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Comparison of three methods of preparing the powder form of yeast extract

Maryam Sadat Mirbagheri Firoozabad [*1], Hamidreza Akhbariyoon [2], Farbod Shirvanifar [3]

[1] *Department of Biology, Faculty of Science, Yazd University, Yazd, Iran*
[2] *Department of Biochemistry, Tarbiat Modares University, Tehran, Iran*
[3] *Department of Biotechnology, Faculty of Biological Sciences and Technology, Shahid Ashrafi Esfahani University, Esfahan, Iran*

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Abstract

Yeast extract obtained by hydrolysis of *Saccharomyces cerevisiae* as a baker yeast provides a high nutritional value for pharmaceuticals, food industries, microbial substrates, and biofuels. Two methods are typical to produce yeast extract, autolysis and hydrolysis. In this study, the rapid and cost-effective process of the hydrolysis method, including the mechanical methods of high temperature and pressure, was utilized to produce the yeast extract. The purpose of this study was to compare three methods, the Spray dryer, freeze dryer, and oven dryer methods, of preparing powder from bakery yeast extract. The effects of the produced yeast extracts were measured on gram-negative and gram-positive bacteria that were given as a bacteria feed to test the quality of products. The extract obtained from the freeze and spray dryer methods accelerated the growth rate of microorganisms. The bacteria *Staphylococcus aureus* and *Escherichia coli* showed better overall growth on the yeast extract attained from the spray dryer method. Hence, the produced spray dryer yeast extracts significantly improved the soluble expression and purification of the protein compared to the commercial extract. Results revealed that the spray dryer method preserves the properties of the yeast extract better than the other methods, and due to low moisture, this method can be used in industry. Nevertheless, all methods are applicable for yeast extract production.

1. Introduction

Yeast extract (Y.E) is a source of nutrition that is produced from yeast cells as the main ingredient. Y.E is classified as a natural flavor and contains large amounts of protein, carbohydrates, lipids, vitamins, minerals, and meaningful substances. Moreover, yeast is a significant source of essential amino acids that humans and mammals need to absorb from food (Berlowska et al., 2017; Milić et al., 2007; Reeds, 2000). Y.E is a processed yeast product, and it is comprised of yeast cell water-soluble components. The most valuable components of yeast extract are nitrogen and vitamins due to their nutritional characteristics (Saksinchai et al., 2001). Therefore, it is widely used in food industries such as poultry feed, pet food, plant foods, protein supplements, and most importantly, as one of the primary food sources of microbiological culture medium. Also, it is widely used in soups, sauces, stews, snack dishes, and canned foods because of its meaty taste (Azar et

*Corresponding author. Tel: + 983531232293
E-mail address: m.mirbagheri@yazd.ac.ir
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al., 2019; Breddam et al., 1991; Hajhosseini et al., 2020; Karamad et al., 2020).

