Production of mushroom protein hydrolysates by enzymatic hydrolysis and their physicochemical properties

Panida Banjongsinsiri[1], Krittalak Pasakawee[1*], Nowwapan Noojuy[1], Takunrat Taksima[1] and Ubonphan Rodsuwan[1]

Abstract

The selected mushrooms (Oyster mushroom, Abalone mushroom and Shiitake mushroom) were hydrolysed with papain enzyme at 50°C for the different time (6, 12, 18 and 24 hours) to get the mushroom protein hydrolysates (Oyster mushroom, OMPH; Abalone mushroom, AMPH; Shiitake mushroom, SHMPH), respectively. The result showed that degree of hydrolysis (%DH), total soluble solids (%TSS), sodium chloride (%NaCl), %nitrogen, %protein and free amino acid content of all mushroom protein hydrolysates (MPHs) increased whereas pH value decreased with the increasing extraction time. The optimum MPHs production related with the suitable hydrolysis time and %DH. The hydrolysis timing to produce the highest %DH of OMPH, AMPH and SHMPH (46.54, 38.15 and 25.75) with papain were 18, 18 and 24 hours and provide the highest total amino acid content (mg/100 ml) at 384, 157 and 110, respectively. Based on this result, there is a possibility that Oyster mushroom, Abalone mushroom and Shiitake mushroom can be produced the vegetable protein extracts using the enzymatic hydrolysis process and rendering the useful ingredient for further food and nutraceutical applications.

Keywords: mushroom protein hydrolysate, hydrolysis production, physicochemical property

1. Introduction

Protein hydrolysate is a foodstuff obtained by hydrolysis of protein molecules and become the important ingredients in food and medicinal products as a result of its functional properties and nutritional values (Solina *et al*., 2007). It can be produced by hydrolysing food materials such as soybean, corn, rice and wheat in acid, alkaline or/and enzyme hydrolysis but the acid and alkaline methods produce toxins and lose nutrients (Howell, 1996). Enzymatic hydrolysis is one of the fast, safe, simple, inexpensive and controllable method of protein digestion producing short-chain peptides and free amino acids (Zarei *et al*., 2014). The hydrolysis conditions such as time, pH, temperature and enzyme concentration influence enzymatic activity resulting in the degree of hydrolysis (DH). Hence these offer possibilities to control the process (Hall and Ahmad, 1992). The DH is the principal parameter to be used in optimization to achieve desired protein hydrolysates (Sujith and Hymavathi, 2011).

[1] Thailand Institute of Scientific and Technological Research (TISTR) 35 Moo 3 Technopolis, Khlong 5, Khlong Luang, Pathum Thani 12120

* Corresponding author, e-mail: krittalak.p@gmail.com

Mushroom is consumed in many countries as a delicacy, have a high nutritional value, especially a peptide/amino acid, provide the specific aroma, taste and texture (Thanasukhon *et al*., 2009; Zhu *et al*., 2015). Mushrooms contain biomolecules with nutritional and medicinal properties which have been recognized as natural sources for the development of medicine and nutraceutical products to diverse beneficial effect on human health (Alves *et al*., 2012; Ren *et al*., 2012). Thus, mushroom is a suitable source for hydrolysed-protein treatment. The main objective of this research was to study the selected mushrooms affected by hydrolysis condition with papain enzyme on the physicochemical properties.

2. Materials and Methods

2.1 Materials and chemicals

Fresh mushrooms (Oyster mushroom; OM, Abalone mushroom; AM and Shiitake mushroom; SHM) were purchased from one of the locally market in Bangkok, Thailand. Papain enzyme was purchased from Siam Victory Chemicals Co., Ltd, Thailand. The standards for amino acid analysis were ordered from Sigma-Adlirch Chemical Co., USA. All chemicals and standards used in this study were analytical grade, except chemicals and standards for amino acid analysis (HPLC grade).

