

Process optimization for the production of Yeast Extract from fresh Baker's yeast (*Saccharomyces cerevisiae*)

Running Title: Yeast extract production process

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Abstract

Yeast extract is widely used in different food industries as a flavoring agent or vitamin supplement. In this study, a process was optimized for the production of yeast extract from Baker's yeast (*Saccharomyces cerevisiae*). A glass vessel stirred fermenter was used for the cultivation of yeast biomass. The effect of various physical and chemical factors was evaluated on the production of yeast cells and optimum conditions for the production of maximum yeast biomass were determined. The optimum growth was obtained at 30°C with pH 4.5 using molasses as a substrate supplemented with urea at 150rpm. Yeast cells were then separated by centrifugation and ruptured and autolysis was observed to be the most feasible method. Among various method employed to dry the yeast extract, spray dryer appeared as most efficient one. Yeast extract obtained after drying was subjected to different analyses and compared with commercial yeast extract. The produced yeast extract was applied in media preparation to grow different microorganisms including yeast, bacteria and fungi and considerable growth was observed. These results indicated that the developed process is a cost effective alternate approach for the production of yeast extract.

Keywords

Yeast extract, Fermentation, *Saccharomyces cerevisiae*, molasses

Introduction

Yeast, *Saccharomyces cerevisiae*, is ubiquitously present in the environment and has great applications in various industries especially in the production of alcoholic beverages and to leaven the bread dough [1,2]. Yeast extract, obtained from the lysis of yeast cells, mainly contains amino acids, vitamins, nucleotides, peptides, carbohydrate, salts and other water soluble components of yeast cells. However, nitrogenous component and vitamins are the vital ingredients of the yeast extracts because of their nutritious properties. It is widely used in food industries as a flavoring agent in sauces, soups, stews, gravies, canned and snack foods. It also possess extensive applications in health sector as a vitamin and protein supplement as well as in microbiological media preparations, in cosmetic industry and for plant nutrient [3].

Yeast extracts commercially available in various forms such a powder, paste or liquid [4,5] and mainly produced by the baker's or spent brewer's yeast, *Saccharomyces cerevisiae*, by autolysis. Though, other yeasts including *Candida utilis* and *Kluyvermyces marxianus* are also used [5]. Since the brewer's yeast is served as inexpensive source of yeast extract production for food and fermentation industries [6], however, yeast extract obtained from brewer's yeast has very undesirable bitter taste due to the absorption of hop constituents and beer solids to the yeast cells during beer fermentation [7] Therefore, de-bittering is required for such yeast creams to reduce the bitter taste in final product [8] Despite this limitation, brewers yeasts have been applied by various researchers for the production of yeast extract [3]. Similarly, baker's yeast has also been investigated by several biotechnologists for the production of yeast extract [9,10]. This study was designed to develop a process of yeast extract production from baker's yeast.

Results and Discussion

Yeast extract is extensively used in different industrial sectors as a flavoring agent or nutrient supplement. In this study, a process was designed and optimized for the production yeast cells (*Saccharomyces cerevisiae*) and extraction of yeast extract from them.

Optimization of production of maximum yeast biomass

There are several physical, chemical and biological factors that affect the growth of microorganism. These factors were optimized by one factor at a time strategy for maximum yeast biomass production.

Temperature is a significant factor that plays a vital role in the growth of microorganism. Yet, each organism requires a specific temperature for their growth [11]. Maximum yeast biomass production was obtained at 30°C (Figure 1). The optimum temperature for the growth was in concordance with the results reported previously for the growth of *Saccharomyces cerevisiae* using cane molasses as a substrate [12–14].

The pH is also a vital factor regarding the growth of microorganisms. It has been reported that pH play an important role in the ionization of enzyme and transport of nutrients across the cell membrane, hence, maintaining the pH at optimal level is critical to ascertain the maximum growth. Out of different selected pH values used for the growth of yeast biomass, the maximum growth was obtained at pH 4.5 (Figure 2). It has been reported that maximum growth of *S. cerevisiae* was obtained at a pH range of 4.5 – 5.5 [12,15].