Manufacturing Y.E is the process of digesting yeast cells using endogenous or exogenous enzymes. Recently, many experiments of these processes have been reported (Bayarjargal et al., 2014; Choi et al., 1998; Mensour et al., 1996). In addition to research on their unique genetics and physiology, they have been historically used for a multiplicity of applications for thousands of years, including bioremediation, baking, and fermentation. Furthermore, nutrient cell components of yeasts known as "*yeast extract*" can be utilized for many essential purposes (Delneri, 2010). Baker's yeast, a species of yeast, has been used to manufacture Y.E. Different methods such as autolysis and hydrolysis have been employed to produce Y.E. These methods aim to disrupt the yeast cell wall and recover soluble fractions. The procedure of harvesting the yeast extract varies, depending on the purpose of production. The yeast extract quality can be improved using these additional methods, however, most of these steps are laborious, time-consuming, costly, and nonessential (Salari et al., 2015; Vieira et al., n.d.). Another valuable substance called beta-glucan can be obtained from the yeast cell wall. Due to its unique properties, beta-glucan is widely used in pharmaceuticals, health, food, dairy, meat, and other products (i.e., desserts, sauces, and beverages). Due to its health-promoting properties, this product affects the physicochemical, rheological, and organoleptic properties of food. These properties highlight the importance of beta-glucan as an effective and valuable ingredient. Most Y.E and beta-glucan extracts are made from baker's yeast. These materials expensive, and much currency is exported annually to import these products. The waste from yeast factories contains utilized yeast, which is transferred out of the factory based on the design of manufacturing companies. This may waste these valuable materials and pollute the environment. As a result, the reuse of spent yeast is essential to prevent waste in a factory (Cefalu et al., 2004). It also supplies a cost-effective range of starting materials for different fields, such as pharmaceutics, production of microorganism substrates, feedstuff industry, or biofuels (Geciova et al., n.d.; Milić et al., 2007; Saksinchai et al., 2001). Moreover, the food industry is interested in the further processing of spent yeast because of its high nutritional value (Cefalu et al., 2004; Ferreira et al., 2010; Jacob et al., 2019). The main reason for using Y.E in a wide range of industries is its high protein content (Dashtban Moghadam et al., 2014). Therefore, this study is conducted to analyze three different methods of preparing powder from bakery yeast extract. The aim of this study is to find a novel, cost-effective, and feasible method of preparing powdered yeast extract. This paper describes the autolysis process and compares methods for drying yeast extract.

2. Materials and methods

2.1. Chemicals and bacterial strain

Isopropyl-β- D-thiogalactopyranoside (IPTG, #I6758) and bovine serum albumin (BSA, #05470) were purchased from Sigma (USA). Yeast extract (#611005) and tryptone (#611004) were supplied by Liofilchem (Roseto Degli Abruzzi, Italy). All components of the growth media were purchased from Liofilchem (Italy) and the Merck company.

2.2. Yeast extracts preparation methods

Baker yeast was used to prepare yeast extract. 250 g of this yeast was dissolved in 1 liter of deionized water and autoclaved for 10 minutes at 110 ° C. Then, it was quickly placed on ice. The solution was then centrifuged at 400 g at 4 ° C for 10 minutes. These steps were repeated several times to completely extract the yeasts and separate them from the insoluble compounds.

Three methods were utilized to dry the obtained extracts. The spray dryer method (using the B-290, Büchi Labortechnik AG, Flawil, Switzerland), oven dryer method at a temperature of 50 ° C for 24 hours, and freeze dryer method (using the Snijders scientific device) were used to obtain the powder.

2.3. Bacterial Strains, Growth Curve Measurement in the presence of yeast extracts

Staphylococcus aureus ATCC6538 and *Bacillus cereus* ATCC11778 as Gram-positive bacteria and *E. coli* ATCC15223 and *Klebsiella pneumonia* as gram-negative bacteria were used to study the growth rate. Each of the extracts was utilized as the only nutrient in the bacterial growth medium, and comprehensive and liquid media were prepared. Extract-free medium (water) was used as a negative control, and a commercial yeast extract (Merck) was utilized for comparison and control.
A single colony of the bacterial strain was grown overnight at 37°C in N.B. The turbidity of the overnight culture was adjusted by adding enriched medium to reach a final OD600 = 0.5. Five milliliters of the overnight culture (OD600 = 0.5) were added in 100 mL of fresh medium containing only the produced yeast extracts, and the growth curve was established by monitoring cell density (CFU/mL) every 2 hours for 28 hours via direct counting by Toma lam. The bacterial growth curve was plotted for 28 hours at 37 °C in a nutrient broth medium (N.B) by a spectrophotometer at 580 nm wavelength using the turbidimetric method.

