Makara Journal of Technology

Volume 14 | Issue 2 Article 4

11-2-2010

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THE EFFECT OF ENZYMATIC HYDROLYSIS ON THE PROPERTIES OF PROTEIN HYDROLYSATE FROM PADDY MUSHROOM

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Abstract

Protein hydrolysate was prepared from Paddy (*Volvariella volvaceae*) mushroom. Hydrolysis uses commercially protease available Protamex ™ with enzyme concentration of 0.1% (w/w). Hydrolysis was performed at three different temperatures (room temperature, 40 °C, and 50 °C) with different incubation periods (60, 90, and 120 minutes). Enzyme inactivation was done in 90 °C for 3 minutes. Yield and degree of hydrolysis ranged from 94.76% to 99.55% and 19.06% to 24.59%. Protein solubility was about 89–11,8%. The longer time of hydrolysis, the darker the color of protein hydrolysate. Protein hydrolysate which has hydrolysis at 50 °C for 120 minutes has the highest protein yield and the best sensory properties: 4.76 (taste liking), 3.68 (aroma liking), and 4.56 (overall liking). However, this protein hydrolysate has the potential for application as an ingredient in formulated diets.

Keywords: enzymatic hydrolysis, mushroom, protease, protein hydrolysate, V. volvaceae

1. Introduction

Protein hydrolysates are mainly applied in the nutritional management of individuals who cannot digest whole/ intact protein. Hydrolysates which are rich in low molecular weight peptides, especially di- and tri-peptides with as little as possible free amino acids, have been shown to have more dietary uses due to their high nutritional and therapeutic values [1]. Extensively hydrolyzed proteins also show reduced immunological reactivity, and can be used in formulas for hyper allergic infants [2]. Furthermore, peptides, being easily absorbed, may be an optimal nitrogen source in sports nutrition, and high biological value peptides are attractive as a general protein supplement in a wide variety of diets [3]. Hydrolyzed proteins may have a high functional value because they may contain peptides with hormonal, neurotransmission and physiological functions, generally known as bioactive peptides [4]. These compounds also have high solubility in acidic solutions and are capable of going through thermal treatment without the formation of precipitates [5].

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Paddy mushrooms (*Volvariella volvaceae)* are one source of food that has a high potential nutritional value of some types of edible mushrooms. Nevertheless, with proper cultivation techniques and better, we always can get the fungus on various seasons throughout the years. This mushroom is delicious and good texture [6]. Paddy mushrooms contain protein, besides water, carbohydrate, and minerals [7]. Although they have a high nutrition value, but their utilization has been limited. However, by a simple process using enzymatic hydrolysis, protein in paddy mushroom can be more valuable.

Application of protease is often an attractive means for obtaining better functional properties of food proteins, without impairing their nutritional value. Enzymatic hydrolysis can produce hydrolysates with well defined peptide profiles, and an extensive review of enzymatic protein hydrolysates in human nutrition was reported in literature [8]. In this present study we use commercial protease Protamex^{TM}. This enzyme is endopeptidase enzyme that is produced from Bacillus which is

developed for hydrolisis in food. This protease has optimal activity at pH 5,5-7,5 and at temperature 35-60 ^{o}C. Due to the its ranged pH, we do not have to adjust pH. It is expected that its hydrolysate is more applicable in utilization.

The aim of the research work is to prepare a protein hydrolysate of paddy mushroom *V. volvaceae* using Protamex enzyme. This report describes its protein solubility, yield, degree of hydrolysis, color, and sensory characteristics.

2. Methods

Substance. Common paddy mushrooms were purchased from a local market in Jember (Indonesia), and they were cultivated in Jember, East Java Province of Indonesia. Protemex TM (Novozyme product), buffer phospat 4% pH 8.2, HCl 1 N liquid, reagent mix-lowry (2 gr Na_2CO_3, 1 mL Na-K tartat and 1 mL $CuSO_4$ 1%), reagen follin, TNBS liquid (0.2 mL reagen TNBS diluted to 10 mL), aquadest, BSA (Bovine Serum Albumin) as standard in soluble protein analyze, and Glycine as standard in DH analyze.

