-2034 and commercial strain was carried out by changing incubation period, temperature and pH. The optimum incubation period, temp. and pH for both strains were 2 days, 30°C, and 5.5, respectively. Sugar concentration, nitrogen source and concentration were also tested. The maximum biomass productivity of *S. cerevisiae* Y-2034 and commercial strain was obtained at 3% sugar, while the maximum nitrogen sources were ammonium phosphate dibasic + ammonium sulfate at 0.423 and 0.635 g N/l, respectively.

Mutation of *S. cerevisiae* Y-2034 was done by Ethyl Methane Sulfonate (EMS) as mutagenesis and revealed that, out of 30 isolated mutants, two (*i.e.* EM-7 and EM-5) exhibited higher fermentation capacity, invertase and maltase activities than *S. cerevisiae* Y-2034 (original strain).

When EM-7 mutant was used in bread making, organoleptic characteristics of the produced loaves had also the highest overall acceptable being 92 compared to original strain *S. cerevisiae* Y-2034 and commercial baker's yeast (being 75 and 79.3, respectively). Also, freshness of these loaves was reduced during storage for 72h by 18.2 % compared to 20% with commercial baker's yeast.

Key words: Baker’s yeast, invertase, maltase, trehalose, fermentation power, corn steep liquor, balady bread, environmental conditions and mutation.

DEDICATTION

I dedicate this work to whom my heart felt thanks; my father, my mother, my dear husband; Hany Mahmoud, my beautiful son; Basem, my sisters and brothers for all support, they lovely offered along the period of my post graduation.

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INTRODUCTION

Balady bread as the main staple food for Egyptian consumers is widely used for its nutritional value and taste. In Egypt, bread alone furnishes more than two-thirds of the total food intake. It is commonly made from wheat flour (at 82% extraction rate), water, yeast and salt (El-Samahy and Tsen, 1981 and Faridi and Rubenthaler, 1984).

Baker's yeast (*Saccharomyces cerevisiae*) is considered as one of the important constituents of baking industry. It plays an essential role in bread quality through leavening wheat flour dough and production of CO_2 via the alcoholic fermentation of sugars (Spencer and Spencer, 1983). It also, contributes to aroma and flavor of baked goods (Stegmann and Huang, 1997).

The quality of commercial baker's yeast is determined by different parameters, *i.e.*, storage stability, high yield of biomass, freeze-thaw resistance, rehydration resistance, color, osmotolerance and fermentation capacity (*i.e.*, the specific rate of carbon dioxide production by yeast into the dough) (Benitez *et al*., 1996 and Randez-Gil *et al*., 1999). The production of CO_2 is largely dependent on the relative strength and action of the different enzymes in the yeast. Baker's yeast contains several enzymes such as invertase, maltase, maltose permease, alcohol dehydrogenase, trehalase, zymase and protease*, etc* (sucrose degradation requires the presence of invertase, while, maltose degradation requires the presence of maltase (α-Glucosidase) and maltose-permease). Therefore, good fermentative

capacity of plain doughs (no-sucrose dough like balady bread) depends on a high potential to ferment maltose (Benitez *et al*., 1996).

Improvement of baker's yeast characteristics is of great importance for bread making because it will affect the quality of the final balady bread loaves. The improvement could be achieved by improving the cultivation conditions, mutagenesis (Shima *et al*., 1999 and Rincon *et al*., 2001), hybridization (Oda and Ouchi, 1991), protoplast fusion (Spencer and Spencer, 1983) and DNA cloning (Reed and Nagodawithana, 1991), followed by selection for bread traits such as fermentation capacity, *etc*.

In Egypt, there is a great demand on baker's yeast for the production of balady bread and other bakery products. There are three factories *i.e.* (Egyptian Starch Yeast and Detergents Company in Alexandria, Egyptian Sugar and Integrated Industries Company in El-Hawamdia, Giza and The General Company of Grand Cairo Bakeries, Al-Salam) produced baker's yeast in two forms: compressed yeast and active dried yeast. The daily amount produced of compressed yeast and active dry yeast from each company are ~15 and 1 ton, respectively. The total amount of baker's yeast produced annually from these factories is 14503 tons, while the quantity required for processing is 49183 tons. Therefore, the needed amount of imports is ~34664 tons with equivalent cost of 12.124 million Egyptian pounds (CAPMS, 2006).

