Biotechnology and Bioprocess Engineering **2005**, 10: 85-90

Downstream Process for the Production of Yeast Extract Using Brewer's Yeast Cells

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Abstract A downstream process was developed for the production of yeast extract from brewer's yeast cells. Various downstream processing conditions including clarification, debittering, and the Maillard reaction were considered in the development of the process. This simple and economic clarification process used flocculating agents, specifically calcium chloride (1%). After the clarification step, a Maillard reaction is initiated as a flavor-enhancing step. By investigating the effects of several operation parameters, including the type of sugar added, sugar dosage, glycine addition, and temperature, on the degree of browning (DB), glucose addition and reaction temperature were found to have significant effects on DB. A synthetic adsorption resin (HP20) was used for the debittering process, which induced a compositional change of the hydrophobic amino acids in the yeast hydrolysate, thereby reducing the bitter taste. The overall dry matter yield and protein yield for the entire process, including the downstream process proposed for the production of brewer's yeast extract were 50 and 50%, respectively.

Keywords: brewer's yeast, yeast extract, downstream process, debittering, process development

INTRODUCTION

Yeast has been utilized in several areas of food production, including brewing, wine, and baking. Brewer's yeast is an inexpensive nitrogen source, and is generally recognized as safe (GRAS). Brewer's yeast also has good nutritional characteristics, and contains plenty of protein, lipids, RNA, vitamins, and minerals.

Yeast extract, produced from yeast cells, consists primarily of amino acids, peptides, nucleotides, and other soluble yeast cell components. Yeast extract can be used as a flavoring agent in soups, sauces, gravies, stews, snack foods, and canned foods. Other applications include vitamin supplements in health foods, and nutrients in microbiological media [1]. Yeast extract is manufactured *via* cellular breakdown induced by either endogenous or exogenous enzymes. Chemical or enzymatic hydrolysis is the most efficient method for solubilizing yeast. Enzymatic hydrolysis is accomplished by either proteolytic enzymes [2,3], cell wall lysis enzymes [4] or culture broth containing *Streptomyces* sp. [5], and produces yeast extract with a low salt content.

We previously reported the enzymatic hydrolysis process for the production of yeast extract, using several kinds of enzymes, including glucanase, protease, nuclease, and deaminase [6-8]. This process, when using food proteins such as hydrolyzed vegetable proteins, is generally composed of two main parts: enzymatic hydrolysis (upstream process) and the downstream process [9-11]. Downstream processing includes solid-liquid separation, clarification, Maillard reaction, debittering, formulation concentration, and finally drying. After the yeast hydrolysis process, solid particles (yeast cells, macro colloids, and suspended matter) and haze-forming solutes are removed. This is called clarification. Several processes, including membrane filtration and the addition of flocculants, can be used to clarify yeast hydrolysate. Additionally, a debittering process is required in commercial production, in order to eliminate the bitter and objectionable taste of yeast hydrolysate. Several debittering processes using polystyrene divinylbenzene adsorbents have been applied in the citrus industry [12]. A combined treatment consisting of membrane filtration and the debittering process, was investigated in the processing of grapefruit juice [13]. The debittering of red grapefruit juice was successfully performed using a dual technique, consisting of ultrafiltration coupled with an XAD-16 adsorption column [14].

In this study, we also report the utilization of waste yeast cells from the beer industry in the production of yeast extract, which can be used as a flavoring foodstuff, mainly focusing on downstream processing. The effects of downstream processing on the final product quality of the yeast extract were evaluated, including the flavor en-

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hancing step, Maillard reaction, and debittering step, and a scheme was proposed to cover the entire process.

MATERIALS AND METHODS

Materials

Dried brewer's yeast cells, *Saccharomyces* sp., were obtained from a beer factory owned by the Doosan Co. (Icheon, Korea). Two types of protease, both manufactured by Novozyme (Bagsvaerd, Denmark), were used in protein hydrolysis: Protamex™ (endoprotease) and Flavourzyme™ (exoprotease). These proteases were utilized according to the method described by Chae *et al.* [6]. Enzyme RP-1 and deamizyme were obtained from Amano Pharmaceuticals (Nagoya, Japan). The synthetic adsorption resins, Diaion™ HP20 (polystyrene divinylbenzene resin) and Amberlite™ XAD7 (aliphatic acrylic polymer resin), were obtained from Mitsubishi Chemical (Tokyo, Japan) and Rohm and Haas (Philadelphia, PA, USA), respectively.

