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Utilization of brewer's yeast cells for the production of food-grade yeast extract. Part 1: effects of different enzymatic treatments on solid and protein recovery and flavor characteristics

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Abstract

Yeast extract was produced from brewer's yeast of a beer factory by combined enzymatic treatments using endoprotease, exoprotease, 5′-phosphodiesterase, and adenosine monophosphate (AMP)-deaminase. Effects of enzyme combination, enzyme dosages and treatment sequence on the recovery of solid and protein, flavor and compositional characteristics were investigated. Exoprotease dosage strongly affected the recovery of protein and degree of hydrolysis (DH) and sensory characteristics. When the yeast cells were treated using optimal combination of endoprotease and exoprotease (0.6% Protamex™ and 0.6% Flavourzyme™), high solid recovery (48.3–53.1%) and the best flavor profile were obtained. Among various treatment sequences using multiple enzymes, treatment with protease followed by nuclease resulted in the highest 5′-guanosine monophosphate (5′-GMP) content. The optimal concentrations of both 5′-phosphodiesterase and AMP-deaminase were found to be 0.03%. After treatments using optimal combination of enzyme, enzyme dosages and treatment sequence for four enzymes, a high solid yield of 55.1% and 5′-nucleotides content of 3.67% were obtained. © 2000 Published by Elsevier Science Ltd.

Keywords: Brewer's yeast; Yeast extract; Enzymatic hydrolysis; Solid and protein recovery

1. Introduction

Yeast has been utilized in the area of food production such as brewing, wine and baking. In Korea, nearly 6000 tons of dried yeast cells were produced from three main beer manufacturers in 1996 (AFLN, 1997). Most of this industrial waste, brewer's yeast cells, is currently utilized as a protein source for animal feed and nutritional supplement after drying because of high protein level (45–60%). Yeast cells contain plenty of protein, lipid, RNA, vitamins, and minerals. The brewer's yeast is an inexpensive nitrogen source and generally recognized as safe (GRAS) and has good nutritional characteristics.

Yeast extract, which is produced from the yeast cells, consists primarily of amino acids, peptides, nucleotides and other soluble components of yeast cells. Yeast extract has been used as a flavoring agent in soup, sauces, gravies, stews, snack food and canned food. Other applications include vitamin supplements in health foods and as a source of nutrients in microbiological media (York and Ingram, 1996). Yeast extract is manufactured by the breaking down of cells using endogenous or exogenous enzymes. There have been many reports on the manufacturing processes (Breddam and Beenfeld, 1991; Roman et al., 1991; Choi and Chung, 1998) which are divided largely into autolysis and hydrolysis. Autolysis by endogenous enzymes occurs naturally in yeasts when they complete the cell growth cycle and enter the death phase. Autolysis process has some disadvantages such as low extraction yield, difficulty in solid–liquid separation due to high content of residue in autolysate, poor taste characteristics as a flavor enhancer, and risk of deterioration due to microbial contamination. In a modified autolysis process referred to as plasmolysis, inorganic salts such as sodium chloride or non-polar organic solvents are often used to accelerate autolysis (Kollar et al., 1991; Nagodawithana, 1992; Belousova et al., 1995). Despite its simplicity, the yeast extract manufactured by plasmolysis may have limited use, since there is a

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growing demand for processed foods containing low salt (Nagodawithana, 1992). Hydrolysis is the most efficient method of solubilizing yeast, and is carried out by hydrochloric acid or proteolytic enzymes. Despite a high production yield, acid hydrolysis is less attractive to the manufacturers because of relatively high capital investment cost, high salt content and high probability of containing carcinogenic compounds such as monochloropropanol and dichloropropanol (Nagodawithana, 1992). Enzymatic hydrolysis is carried out by either proteolytic enzymes (Knorr et al., 1979), cell wall lysis enzyme (Rayan and Ward, 1988), or culture broth of *Streptomyces* sp. (Lim, 1997), and produces yeast extract low in salt content.