When the effect of different carbon sources was determined on the growth of yeast cells by culturing them using different carbon sources, the highest growth yield was obtained with molasses (100 g/l) as compared to other screened media. (Table 1). Previously, several

researchers reported the maximum production of *S. cerevisiae* using cane molasses as a substrate [12,16],

The effects of different nitrogen sources i.e., yeast extract, urea, NH_4Cl and Na_2NO_3 was also observed on the production of biomass (*Saccharomyces cerevisiae*). It was observed that the growth of biomass was optimum when yeast extract was supplemented as nitrogen source (Table 2). It has been reported that *S. cerevisiae* is able to utilize a wide variety of nitrogen sources including organic and inorganic, nevertheless, various nitrogen sources were found optimum for the growth of *S. cerevisiae* including urea [17] and yeast extract [18] previously. The maximum growth was obtained at 150 rpm as shown in figure 3, commonly used for the growth of *S. cerevisiae* [19].

Cell rupturing process

After fermentation, the whole content from fermenter was subjected to rotary vacuum filter to obtain yeast biomass cakes. Following this, different strategies were opted for the lysis of yeast cells. Autolysis was observed as a most efficient method with highest brix (Table 3). This result is consistent with the reports of others [20]

Drying

The soluble content was subjected to drying by employing different methods. All procedures were analyzed and it was found that spray dryer gives best results (Table 4). Instead, spray dryer is an extensively used method for the drying of yeast extract [3].

Chemical analysis and comparison of produced yeast extract with commercial available yeast extracts

Total solid content was determined by hand refractometer and both commercial and produced yeast showed 5 Brix. Protein content in both the yeast extracts was estimated by Bradford's

method and both were found to have 13.75 and 13.5 mg/ml of proteins, respectively. Thin layer chromatography is one of cheapest and reliable method for quantitative and qualitative analysis of amino acid. The results of thin layer chromatography showed that commercial and produced yeast extract have the same number of amino acids (Figure 4). The chromatogram depicted that the produced yeast extract possessed elevated concentrations of amino acids as compare to commercial yeast. The amino acids were identified as leucine, isoleucine, histidine, aspartic acid, arginine, lysine, and glutamine.

Assessment of Biological activity

The growth of different bacteria and fungi was evaluated in the media prepared by produced yeast extract. Considerable growth was observed in the slants with prepared yeast extract that was comparable with the growth of organisms in the media with commercial yeast extracts. In addition, the organisms showed late growth in commercially prepared media (After 24 h) whereas the early growth (within 16-24) was obtained in the media with produced one indicated that the produced yeast extract is an efficient alternate to be applied in the preparation of microbiological media.

Materials and methods

Organism and inoculum preparation

Yeast (*Saccharomyces cerevisiae*) was purchased from Sigma-Aldrich and maintained on Yeast Peptone Dextrose (YPD) agar containing 3.0 g yeast extract, 5.0 g peptone, 10.0 g Dextrose and 20.0g agar (pH 5.5) at 4°C. The inoculum was prepared by transferring a single colony to 250 ml Erlenmeyer flask containing 50 ml YPD broth medium and incubated at 30°C at 200 rpm in a rotary shaker for 24 h.

Optimization of Fermenter production of yeast biomass

Stirred tank fermenter (7.5 L) was used for the production of yeast cells. Different factors such as media (Yeast extract, Potato Dextrose, Sabouraud Dextrose Broth, Yeast extract Peptone Dextrose plus Adenine, molasses, whey), nitrogen sources (urea, peptone, yeast extract, NH_4Cl , Na_2NO_3), temperature (20-40°C), pH (3-6) and agitation (50-200 rpm) were evaluated for their optimum levels for the maximum production of yeast biomass. Five liter fermentation media with 25% inoculum was used for each fermentation batch. The fermenter vessel was sterilized under 121°C temperature for 15 min. Sterilized solution of 1N NaOH and 1N HCl was used for pH control, whereas silicon oil was used to control foaming during fermentation. The fermentation was conducted for a period of 12 h for each batch unless mentioned.