2.2 Production of mushroom protein hydrolysate

The enzymatic hydrolysis was processed according to a previously described method of Thanasukhon (2009). Fresh mushrooms were cleaned with tap water and dried in tray dryer at 50 ± 2°C for 17 ± 2 hours. The moisture content (≤ 5%) of all samples was measured for process control using the AOAC procedure (2000). Each dried mushroom was ground with blender for 1 minute and stored in polyethylene bag until used. Ground mushroom was mixed with water at the ratio of 1:20 (w/v) and adjusted to pH 6.5 with 0.1M NaOH. The mixture was continuously pre-heated at 50°C for 15 minutes using a shaker and then hydrolysed via papain enzyme at a concentration of 15% (w/w). The production was followed by varying hydrolysis time to 6, 12, 18 and 24 hours at 50°C. After hydrolysis, the enzymes were deactivated at 95°C for 20 minutes. Each mixture was centrifuged at 7,000 rpm. The supernatant was precipitated and kept in closed-glass bottle at 4 - 10°C for overnight. Base on this production, mushroom protein hydrolysates from Oyster mushroom, Abalone mushroom and Shiitake mushroom were labeled as OMPH, AMPH and SHMPH, respectively.

2.3 Analytical methods

The degree of hydrolysis (DH) of mushroom protein hydrolysates (MPHs) via the papain was determined according to protocol of Netto and Galeazzi (1998). This method is based on the amount of nitrogen released by protein hydrolysis in the presence of a precipitate

agent, such as, trichloroacetic acid (TCA). TCA solution (20% w/v) was added into an equal amount of sample at ratio 1:1. After 1 hour of precipitation at room temperature, the sample was centrifuged at 10,000 rpm for 10 minutes to remove the insoluble protein. The supernatant was collected to analyze nitrogen content using the combustion method (FP-528 LECO, MI, USA). %DH was calculated as following equation (1).

equation (1)

Each MPH was analyzed for chemical compositions such as %total soluble solid content (%TSS), pH value and %sodium chloride (%NaCl) using the instruments as a hand-held solid refractometer (PAL-1, ATAGO, Japan), a pH meter (Seven Easy, Mettler Toledo, Switzerland) and a salinity refractometer (N-1E, ATAGO, Japan), respectively.

The total nitrogen content was determined by the thermal conductivity detection method using a combustion analyser (FP-528 LECO, MI, USA). The nitrogen content was then converted to equivalent protein using the Windows®-based operating software with a default protein factor of 6.25.

The color of MPHs was measured by a colorimeter (Hunter Lab, Color Quest XE, USA) using the CIE system (Diffuse/8° transmission). Sample (20 mL) was added into a rectangular transmission cell with 10.0 mm path length, then placed at the sphere at the front of the transmission compartment. The color was expressed as L*(0-100), a*(+/-) and b*(+/-) parameters indicating darkness/lightness, redness/greenness and yellowness/blueness, respectively. The colorimeter was calibrated with black and white standard plate.

The analysis of free amino acids in each MPH was performed at Central Instrument Facility, Faculty of Science, Mahidol University, Bangkok, Thailand. Amino acid composition was determined by high performance liquid chromatography system (Water Alliance 2695 with heater Jasco FP 2020 fluorescence detector) and hypersil gold column C18 (4.6x150 m.m., 3 µm) using the following method of AOAC procedure (2000). Each amino acid was identified by comparing the samples with a standard analyzed under the same conditions and quantified by the calibration curve of the authentic compound.

2.4 Statistical analysis

All of the experiments were carried out in triplicate. The data analyses were performed using the Microsoft EXCEL. Analysis of variance (ANOVA) was done to determine the significance of the main effects and using Duncan's test with confidence level as $p<0.05$. The chemical and color properties were reported as mean ± standard deviation.