Flavoring enhancers, monosodium glutamic acid (MSG) and nucleotides such as 5′-guanosine monophosphate (5′-GMP) and 5′-inosine monophosphate (5′-IMP) are well known in the food processing. The nucleotides can be produced by degradation of ribonucleic acid (RNA). When yeast suspension containing RNA is incubated with 5′-phosphodiesterase, RNA extracted from yeast cells can be degraded into four 5′-nucleotides: 5′-GMP, 5′-uracil monophosphate (5′-UMP), 5′-cytosine monophosphate (5′-CMP), and 5′-adenosine monophosphate (5′-AMP). Of these, only 5′-GMP possesses flavoring properties.

In this study, we report the utilization of the waste yeast cells from beer industry for the production of a flavoring foodstuff. We produced yeast extract from dried brewer's yeast cells by enzymatic treatment. The strategy was to hydrolyze the intact yeast protein into low molecular weight peptides and amino acids by proteolytic enzymes, and then hydrolyze RNA for the release of 5′-nucleotides including 5′-GMP, 5′-AMP, 5′-UMP and 5′-CMP by 5′-phosphodiesterase. Even though 5′-AMP has no flavor-potentiating property, it can be converted into a flavor-enhancing compound 5′-IMP. The last step was to covert the 5′-AMP resulted from RNA degradation into 5′-IMP by AMP-deaminase. In the hydrolysis of yeast cells using multiple enzymes, the effects of protease type (endoprotease and exoprotease), enzyme dosages and treatment sequence on solid and protein recovery and flavor characteristics were investigated.

2. Methods

2.1. Materials

Dried brewer's yeast cells, *Saccharomyces* sp., were obtained from a beer factory of Doosan (Icheon, Korea). Two types of protease manufactured by Novo Nordisk A/S (Bagsvaerd, Denmark) were used in the protein hydrolysis: Protamex™ (endoprotease, 398.7 mAU/g) from *Bacillus licheniformis* and Flavourzyme™ (reported as an enzyme complex with high exoprotease (1046.1 LAPU/g) and low endoprotease activity (92.3 mAU/g) from *Aspergillus oryzae*, whose activities were assayed in a previous report (Chung et al., 1999). Protein FN™ (Daiwa Kasei, Osaka, Japan) which has both endoprotease and exoprotease activities was also used. Enzyme RP-1, a 5′-phosphodiesterase from *Penicillium citrinum*, was obtained from Amano Pharmaceutical (Nagoya, Japan). As an AMP-deaminase, Deamizyme (Amano Pharmaceutical) originating from *Aspergillus melleus* was used.

2.2. Enzymatic hydrolysis

2.2.1. Protease treatment

Dried brewer's yeast was suspended in 120 ml distilled water to achieve a concentration of 20% (w/w). The yeast suspensions were placed in 250 ml glass vessels immersed in a temperature-controlled water bath. Initial pH was adjusted to 6.5 by NaOH (10 N) and the solution was heated at 95°C for 5 min. After the heat treatment followed by cooling, both Flavourzyme (0.6–2.0%) and Protamex (0.6–2.0%) were added together. In all cases, enzyme dosage was determined on a protein content basis. The reaction mixture was stirred by a four-blade impeller (5 cm diameter, 1 cm height) at a speed of 200 rpm. The hydrolysis was carried out at 50°C for 12 h. To determine solid and protein contents of the hydrolysate, 2 ml aliquots of reaction mixture were taken, heated at 95°C for 5 min to inactivate enzyme, and centrifuged ($10,000 \times g$, 4°C) for 20 min.

2.2.2. Nuclease treatment

After protein hydrolysis with Protamex (0.6%) and Flavourzyme (0.6%) at 50°C for 12 h, followed by heat treatment to inactivate residual protease activity, enzyme RP-1 (5′-phosphodiesterase) was added to the reaction mixture at concentrations of 0.015%, 0.03%, 0.04%, 0.05% and 0.06% to hydrolyze RNA. The enzyme dosage was determined based on the solid content of hydrolysate. Initial pH was adjusted to pH 5.5 (an optimum pH of enzyme RP-1) by 5 N NaOH. The nuclease treatment was performed at 60°C for 3 h. Additionally, the reaction mixture was treated by Deamizyme (0.03%) at pH 5.5 and 45°C for 2 h. After all enzymatic treatments, the hydrolysate was heated (95°C, 5 min), centrifuged, and finally the supernatant (yeast extract) was used for further analysis.