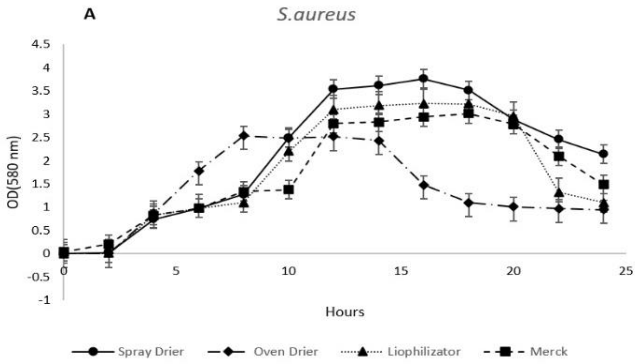
2.4. Protein expression using extracts

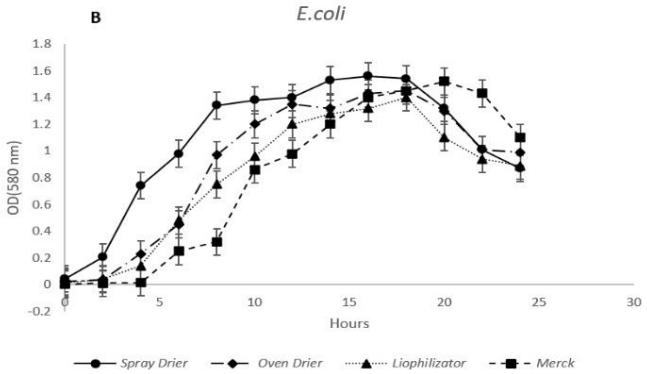
Uricase enzymes were expressed and purified according to Dashtban et al.'s method (Dashtban Moghadam et al., 2014). Each of the extracts was utilized as a component in a 2xYT medium. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed in 12.5% polyacrylamide gel, according to Laemmli (Laemmli, 1970). All samples were prepared by dilution with an equal volume of Laemmli sample buffer (62.5 mM Tris-HCl, 25% [w/v] glycerol, 2% [w/v] SDS, 0.01% [w/v] bromophenol blue, 5% [v/v] b-mercaptoethanol) and heating at 95°C for five minutes. Gels were run at 200 V until the dye front reached the bottom of the gel. Tris/glycine/SDS (25 mM Tris, 192 mM glycine, 0.1% [w/v] SDS) was used as the running buffer. The protein concentration was measured at 595nm with the Bradford method using Coomassie G-250 and bovine serum albumin as the standard (Bradford, 1976; Ghaemmaghami et al., 2003; Parsazad et al., 2020).

3. Results and Discussion

3.1. Effect of each type of yeast on the bacterial growth media

As mentioned above, yeast extract is comprised of amino acids, peptides, carbohydrates, salts, Nitrogen components, and vitamins, which are critically important for bacterial growth in media (Milić et al., 2007). For that reason, in this study, we produced yeast extract by utilizing *Saccharomyces cerevisiae* (baker's yeast). [Fig. 1].