3. Results and Discussion

3.1 Degree of hydrolysis (DH)

The rate of hydrolysis is an important parameter as referred to an enzymatic activity and resulted in the degree of hydrolysis (DH), as shown in Figure 1. The highest %DH of MPHs observation indicates the maximum cleavage of peptides. The curve of OMPH, AMPH and–SHMPH showed an increased significant difference in hydrolysis rate for the first 0 - 12 hours ($p<0.05$) and remained steadily rate from 12 - 24 hours. Based on this result, it could be concluded that the longer time of hydrolysis, the higher of %DH. It is possible that the longer time facilitates the contact of enzyme and substrate. The more amount of hydrolyzed peptide bond will be cleaved (Palupi *et al.*, 2010) resulting the increasing the peptides solubility in TCA solution (Haslaniza et al., 2010) and making %DH increased.

As shown in figure 1, the trend of %DH of OMPH is the highest throughout hydrolysis process comparing with that of AMPH and SHMPH. The highest %DH of OMPH, AMPH and SHMPH were 46.54%, 38.15% and 25.75%, that occurred at 18, 18 and 24 hours hydrolysis, respectively.

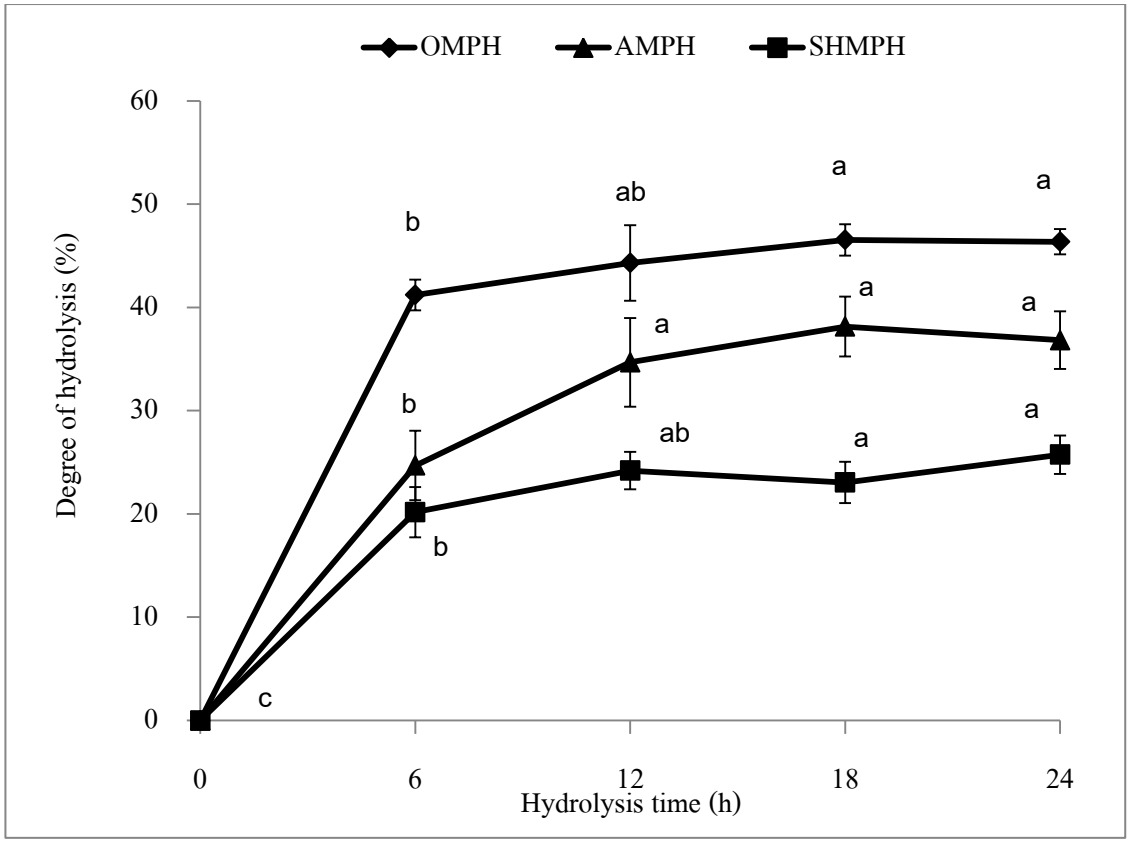

Figure 1 Degree of hydrolysis of mushroom protein hydrolysates from Oyster mushroom (OMPH), Abalone mushroom (AMPH) and Shiitake mushroom (SHMPH) at 50°C within 0–24 hours.

[a-c]Different superscripts of each MPH indicate significant differences ($p \leq 0.05$).

3.2 Chemical properties

As shown in table 1, each MPH showed the decreasing of pH value from 6.46 to 4.42 ($p<0.05$) with the increased hydrolysis time. In contrast, the hydrolysis time increasing exhibited the increased %TSS of MPHs ($p<0.05$). In addition, %NaCl of most MPHs also increased with the increased hydrolysis time except OMPH. During the enzymatic hydrolysis, plant materials are hydrolysed into small molecular weight of peptides, amino acids and ammonia by the proteases (Howell, 1996). Reduction of the organic matter concentration in the hydrolysis resulted in the increased soluble solid content (Shiau and Chai, 1999). Some alpha-amino acids might be produced in the hydrolysis process, while the degree of ionization of free carboxyl- and amino- groups are formed effecting on the drop of pH value (Shiau and Chai, 1999). In addition, salt may be formed during hydrolysis, resulting in the increasing of NaCl content (BD Biosciences, 2009).

