Production of Baker's Yeast Using Date Juice
利用枣汁生产面包酵母

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摘 要 面包酵母是一类用来提高面包质量的重要添加剂。目前,不同国家主要采用分批培养、补料分批培养或连续培养的方式来生产面包酵母。酿酒酵母是用来发酵面团中淀粉的理想微生物,除了提升食品香味,增加口感之外,这一发酵过程可以产生多种维生素和蛋白质。用于生产酵母生物量的主要成分包括各种碳源,如甜菜糖蜜和甘蔗糖蜜等。由于甜菜糖蜜可用于高产率地生产乙醇,加之其带来的生物环境污染和废水处理问题,因此需要考虑用其他糖类来生产面包酵母。其中一个代替性糖源是枣。由于各种原因,伊朗每年都浪费大量的枣。研究了用枣作为培养基碳源的可行性。将废枣榨成汁,然后研究了酵母的产量和生长速率。结果发现,在 pH3.4,温度 30℃,通风量 1.4vvm,发酵罐搅拌速度 500r/min 时,酵母对底物的产率接近 50%。

关键词 面包酵母,枣汁,酿酒酵母,生物量,生长速率
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Abstract Baker's yeast is an important additive among the products which improves bread quality and for present time is being produced in different countries by batch, fed batch or continuous cultures. *Saccharomyces cerevisiae* is used in fermentation of starch in dough, giving a favourable taste and produces a variety of vitamins and proteins. The main ingredient in yeast production is carbon source such as beet molasses, cane molasses, and so on. Since beet molasses has other major function as in high yield alcohol production and also due to the bioenvironmental issues and related wastewater treatment, the use of other carbohydrate sources may be considered. One of these carbohydrate sources is date which is wasted a great deal annually in this country (Iran). In this study, the capability of date to act as a suitable carbon sources was investigated. The waste date turned into juice and consequently production and growth rate of *Sacchromyces cervisiae* were studied with this juice. A maximum possible yield of 50% was obtained by the optimum medium (P3), at pH 3.4, 30℃, 1.4vvm aeration rate and agitation of 500r/min.

Key words baker's yeast, date juice, *Saccharomyces cerevisiae*, biomass, growth rate

Many of developing countries where major nutritional problems exist, produce an excess of materials high in carbohydrates that can be utilized in fermentation processes to produce microbial protein which in turn can be used to upgrade both food and feeds. Agricultural waste materials are an attractive renewable source for bioconversion to ethanol and biomass. Current research and development is being directed towards the substitution of the higher-cost sugar feedstocks with the low-cost lignocellulosic biomass. This idea has two advantages: 1. to obtain product with high value and 2. to decreases the environmental impact.

Date contains an average of 65% sugar, that most of it is glucose and fructose. The sugar can be easily extracted from dates by water and it also contains substantial level of nutrients required for the growth of microorganisms.

Saccharomyces cerevisiae has several specific advantages over plants and animals as protein producers and is used for production of glutathione, biosorption of heavy metals[1,2], higher level of flavouring compounds[3], asparagines[4], synthesis of food flavour like methyl benzoate[5], and biotransformation of organic compounds[6].

General baker's yeast is *Saccharomyces cerevisiae* that grows in the present of beet and cane molasses[7]. Sugar sources that baker's yeast can consume are: fructose, mannose, galactose, sucrose, maltose and hydrolyzed lactose[8]. Ethanol also is consumed by *Saccharomyces*

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cerevisiae as a carbon source[9].

Studies show production of baker's yeast in sugar substrate culture media that growth take place in two phases, first phase is related to acquire energy production (glycolysis) and respiration effect is low, while the second phase is related strongly to kind of culture media and strain[10,11].

The present study was undertaken in order to access the possibility of using dates as the substrate for fermentation processes to produce baker's yeast. The effect of corn steep liquor as a growth promotion agent, pH, temperature, aeration, mixing and different kind of media was investigated.

1 Materials and Methods

1.1 Culture medium

Date juice was used as a culture medium.