2.3. Analytical methods

The solid content of yeast hydrolysate was determined using a Brix meter (Atago, Japan). Total crude protein content (TN × 6.25) was estimated from total nitrogen (TN) measured by the Kjeldahl method (AOAC, 1980). For determination of degree of hydro-

lysis (DH), equal volumes of the hydrolysate solution and 20% trichloroacetic acid (TCA) solution were mixed, centrifuged ($10,000 \times g$ at 4°C) for 10 min, and then the total protein content of supernatant was measured. DH was determined by the TCA-soluble protein fraction in total protein content of yeast hydrolysate (Basappa et al., 1986). Free amino acid composition was determined by reverse phase chromatography (Godel et al., 1992) using an HPLC (Waters, Milford, MA). The contents of 5′-nucleotides (5′-GMP and 5′-IMP) were measured using an HPLC (Waters, Milford, MA) with an Econosphere C_{18} column (Alltech, Deerfield, IL) and a UV detector at 254 nm. For elution, a mixture of distilled water, 2.5%(v/v) acetonitrile, 0.0025 M tetrabutyl ammonium phosphate, 0.065 M KH_2PO_4 was used. The flow rate was 1.0 ml/min. Molecular weight distribution was determined by gel permeation chromatography using Shodex™ Protein KW-802.5 column (Showa Denko, Tokyo, Japan) (Richter et al., 1983). All data for solid and protein recovery and DH represent average values of triplicate measurements. Standard errors of the mean (SEM) for each sample were calculated and reported.

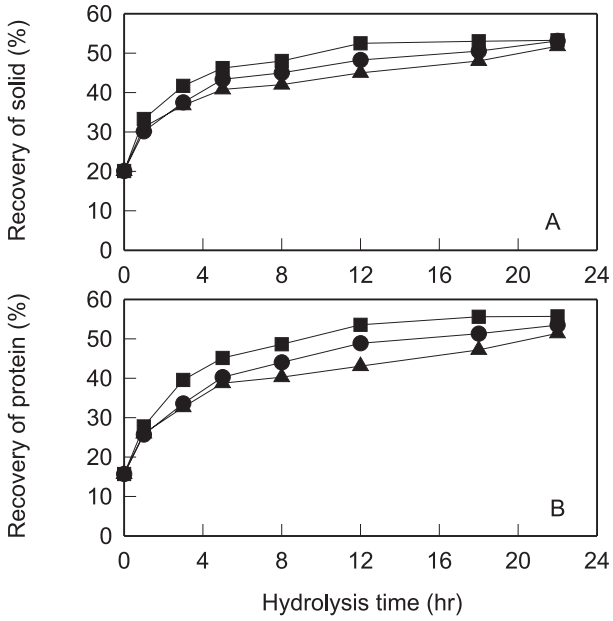

Fig. 1. Time courses of recovery of solid (A) and recovery of protein (B) using different enzyme combinations and dosages. ■ – ■, Protamex (0.6%) and Flavourzyme (2.0%); ● – ●, Protamex (0.6%) and Flavourzyme (0.6%); ▲ – ▲, Protein FN (0.5%).

3. Results and discussion

3.1. Effects of protease treatment on recovery of solid and protein

The extent of breakdown and release of yeast cellular components was determined by measuring the soluble solid content and total nitrogen content. The total nitrogen corresponds to hydrolyzed proteins, peptides, free amino acids and non-protein nitrogen. Crude protein content can be determined from total nitrogen using a Kjeldahl N factor ($P(\%) = TN(\%) \times 6.25$). The protein content of the raw material (dried yeast cell) was determined as 49.2%.

It has been generally accepted that when an exoprotease is used in conjunction with endoprotease, more acceptable taste and higher DH result because it produces small non-bitter peptides (Ge and Zhang, 1996; Howell, 1996). In the present study, the yeast cells were treated with a combination of two types of proteolytic enzymes: endoprotease (Protamex) and exoprotease (Flavourzyme) or a single enzyme (Protein FN) which has both activities. The influence of different enzyme combinations and dosages on the recovery of solid and protein are shown in Fig. 1. Each result represents an average value from triplicate measurements. The yeast cells were treated with Protamex (0.6%) and Flavourzyme (0.6%); Protamex (0.6%) and Flavourzyme (2%); Protein FN (0.5%). The release of solid and protein increased with increasing incubation time. Among the three treatments, Protamex (0.6%) and Flavourzyme (2%) exhibited the highest solid and protein recovery, which reached 52.5% and 53.6%, respectively, at 12 h hydrolysis. With low Flavourzyme (0.6%), the solid recovery reached 48.3% and 53.1% after 12 and 22 h, respectively. For comparison, Protein FN (0.5%), whose endoprotease and exoprotease activities were 17.7 (mAU/g) and 311.62 (LAPU/g), respectively (Chung et al., 1999), was used and showed moderate yields. The increase in recovery of solid and protein must have resulted from the release of intracellular components and degradation of yeast protein.