Removal of water from yeast cell biomass

After optimum production of yeast biomass, the whole content from the fermenter was transferred to the rotary vacuum filter in order to remove the water from wet yeast cells and the yeast cells were obtained in the form of small cakes. These cakes were then further processed for cell lysis in next step.

Cells disruption/lysis

Different techniques were adopted by keeping in view the financial feasibility. These methods were sonication, use of liquid nitrogen, acetone and toluene, bead mill and autolysis [3,21]. Results were recorded in term of Brix. After the rupturing of the cells, the whole material were subjected to centrifugation (Avanti J-26 XP centrifuge) for 10 min at 6000 rpm at 10 °C and supernatant was collected and stored at 4 °C till further use.

Final Drying

Various methods were screened to dry the yeast extract obtained after separation of cell debris. These methods including Rotary vacuum filter, Spray dryer (Spray Dryer SD-05), Rolling bed filter, Fluidize Bed dryer and Pneumatic dryers.

Chemical analysis and comparison of produced yeast extract with commercial yeast extract

After getting the product in its final crystalline powder form, some compositional tests were performed. For the estimation of total solid content, hand refractometer was employed and the total solid content was measured in term of brix by placing one drop of supernatant at hand refractometer [22]. Protein content was measured by using Bradford protein assay [23]. The presence of amino acids was investigated by thin layer chromatography (TLC) technique in both commercial and produced yeast extract by using the method of Brenner and Niederwieser [24].

Assessment of microbial growth using prepared yeast extract

An experiment was performed to compare the biological activity of prepared yeast extract from yeast biomass (*Saccharomyces cerevisiae*) with different yeast extracts available in market. Growth media was prepared by dissolving malt extract (2g), agar (2g) and prepared or commercial yeast extract (2g) in 100 ml of distilled water. Media was inoculated with *Geotrichum candidum*, *Aspergillus niger*, *Escherichia coli* and *Bacillus subtilis* separately and incubated at 30 °C for fungi or at 37 °C in case of bacteria for 24 h.

Conclusion

The developed process for the production yeast extract have several advantages such as no chemicals or enzymes were added during extraction process which resulted in the reduction of cost and number of steps of down streaming process. The chemical analysis showed that prepared yeast extract was comparable with commercial one in term of its properties. In the end, application of produced yeast extract in microbiological media revealed its suitability for the growth of different microorganisms. With these promising results, this process could be an efficient alternate for the lab or commercial scale production of yeast extract specifically for microbial growth.

Conflict of interest

The authors have no conflict of interest to declare.

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Tables

Table 1: Growth yield of yeast biomass by using different carbon sources

S. no.	Media	Growth yield (g/L)
1	YPD	70
2	SDB	40
3	YPAD	60
4	Molasses	100
5	Whey	50

Table 2: Effect of different nitrogen sources on biomass production

S. no.	Nitrogen Sources	Biomass Production (g/L)
1	Yeast Extract	12.00
2	Peptone	10.00
3	Urea	0.5 0
4	NH ₄ Cl	1.00
5	Na ₂ NO ₃	1.00

Table 3: The effect of different methods on the lysis of yeast cells

S. No	Process	Brix (°Bx)
1.	Use of Acetone and Toluene	15
2.	Sonication	13
3.	Pastel and mortar	11
4.	Liquid Nitrogen	17
5.	Autolysis	19

Table 4: Different methods used for the drying of produce yeast extract

S. no.	Methods of drying	Flow rate of liquid (L/h)
1	Rotary vacuum filter	0.1
2	Spray dryer	0.2
3	Rolling bed filter	1.5
4	Fluidize Bed dryer	0.9
5	Pneumatic dryers	1.7

Figure Legend

Figure 1: Effect of different temperatures on the growth rate of yeast cells: X-axis represents temperature in degree Celsius, Y-axis represents growth rate in gram/liter

Figure 2: Effect of various pH on the growth rate of yeast cells: X-axis represents pH, Y-axis represents growth rate in gram/liter

Figure 3: Effect of agitation on the growth rate of yeast cells: X-axis represents agitation rate (rpm), Y-axis represents growth rate in gram/liter

Figure 4: Amino acid profiling of produced and commercial yeast extracts by Thin Liquid Chromatography (TLC) plate.

