RELATIONSHIP BETWEEN BITTERNESS OF BREWER’S YEAST HYDROLYSATE AND HYDROPHOBIC AMINO ACID CONTENT

Nguyen Thi Thanh Ngoc¹, Quan Le Ha²,*

¹East Asia University of Technology, A2CN8, Tu Liem Industrial, Hanoi, Vietnam
²School of Biotechnology and Food Technology, Hanoi University of Science and Technology, 1 Dai Co Viet, Hanoi, Vietnam

*Email: ha.quanle@hust.edu.vn; ngoc.nguyen@polyco.com.vn

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ABSTRACT

Brewer’s yeast spent, obtained after the main fermentation stage, is a rich-in-protein source (protein content accounts for 48 - 50% dry matter). In order to use efficiently this source, it was hydrolysed by different methods. Protein hydrolysate products are normally mixtures of peptides and amino acids. Protein hydrolysates have a wide range of applications in food. It can be used as emulsifying agents in a number of applications such as salad dressings, spreads, ice cream, coffee whitener, cracker, and meat products like sausages. However, bitterness in hydrolysates is one of the major undesirable aspects for various applications in food processing. In this study, we used enzymatic mixture alcalase and flavourzyme, yeast treatment methods to hydrolyse brewer’s yeast. The hydrolysate and fractions of protein hydrolysate obtained after filtration with 10 kDa and 3 kDa filters were used for determination of bitterness and hydrophobic amino acids content. The bitter taste of hydrolysate was determined by sensory method (using quinine standard) and amino acid content was analysed by HPLC method. The result showed the close relationship between bitter taste and hydrophobic amino acid content. The bitter taste of protein hydrolysate was reduced as the hydrophobic amino acid content decreased. When the bitter taste (equivalent to quinine concentration) decreased from 16.25 μmol/l to 3.59 μmol/l, the total content of hydrophobic amino acids in protein hydrolysate reduced from 1653 μg/ml to 932 μg/ml.

Keywords: bitterness, enzymatic hydrolysis, amino acids, protein hydrolysates, brewer’s yeast spent.

1. INTRODUCTION

The bitter taste of protein hydrolysate is formed due to the presence of hydrophobic amino acids or peptides contain hydrophobic amino acid residues [1, 2, 3]. Bitterness increased as hydrophobic amino acid content increased [3]. Several oligopeptides such as Trp-Phe, Trp – Pro, Leu – Pro – Trp caused bitterness in brewer’s yeast hydrolysate [4]. There are many studies...
reporting that some amino acids having sweet taste were glycine, alanine, threonine, valine, serine, lysine and proline [5], the amino acids causing bitter taste were isoleucine, leusine, arginine, cysteine, methionine, phenylalanine, tryptophan and histidine [4, 5, 6]. The peptides, that had molecular weight > 10 kDa and contained Pro, Leu, Tyr, Phe, Ala were bitter [2].

Debitterizing methods can be divided into two categories which are physical chemical debitterizing methods and enzymatic debitterizing methods. Physical chemical debitterizing methods for debittering of protein hydrolyzates include selective separation such as treatment with activated carbon, extraction with alcohol, isoelectric precipitation, chromatography on silica gel, hydrophobic interaction chromatography. Enzymes were used for debitterizing that are aminopeptidases [7].

The bitter taste of hydrolysate was determined by sensory method, according to molecular weight peptides [1, 3, 8] or exist hydrophobic amino acids in hydrolysate by HPLC [3]. Sensory panels were trained to evaluate the taste intensity with standard solution such as caffeine, quinine and quinine HCl, caffeine threshold ≤ 150 mg/L, quinine threshold ≤ 6 µM [1, 10]. To study the relationship between bitter taste of brewer’s yeast hydrolysate with hydrophobic amino acid contents, hydrolysis experiments were carried out with combination methods of disrupting yeast cell membranes (autolysis, autoclaving, ultrasound) and hydrolyzing by mixture of alcalase and flavourzyme. Bitterness and hydrophobic amino acid (HAA) contents of brewer’s yeast hydrolysis were determined to find out the relashionship between them.

2. MATERIALS AND METHODS

2.1. Materials

The spent brewer’s yeast Saccharomyces used as a substrate was donated by Sai Gon Ha Noi beer company. Flavourzyme and alcalase were obtained from Novozymes, Denmark. Their proteolytic activity were 289U/g and 328 U/g, respectively (One Unit of protease activity was determined as amount of enzymes hydrolyzing casein to produce amino acids equivalent to 1 µmol tyrosine at 30 °C in 1 min).

