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Characterization of yeast protein hydrolysates by single enzyme treatment for promising alternative protein source

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Abstract

Yeast protein can be a nutritionally suitable auxiliary protein source in livestock food. The breakdown of proteins and thereby generating high-quality peptides, typically provides nutritional benefits. enzyme hydrolysis has been effectively used; however, studies on the potential applications of different types of enzymes to produce yeast protein hydrolysates remain limited. This study investigated the effects of endo- (alcalase and neutrase) and exotype (flavourzyme and prozyme 2000P) enzyme treatments on yeast protein during the production of enzymatic protein hydrolysates. Endotype enzymes facilitate a higher hydrolysis efficiency in yeast proteins than exotype enzymes. The highest degree of hydrolysis was observed for the protein treated with neutrase, which was followed by alcalase, prozyme 2000P, and flavourzyme. Furthermore, endotype enzyme treated proteins exhibited higher solubility than their exotype counterparts. Notably, the more uniform particle size distribution was observed in endotype treated yeast protein. Moreover, compared with the original yeast protein, the enzymatic protein hydrolysates possessed a higher content of β-sheets and random coil structures, indicating their higher structural stability. Regardless of enzyme type, enzyme treated protein possessed a higher total free amino acid content

including essential amino acids. Therefore, this study provides significant insights into the production of enzymatic protein hydrolysates as an alternative protein material.

Keywords: yeast protein; endoprotease; exoprotease; hydrolysis; alternative protein

Introduction

Proteins play a significant role in regulating numerous physiological processes including the endocrine, immune, circulatory, nervous, and digestive systems (Minkiewicz et al., 2008; Sobczak et al., 2023). Animal proteins are renowned for their high quality and ability to provide adequate and balanced amino acids (AAs); however, their functionality is limited by resources and processes (Wu, 2022). From a nutritional perspective, the incorporation of different-sourced proteins like plant-based proteins as well as the production of bioactive peptides by enzyme treatment can sufficiently meet human health requirements by providing an ample supply of essential AAs (EAAs) or enhancing amino acid absorption (Jeon et al., 2023; Kumar et al., 2022).

Efforts to replace livestock products are continuing because of the growing population and the lack of supply of animal proteins (Gerber et al., 2007). Despite the growing interest and research of plant protein-based alternatives, they have nutritional limitations that cannot completely replace animal proteins. Recently, yeast became preferable alternative protein sources in accordance with its well-established production process such as rapid growth and ease of harvest, high protein content, and low contamination risk (Lapeña et al., 2020; Ø verland & Skrede, 2017). In addition, yeast proteins, a type of single cell protein, can provide balanced amino acid composition with high solubility and water-holding capacity (Puligundla et al., 2020), which are important characteristics in playing an auxiliary role in livestock food.

The chemical or biological breakdown of proteins presents a promising approach for generating high-quality small and large peptides in the diets of livestock, poultry, and fish, providing both nutritional benefits and crucial physiological or regulatory functions (Hou et al., 2022; Da Silva et al., 2018). Compared to chemical hydrolysis giving nonspecific breakdown into peptides and AAs, the enzymatic approach facilitates a highly precise and

63 controlled cleavage of specific amide bonds (Oshimura & Sakamoto, 2017). In addition, it
64 operates under mild reaction conditions, producing limited unwanted by-products (Czelej et
65 al., 2022). For example, enzymatic protein hydrolysates play a significant role as supplements
66 in livestock production. Numerous studies have investigated their impact on the growth
67 performance and hematological parameters of beef cattle, as well as their effects on the health
68 and performance of dairy cows, digestive function in cattle, and immune responses in calves
69 (Gunun et al., 2022; Kim et al., 2011; Nocek et al., 2011; Salinas-Chavira et al., 2015;
70 Stefenoni et al., 2020). Although substantial researches have been conducted on the use of
71 various protein sources, (Baker et al., 2022; Jach et al., 2022; Pang et al., 2022; Shurson,
72 2018), enzymatic hydrolysis of yeast protein remain relatively insufficient.
73 Based on positional specificity, proteolytic enzymes are categorized into two primary
74 groups: endopeptidases and exopeptidases. Endopeptidases target internal bonds within
75 polypeptides, whereas exopeptidases cleave near the C- or N-terminus (Gurumallesh et al.,
76 2019). So far, enzymatic hydrolysis has been widely performed and their functional or
77 structural alterations were reported (Etedmadian et al., 2021; Chalamaiah et al., 2012;
78 Gajanan et al., 2016; Dumitrașcu et al., 2023). Among them, several researches are
79 employing various commercial proteolytic enzymes, including alcalase, neutrase, protamex,
80 flavourzyme, pronase, and kojizyme of microbial origin; papain and bromelain sourced from
81 plants; and pepsin, trypsin, chymotrypsin, and pancreatin derived from animals. However, it
82 is still required to compare the hydrolysis effect using different groups of enzyme on
83 structural and functional characteristics of yeast protein. Therefore, this study aimed to
84 evaluate the effects of treatments using biological enzymes, either individually or in
85 combination (endo- and exotypes), on the properties of yeast proteins during the production
86 of enzymatic protein hydrolysates and provide fundamental data for exploring their potential
87 application as an alternative animal protein

Materials and Methods

Materials

Yeast protein was kindly supplied by Amored fresh (Seoul, Korea). Proteolytic enzymes, including endopeptidases; alcalase 2.4 L FG (from *Bacillus licheniformis*) and neutrase 0.8 L (from *Bacillus amyloliiensquefaciens*) and exopeptidase; flavourzyme 1000 L (from *Aspergillus oryzae*) from Daesang Corporation (Seoul, Korea) and prozyme 2000P from Bision Biochem Corporation (Seongnam, Korea) were supplied. Trichloroacetic acid (TCIchemical, Seoul, Korea) and bicinchonic acid (BCA) protein assay kit (Thermo Scientific, Seoul, Korea) were also used.