Likewise, %protein and %nitrogen of OMPH, AMPH and SHMPH are not significantly different throughout the hydrolysis time. The range of %protein of OMPH, AMPH and SHMPH were 0.60 – 0.73, 0.32 – 0.36 and 0.63 – 0.77 whereas %nitrogen of them were 0.10 – 0.12, 0.05 – 0.06 and 0.10 – 0.12, respectively. Nitrogen contents relating to protein contents, are the important parameter used for grading the quality of hydrolysed products.

The changes of chemical composition of MPHs depend on the hydrolysis time. While the hydrolysis time increased, the results showed the increasing of TSS, NaCl, nitrogen and protein level with the decreasing of pH value in agree with Yanfang *et al.* (2009).

3.3 Color

Color parameters of MPHs from Oyster mushroom, Abalone mushroom and Shiitake mushroom with different hydrolysis time are L*, a* and b* values shown in Table 1. L*, a* and b* values of MPHs had significant differences ($p<0.05$) at the different hydrolysis time. Obviously, the color of SHMPH exhibited the decreasing of lightness (L* value) and the increasing of redness (a* value) and yellowness (b* value) while the hydrolysis time increased. In general, the liquid of AMPH, OMPH and SHMPH had a yellow-brown color throughout the hydrolysis time. The color change of SHMPH is in agreement with the research of Palupi *et al.* (2010). The slightly brown of sample could be assumed that proteins in mushroom were extensively hydrolysed with protease using the longer of hydrolysis time, its color change can be attributed to the reaction between primary amine groups and glucose occurring in Maillard reaction. This reaction will produce Maillard products having dark color. Furthermore, Maillard reaction is influenced by several factors, such as substrate concentration, hydrolysis temperature and time.

In contrast, L* value of OMPH and AMPH increased but a* and b* values of them decreased when the hydrolysis time increased. It was shown that the color of OMPH and AMPH have a trend to be lighten with the increasing of hydrolysis time. It may be due to the pigment compounds, anthraquinones in the oligomer form, being broken down by hydrolysis reaction to be in the reduced form (anthranol, anthrone, anthrahydroquinone, and oxanthrone derivatives) which it is colourless (Velíšek and Cejpek, 2011). Moreover, the differences in peptide size and amino acid composition of MPHs might be responsible for the color change. In general, color does not influence the functional properties of MPHs but it is an important property to apply in food.