2.2. Methods

2.2.1. Technological methods

Washing process spent brewery’s yeast: It was washed 1 time with NaOH 0.1 N for removing polyphenols and 3 times with cold water for removing remained solids, and then centrifuged at 4000 rpm at 4 °C for 15 min using a thermo Fisher (USA) to recover solids, which were material for our studies.

Disrupting yeast cell membranes: The pretreatment method for disrupting yeast cell membranes was chosen from our previous studies: Autolysis at 50 °C, pH 5.5 in 24 hours; autoclave at 121 °C in 20 minutes; treating with ultrasound at 50 °C, 50/90 Hz (QSONICA) in 10 minutes.

Hydrolysis process: Sludge of treated yeast was adjusted to pH 7.5 (HI 2211 pH/ORP meter) with using NaOH 0.2 N, the ratio of yeast: water was 1:1.5 (w/w) then added with enzyme mixture (flavourzyme 8.5 U/g and alcalase 7.2 U/g) and performed hydrolysis using IKA Eurostar (USA) agitator with agitation 500 rpm at 51 °C in 9 hours. After hydrolysis, the sample
was inactivated by 0.5 M TCA and removed the sludge by using centrifuge (6000 rpm, 4 °C for 10 min), hydrolysate was recovered in order to determine amino acid content (by HPLC) and bitterness of hydrolysate (by sensory method).

2.2.2. Analysis methods

**HPLC (High Performance Liquid Chromatography) method**

Amino acid content in hydrolysate was measured by adopting HPLC Agilent 1200, USA, using reverse – phase column of Zorbax AAA agilent, mobile phase: sodium phosphate buffer 40mM pH 7.8 and solution-acetonitrile:methanol: water 45:45:30; with velocity 1 mL/min, at temperature 25 °C. OPA reagent was used to react with sample before analysis in 2 minutes. OPA reagent and amino acid standard were purchased from Sigma, USA.

**Sensory method**

Sensory evaluation for bitter taste of the yeast protein hydrolysate was conducted by a panel consisting of 07 female and 05 male between the ages of 22 and 40. The panel members were trained for a period of 1 month, four times per week, with using quinine as standard (S6672804614, Merk, Germany). Quinine threshold was determined 4 µmol/l, calibration curve equation of quinine was determined: \( y = 9.6674x - 9.126 \) \( (R^2 = 0.9593) \), where: \( y \) - Quinine concentration (µmol/l); \( x \) - Bitter taste point (0 -10). A Fisher’s exact test was performed to determine the significance of differences of detection thresholds. The differences in bitterness intensity between the hydrolysate- samples were tested by Analysis of variance (ANOVA). The level of significance was chosen as p-value < 0.05. All analyses were performed with XLSTAT (Addinsoft XLSTAT v 2010.5.01).

3. RESULTS AND DISCUSSION

3.1. Relationship between bitterness and hydrophobic amino acid content in brewer’s yeast hydrolysate obtained by using mixture enzyme and combinating with pretreatment of yeast cell

It was carried out with 4 variants for obtaining yeast hydrolysates (M1-M4). The experiments were arranged according to Tables 1. The analysis results of HAA content and bitterness of hydrolysate are illustrated on Fig.1 and Table. 2, respectively.

**Table 1.** Experimental variants for obtaining yeast hydrolysates

| **M1:** Yeast was hydrolysed by using mixture of alcalase and flavourzyme, without pretreatment |
| **M2:** Yeast cell was pretreated with simultaneous combination (autolysis, autoclave and ultrasound) and then yeast sludge was hydrolysed by using mixture of alcalase and flavourzyme. |
| **M3:** Yeast cell was pretreated with simultaneous combination (autolysis and autoclave) and then yeast sludge was hydrolysed by using mixture of alcalase and flavourzyme. |
| **M4:** Yeast cell was autolysed and then it was hydrolysed by using mixture of alcalase and flavourzyme. |
The result in the Fig. 1 has shown that alanine and leucine content is higher in all hydrolysate samples, accounting for over 50% of hydrophobic amino acids. In Table 2, Fisher’s exact test was used to compare the differences of bitterness between samples with and without pretreating yeast cell. It was indicated that M1 belongs to (a) group and the other samples M2, M3, M4 belong to (b) group, it means that there is a difference from the bitterness between M1 M2, M3 and M4. This result is demonstrated by the reducing of valine + methionine, phenyl and isoleucine content in Figure 1 and Figure 2. Bitter taste of M4 was the lowest so we have chosen M4 (13.431 μmol quinine/l) for further study.