Enzymatic hydrolysis of yeast protein

Two different types of commercial enzymes, namely, endo- (alcalase and neutrase) and exo- (flavourzyme and prozyme 2000P), were selected to hydrolyze the yeast protein. The details of these enzymes are presented in Table 1. Using 10% (w/v) suspension at 55℃ using distilled water with the yeast protein, hydrolysis was conducted for 8 h at an enzyme/substrate ratio of 1 g enzyme/100 g protein (Suh et al., 2017 and Xia et al., 2021). pH values were determined at 0, 1, 2, 4, 6, and 8 h. The samples were freeze-dried at −80℃ and stored at room temperature before use for further studies.

Degree of Hydrolysis (DH)

The DH of the protein hydrolysate was measured by following Park and Yoon (2018) with slight modification. In brief, one percent (w/v) of hydrolysate (with pH adjusted to 7) was followed by the addition of the same amount of 20% trichloroacetic acid solution. After conducting the reaction at room temperature for 30 min, centrifugation (Eppendorf 5910 R,

Germany) was performed at 3,500 rpm at 4℃ for 20 min, and a supernatant was obtained. The absorbance of the supernatant was measured at a wavelength of 562 nm using an ultraviolet–visible spectrophotometer based on the BCA method (Smith et al., 1985) and the DH value was calculated (Ha, Kim, & Yoo., 2019).

$$\text{DH}\ (\%) = \frac{(W_h - W_0)}{W_h}\ A562\ x\ 100 \qquad (1)$$

Here, W_o and W_h represent the absorbance of yeast protein before and after hydrolysis, respectively.

Determination of solubility

An 1% (w/v) protein solution was incubated at a room temperature for 30 min at different pH from 2 to 12, which were adjusted using a 1 M HCl and 1 M NaOH solution, and centrifuged at 13,000 rpm for 25 min (Chen et al., 2018; Wang et al., 2021). The protein content in the supernatant was measured using the BCA protein assay kit (Pierce BCA protein Assay Kit, Thermo Scientific, Rockford, IL, USA). Protein solubility was expressed as a percentage value of soluble protein concentration to the total protein concentration of the sample.

Particle size distribution (PSD)

PSD was determined using a Mastersizer 3000 static laser light diffraction unit (Malvern Panalytical Ltd., Malvern, UK) across a size range of 0.01–3500 μm by employing a red laser (633 nm) and blue light source (470 nm). Particle size is expressed as average passing values from the results presented in a volume-based Particle Size Distribution (PSD) analysis using the Mastersizer 3000 software. The distribution width, often represented by the span, is calculated as $(D_{90} - D_{10})/D_{50}$, where D_{10}, D_{50}, and D_{90} denote the 10th, 50th, and 90th percentiles of the distribution, respectively (Istianah et al., 2024; Qin et al., 2023).

Fourier-transform infrared (FTIR) spectroscopy

To analyze the chemical structure, the dried yeast protein was positioned on a Fourier-transform infrared spectroscopy (FTIR) plate (Nicolet iS5, Thermo Fisher Scientific, Waltham, MA, USA). Light absorption across wavelengths from 550 to 4,000 cm^{-1} was collected, and FTIR spectra were recorded using a spectrometer fitted with an iD7 ATR accessory with a ZnSe crystal (4000–400 cm^{-1}) at 25℃. The equipment was operated at a scan speed of 0.2 cm/s, and at 16 scans with a resolution of 4 cm^{-1}. Background reference values were calculated using a standard log transformation of the sample and single spectra to remove the background signal. Their second-order derivative spectra were also obtained by using Origin Pro software (OriginLab Co., MA) after smoothing through the Savitzky–Golay algorithm employing nine data points from the analysis. The proportion of each secondary structural component is presented as a percentage, which is obtained by dividing the area of a single Amide I band component by the sum of the areas of all the amide band components.

Composition of free amino acids (AA)

The analysis of AAs within the yeast-protein extract was conducted using a Dionex Ultimate 3000 high-performance liquid chromatography system from Thermo Fisher Scientific, coupled with a 1260 Infinity fluorescence detector from Agilent Technologies (Waldbronn, Germany). The analysis method was based on the approach outlined by Min et al. (2023) and Yoon et al. (2019) with slight modifications. After the sample derivatization using o-phthalaldehyde (OPA) and 9-fluorenylmethoxycarbonyl (FMOC), 0.5 µL samples were injected into an Inno-C18 column (4.6 × 50 mm, 5 µm, Youngin Biochrom, Korea) at 40℃. Fluorescence detection was performed at excitation and emission wavelengths of 340 and 450 nm for OPA and 266 and 305 nm for FMOC, respectively. The primary and

secondary AAs were identified using the OPA and FMOC derivatives, respectively. The mobile phases were as follows: 40 mM sodium phosphate (pH 7) as solvent A, 10:45:45 (v/v) mixture of distilled water, acetonitrile, and methanol as Solvent B. A gradient program was employed at a flow rate of 1.0 mL/min, starting with 5% Solvent B for 3 min, followed by a gradient from 5% to 55% Solvent B in 24 min and then from 55% to 90% Solvent B in 25 min. This concentration was maintained for 6 min before reverting from 90% to 5% Solvent B over 3.5 min, with a maintenance period of 0.5 min at 5% Solvent B.

Statistical analysis

Statistical analysis was performed using MINITAB version 21. All measured parameters were assessed using one-way analysis of variance, followed by Tukey's post-hoc test to identify significant differences among the individual means. Statistical significance was determined at $p < 0.05$.