Enzymatic Hydrolysis

The conditions for enzymatic hydrolysis were same as in previous reports [6], and proceeded as follows: dried brewer's yeast was suspended at a concentration of 20% (w/w) in 1,200 mL of distilled water. The yeast suspensions were placed in 2,500-mL glass vessels, which were immersed in a temperature-controlled water bath. Initial pH was adjusted to 6.5 with 10 N NaOH, and the solution was heated at 95°C for 15 min. After cooling, 0.6% Flavourzyme and 0.6% Protamex were added simultaneously. In all cases, the protease dosage was determined on a protein content basis. The reaction mixture was then stirred with a four-blade impeller (10 cm diameter, 3 cm height) at a speed of 200 rpm. Hydrolysis was carried out at 50°C for 12 h. In order to determine the solid and protein contents of the hydrolysate, 10 mL aliquots of reaction mixture were taken, boiled at 95°C for 5 min to inactivate the enzymes, and centrifuged (10,000 × *g* at 4°C) for 20 min.

After protein hydrolysis with the proteases, and then heat treatment to inactivate any residual protease activity, enzyme RP-1 (5'-phosphodiesterase) was added to the reaction mixture at a concentration of 0.03%, based on the solid hydrolysate contents, in order to hydrolyze the RNA. The initial pH was adjusted to pH 5.5 with 5 N NaOH. Nuclease treatment was performed at 60°C for 3 h. Additionally, the reaction mixture was treated with deamizyme (0.03%) at pH 5.5 and 45°C for 2 h. After all enzymatic treatments had been administered, the hydrolysate was heated (95°C, 5 min), and was later utilized in downstream processing.

Clarification

Various chemicals, including calcium chloride, magnesium sulfate, phytic acid, and aluminum chloride were tested for their efficacy as clarifying agents. The agents were added at concentrations of 0.2% (w/w) and 1% (w/w) in order to accelerate the flocculation of the yeast hydrolysate (100 g), causing it to settle down. The mixture of the hydrolysate and agents was stirred at 100 rpm for 30 min, then allowed to settle down without mixing for 30 more min. After the treatment, the mixture (40 g) was centrifuged at 3,000 × *g* for 30 min. In order to measure the degree of browning (DB), the optical density (OD) of the supernatant was measured at 490 nm (OD_{490}). The remaining mixture (about 60 g) was then filtered using filter paper (Whatman No. 2), and Celite 545 (5 g) as a filter-aid. In order to measure the degree of clarification (DC), the OD of the filtrate at 600 nm (OD_{600}) was monitored. DB and DC were determined according to the following equations:

$$DB = \frac{OD_{490}^{\text{treatment}} - OD_{490}^{\text{control}}}{OD_{490}^{\text{control}}} \times 100(\%) \quad (1)$$

$$DC = \left(1 - \frac{OD_{600}^{\text{treatment}}}{OD_{600}^{\text{control}}}\right) \times 100(\%) \quad (2)$$

Maillard Reaction

The yeast hydrolysate was placed in 500-mL Erlenmeyer flasks. For the Maillard reaction of the yeast hydrolysate, glucose and high fructose corn syrup (HFCS) were added, at concentrations of 0.05 and 0.5% to the yeast hydrolysate. Glycine (0.05%) was simultaneously added as well. The reaction mixture was boiled at 100°C and 121°C for 2 h in an autoclave. After the heat treatment, the reaction mixture was cooled at room temperature, and centrifuged for 30 min at 6,000 × *g*. The absorbance of the supernatant was measured at 490 nm (OD_{490}) in order to determine the DB, by which the degree to which the Maillard reaction had been effective was determined.