3.2. Effects of dosages of endoprotease and exoprotease on recovery

The recovery of solid and protein with different concentrations of Protamex and Flavourzyme, which were added at final concentration of 0.2%, 0.6%, and 1.0% (w/w), respectively, is illustrated in Fig. 2. After the treatment for 12 h, no significant difference in solid recovery (within the range 55–57%) was found with nine treatments using different dosages. However, the protein recovery strongly depended on the enzyme dosages. Protein recovery was more responsive to Flavourzyme than Protamex, indicating that exoprotease rather than endoprotease is a key factor in yeast protein hydrolysis. A similar effect was observed with DH (Fig. 2(c)). The exoprotease had a more significant effect on DH than the endoprotease, and DH was affected by the endoprotease dosage (Adler-Nissen, 1986).

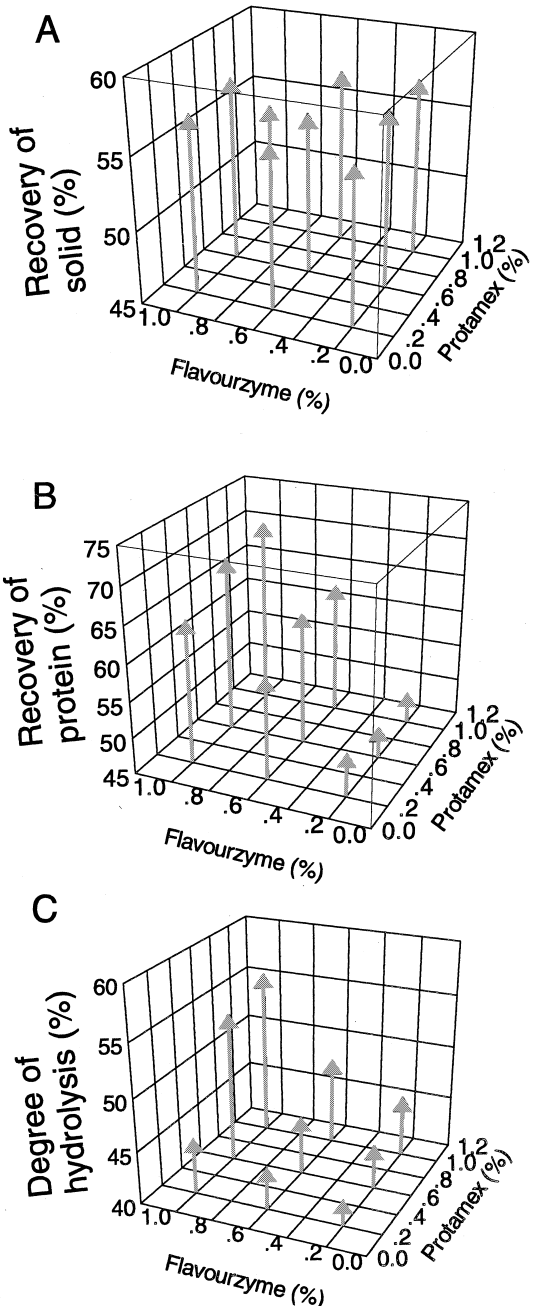

Fig. 2. Effects of dosages of Flavourzyme and Protamex on recovery of solid (A), recovery of protein (B) and DH (C). All enzyme combinations were incubated with 20% (w/w) brewer's yeast for 12 h.