Results and discussion

Protein hydrolysis and pH measurements after protease treatment

The DH represents the percentage of cleaved peptide bonds in a protein hydrolysate and is a predominant parameter for distinguishing the structural variations among different hydrolysates (Yi et al., 2021). In this study, yeast protein gave over 80% hydrolysis yield after 8 h of enzyme treatment, regardless of enzyme types (Figure 1A). The hydrolysis levels decreased in the order of neutrase, alcalase, prozyme 2000P, and flavourzyme, indicating endotype enzymes facilitate a higher hydrolysis efficiency in yeast proteins than exotype enzymes. The higher efficiency of endotype enzymes might be because of stronger product inhibition from exo products or lower activation energy for endo product (Furusawa et al., 2008). Considering the endotype enzyme treatments, the DH of neutrase (90.02%) was higher

than that of alcalase (88.72%), which was consistent with the results of studies involving casein protein hydrolysate with the same enzyme employed in this study (Kim et al., 2021). After exotype enzyme treatments, the DH of prozyme 2000P (86.62%) was higher than that for flavourzyme (84.83%).

Meanwhile, regardless of enzyme types, the hydrolysis of yeast protein using endo and exo proteases was rapidly started right after the enzyme addition. It was indirectly proved by the changes in pH levels over time (Figure 1B). For example, the initial pH of yeast protein (about 7.09) rapidly decreased within 1 h, and the pH variations became less significant over time, which is generally observed during protein hydrolysis, suggesting that rapid degradation within a short period may exert a positive industrial impact on peptide production (Suh et al., 2017). This decrease may be attributed by the protein degradation, leading to the accumulation of acidic AAs or the subsequently formed carboxyl groups (Gam et al., 2019; Ryu et al., 2015). Thus, the proteolysis of yeast protein might positively affect the final protein qualities, however be differently affected by the enzyme types.

Analysis of protein solubility

Protein solubility, one of the typical criteria for measuring protein qualities, plays a crucial role in determining physicochemical properties, processing, nutritional profiles, etc. (Grossmann & McClements, 2023; Hellebois et al., 2021). Also, it largely affects formulation of products and their stabilities (Vihinen, 2020). Various intrinsic and extrinsic factors including molecular weight, specific AA composition, average charge, pH, and ionic strength collectively affect protein solubility (Diaz et al., 2010; Grossmann et al., 2019). In the present study, Figure 2 illustrates the solubility of yeast proteins across diverse pH ranges. The yeast protein sample demonstrated the highest solubility at alkaline pH 12. Also, their solubility became notably high at acidic pH 2. Owing to the presence of a net negative or positive

charge on a protein at high or low pH level (i.e. furthest above and below pI), a large amount of water might interact with the protein (Pelegrine & Gasparetto, 2005). Moreover, after enzyme treatment, the yeast protein exhibited a significant increase in protein solubility regardless of pH range, demonstrating an enhancement of more than three folds. The higher solubility of the protein hydrolysates than the initial proteins can be predominantly attributed to the liberation of polar functional gropus owing to the cleavage of peptide bonds. Especially, samples treated with neutrase exhibited the highest solubility among the enzyme-treated variants. Furthermore, as similarly to the hydrolysis results, samples treated with endotype enzyme including neutrase and alcalase demonstrated higher solubility than those processed with exotype enzymes. These findings are correlated with the results obtained from the hydrolysis of whey protein (Cui et al., 2021; Kim et al., 2022). In general, the protein solubilities are affected by both hydrophobic interactions among proteins and ionic interactions between protein and water (Cui et al., 2021; Xiong et al., 2023). Thus, hydrophilic structures that were previously concealed in the native structure of the aqueous solvent were revealed after enzyme treatment, which are increasing protein solubilities (Beaubier et al., 2021). The proteolysis of yeast protein might positively affect the final protein qualities in terms of enhancing solubility, however be differently affected by the enzyme types.

Effects of hydrolysis on the particle size

The particle size of food ingredients including protein samples is another important parameter indicating protein qualities. In general, a decrease in the particle size increases nutrient digestibilities by increasing available surface area (Blasel et al., 2006; Lyu et al., 2022). Table 2 illustrates the distribution of particle size of the protein hydrolysate after enzyme treatment. Yeast protein showed the average particle size (D_{50}) of 12.80 μm with 3.71 μm D_{10}

and 24.80 μm of D_{90}. Enzyme treatment of yeast protein gave a decrease in the D_{50} value, which is generally observed from the hydrolysates of food proteins (Cui et al., 2021; Hao et al., 2022). The reduction in the protein sizes after enzymatic hydrolysis might be attributed to the disruption of protein structure, allowing smaller peptides to be more readily solubilized in the solution, thus correlating with an increase in peptide solubilities. The particle sizes were decreased in the order of prozyme 2000P, alcalase, neutrase, and flavourzyme, indicating the size reduction was not considerably affected by enzyme types. Alcalase in endo-type protease and prozyme 2000P in exo-type protease exhibited a lower particle size (9.96 μm; 9.44 μm, respectively), suggesting that the specific introduction of each enzyme or utilization of combinations of different enzymes were required, based on the diverse substrate compositions. Also, the limited particle size reduction (i.e. sizes in the μm range) could be attributed to the extent and duration of hydrolysis, which can lead to either further breakdown or aggregation of particles (Shen et al., 2020; Hao et al., 2022).

Although the particle size did not show consistency according to the employed enzyme types, the span values of endo- and exotype enzyme treatments indicating variations in D_{10} and D_{90} values were approximately 1.68 and 1.81, respectively. These smaller span values imply a higher degree of dimensional uniformity in the yeast protein after hydrolysis with a more consistent particle size distribution (Jewiarz et al., 2020). Thus, with their lower span values, endotype enzymes treatment of yeast protein might contribute to a more uniform particle distribution, emphasizing their ability to promote particle uniformity.