Debittering by Adsorption Resins

The supernatant of yeast hydrolysate obtained by centrifugation (6,000 × *g*, 30 min) was mixed with adsorption resins for debittering. Batch-type adsorption was carried out using Diaion HP20 and Amberlite XAD7 in 250-mL Erlenmeyer flasks. Pre-washed resins were added at 5, 10 and 25% (w/w) of the yeast hydrolysate. The flasks containing the mixture were stirred at 150 rpm for different treatment times (0.25~2 h) in a shaking incubator. The treatment temperatures were either 30°C or 50°C. After the resin treatment, the OD_{490}, OD_{600}, and the free amino acid compositions of the raffinate were determined. For the column adsorption operation, HP20 resin (about 100 mL) was washed with distilled water and packed in a glass column (diameter 25 mm), making a resin volume of 100 mL. The reacted yeast hydrolysate, at a volume 3-fold higher than the resin volume (3 RV) was passed through the adsorption column at a space

velocity (SV) of 5 (volume flow rate of 500 mL/h). After the passing of the yeast hydrolysate, 3 RV of distilled water was used as an elution solvent. Resin-treated hydrolysate (the early fraction before elution by distilled water) and distilled water-eluted solution (the late fraction) were taken separately for free amino acid analysis.

Polishing

After debittering, NaCl was added to the eluted hydrolysate as a preservative, creating a final concentration of 17% (w/w) of the final solution product of the yeast extract (YE-L). The salt-added product was placed in a storage vessel at 10°C for 7 days, for purposes of flavor stabilization. In order to produce a paste product of the yeast extract (YE-P), the yeast hydrolysate was concentrated to 75% (w/w) in a rotary vacuum evaporator (Eyela, Tokyo, Japan) at 80°C.

Analytical Methods

Free amino acid composition was determined by reverse phase chromatography [15] using HPLC (Waters, Milford, MA). OD_{490} and OD_{600} were measured using a spectrophotometer (UV mini 1240, Shimadzu, Japan). All the experimental data represent mean (average) values from triplicate measurements.

RESULTS AND DISCUSSION

Clarification

Beer is one of the most common products requiring clarification during its manufacture [16]. Rough beer must be filtered to remove the yeast and colloidal particles which are responsible for haze. In general, clarification ensures the biological stability of the final products [17]. In order, then, to select the proper flocculating agent and treatment dosage, we evaluated various flocculating agents by measuring their optical densities at 490 and 600 nm (OD_{490} and OD_{600} indicate the degree of browning and clarification, respectively). Among the materials we tested, aluminum chloride exhibited the highest effectiveness in lowering OD_{600} (increasing DC), as is shown in Fig. 1. However, aluminum chloride is not generally accepted to be safe for use as a food additive in Korea. Calcium chloride, however, also exhibited excellent efficiency with regard to the clarification of the yeast hydrolysate. Therefore, calcium chloride was selected for the process, and the treatment dosage was determined to be 1% (w/v) of the hydrolysate mixture, making the DC about 52%. Membrane filtration is currently considered to be a very promising technology for this purpose, due to its ability to perform wine clarification/filtration/hygienization in one single step, in continuous operation with clean-in-place (CIP) strategies. Microfiltration (MF) is typically used as a membrane filtraton-based clarification process [18-20]. However, this operation is associated presently with prohibitively high costs [21,22]. Alternatively, clarification using flocculating agents has several advantages, including the simplicity of the process equipment, and low operating costs.

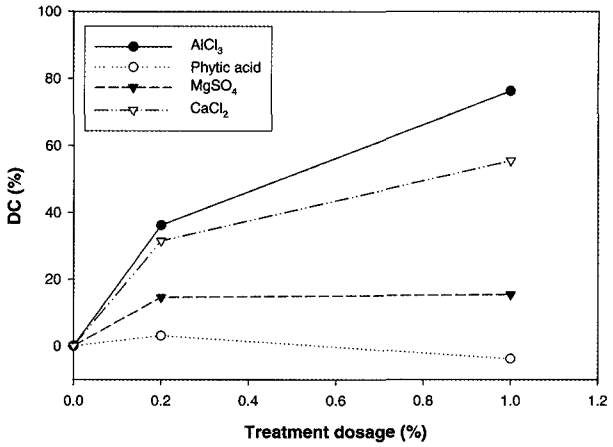

Fig. 1. The effects of various clarifying agents and treatment dosage on the degree of clarification.