The sensory characteristics of the yeast extract produced by the protease treatment were evaluated using a ranking test (Meilgaard et al., 1987). A panel of 10 people screened nine hydrolysates (the same samples of Fig. 2) by evaluating taste characteristics. In the first screening, four hydrolysates that ranked highly in overall acceptance were selected for a second screening based on odor, color, taste, and overall acceptance. In the second screening, the hydrolysate treated with Protamex (0.6%) and Flavourzyme (0.6%) was found to have the best flavor and overall acceptance. In addition, this combination gave acceptable yields of solid and protein. In determining enzyme dosage for the production of food-grade yeast extract, the sensory properties of hydrolysate are important parameters. Therefore, based on production yield and sensory characteristics, 0.6% of Protamex and 0.6% of Flavourzyme were selected as optimum levels for further process conditions.

3.3. Effects of enzyme treatment sequence

To find the best hydrolysis sequence, the yeast cells were treated with different sequences of protease and nuclease (5′-phosphodiesterase) (Fig. 3). Nuclease treatment (N-treatment in Fig. 3) exhibited the lowest solid recovery (29.7%) and protein recovery (17.2%). When treated with protease (P, NP, and PN in Fig. 3), the solid recovery and protein recovery increased up to 41.1–42.9%. This indicated that protease treatment is very important in solubilization and recovery of cellular components. Although the protease-treated hydrolysates (P, NP, and PN) showed little difference in solid recovery and protein recovery, 5′-GMP content in yeast extract was strongly affected by the enzyme treatment sequence. The initial 5′-GMP content in the raw material was 0.02%. Yeast cells are partially autolysed at high temperature after completion of brewing to avoid release of viable strains. This initial concentration of 5′-GMP is likely due to this partial autolytic treatment. The P-treatment (treatment with protease only) gave the lowest 5′-GMP content (0.03%), which represents a 50% increase in the value from the initial 5′-GMP content (0.02%). The 5′-GMP content after the treatment with nuclease only (N) and nuclease followed by protease (NP-treatment) increased up to 0.050–0.051%. The PN-

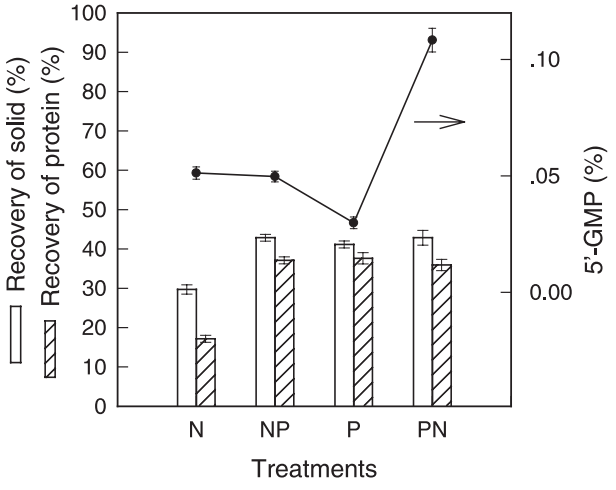

Fig. 3. Effects of enzyme treatment sequence on solid recovery, protein recovery and 5′-GMP content. N, nuclease (5′-phosphodiesterase) treatment; NP, treatment with nuclease followed by protease; P, protease treatment; PN, treatment with protease followed by nuclease. Error bars indicate the standard error of the mean (SEM).

treatment (treatment with protease followed by nuclease) showed the highest 5′-GMP content (0.11%). When the yeast cell suspension is treated with protease before nuclease, the protease breaks down the insoluble protein and protein-bound intracellular molecules to small molecules, facilitating action of enzymes such as nuclease. Therefore, the treatment of protease followed by nuclease appeared to be desirable for yeast hydrolysis.

3.4. Optimization of nuclease treatment

To obtain optimum nuclease concentration for 5′-GMP formation, the hydrolysate prepared with Protamex (0.6%) and Flavourzyme (0.6%) for 20 h was treated with 5′-phosphodiesterase (enzyme RP-1) at different concentrations (0.015–0.06%), on a solid weight basis. The solid and protein recovery and 5′-GMP content increased as the nuclease dosage increased, but reached a maximum value at 0.03% enzyme RP-1. At different AMP-deaminase (Deamizyme) concentrations, there were no significant differences in solid and protein recovery, while the highest concentration of 5′-IMP was observed at 0.03% enzyme RP-1. Therefore, the optimum dosages of both 5′-phosphodiesterase and AMP-deaminase were found to be 0.03%.