Structural changes in yeast protein treated with various enzyme

The FTIR spectra (Figure 3A) reveal the yeast protein contains characteristic peaks indicating Amide A, Amide I, Amide II, and Amide III. For example, a distinctive peak at 3,280 cm^{-1} corresponds to the N–H stretching vibration, which is a key absorption feature

associated with Amide A (Haris, 2013; Zhou et al., 2016). The presence of amide I and Amide II in yeast protein and its hydrolysate was confirmed by the appearance of peaks at 1,630 and 1,520 cm^{-1}, respectively. The Amide I peak is attributed to the stretching vibration of C=O bonds and the Amide II peak is N–H and C–H stretching vibrations. In particular, Amide I exhibit the strongest transmission band and is highly sensitive to the secondary structure, reflecting diverse hydrogen-bonding environments associated with α-helix, β-sheet, turn, and unordered conformations (Prajapati et al., 2021). Furthermore, the bands at 2930 cm^{-1} correspond to $–CH_2$ groups (Gbassi et al., 2012).

In order to clarify the changes in the secondary structure of the yeast protein, the relative proportions of secondary structures within yeast protein after enzymatic hydrolysis were investigated (Table 3 and Figure 3B). Yeast proteins were characterized by a predominant presence of α-helix structures (i.e. about 53.30%) with 36.55% β-sheet and 10.14% β-turns. Conversely, enzymatic hydrolysis considerably altered the secondary structure of yeast proteins, exhibiting reduction in α-helix structures with β-turns, but increase in β-sheets, which shows an important feature of plant-based proteins (Carbonaro et al., 2012). The β-sheet was highly stable, whereas the α-helix and β-turn were highly flexible, exhibiting loose secondary structures (Wang et al., 2022; Xu et al., 2016). Thus, high content of the β-sheet structure provides resistance to protein breakdown in the digestive tract, which is advantageous to muscle forming (Berrazaga et al., 2019). In summary, the enzymatic hydrolysis of yeast protein increase flexibility and stability differently, but the levels may vary depending on the type of enzyme used for the treatment.

Effects of enzymatic hydrolysis on free AAs

The profiles of free AAs in the yeast proteins are presented in Table 4. Yeast protein contained 313.92 mg/kg total free amino acids with about 55% essential amino acids. Lysin is

288 the highest amounts by following glutamic acid, implying that yeast protein can be used as an
289 alternative to animal protein, possessing higher levels of lysine and valine than plant proteins
290 (Day et al., 2022). After enzymatic hydrolysis of the yeast protein, the amounts of free amino
291 acids considerably increased; however, the exotype treatment showed much higher value than
292 endotype treatment. For example, the yeast protein hydrolysates treated by exo-proteases
293 contained over 200,000 mg/kg total amino acids content. Among them, there are over 38,000
294 mg/kg aromatic amino acids, above 110,000 mg/kg hydrophobic amino acids, about 140,000
295 mg/kg essential amino acids. While, the yeast protein hydrolysates from endo-protease
296 treatment showed only about 5,232~11,161 mg/kg total amino acids. EAAs are indispensable
297 in human body as they cannot be synthesized de novo or produced at a sufficient rate to meet
298 the body's requirements. Furthermore, dietary EAAs play a pivotal role as catalysts for
299 skeletal muscle protein synthesis, thus holding significance in feed supplements utilized in
300 livestock farming (Church et al., 2020). Hence, obtaining EAAs through dietary protein is
301 imperative.Meanwhile, free amino acid profiles became different after hydrolysis.
302 Interestingly, leucine was the major free amino acid observed in proteins regardless of
303 enzyme types such as 1st ranked in flavourzyme (32,462.11 mg/kg), prozyme 2000P
304 (34,292.80 mg/kg), and alcalase (1946.93 mg/kg), and 2nd ranked in neutrase (14413.05
305 mg/kg). Owing to its regulatory effects on muscle protein synthesis and lipid deposition,
306 leucine can enhance the proportion of lean meat and reduce fat deposition, improving the
307 feed utilization efficiency to produce high-quality pork products (Rieu et al., 2003; Zhang et
308 al., 2020). Proteins treated with alcalase exhibit a significant generation of glutamic acid
309 (2341.31 mg/kg), which can contribute to enhance the flavor in alternative food and feed
310 industry (Lipnizki et al., 2010). Also, yeast proteins treated with the exotype enzyme
311 possessed considerably higher concentrations of lysine and valine than the original yeast
312 protein. This result suggests that yeast proteins treated with exotype enzymes can be viable

alternatives to animal proteins. In addition, the concentration of hydrophobic AAs including phenylalanine, leucine, isoleucine, tyrosine, tryptophan, valine, methionine, and proline (Widyarani et al., 2016) significantly increased compared with that of the control group, except for neutrase treatment. Among the treated samples, the largest increase in the concentration of hydrophobic AAs was observed for flavourzyme-treated proteins. These increased amounts of hydrophobic AAs could serve as excellent sources of antioxidants and antihypertensive agents (Khushairay et al., 2023). Although neutrase generates the least amounts of TAAS, the ratio of EAAs to TAAs was the highest in the yeast protein, reaching 71.80%. According to the ideal model proposed by the Food and Agriculture Organization and the World Health Organization, the reference value for high-quality protein should be over 40% (Li et al., 2022). Therefore, proteins treated with neutrase, flavourzyme, and prozyme 2000P were confirmed to be of high quality compared with the control group.