Table 1. Physiochemical properties and color of mushroom protein hydrolysate from Oyster mushroom (OMPH), Abalone mushroom (AMPH) and Shiitake mushroom (SHMPH).

Physiochemical properties	OMPH (hydrolysis time: hrs)			
	6	12	18	24
%TSS	2.80 ± 0.07^{c}	2.90 ± 0.03^{b}	2.90 ± 0.00^{b}	3.10 ± 0.00^{a}
pH	5.85 ± 0.03^{a}	4.48 ± 0.02^{b}	4.50 ± 0.01^{b}	4.42 ± 0.01^{b}
%NaCl^{ns}	2.60 ± 0.00	2.60 ± 0.00	2.60 ± 0.00	2.60 ± 0.00
%Protein^{ns}	0.60 ± 0.04	0.73 ± 0.05	0.61 ± 0.03	0.60 ± 0.04
%Nitrogen^{ns}	0.10 ± 0.01	0.12 ± 0.01	0.10 ± 0.018	0.10 ± 0.01
L*	82.59 ± 0.01^{d}	83.46 ± 0.01^{c}	84.03 ± 0.03^{b}	84.47 ± 0.00^{a}
a*	3.55 ± 0.01^{a}	2.98 ± 0.01^{b}	2.57 ± 0.01^{c}	2.52 ± 0.00^{d}
b*	46.90 ± 0.04^{a}	44.15 ± 0.03^{b}	42.89 ± 0.02^{c}	42.60 ± 0.03^{d}
Physiochemical properties	AMPH (hydrolysis time: hrs)			
	6	12	18	24
%TSS	2.50 ± 0.03^{c}	2.70 ± 0.00^{b}	3.10 ± 0.32^{a}	3.10 ± 0.03^{a}
pH	6.46 ± 0.02^{a}	4.60 ± 0.02^{b}	4.56 ± 0.04^{b}	4.54 ± 0.02^{b}
%NaCl	2.30 ± 0.00^{d}	2.80 ± 0.00^{c}	2.90 ± 0.00^{b}	3.00 ± 0.00^{a}
%Protein^{ns}	0.32 ± 0.02	0.36 ± 0.05	0.34 ± 0.03	0.34 ± 0.03
%Nitrogen^{ns}	0.05 ± 0.00	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.00
L*	72.62 ± 0.01^{b}	73.92 ± 0.03^{d}	75.62 ± 0.02^{c}	77.19 ± 0.01^{a}
a*	8.56 ± 0.00^{a}	5.54 ± 0.01^{b}	5.33 ± 0.01^{c}	4.94 ± 0.01^{d}
b*	49.65 ± 0.02^{a}	43.77 ± 0.02^{c}	43.51 ± 0.02^{d}	44.35 ± 0.01^{b}

Physiochemical properties	SHMPH (hydrolysis time: hrs)			
	6	12	18	24
%TSS	2.20 ± 0.03^{d}	2.50 ± 0.09^{c}	2.80 ± 0.03^{b}	3.20 ± 0.07^{a}
pH	6.02 ± 0.04^{a}	5.89 ± 0.02^{b}	5.89 ± 0.01^{b}	5.87 ± 0.03^{b}
%NaCl	2.20 ± 0.00^{d}	2.40 ± 0.00^{c}	2.60 ± 0.00^{b}	2.80 ± 0.00^{a}
%Protein^{ns}	0.63 ± 0.04	0.69 ± 0.02	0.77 ± 0.03	0.72 ± 0.02
%Nitrogen^{ns}	0.10 ± 0.01	0.11 ± 0.00	0.12 ± 0.01	0.12 ± 0.00
L*	70.82 ± 0.01^{a}	64.73 ± 0.01^{b}	64.31 ± 0.03^{c}	64.19 ± 0.00^{d}
a*	14.49 ± 0.01^{d}	18.29 ± 0.00^{c}	19.65 ± 0.02^{b}	19.83 ± 0.01^{a}
b*	66.99 ± 0.00^{d}	70.44 ± 0.03^{c}	72.15 ± 0.03^{b}	72.49 ± 0.02^{a}

Note : $^{a-d}$Different superscripts in the same row of each MPH indicate significant differences ($p \leq 0.05$).