3.5. Compositional characteristics of yeast extract

The compositional characteristics of yeast extract, prepared by the combined treatment of protease, 5′-phosphodiesterase and AMP-deaminase, are summarized in Table 1. The crude protein content and solid content were 9.25% and 18.2%, respectively. The molecular weight distribution analysis (ranging 120–1355 Da) indicated that the extract contained mostly low molecular weight peptides. On a solid weight basis, the total amino acid content, 5′-GMP and 5′-IMP contents were 25.9%, 2.74% and 0.93%, respectively. The total 5′-nucleotide content (GMP + IMP = 3.67%) was comparatively higher than other results obtained previously. Kim et al. (1999) reported that a high 5′-nucleotide content (3.2%) could be obtained using high RNA containing *Saccharomyces* strain. In addition, as shown in Table 2, a high solid recovery of 55.1% was obtained by the combined treatment with two proteases, 5′-phosphodiesterase and deaminase. This solid recovery was also higher than that (42.0%) reported previously (Lee et al., 1993). Free amino acids in the extracts are known to exert a major influence on flavor. The free amino acid composition is shown in Table 3. Most essential amino acids were contained in the yeast extract except for sulfur-containing amino acids such as cystine and methionine, which were limiting amino acids. This is in agreement with the result of Moresi et al. (1995).

Table 1
Chemical properties of yeast extract prepared with proteases, 5′-phosphodiesterase and AMP-deaminase

Properties	Value[a]
Total nitrogen (%)	1.48 ± 0.08
Crude protein (%, TN × 6.25)	9.25 ± 0.21
Solid (%)	18.2 ± 0.5
Molecular weight distribution (Da)	120–1355
DH (%)	44.8 ± 1.3

[a] All values are mean ± standard error of the mean (SEM).

Table 2
Comparison of yield and characteristics of yeast extracts derived from different processes

Process	Solid recovery (%)	5′-nucleotides (%) (5′-GMP + 5′-IMP, dry basis)	Reference
Enzymatic	55.1	3.67	This work
	42.0	3.00	Lee et al. (1993)
Autolysis	35.1	2.48	Lee et al. (1993)

Table 3
Free amino acid composition of yeast extract prepared with proteases, 5′-phosphodiesterase and AMP-deaminase

Essential amino acids	Yeast extract profile, A (g/100 g protein)	FAO/WHO profile [a], B (g/100 g protein)	Ratio (A/B)
Isoleucine	2.89	4.0	0.72
Leucine	4.97	7.0	0.71
Lysine	8.81	5.5	1.60
Cystine + Methionine	0.67	3.5	0.19
Phenylalanine + Tyrosine	3.82	6.0	0.64
Threonine	2.21	4.0	0.55
Tryptophan	0.98	1.0	0.98
Valine	5.87	5.0	1.17
Non-essential amino acids	**Yeast extract profile (g/100 g protein)**		
Alanine	5.90		
Arginine	1.90		
Asparagine	4.85		
Aspartic acid	2.78		
Glutamic acid	7.80		
Glycine	3.27		
Histidine	7.29		
Serine	3.04		

[a] Recommended by FAO/WHO of United Nations (FAO/WHO, 1973).

4. Conclusions

In the hydrolysis of yeast protein, exoprotease dosage strongly affected the recovery of protein and DH and sensory characteristics. The combined treatment of

endoprotease and exoprotease (0.6% Protamex and 0.6% Flavourzyme) resulted in high solid recovery (48.3–53.1%) and the best flavor profile. Among various treatment sequences using multiple enzymes, the treatment with protease followed by nuclease was desirable for yeast hydrolysis in terms of 5′-GMP content and solid and protein recovery. The optimal concentrations of both 5′-phosphodiesterase and AMP-deaminase were found to be 0.03%. The treatments using optimized conditions produced yeast extract that contained mostly low molecular weight peptides and free amino acids, with a high solid recovery of 55.1%. Consequently, a three-step process using four enzymes was developed for the production of yeast extract utilizing brewer's yeast.

References

AFLN, 1997. Korea Food Yearbook. Agriculture Fishery and Livestock News, Seoul, Korea, pp. 468-484.

AOAC, 1980. Official Methods of Analysis, 13th ed. Association of Official Agricultural Chemists, Washington, D.C.