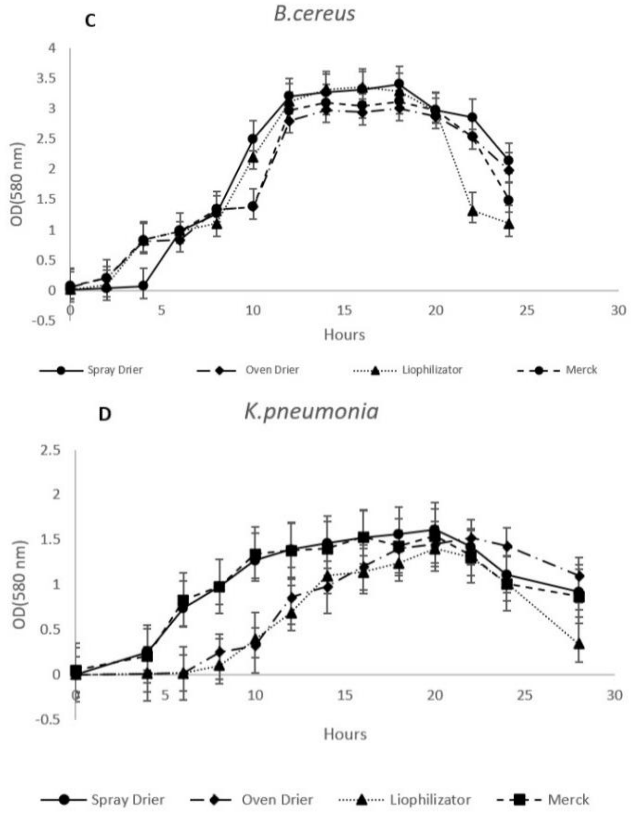


Fig. 1. Bacterial growth curve on 3 types of produced yeast extracts and control sample A) *S.aureus* B) *E.coli* C) *B.cereues* D) *K.pneumonia*.

The growth of the bacteria on a solid medium (including agar) showed that after a 24 hours incubation at 37 °C, all the studied bacteria had a considerable growth compared to N.A, as a control media. Only *Klebsiella pneumonia*, a gram-negative bacterium, has slow growth and gave colonies in 30 hours.

The results revealed that in the spray drying method, the dried yeast extract increases the growth rate of bacteria, compared to the other two yeast extracts. This could be due to the method of drying, which preserves most of the minerals. Therefore, the chart reached the logarithmic stage earlier and entered the stationary phase later. Then the, freeze-drying and oven drying yeasts reached these results, respectively. Also, the results showed that there was significant growth in the presence of all three yeast extracts in comparison to the commercial yeast extract (Merck). Also, the *E.coli* and *S.aureus* from the spray dried yeast extract grew faster than industrial ones.

3.2. Effect of yeast extract on protein expression

As mentioned in the introduction, using yeast extract as a growth factor in bacterial media, would enhance bacterial growth; and consequently, could increase protein expression (Milić et al., 2007; Vieira et al., n.d.; Zarei et al., 2016). In this study, the role of three methods of preparing yeast extract were further studied because of their effect on protein expression and purification. Uricase enzyme was overexpressed in BL21 *E. coli* cells. The SDS-PAGE of purified uricase revealed a single highly pure band with a molecular mass of almost 47 kDa [Fig. 2].