Conclusions

The hydrolyzed yeast protein could be utilized as a promising auxiliary protein source in livestock food in terms of their nutritional benefits. In this study, the quality of the yeast protein hydrolysates was compared after the enzymatic hydrolysis using two endotype (alcalase and neutrase) and two exotype (flavourzyme and prozyme 2000P) enzymes. The results indicated that the proteins treated with endotype enzymes exhibited higher DH and solubilities and gave more uniform particle size distributions than than those treated with exotype enzymes. The analysis of the secondary structure of the proteins revealed a decrease in the α-helix content and an increase in the β-sheet content upon hydrolysis, indicating an improvement in structural stability, regardless of enzyme types. AA profiling also demonstrated that enzyme treatment enhanced generations of free amino acids, and mostly high-quality proteins upon hydrolysis were produced. Overall, efficient processing of yeast

protein through enzymatic hydrolysis could contribute to the development of sustainable and efficient alternative protein materials for food production and animal feed industries.

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Declaration of interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study.

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Figure captions

Fig. 1. Degree of hydrolysis of yeast protein after enzyme treatment (A); pH changes in yeast protein with various enzyme treatments over time (B)

Fig. 2. Degree of protein solubility of yeast protein with pH changes after enzyme treatments

Fig. 3. Fourier-transform infrared spectra of yeast protein after enzyme treatments (A) and the deconvolution of amide Ⅰ range (B)

642 **Tables and Figures**

643 **Table 1**. List of endo- and exotype proteases used in this study

	Enzyme	Type	Optimal condition	Ref.
1	Alcalase 2.4 L FG	Endo	pH 6.5–8.5 55℃–70℃	Noh et el., 2013
2	Neutrase 0.8 L	Endo	pH 6–9 30℃–60℃	Zhang et al., 2022
3	Flavourzyme 1,000 L	Exo	pH 5–7.2 50℃–55℃	Hau et al., 2022
4	Prozyme 2000P	Exo	pH 5–5.5 55℃–60℃	Kim et al., 2022

644

645 **Table 2. Particle size of yeast protein by endo- or exo- protease treatment**

Sample		Diameter (μm)			Span
		D_{10}	D_{50}	D_{90}	
Yeast protein		3.71 ± 0.01[c]	12.80 ± 0.10[a]	24.80 ± 0.10[b]	1.65 ± 0.01[d]
Endo protease	Alcalase	3.79 ± 0.01[b]	9.96 ± 0.00[d]	20.40 ± 0.05[d]	1.67 ± 0.01[c]
	Neutrase	3.90 ± 0.00[a]	11.10 ± 0.00[c]	22.70 ± 0.00[c]	1.69 ± 0.00[b]
Exo protease	Flavourzyme	3.80 ± 0.00[b]	11.85 ± 0.05[b]	25.10 ± 0.10[a]	1.80 ± 0.00[a]
	Prozyme 2000P	3.66 ± 0.01[d]	9.44 ± 0.01[e]	20.7 ± 0.01[d]	1.81 ± 0.00[a]

646

647 **Table 3.** Deconvoluted FTIR peak areas of yeast protein treated with various enzymes

Sample		Percentage (%)		
		α-helix	β-sheet	Turns and band
Yeast protein		53.30 ± 0.03[a]	36.55 ± 0.03[d]	10.14 ± 0.01[a]
Endo protease	Alcalase	44.95 ± 3.60[b]	53.36 ± 0.08[c]	1.69 ± 0.06[c]
	Neutrase	26.22 ± 0.20[d]	64.50 ± 0.05[b]	9.28 ± 0.05[b]
Exo protease	Flavourzyme	46.38 ± 0.05[b]	51.79 ± 0.70[c]	1.83 ± 0.03[c]
	Prozyme2000P	30.13 ± 0.40[c]	68.73 ± 0.05[a]	1.13 ± 0.02[d]

648 Data are expressed as mean ± standard deviation (n = 3).

649 Means with different letters within the same row are significantly different at $p < 0.05$.

650 N.D. Not detected

651 **Table 4**. Free amino acid profile (mg/kg) of yeast protein after hydrolysis treatment with

652 different enzymes (endo- and exotype)

Free amino acids		Endo protease		Exo protease	
	Control	Alcalase	Neutrase	Flavourzyme	Prozyme 2000P
Aspartic acid	15.10 ± 0.03^{d}	799.84 ± 63.91^{c}	69.36 ± 1.96^{d}	10980.45 ± 112.41^{a}	5981.38 ± 182.86^{b}
Glutamic acid	42.19 ± 0.68^{d}	2341.31 ± 139.60^{c}	99.25 ± 1.72^{d}	15045.57 ± 164.85^{a}	7317.84 ± 167.81^{b}
Asparagine	1.32 ± 0.19^{d}	593.82 ± 26.01^{c}	54.89 ± 2.58^{d}	10875.72 ± 116.03^{a}	8017.27 ± 216.41^{b}
Serine	4.21 ± 0.04^{d}	606.73 ± 34.73^{c}	120.14 ± 2.58^{d}	13716.34 ± 121.55^{a}	9928.50 ± 255.77^{b}
Glutamine	2.52 ± 0.13^{d}	166.40 ± 9.62^{c}	33.96 ± 2.29^{d}	9184.48 ± 90.40^{a}	6156.48 ± 163.99^{b}
Histidine	3.87 ± 0.09^{c}	112.77 ± 7.16^{c}	56.69 ± 4.41^{c}	7584.09 ± 156.37^{a}	6857.71 ± 275.53^{b}
Glycine	7.51 ± 0.22^{d}	138.33 ± 10.83^{c}	40.43 ± 1.49^{d}	5736.49 ± 16.02^{a}	3095.87 ± 76.12^{b}
Threonine	2.72 ± 0.13^{c}	291.24 ± 15.23^{c}	89.03 ± 5.14^{c}	15445.25 ± 162.29^{a}	14161.13 ± 388.80^{b}
Citrulline	3.38 ± 0.08^{b}	7.17 ± 0.39^{b}	8.27 ± 0.25^{b}	52.68 ± 7.38^{a}	52.47 ± 1.01^{a}
Arginine	11.24 ± 0.17^{b}	260.00 ± 9.32^{b}	160.59 ± 8.17^{b}	21116.10 ± 144.10^{a}	20836.41 ± 390.38^{a}
Alanine	22.52 ± 0.23^{e}	755.01 ± 35.98^{c}	378.09 ± 2.84^{d}	15255.88 ± 102.09^{a}	11158.88 ± 241.07^{b}
Tyrosine	16.13 ± 0.20^{c}	680.82 ± 20.57^{c}	248.60 ± 5.95^{c}	16061.61 ± 422.56^{a}	13553.08 ± 457.60^{b}
Valine	5.01 ± 0.25^{d}	428.94 ± 16.73^{cd}	662.75 ± 7.53^{c}	20025.00 ± 258.62^{a}	18670.87 ± 430.27^{b}
Methionine	2.33 ± 0.45^{c}	434.37 ± 17.49^{b}	261.73 ± 7.36^{b}	6401.54 ± 75.83^{a}	6527.38 ± 163.48^{a}
Tryptophane	20.17 ± 0.64^{c}	104.79 ± 9.93^{c}	N.D.	3586.82 ± 56.27^{a}	3432.12 ± 104.89^{b}
Phenyl-alanine	18.61 ± 0.48^{d}	786.07 ± 22.89^{c}	916.04 ± 11.68^{c}	19174.72 ± 242.16^{b}	21442.14 ± 564.94^{a}