Maillard Reaction

The Maillard reaction has been used to produce foods that look and taste attractive for thousands of years. The food industry relies on the application of the Maillard reaction to produce many foods, including coffee and bakery products [21]. The Hodge scheme remains in wide use today [24]. In essence, it is predicated on the fact that a reducing sugar (like glucose) can condense with a compound possessing a free amino group (such as an amino acid), yielding a condensation product. Various factors influence the Maillard reaction [25], and can be divided into food processing and storage variables. These include the nature of the reactants (the composition of the raw materials), the temperature-time combination used during heating and storage, pH, the water activity of the food, the presence of oxygen or metals, and the presence of any reaction inhibitors, such as sulfur dioxide [26].

The extent of the Maillard reaction of the yeast hydrolysate was determined by measuring the OD_{490}, which indicates the degree of browning (DB). When the yeast hydrolysate was treated at 100°C for 2 h, the OD_{490} did not exhibit a great deal of variation according to the type of sugar added, sugar dosage, or glycine addition (data not shown). However, the DB exhibited significant variation according to treatment time at 121°C as shown in Fig. 2. When the yeast hydrolysate was treated at 121°C, a significant effect of glucose (Glc) addition on the DB was observed. Glycine (Gly) addition, however, still exerted no significant effects on DB. It has been established that the rate of brown pigment formation is proportional to the square of amino content, reducing sugar concentration, and treatment time. The fact that glycine addition had no effect on this process can be explained by that the fact that the yeast hydrolysate already had plenty of

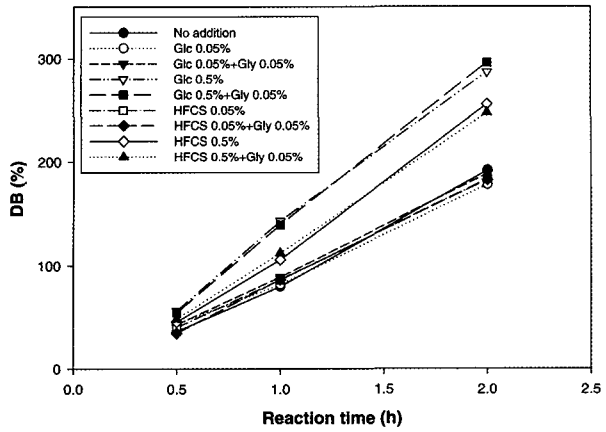

Fig. 2. The change of degree of browning by addition of different type of sugars, sugar dosage, and glycine addition at 121°C.

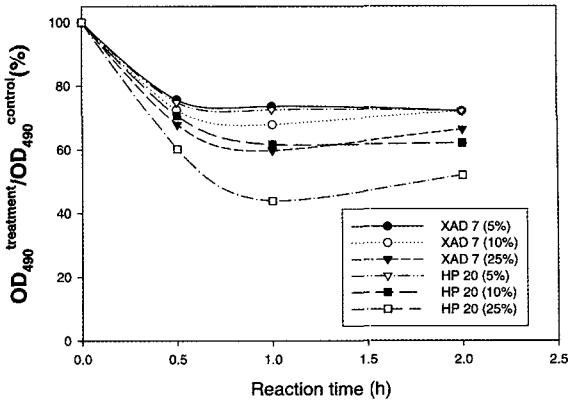

Fig. 3. Adsorption performance measured at OD_{490} for the treatment involving XAD7 and HP20 resins at 30°C.

amino compounds, so amino groups had little effect on the Maillard reaction, especially as compared to the effects of added sugars. The reactivity of browning was found to be contingent on the type of sugar, in the order of pentose > hexose > sucrose. The reaction rate (the rate of OD_{490} change) at 121°C increased up to 5.9 times that observed in an experiment which was carried out at 100°C. The main advantage of high-temperature treatment is hydrolysate sterilization. When the hydrolysate was treated at a high temperature (121°C), a bitter taste appeared. Thus, it seemed desirable to shorten the treatment time. The degree of precipitation in storage was also examined at different temperatures. After the high heat treatment (121°C) of the proteinous materials and hydrolysis products, the supernatant after centrifugation did not precipitate, even when stored at 4°C. However, after only mild heat treatment (80°C), precipitation occurred, even when stored at room temperature. This is probably due to insufficient polymerization and the existence of unstable intermediates and their denaturation while in storage.