Date juice preparation: wasted dates were ground by using an electrical meat grinder prior to soak it in approximately six times of their weight in cold tap water for 12 hours. The mash was pressed and filtered several times through cheesecloth. The extracted juice contained 14% w soluble solids. The extract was stabilized by rapid heating to 95℃ for five minutes. It was then dispensed into polyethylene containers, and stored at −20℃ until it was required for use. Immediately prior to each experiment an appropriate quantity of date extract was thawed and diluted with distilled water to the desired concentration of soluble solids which was determined by refractometer.

The culture media contains: aluminium sulphate 8g/L, Na_2HPO_4 5g/L, $MgSO_4$ 0.25g/L, EDTA 0.1g/L, CSL 7% and dates extract with 20g/L sugar.

1.2 Microorganism

The yeast strains *Saccharomyces cerevisiae* SFO6 was purchased from Iran Mayeh Company (Iran). The strain was maintained on agar slant having the following composition: Dextrose 20g/L, bactopeptone 10g/L, yeast extract 5g/L, agar 20g/L. A 24h growth of the yeast was preserved at 4℃.

1.3 Inoculums

The inoculums were prepared by a single transfer in dextrose broth followed by transfer to a 5% soluble solids date juice which had been sterilized using Millipore (0.45μm) filter. All incubations were done by using a shaking incubator at 30℃ ± 1℃ for 24h. A 10% inoculum was then introduced directly into the fermentation flaks or fermenter. Inoculum concentration of yeast was adjusted to give initial concentration of 3g/L.

1.4 Fermentation

Batch fermentation was performed in Erlen Myer flasks in an aerobic shaker and 5L fermenter. Most of the growth experiments were performed in identical 1L flaks each contains 500mL of medium includes 5% soluble solids date juice enriched with chemicals to get optimum medium. The flaks were closed with cotton-wool plugs and sterilized at 121℃ for 15minutes. Incubation carried out in an orbital shaking (200r/min) maintained at 30℃ ± 1℃.

For other fermentations 3L of the medium was introduced into a 5L fermenter (CHEMAP) and sterilized at 121℃ for 30 minutes. The temperature was kept at 30℃ and agitation was controlled to 500r/min and air flow at 5L/min. the initial pH of the juice was 6 ~ 7.

1.5 Analytical methods

Biomass was measured in terms of dry weight. Yeast cells were harvested by centrifugation for 10min at 10000r/min and then washed twice with distilled water and weighed after 24h at 100℃. Sugar concentration was determined by employing the DNS method used for reducing sugars[12].

Ethanol was estimated by dichromate colorimetric method[13], which is based on the complete oxidation of ethanol by dichromate in the presence of sulphuric acid to form acetic acid.

Nitrogen was measured by semi micro kjeldal method[14] and yeast protein was calculated by using a factor of N × 6.25. Atomic adsorption instrument was used to determine the concentration of Na, K, Ca, Fe, Cu and Mg in dates extract.

1.6 Yeast Activity test

Widely-used measure of yeast activity in dough is the volume or pressure of gas generated by dough proofing in a closed container (gassing cover). Pressure gages and liquid manometers have largely been the placed by automated apparatus such as the Risograph and Fermentograph. The total pressure or volume of gas generated during to 2 ~ 3 hours of proofing is often reported and cumulative volume versus time plots (gassing curves)[15]. In this study the activity of baker's yeast has been measured by Lean dough method. The data shows that, the obtained baker's yeast has been having remarkable activity by comparison the other yeasts Table 6.

Table 6 Baker's activity of dough

Baker's yeast type	CO_2 volume after 1.0h/ccm	CO_2 volume after 2.0h/ccm	CO_2 volume/ccm
Sermipan Co. Netherlands	490	1000	1490
Lesaffre Co. France	475	990	1465
Iran Mayeh Co.	450	975	1425
Present study	400	950	1350

Temperature: 35℃; yeast/ flour ratio (g/g): 0.75/140

2 Results and Discussions

Date extract is analysed and the results are shown in Table 1.