Isoleucine	1.56 ± 0.22^{b}	163.72 ± 41.32^{b}	294.17 ± 4.40^{b}	16431.76 ± 191.37^{a}	16143.14 ± 368.14^{a}
Leucine	2.36 ± 0.21^{d}	1946.93 ± 83.95^{c}	1413.05 ± 31.50^{c}	32462.11 ± 419.81^{b}	34292.80 ± 862.93^{a}
Lysine	117.01 ± 3.72^{c}	543.53 ± 30.93^{c}	325.51 ± 28.62^{c}	30678.46 ± 183.12^{a}	23492.51 ± 445.84^{b}
Proline	14.15 ± 0.36^{b}	N.D.	N.D.	1091.38 ± 13.04^{a}	935.35 ± 152.86^{a}
AAAs[1)]	54.91 ± 0.32^{b}	1571.68 ± 32.02^{b}	1164.65 ± 14.07^{b}	38823.15 ± 716.29^{a}	38427.33 ± 1119.54^{a}
HAAs[2)]	80.33 ± 1.12^{c}	4545.64 ± 178.91^{b}	3796.34 ± 55.57^{bc}	115234.95 ± 1651.70^{a}	114996.88 ± 3067.61^{a}
EAAs[3)]	171.32 ± 3.05^{c}	4377.98 ± 195.83^{c}	3757.24 ± 78.70^{c}	145388.22 ± 1165.98^{a}	138492.41 ± 3401.26^{b}
TAAs[4)]	313.92 ± 3.07^{d}	11161.78 ± 553.29^{c}	5232.57 ± 93.61^{cd}	270906.45 ± 2484.28^{a}	232053.32 ± 5810.07^{b}
EAAs/TAA (%)	54.57 ± 0.5^{c}	39.23 ± 0.25^{e}	71.80 ± 0.48^{a}	53.67 ± 0.09^{d}	59.68 ± 0.04^{b}