Figure 1

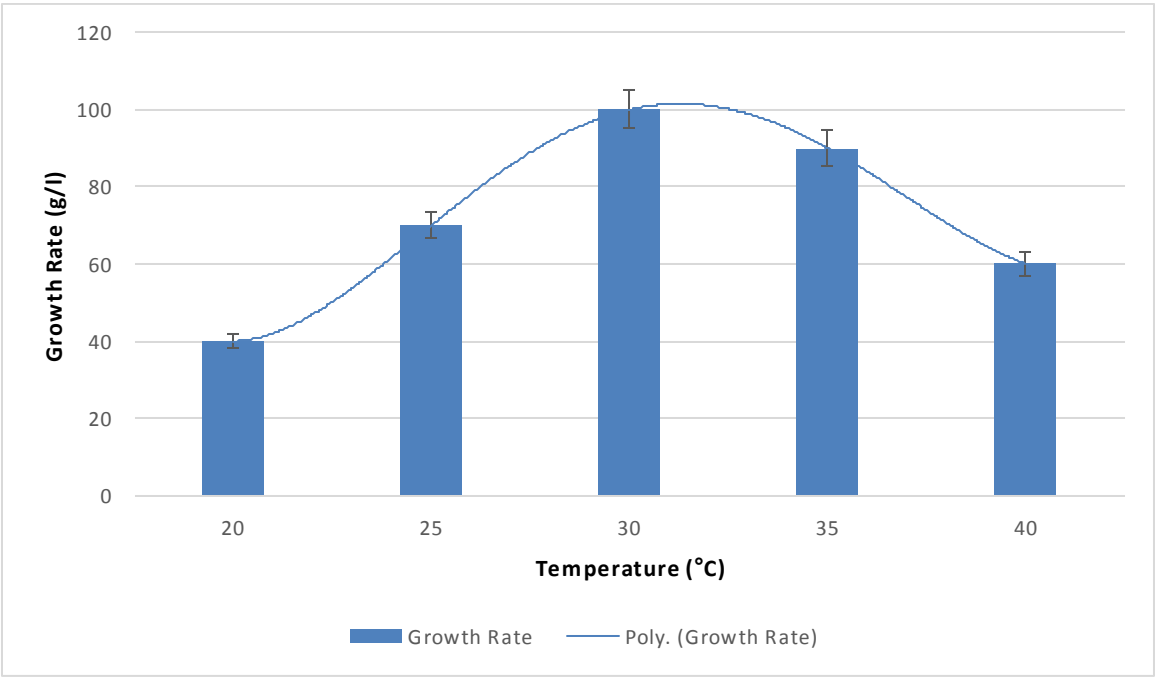


Figure 2

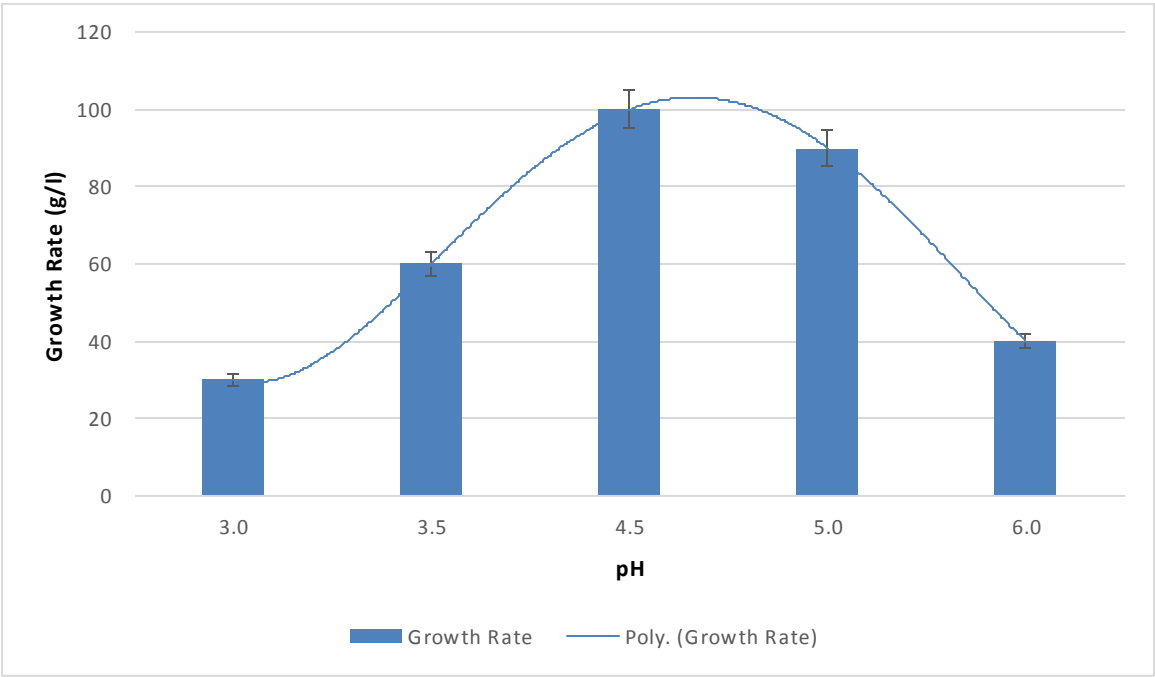