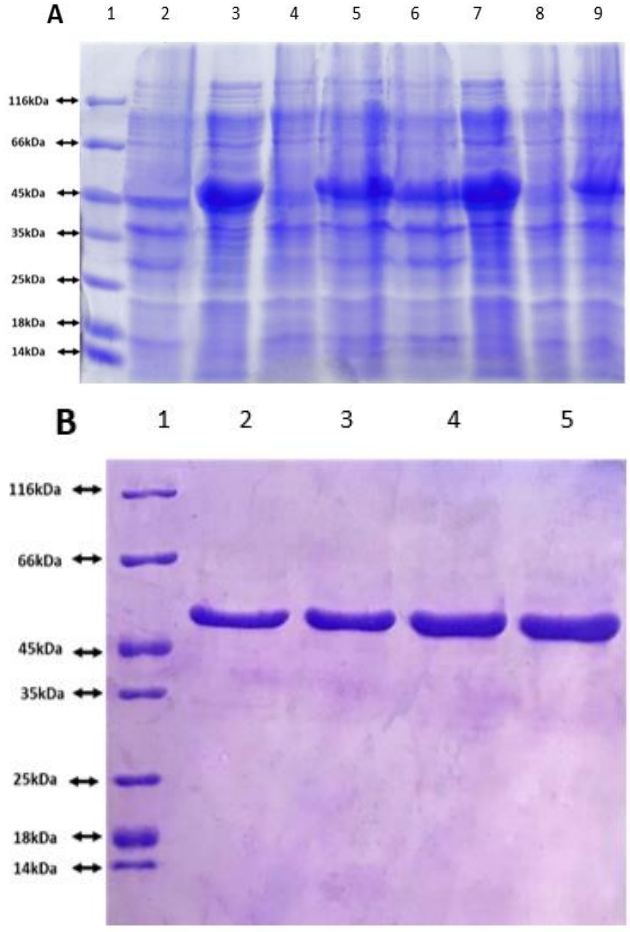


Fig. 2. SDS-PAGE analysis of effect of different type of yeast extract on A) protein expression including: 1- Protein marker 2- Sample pellet 3- Sample soluble 4- Freeze drier pellet 5- Freeze drier soluble 6- Spray drier pellet 7- Spray drier soluble 8- Oven drier pellet 9- Oven drier soluble and B) purification: 1- Protein marker 2- Sample 3- Freeze drier 4- Spray drier 5- Oven drier.

Fig. 2 shows that spray dried yeast extract has the most positive effect on uricase expression and purification. The result was the same as for the bacterial growth mentioned above. Yeast extract is obtained from bakery or brewery yeast and contains beneficial compounds with wide application in the food industry. Zarei et al, detected protein (30%), fat (0.42%), sodium chloride (0.67%), ash (12.18%), and total volatile nitrogen (9.2%) with the moisture of 4.72% in yeast extract produced by baker yeast (Amorim et al., 2016). Depending on the cell disruption method and following processing steps of the yeast extract, different amounts of amino acids are found in the yeast extract product (Ghaemmaghami et al., 2003; Parsazad et al., 2020). The cell disruption methods for yeast extract production include autolysis and hydrolysis. Autolysis is a natural event after cell death, where the yeast cell is digested by enzymes, and there have been reports of changes in temperature, pH, and osmotic pressure for autolysis (Berlowska et al., 2017; Podpora et al., 2015). Even though the autolysis processes increase the risk of microbial contamination or low extraction of the product due to the longer incubation time, this method is mostly used in industry (Berlowska et al., 2017; Podpora et al., 2015). Bayarjargal et al. obtained total solids of the yeast extract with an oven drier, drying at 105oC until a constant mass was achieved (Mensour et al., 1996). Their research showed temperature, influenced the solids content of yeast extracts. The yields of final products at 45oC were higher than 50oC in both autolysis hydrolysis of the enzyme (Dashtban Moghadam et al., 2014).

Depending on the disruption method chosen, amino acids are released into the extract. Autolysis is superior to hydrolysis for protein extraction. Jacob et al. showed the ultrasonic with its up-scaling is possible and considered to be equivalent to the cell mill. Apart from differences in individual amino acids, the variability of the yeast extracts is also reflected in the different protein fractions (Ghaemmaghami et al., 2003). However, the use of enzymes is expensive, and the use of acids and alkalis also challenge the purification process. In this study, we used a faster method, in which was the use of high temperature and pressure, followed by rapid cooling was repeated work several times in a row, resulting in the preferred hydrolysis of yeasts. Zarei et al. used a spray dryer, and Bayarjargal et al. used an oven drier for yeast extract production (Bayarjargal et al., 2014; Zarei et al., 2016).

Yeast extract is used as a growth factor in media, including LB agar, YPD agar (Babayan et al., 1985; Kanegae et al., 1989). Utilization and investigation of the effect of different types of yeast extract in the 2xYT medium produced in this research on protein expression and purification revealed that all of the yeast extracts increased protein expression, which confirmed other research (Mirbagheri et al., 2012; Mirbagheri et al., 2011).

4. Conclusions

The focus of this research was on evaluating the quality and function of three different drying methods for producing yeast extract as the sole carbon source for microorganisms. The results showed that all three types of yeast extract producing methods produced good quality yeast extract. Although, the growth rate of the studied microorganisms in the spray dryer method is better than other methods due to the preservation of essential amino acids. Results showed that although all three methods are acceptable for yeast extract production, the spray drying method preserves the properties of yeast extract better than other methods, and due to low moisture, this method can be used in industry.

Conflict of Interest

The authors declare no conflict of interest.

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Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors. This article does not contain any studies with human participants or animals performed by any of the authors.

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