[1)] AAAs: aromatic amino acids

[2)] HAAs: hydrophobic amino acids

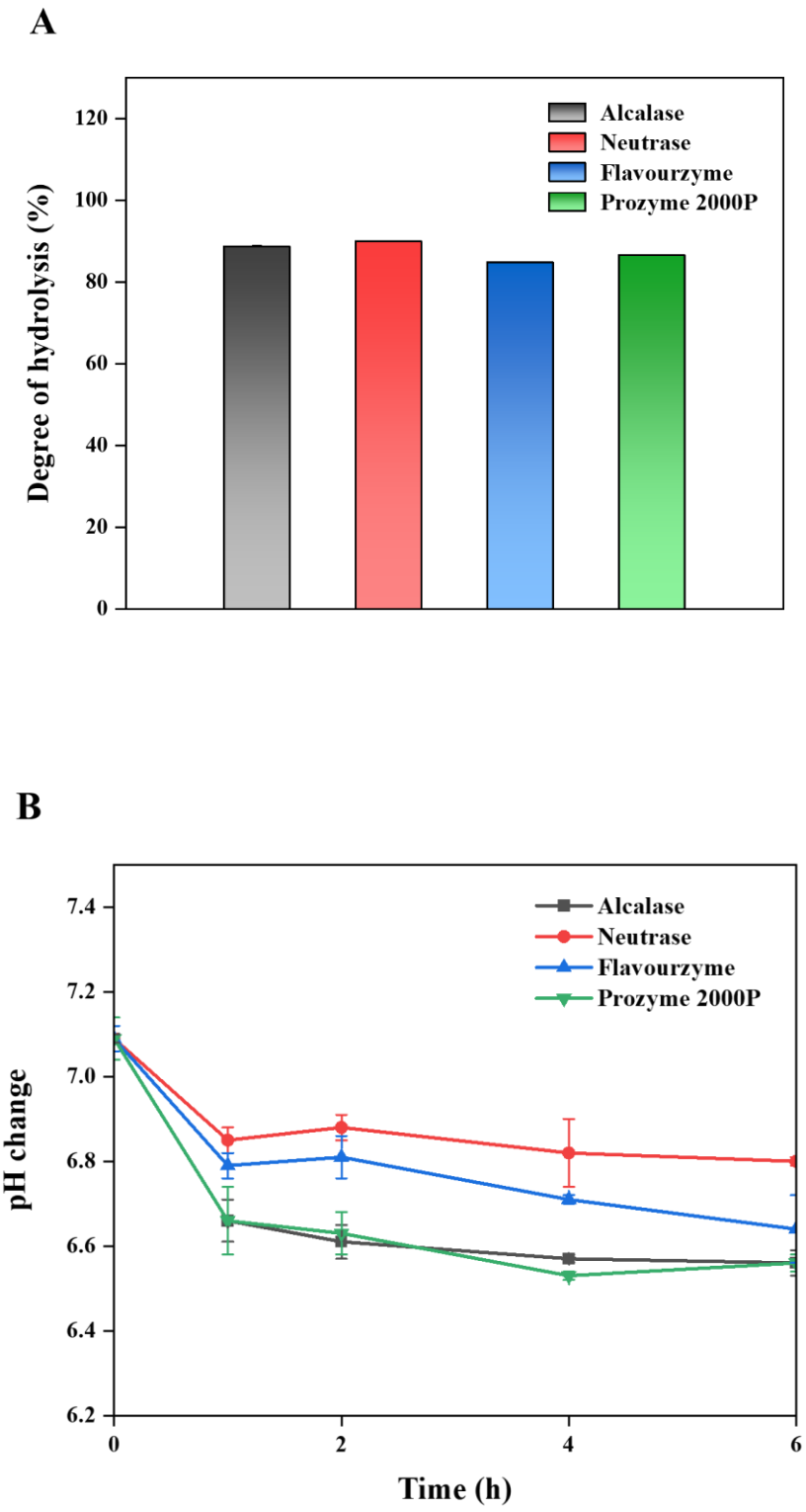
[3)] EAAs: essential amino acids

[4)] TAAs: total amino acids

Data are expressed as mean ± standard deviation (n = 3).

The means indicated with different letters within the same column are significantly different at $p < 0.05$.

660 **Fig. 1**



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662

663

Fig. 2

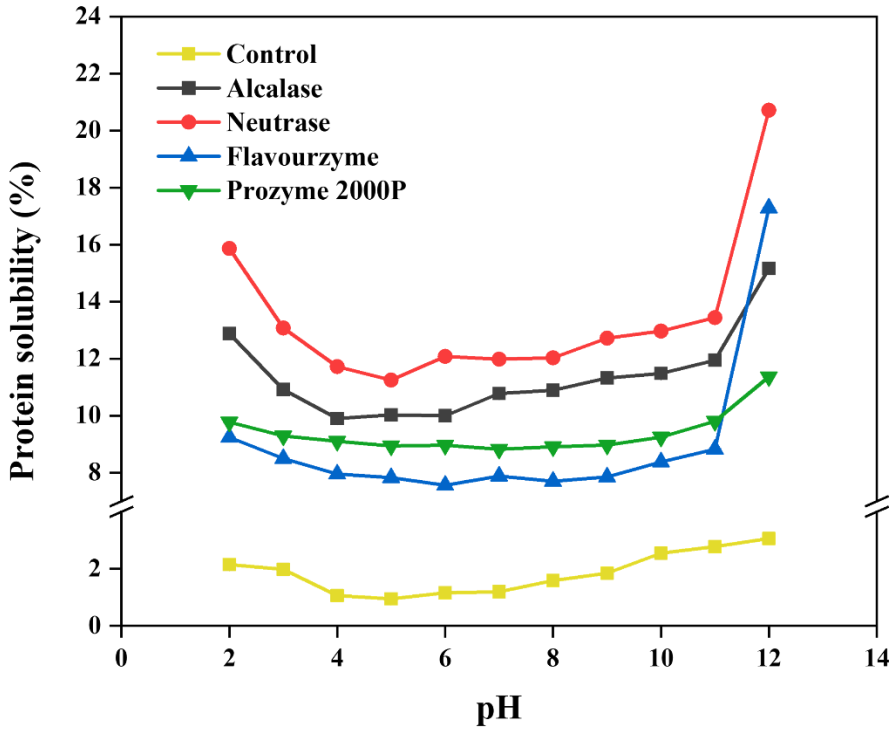
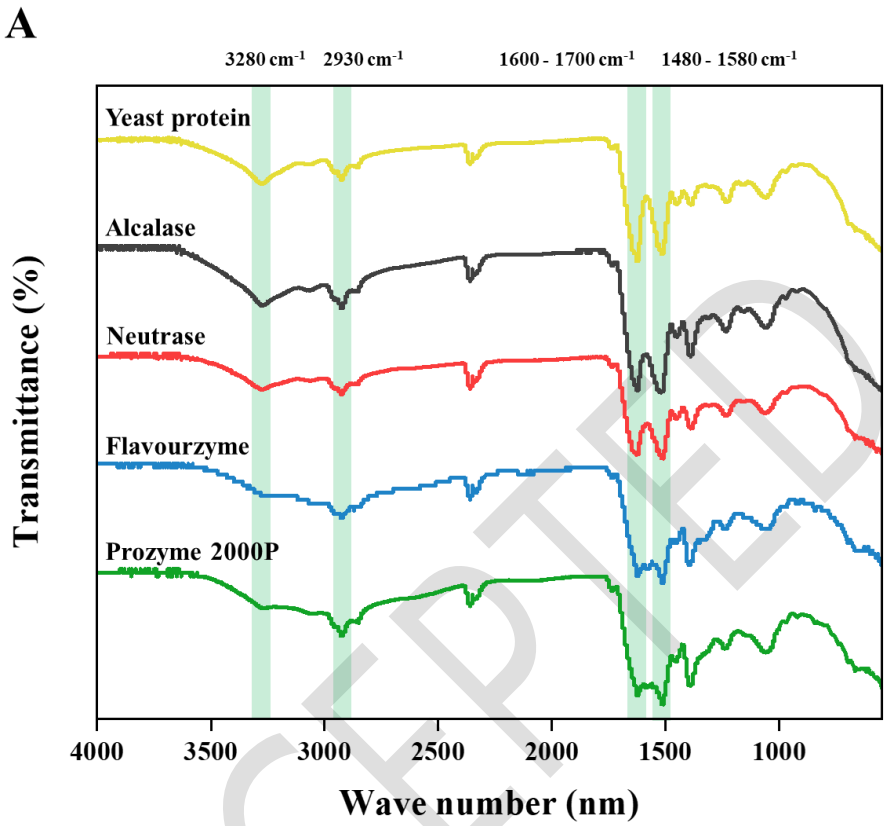
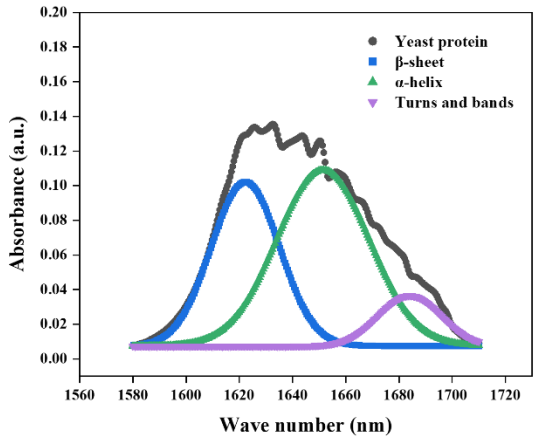
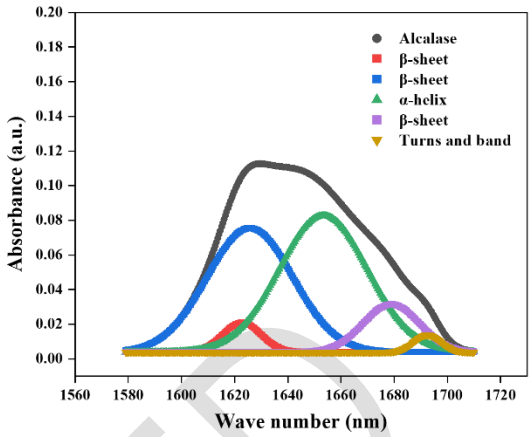