Protein hydrolyzates preparation. Fresh mushrooms were cleaned from dirt by using flowing water. After that, mushrooms were cut into small pieces and then blanched using steam blanching with temperatures 93-94 °C for three minutes. The purpose of blanching was for inactivation of enzyme polyphenol oxidase, breaking the network of cells that protect the protein so that the substance will come out and simplify protein denatured proteins that accelerate the hydrolysis process. To prepare protein hydrolysate, fresh paddy mushrooms were crushed using a blender and suspended in distilled water with ratio 1:1 (w/v) at room temperature. The slurry was hydrolysis using 0.1% (w/w) Protamex enzyme. Incubation was done at room temperature, 40 °C, and 50 °C with interval time of 60, 90, and 120 minutes. The enzyme was inactivated by keeping the mixture in boiling water bath for 3 minutes. The slurry was cooled to a room temperature and kept in a refrigerator until it was ready to be analyzed.

Protein Solubility. The amount of soluble protein in the filtrate was determined by the method of Lowry et al. with bovine serum albumin as the standard [9]. Absorbance was measured at wavelength of 750 nm. The solubility was expressed as a percentage of total protein concentration.

Yield. The yield was calculated by comparing the weight of hydrolysate with mushroom suspension. The result showed as percentage of yield.

Degree of hydrolysis. The degree of hydrolysis was determined spectrophotometrically by the trinitrobenzene sulphonic acid method, as described by Adler-Nissen that was measured at absorbance 340 nm [10].

Color. Color measurement of samples was determined in triplicate using color reader (Minolta camera, Ltd, Tokyo, Japan). The color values were recorded as L = lightness (0 = black, 100 = white).

Sensory properties. Test parameters of sensory properties were taste, aroma, and overall liking of protein hydrolyzate. Score of liking ranges from 1 (don't like very much) to 7 (like very much).

3. Results and Discussion

Protein solubility. Protein solubility gradually increases as well as the longer time of hydrolysis (Table 1). These phenomena were shown by all proteins that have hydrolysis at different temperatures. The highest protein solubility 110% was achieved at 50 °C after 120 minutes. Some of samples have a value above 100%. It was suggested that there were other components in hydrolysates that contribute in absorbance 340 nm. In the previous study, the solubility above 100% that was analyzed by Lowry method also showed that hydrolysate of soy protein was isolated and hydrolysis in pH 4,5 used bromelain enzyme [11].

Solubility is one of the most important functional properties of protein hydrolysate [12]. Good solubility of protein is required for many functional applications, especially for making emulsion, foams, and gels. The high solubility of protein hydrolysates facilitates potential application in a lot of food formulation.

Yield. The yield of *V. volvaceae* protein hydrolysate in Table 1 ranges from 94.762% to 99.551%. The great value of its yield is expected from its usefulness in food application. The relation between protein solubility and yield at 50 °C shown has R^2 = 0,989 (Fig. 1). It is generally accepted thatthe higher protein solubility, the

Table 1. Protein Solubility and Yield of Protein Hydrolysate of Mushroom *V. volvaceae*

Temperature of hydrolysis (°C)	Time of hydrolysis (minutes)	Protein solubility (%)	Yield (%)
Room temperature	60	90.80	94.762
	90	102.00	95.576
	120	104.00	99.442
40	60	89.60	96.413
	90	102.00	99.375
	120	118.00	99.543
50	60	91.20	98.056
	90	106.40	99.449
	120	110.00	99.551

higher yield of protein hydrolysate. The lower R^2 value between protein solubility and yield at room temperature and 40 °C (data not shown) due to protein solubility at room temperature and 40 °C is lower than at 50 °C so there are other components that contribute to the yield.

Good solubility and high yield of paddy mushroom protein hydrolysates are expected to be profitable in food industry.

Degree of Hydrolysis. Degree of hydrolysis *V. volvaceae* protein hydrolysate is about 19.064%-24.590% (Fig. 2). This result was lower than Ovissipour's work that used Alacalase for hydrolysis protein of viscera fish [13]. In contrast, our result was better than Feliciano done using pepsin in Amaranthus and Bluckwheat protein hydrolysis [14]. The difference in DH suggested the difference enzyme type which is used in protein hydrolysis. In Ovissipour et al. [13], they work using Alcalase that has high degree of hydrolysis compared to neutral or acidic enzymes [12,15-17]. Protamex is endopeptidase enzyme that is produced from Bacillus which is developed for hydrolisis in food. This protease has optimal activity at pH 5,5-7,5 and at temperatures 35-60 ^{o}C [18]. However, protamex is neutral enzyme protease.