The Results of shake flask experiment (Fig. 1) show that *Saccharomyces cerevisiae* didn't produced in high cell concentration and high conversion rate in 24h fermentation. So we decided to investigate the optimum culture medium to obtain high cell concentration. The best culture medium contained, date juice, 20g/L sugar and was fortified with $(NH_4)_2SO_4$, 8.0g/L; NaH_2PO_4, 5g/L; $MgSO_4$, 0.5g/L; EDTA, 0.1g/L; 7% CSL; Urea, 7g/L, pH = 3.4 and 30℃. The total biomass was obtained 7.05g/L with this culture medium. Then optimum operating condition for getting maximum yield in 5L fermenter was obtained.

Table 1 Analysis of dates extract

Total reducing sugar	Sucrose	Mineral nitrogen	Protein	P	Total nitrogen	Ca	K	Na	Fe	Cu	Total ash
50 ~ 70	3.58%	0.17	1.06	0.28	0.18 ~ 1.27	0.54	21 ~ 25	0.9	0.16	0.012	1.3

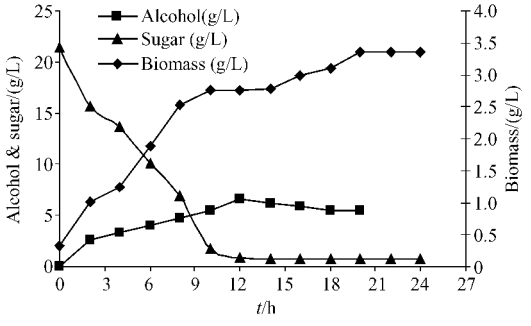

Fig. 1 Batch growth rate of *Saccharomyces cerevisiae* in shaking flask

2.1 Effect of pH

Five flasks were prepared; each contained 500mL of 5% soluble solid to study the effect of initial pH on the growth of the *Saccharomyces cerevisiae*. Phosphate buffer was used to adjust the medium pH to 2.8, 3.2, 3.4, 3.6, 3.89 and 4 respectively. The results presented in Table 2 show that lowering the initial pH to 3.4 lead to the highest cell production. In fermentation process microorganism should be used in acidic pH to decrease the level of contamination.

2.2 Effect of temperature

Growth temperature is an important factor in *Saccharomyces cerevisiae* production process. The results in Table 3 show that the highest production was obtained at 30℃.

Table 2 Effect of pH on yeast growth

Initial pH	Biomass/(g/L)	Yield/%
2.8	3.69	17.5
3.2	4.25	20.36
3.4	5.26	25.3
3.6	4.85	23.55
3.8	4.52	21.83
4.0	4.3	20.81

Table 3 Effect of temperature on yeast growth

Temperature/℃	Biomass/(g/L)	Yield/%
20	5.05	24.41
25	5.70	27.76
30	7.05	34.33
35	4.3	20.65
40	0.72	2.5

2.3 Effect of Corn Steep Liquor (CSL)

Seven different concentration of CSL were added to the date juice and inoculated with *Saccharomyces cerevisiae* and incubated with shaking at 30℃ and pH 3.4 for 24h., the results show that highest biomass produce in 7% CSL (Fig. 2). CSL can be used both as a nitrogen source and a source of co-factors for growth promotion.

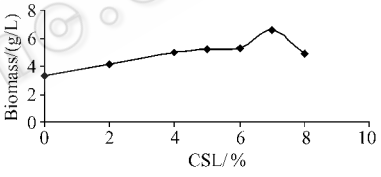

Fig. 2 Effect of CSL concentration on biomass in batch reactor (T = 30℃, pH = 3.4)

2.4 Growth of *Saccharomyces cerevisiae* in 5L fermenter

After obtaining the optimum culture media and operation condition from shaking flask, 3L of medium was introduced into 5L fermenter with Roshton turbine mixer, four baffles and aeration.

The agitation was controlled at 500r/min, pH adjusted to 3.4 by citrate buffer and temperature at 30℃.