Control
Alcalase
Neutrase
Flavourzyme
Prozyme 2000P
Protein solubility (%)
pH
0
2
4
6
8
10
12
14
16
18
20
22
24

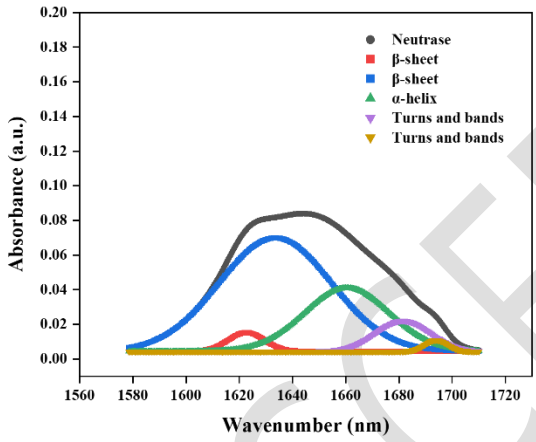
Fig. 3

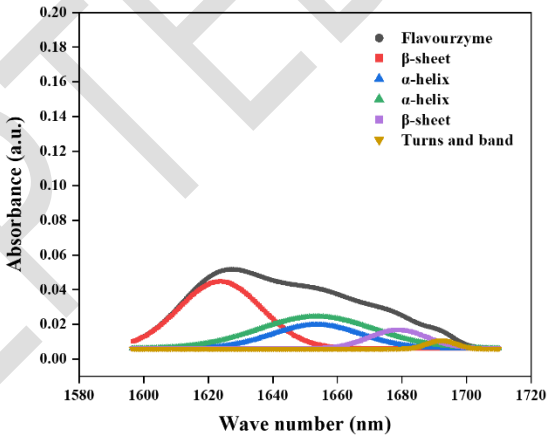


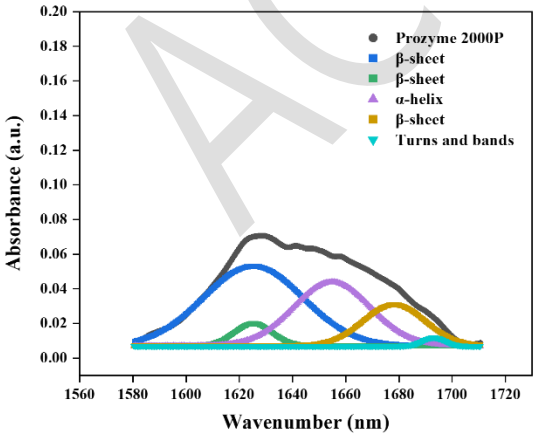
B


Yeast protein
β-sheet
α-helix
Turns and bands
Absorbance (a.u.)
Wave number (nm)


Alcalase
β-sheet
β-sheet
α-helix
β-sheet
Turns and band
Absorbance (a.u.)
Wave number (nm)


Neutrase
β-sheet
β-sheet
α-helix
Turns and bands
Turns and bands
Absorbance (a.u.)
Wavenumber (nm)


Flavourzyme
β-sheet
α-helix
α-helix
β-sheet
Turns and band
Absorbance (a.u.)
Wave number (nm)


Prozyme 2000P
β-sheet
β-sheet
α-helix
β-sheet
Turns and bands
Absorbance (a.u.)
Wavenumber (nm)