The similarity of this study with others is the higher degree of hydrolysis happening at higher temperature [13,16]. In addition, the longer time of hydrolysis, the longer was the contact of enzyme subtrate, so the amount of hydrolyzed peptide bond will be more and more. To conclude, the higher temperature and the longer time of hydrolysis, the higher of DH as the result.

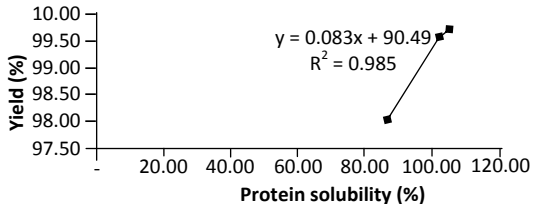

Fig. 1. Relation between Protein Solubility (mg/ml) and Recovery (%) of Protein Hydrolysate, 50C (♦)

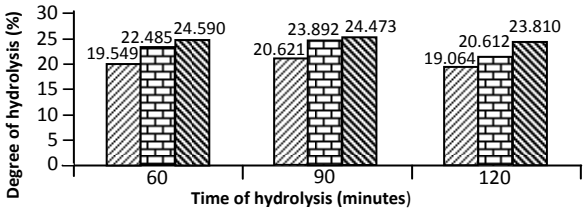

Fig. 2. DH (%) of *V. volvaceae* Protein Hydrolysate at Different Temperatures (°C) and Hydrolysis Time (min). Room Temperature (▨), 40 °C (▤), and 50 °C (▧)

Color. The color of *V. volvaceae* protein hydrolysates at all incubation temperature gets darker, and the time gets longer for hydrolysis (Fig. 3). It is assumed that the longer time of hydrolysis, the bigger the production of peptide is. Itis accepted there are more primary amine groups which can react with glucose, resulting in Maillard reaction that the more intensive it is, the more Maillard products which have dark color are produced. Paddy mushrooms *V. volvaceae* contain carbohydrate 2.68% [7]. The same phenomenon also occurs in the higher incubation temperature. Maillard reaction is influenced by many factors, including reactant concentration, temperature, time, initial pH, and water activity [19].

Sensory properties. Protein hydrolysate that has hydrolysis at room temperature has lower sensory properties than the one having hydrolysis at 40 °C and 50 °C. The bitterness of a peptide is related to the sequence and polarity of the comprising amino acids, size of the peptide, presence of amino acids that inhibit flavor, pH, and temperature [20-22]. The main aminoacids described as the source of the bitter taste from peptides are: phenylalanine (PHE), tryptophane (TRP), tyrosine (TYR), isoleucine (ILE), proline (PRO) and histidine (HIS) [21-23].

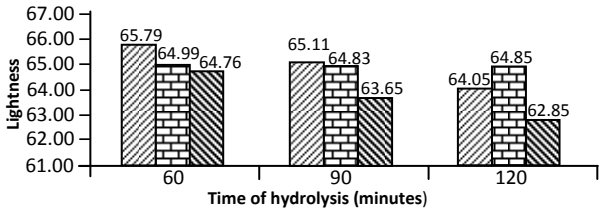

Fig. 3. Lightness of *V. volvaceae* Protein Hydrolysate In Different Temperatures (°C) and Time of Hydrolysis (min). Room Temperature (▨), 40 °C (▤), and 50 °C (▧)

Table 2. Sensory Properties of Protein Hydrolysate of Paddy Mushroom (*V. volvaceae*)

Temperature of hydrolysis (°C)	Time of hydrolysis (min)	Taste liking	Aroma liking	Overall liking
Room temperature	60	2.60	2.56	2.84
	90	3.04	2.76	3.28
	120	4.36	3.48	4.40
40	60	4.24	3.28	4.08
	90	4.44	3.16	4.28
	120	4.60	3.12	4.16
50	60	4.60	3.64	4.44
	90	4.56	3.36	4.40
	120	4.76	3.68	4.56

Protein hydrolysate of paddy mushrooms that has the highest protein solubility and yield (110% and 99.5%) also has the best sensory properties for taste, aroma, and overall liking. It is accepted that protein hydrolysate that has hydrolysis at 50 °C for 120 minutes has the best properties than others.

4. Conclusion

Correlation between protein solubility and yield is positive. However, yield increases as well as protein solubility increases. Protein hydrolysate of paddy mushrooms that has hydrolysis at 50 °C for 120 minutes has the best properties. Protein hydrolysate of paddy mushrooms has good properties (high solubility, yield, sensory liking) that would be applicable in food diets and food industry.

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