Debittering

It is generally accepted that, when an exoprotease is used in conjunction with an endoprotease, the result is a more acceptable taste and a higher DH, as this tends to yield small non-bitter peptides [27,28]. In the present study, the yeast cells were treated with a combination of two types of proteolytic enzymes: endoproteases (Protamex) and exoproteases (Flavourzyme). In terms of upstream processing, this was expected to reduce bitter taste [6,29]. Debittering can also be achieved as a part of a downstream process, as we determined in this study.

Adsorption chromatography offers a simple and reliable technique for the debittering and/or decoloring of a variety of biological products. In order to develop a desirable debittering process for yeast extract, we tested a host of widely-used adsorption chromatography resins, such as HP20 and XAD7, at different temperatures (30°C and 50°C). OD_{490} was used as an index of adsorption, much

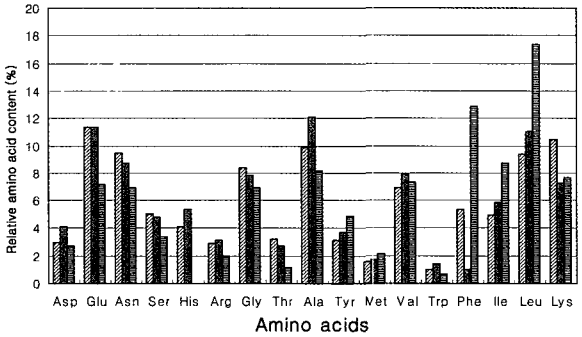

Fig. 4. The change of amino acid composition by column-type debittering process using HP20 resin. (▨) Supernatant after centrifugation, (▩) treated fraction (the early fraction), (▤) water eluted fraction (the late fraction).

in the same fashion as DC and DB were used. In the batch-type adsorption chromatography of the hydrolysate the HP20 resin exhibited slightly higher adsorption activity (OD_{490} reduction) than did XAD7, as shown in Fig. 3. This might be because the HP20 resin possesses a more hydrophobic backbone (polystyrene divinylbenzene) than does XAD7 (aliphatic polymer backbone). At 30°C, maximum adsorption was measured after 1 h of treatment using 25% HP20. At 50°C, maximum decoloring was observed after 0.5 h of treatment (data not shown). HP20 was finally selected as the adsorption resin to be used for further study.

The adsorption resin was used to debitter the yeast hydrolysate. Therefore, we also analyzed the amino acid composition of the yeast hydrolysate treated with the HP20 resin. In order to determine the optimal treatment conditions, and to ascertain whether the amino acid profile had been changed by the adsorption process, the HP20 resin was packed in a column, and debittering was performed at different temperatures (30°C and 50°C). However, the amino acid content was not affected by the treatment temperature (data not shown).

On the other hand, a column-type operation using HP20 resin was performed at 30°C. As shown in Fig. 4,