Adler-Nissen, J., 1986. Enzymatic Hydrolysis of Food Proteins. Elsevier Applied Science Publisher, New York.

Basappa, S.C., Jaleel, S.A., Murthy, A.S., Murthy, V.S., 1986. Preparation of yeast hydrolysate – a flavoured food adjunct. Ind. Food Industry 5, 23–26.

Belousova, N.I., Gordienko, S.V., Eroshin, V.K., 1995. Influence of autolysis conditions on the properties of amino-acid mixtures produced by ethanol-assimilating yeast. Appl. Biochem. Microbiol. 31, 391–395.

Breddam, K., Beenfeld, T., 1991. Acceleration of yeast autolysis by chemical methods for production of intracellular enzymes. Appl. Microbiol. Biotechnol. 35, 323–329.

Choi, S.J., Chung, B.H., 1998. Simultaneous production of invertase and yeast extract from baker's yeast. Kor. J. Biotechnol. Bioeng. 13, 308–311.

Chung, Y., Chae, H.J., Kim, D.C., Oh, N.-S., Park, M.J., Lee, Y.S., In, M.-J., 1999. Selection of commercial proteolytic enzymes for the production of brewer's yeast extract. Food Eng. Progress 3, 159–163.

FAO/WHO, 1973. Ad hoc expert committee, Technical Report No. 522. World Health Organization, Geneva, Switzerland.

Ge, S.J., Zhang, L.X., 1996. The immobilized porcine pancreatic exoproteases and its application in casein hydrolysates debittering. Appl. Biochem. Biotechnol. 59, 159–165.

Godel, H., Seltz, P., Verhoef, M., 1992. Automated amino acid analysis using combined OPA and FMOC-Cl precolumn derivatization. LC GC International 5, 44–49.

Howell, N.K., 1996. Chemical and enzymatic modifications. In: Nakai, S., Modler, H.W. (Eds.), Food Proteins: Properties and Characterization. VCH Publishers, New York, pp. 262–263.

Kim, J.-S., Kim, J.-W., Shim, W., Kim, J.-W., Park, K.-H., Pek, U.-H., 1999. Preparation of flavor-enhancing yeast extract using a *Saccharomyces cerevisiae* strain with high RNA content. Kor. J. Food Sci.Technol. 31, 475–481.

Knorr, D., Shetty, K.J., Hood, L.F., Kinsella, J.E., 1979. An enzymatic method for yeast autolysis. J. Food Sci. 44, 1362–1365.

Kollar, R., Sturdik, E., Farkas, V., 1991. Induction and acceleration of yeast lysis by addition of fresh yeast autolysate. Biotechnol. Lett. 13, 543–546.

Lee, S.-K., Park, K.-H., Pek, U.-H., Yu, J.-H., 1993. Production of brewer's yeast extract by enzymatic method. Kor. J. Appl. Microbiol. Biotechnol. 21, 276–280.

Lim, U.-K., 1997. Effects of addition of culture broth of *Streptomyces faecalis* MSF for the preparation of yeast extracts containing savory compound related to RNA. Kor. J. Appl. Microbiol. Biotechnol. 25, 512–519.

Meilgaard, M., Civille, G.V., Carr, B.T., 1987. Sensory Evaluation Techniques. CRC Press, Boca Raton (Chapters 6 & 7).

Moresi, M., Orban, E., Quaglia, G.B., Casini, I., 1995. Effect of some physio-chemical treatments on the kinetics of autolysed-yeast extract production from whey. J. Sci. Food Agric. 67, 347–357.

Nagodawithana, T., 1992. Yeast-derived flavors and flavor enhancers and their probable mode of action. Food Technol. 46, 138–144.

Rayan, E., Ward, O.P., 1988. The application of lytic enzymes from *Basidiomycete aphyllpphoroies* in production of yeast extract. Process Biochem. 23, 12–16.

Richter, W.O., Jacob, B., Schwandt, P., 1983. Molecular weight determination of peptides by high-performance gel permeation chromatography. Anal. Biochem. 133, 288–291.

York, S.W., Ingram, L.O., 1996. Ethanol production by recombinant *Escherichia coli* KO11 using crude yeast autolysate as a nutrient supplement. Biotechnol. Lett. 18, 683–688.