Figure 3

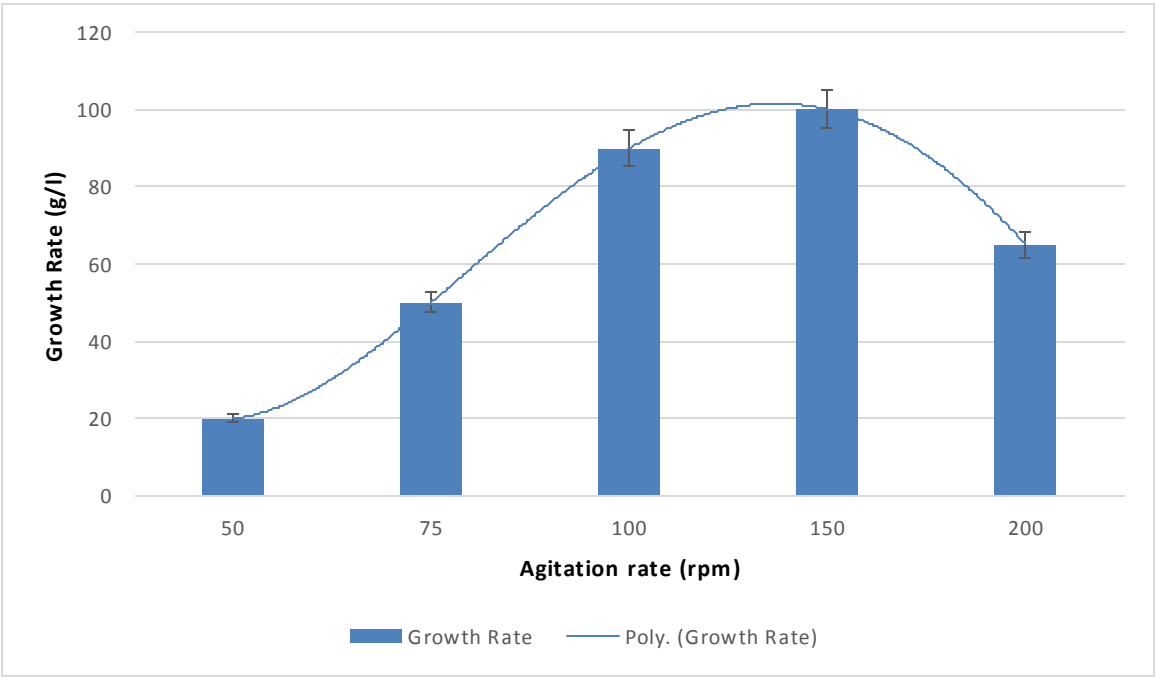


Figure 4