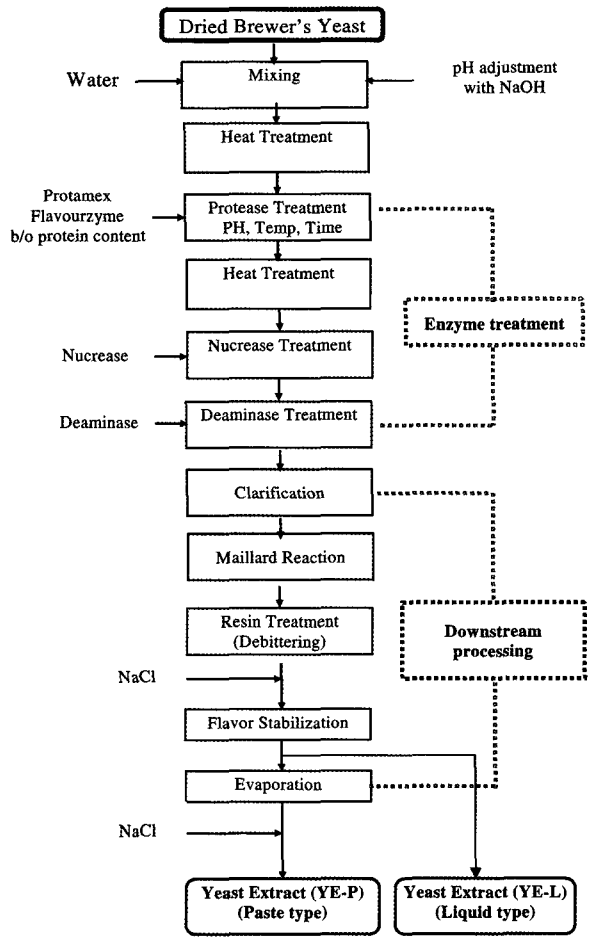

Fig. 5. The entire process diagram for yeast extract production.

the relative contents of relatively hydrophobic amino acids (Trp, Phe, Leu, *etc.*) remaining in the hydrolysate exhibited a high degree of variation, according to treatment. Moreover, the solubility of a protein is also related to the content of its hydrophobic amino acids, including tyrosine, phenylalanine, and leucine [30]. Some precipitation can occur during evaporation of the protein products, as the precipitate includes free amino acids with very low solubility, such as tyrosine [10]. Additionally, we found that the amino acid composition changed according to elution volume. The late fraction, which was eluted by distilled water, was found to contain a greater quantity of hydrophobic amino acids as compared to the early fraction. This result suggested that the debittering using the adsorption resin was, indeed, quite effective. This result also indicated that hydrophobic amino acids could be removed by adsorption chromatography, such that the bitterness was reduced substantially. Consequently, a treatment temperature of 30°C and a space velocity of 5 were judged to be adequate for the debittering of the yeast hydrolysate using the HP20 resin. The application of synthetic polymer resins in the debittering process has been reported predominantly in the citrus industry [13,14]. Most of these applications involve combined treatment methods consisting of membrane filtration coupled with adsorption technology. In the present study, clarification by membrane filtration was replaced by a simple process using flocculating agent; therefore, we proposed a combined process of clarification by flocculation and debittering *via* HP20 adsorption column. Debittering with the adsorption resin proved adequate as a part of the downstream processing of yeast extract production.

The Entire Process Scheme

As previously noted, a downstream processing scheme was suggested in this study for the commercial production of yeast extract. By combining the previously-reported yeast hydrolysis process (upstream process) [6] and the downstream process studied here, an entire process scheme could be devised, which is shown in Fig. 5. After the whole process is completed, two types of yeast extract product result: liquid type (YE-L) and paste-type (YE-P) yeast extracts. The total solid contents of YE-L and YE-P were measured to be 29 and 75%, respectively, and their total nitrogen contents were 1 and 4.6%, respectively. The overall dry matter yield and protein yield after resin treatment were 50 and 50%, respectively. The process consists of a three-step enzymatic process, which involves four enzymes (endoprotease, exoprotease, nuclease and AMP-deaminase), and a three-step downstream process, including clarification, Maillard reaction, and debittering by adsorption. The yeast extract manufacturing process suggested in this study was also successfully performed, and generated products as described

CONCLUSION

Various downstream processing conditions were considered in the design of an entire process scheme. These conditions included clarification, debittering, and the Maillard reaction. Clarification was performed using flocculating agents, specifically calcium chloride (1%). As a flavor-enhancing step, the Maillard reaction was performed subesequent to the clarification step. When the yeast hydrolysate was treated at 121°C, a significant effect on DB was seen due to glucose (Glc) addition. Glycine (Gly) addition, however, proved to have no significant effects on DB. The reactivity of browning depended on the type of sugar added, in the order of pentose > hexose > sucrose. For debittering, HP20 was finally selected for use as an adsorption resin. The relative contents of relatively hydrophobic amino acids (Trp, Phe, Leu, *etc.*) remaining in the hydrolysate exhibited a great degree of variation according to treatment.

The process consists of a three-step enzymatic (upstream) process involving four enzymes (endoprotease, exoprotease, nuclease and AMP-deaminase), and a three-step downstream process, which includes clarification, Maillard reaction, and debittering. The suggested process was successfully performed as a yeast extract manufacturing process, in which the overall dry matter and protein yields were 50 and 50%, respectively.

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[Received November 19, 2004; accepted January 7, 2005]