Table 2. Medium bitterness of hydrolysate samples (M1-M4).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Medium point of bitterness (µmol/l)</th>
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<tbody>
<tr>
<td>M.1</td>
<td>2.625 ± 0.34(b) 16.251</td>
</tr>
<tr>
<td>M.2</td>
<td>2.373 ± 0.51(a) 13.812</td>
</tr>
<tr>
<td>M.3</td>
<td>2.358 ± 0.42(a) 13.673</td>
</tr>
<tr>
<td>M.4</td>
<td>2.333 ± 0.37(a) 13.431</td>
</tr>
</tbody>
</table>

Figure 1. Hydrophobic amino acid content of hydrolysate samples (M1-M4) were determined by HPLC.

Figure 2. Chromatogram (HPLC) of hydrolysate samples M1-M4.

3.2. Influence of 10 kDa, 3 kDa filtration for yeast hydrolysate on bitterness and hydrophobic amino acid content

The hydrolysate M4 was filtrated through 5 µm membrane to obtain hydrolysate M5 and was segmented into M6, M7 fractions by using cut-off membrane MWCO 3 kDa và 10 kDa. The hydrophobic amino acid contents and bitterness of hydrolysate M4, and all fractions are illustrated on Fig. 3 and Table. 3.
In the Fig. 3, it was shown that Leu content was the highest values in all fractions M5, M6, M7 and lower in small amount in comparison with M4. But Gly and Ala content in M5, M6, M7 were reduced significantly. In particular, Ala content of M6 and M7 hydrolysate were reduced 71.36 % and 83.63 %, respectively.

In the Table 3, bitterness between samples has shown that M7 was (a) group, M6 was (b) group and M5, M4 were (c) group. There is a difference from the bitterness between M7, M6, and M5, M4. Bitter taste of M7 hydrolysate was the lowest, reached 3.587 µmol quinine/l.
Fig. 5 showed that bitterness of hydrolysate was decreasing with the decrease of quinine concentration (from 16.25 µmol/l to 3.59 µmol/l). The total content of hydrophobic amino acid decreased from 1653 to 932 µg/ml. This indicates a propositional correlation between bitterness and HAA content. These results are consistent with studies of Teruyoshi Matoba & Tadao Hata [3]; Peter Amala Sujith and Hymavathi T. V. and Izawa N. [1]. As the result has shown, bitter taste of hydrolysate rapidly decreased when it was segmented by the membrane filtration, this reduction may be caused by decrease of HAA content or removing of peptides containing HAA residues.

4. CONCLUSIONS

Bitterness of brewer’s yeast hydrolysate had propositional correlation with the hydrophobic amino acid content. It was more clear when pretreatment process of yeast cells was used before hydrolysis. In particular, bitter taste of hydrolysate decreased from 16.25 µmol/l (M1) to 13.43 µmol/l (M4) as hydrophobic amino acid content decreased from 1653 µg/ml (M1) to 1428 µg/ml (M4). The study also indicated that their relationship still true when M4 hydrolysate was segmented into fractions by membrane filtration, bitter taste of hydrolysate decreased from 13.43 µmol/l (M4) to 3.59 µmol/l (M7) as hydrophobic amino acid content decreased from 1428 µg/ml (M4) to 932 µg/ml (M7).

REFERENCES

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TÓM TẮT

NGHIỆN CỦ MÔI LIÊN HỆ GIỮA VỊ ĐĂNG CỦA DỊCH THỦY PHÂN NAMILY MEN BIA VÀ THÀNH PHẦN AXIT AMIN KÌ NƯỚC

Nguyễn Thị Thanh Ngọc1, Quan Lê Hà2.*

1Trường Đại học Công nghệ Đông Á, A2CN8, khu công nghiệp vừa và nhỏ Từ Liêm, huyện Từ Liêm, Hà Nội
2Viện Công nghệ Sinh học và Công nghệ Thực phẩm, Trường Đại học Bách Khoa Hà Nội, số 1 Đại Cồ Việt, Hà Nội

*Email: ha.quanle@hust.edu.vn; ngoc.nguyen@polycu.com.vn


Từ khóa: vị đắng, thủy phân bằng enzym, amino acid, dịch thủy phân protein, bã nam men bia.