In addition, baker’s yeast in Egypt faces many problems in quality and quantity such as low productivity, low fermentation power, short shelf life, and contamination with other microorganisms as well as low resistance to freezing. Therefore, the present work aimed to:

1- Select the good quality baker’s yeast strains.

2- Study the optimal environmental conditions for the production of baker's yeast at high quality characteristics.

3- Improve the quality characteristics of the selected strains by mutagenesis treatment.

4- Study the quality characteristics of the mutants and their application in balady bread-making

REVIEW OF LITERATURE

Yeast has been used for leavening doughs for several thousands years. Evidence of this is found in ancient Babylonian wall carvings dating back to 2000 B.C. (Reed and Nagodawithna, 1991). The need for a regular supply of yeast for bakeries drove the development of the baker's yeast manufacturing industry in the 1800's (Evans, 1990).

Baker's yeast is used in bread making for three basic reasons: leavening action, due to carbon dioxide production; flavor development as consequence of the alcohol, esters, and flavor precursors formed; and dough development. The later factor results in turn, from several actions; the lowering of pH due in part to carbon dioxide in the dough aqueous phase, the effect of alcohol produced by yeast on the interfacial tension of various dough components, physical work on dough due to carbon dioxide expansion, and slackening effects resulting from yeast reductases acting on such substrates as thioacetic acid from flour or glutathione from yeast (Cooper and Reed, 1968 and Yabaya and Jatau, 2009).

1. Production of baker's yeast

a. Strain selection

The characteristics required for good baking yeast are slightly different from those of other yeasts, since baking yeasts are grown under aerobic conditions and transferred to essentially microaerophilic conditions for the dough fermentation. To perform satisfactory, the yeast must primarily produce a maximum amount of CO_2. Moreover, good stability, both genetical and physical consistent performance, low

cost and absence of objectionable features are also included (Harrison, 1971).

Spencer and Spencer (1983) stated the desired characteristics of baker's yeast as (a) quality, in terms of dough fermentation, storage stability, and osmotic resistance to salts and sugars, and (b) quantity, or yield of biomass per unit of substrate.

Also, Oda and Ouchi (1989a) reported the necessary properties for fast dough fermentation as: (i) high potential glycolitic activity; (ii) ability to adapt rapidly to changing substrates; (iii) high invertase activity (or other hydrolytic enzymes); (iv) high potential maltose fermentation; and (v) ability to grow and synthesize enzymes and coenzymes under anaerobic conditions. Osmotic stability in the presence of high sugar concentrations and pH tolerance (because of the use of acidified baking aids) are also desirable. However, Mostafa (2000) reported that, biomass of baker's yeast produced at the highest efficiency and productivity does not necessarily has the most desirable properties for baking.

1. Baker's yeast yield

The goal of baker's yeast production should be to maximize growth and minimize alcohol fermentation. For this reason, sugar levels have to be maintained very low to avoid catabolite repression and, consequently, the growth rate. When this is not so, ethanol is formed and yeast yield is drastically reduced (Reed and Nagodawithana, 1991).

The yield of biomass from fermentable carbohydrate is a key commercial parameter to be measured in baker's yeast strains since it is

directly related to the cost of the major raw material, molasses (Jenson, 1996 and Perez-Torrado *et al*., 2005). It would be most desirable to obtain both high productivity (biomass produced per unit of time) and yield.

Layokun *et al.* (1986) obtained a yield of *Saccharomyces cerevisiae* cultivated on cashew apple juice ranged from 0.39 to 0.5 g cells /g reducing sugar as well as Vazquez *et al.* (1993) obtained the highest mean biomass yield from *Candida utilis* and *Saccharomyces cerevisiae* being 0.48 g and 0.45 g /g glucose utilized in the medium, respectively.