A 5% inoculum was then introduced directly into the fermemter from selected flask with high activity. The results show that the yields and cell production are 42.7%, 49.77% and 8.53g/L and 10.65g/L after 24 and 48h of fermentation with different aeration respectively (Table 4). Also the results show that the optimum aeration is 5L/min (1.7vvm) and maximum biomass production increases with aeration time from 24 to 48 h. The optimum glucose concentration was obtained 20g/L that is corresponds to a specific growth rate $0.24h^{-1}$. If glucose concentration increased more than critical value, ethanol production increased very rapidly and it could be an inhibitor for growth of yeast (Table 5). The batch kinetics of bakers' yeast production from date juice was studied in details. Fig. 3 shows batch kinetics of date juice bioconversion to ethanol

and cell growth by *Saccharomyces cerevisiae*. Most of initial glucose and fructose (20g/L) was metabolized by the yeast within 12h resulting in the formation of 5g/L ethanol. In this phase the biomass produced was about 6g/L. In the second phase initial ethanol (5g/L) was metabolized by the yeast also within 8h resulting in the formation of 8.7g/L biomass. Growth rate reached a maximum value of $0.24h^{-1}$ in first phase and $0.18h^{-1}$ in second phase (Fig. 4 and 5) and Monod equation was obtained.

For glucose : $\mu_1 = 0.24\ S/(2.572 + S)$ (1)

For ethanol : $\mu_2 = 0.18\ E/(0.791 + E)$ (2)

S : Glucose concentration E : Ethanol concentration

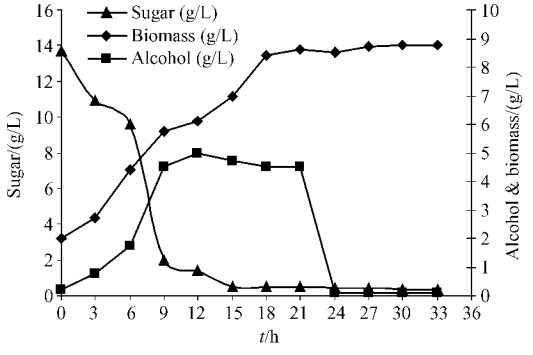

Fig. 3 Batch growth rate of *Saccharomyces cerevisiae* in 5L fermenter

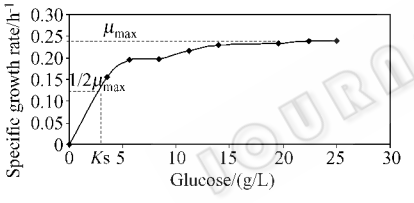

Fig. 4 Specific growth rate *vs*. glucose concentration in the first phase

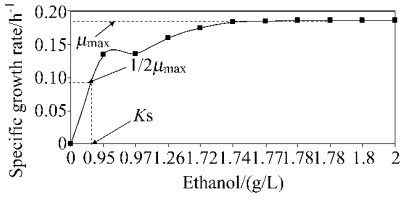

Fig. 5 Specific growth rate *vs*. ethanol concentration in the second phase

(Fig. 4 and 5) show that specific growth rate didn't decrease sharply after 11h and 18h of fermentation according to less production of inhibitor.

Table 4 Effect of aeration and time of aeration on biomass production

Aeration rate /(L/min)	Biomass after 24h/(g/L)	Biomass after 48h/(g/L)	Yield/%
2	6.75	5.93	31.20
3	6.79	6.99	32.33
4	8.33	9.13	42.7
5	8.53	10.65	49.77
6	6.53	8.78	41.24

The peak of specific glucose and ethanol consumption rate were observed within the maximum substrate utilization during the exponential phase.

Table 5 Effect of sugar concentration on biomass concentration

Sugar Conc./(g/L)	Biomass/(g/L)
7.0	3.87
12.0	4.88
16.0	5.68
20.0	6.34
26.0	5.47

3 Conclusions

Dates extract can be used as a rich source of glucose and fructose (70% of reducing sugars) and yeast can consume it easily. Juice was extracted from date by water and this juice was considered as a good choice for baker's yeast production. According to our study the optimum sugar concentration for baker's yeast production was 20g/L. Date extract as a substrate was used by baker's yeast and our data shows a kg of baker's yeast was produced per 3kg of date waste.

Optimum temperature, pressure, pH and aeration rate were obtained 30℃, 1atm 3.4 and 5L/min respectively. After 48h concentration of biomass was reached 10.65g/L according to CSL 7% concentration. The yield base on optimum parameters was obtained 50%.

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