The biomass production of *S. cerevisiae* cultivated by molasses was determined by Amin (1978). The author reported that, yield coefficient of the yeast was increased by increasing the successive cultivation times indicating more than 79% at the end of the first stage and 109 % after 15 transfers.

Omobuwajo *et al*. (1987) obtained cell biomass productivity (at a reducing sugar level of 7 g/L of hydrolyzed corn straw) of 5.0 g/L medium by *C. intermedia* and 3.5 g/L medium by *Saccharomyces cerevisiae.* Also, Selim *et al.* (1991) obtained a yield of 12.75 g dry/L medium when *S. cerevisiae* was grown on Egyptian vinasse (by batch fermentation process).

In ideal conditions, it was reported that, 245 g dry weight of yeast could be obtained from I kg of molasses containing 480 g sucrose (Rosen, 1987) which represented a substrate yield of almost 50% on a weight basis. Such yield however, could only be achieved in aerobic conditions, and much lower biomass yields were obtained in

fermentative conditions when most of the carbohydrate converted to ethanol.

Litchfield (1992) produced about 10 to 15 g dry weight yeast/ L medium from *Saccharomyces cerevisiae* under typical batch process, however in conventional fed-batch process it was about 35 - 45 g yeast (dry wt.)/ Liter.

Abramov *et al.* (1995) obtained 43.6 g biomass (wet) /L medium by growing *S. cerevisiae* in molasses medium mixed with geothermal water as a cheap mineral source.

Almeida and Pais (1996) obtained final biomass ranging from 9.3 to 13.2 g (dry) /L medium when they compared between some *S. cerevisiae* and *T. delbrueckii* strains. The maximum yield was obtained by *S. cerevisiae* IGC 5319 strain after cultivation in YPS medium (2% yeast extract, 4% peptone, 2% sucrose, 0.2% KH_2PO_4, and 0.1% $MgSO_4.7H_2O$) at 30°C.

Liu *et al.* (1996) improved the productivity of two baker's yeast strains (commercial dry baker's yeast ICM10 and ICM-11) from 0.230 to 0.241 and from 0.277 to 0.327 g, respectively by cultivation using a fed-batch process with a novel airlift loop reactor (glucose concentration was maintained at about 5 g/L, pH 4.5 and at 30°C). The results showed that, the yield of yeast cells was much higher in forced periodic operations with a novel airlift loop reactor than in steady-state operations with a traditional airlift loop reactor.

Using a defined mineral medium, biomass concentration of 130 g dry /l was reproducibly attained by Van Hoek *et al.* (2000) with baker's yeast.

Araud (2004) obtained the smallest baker's yeast yield coefficient (151.2%) during the first hour of cultivation (by using molasses) while, the greatest one (223.3%) was recorded during the fourth hour of cultivation. He cultivated the same strain on a supernatant resulted from pretreatment of barley straw and the yield coefficient was very low and ranged between 28-35.9%. Meanwhile, the yield coefficient was rather high (77.3-84%) and increased as a result of yeast cultivation in glucose solution (as a carbon source) presented by acid hydrolysis of residual cellulose of pretreated barley straw. The maximum yield coefficient (134.5-143.8%) was obtained when the yeast was cultivated in glucose hydrolyzate (produced by enzymatic hydrolysis of residual cellulose of pretreated barley straw).

Stavros *et al.* (2006) reported that, daily maximum biomass yield in batch aerobic fermentation of *S. cerevisiae* was 14.9 g dry/l. However, it was 11.2 g dry/ l with continuous aerobic fermentation.

2. Invertase activity

Sucrose is first hydrolyzed sugar by the extracellular invertase (β-fructofuranosidase, E.C. 3.2.1.26) into monosaccharides, which are then taken up into the yeast cell (Sanjay and Sugunan, 2005 and Uma *et al*., 2010). In *S. cerevisiae* the ability to hydrolyze sucrose in conferred by any one of six polymeric sucrose genes (denoted SUC1 to SUC6) that reside at loci distributed throughout the genome. This enzyme is separable into two isoenzymes, which are located in different parts of the cell (Gascon and Ottolenghi, 1967): an external form (270.000 Mw) located on the outside of the plasma membrane (Sutton and Lampen,