^{ns}No significant differences in the same row of each MPH.

3.4 Free amino acids composition

The amino acid compositions of MPHs are shown in Table 2. The total amino acid content of OMPH (341 - 384 mg/100 ml) was highest when compared with the AMPH (119 – 157 mg/100 ml) and SHMPH (80 - 110 mg/100 ml). The changing of amino acid level affected by DH could also modulate the biological activity of the peptides formed during hydrolysis (Jamdar *et al.*, 2010). These different observations are possibly be the amino acid composition and solubility of peptides (Netto and Galeazzi, 1998). As a result of enzymatic hydrolysis, precipitation and separation of the insoluble fraction, the obtained protein hydrolysates might have the different amino acid composition from the different hydrolysis time.

In terms of individual amino acids, the most abundant amino acids found in all three MPHs were glutamic acid (20 – 40%), this result was in agreement with the reports of Chirinang and Intarapichet (2009). It could be explained that Glutamate has been deaminated to Glutamic acid during the hydrolysis (Aaslyng *et al*., 1998). Lysine, a vital amino acid for children lacking protein, was not found in Oyster mushroom and Abalone mushroom. However, it can be found in both MPHs after 12 hours hydrolysis compared to the decreasing of arginine content. In chemoenzymatic peptide synthesis, arginine was cleaved by papain enzyme, while lysine was synthesized using the N-protecting groups viz. the tert-butoxycarbonyl and carboxybenzyl groups (Yazawa and Numata, 2014).

Table 2 Free amino acid composition of mushroom protein hydrolysates from Oyster mushroom (OMPH), Abalone mushroom (AMPH) and Shiitake mushroom (SHMPH).

Amino acids	OMPH				AMPH				SHMPH			
(mg/100ml)	6	12	18	24(hrs)	6	12	18	24(hrs)	6	12	18	24(hrs)
Aspartic acid	19.81	21.01	20.68	20.95	2.66	4.41	4.50	3.76	3.16	3.94	1.16	0.77
Serine	21.66	25.80	25.82	23.99	10.63	13.58	14.06	13.31	3.76	5.82	3.01	3.12
Glutamic acid	44.00	47.70	46.32	47.78	31.22	32.06	36.10	36.33	14.23	17.68	20.61	21.55
Glycine	9.26	12.76	13.17	11.88	6.01	7.27	7.68	7.41	1.94	2.52	2.73	2.77
Histidine	10.07	11.11	11.30	11.20	4.10	4.93	5.23	5.06	1.23	1.74	1.74	1.73
Arginine	32.47	22.55	22.77	24.69	5.54	-	-	-	6.45	9.70	9.42	9.55
Threonine	23.98	25.77	25.99	25.25	11.58	13.04	13.77	13.40	5.56	7.38	7.56	8.03
Alanine	31.35	33.55	33.17	32.36	11.85	13.30	14.68	14.23	9.12	11.47	12.46	12.92
Proline	7.63	10.94	11.39	10.54	4.44	6.63	7.76	7.90	0.89	1.59	1.71	1.71
Tyrosine	23.06	22.82	23.68	22.97	2.65	3.64	3.66	2.51	2.75	3.52	3.44	3.62
Valine	27.83	28.58	29.04	28.47	10.22	11.66	12.69	1200.	7.43	9.84	10.21	10.61
Methionine	5.69	4.87	5.03	4.92	-	-	-	-	-	-	-	-
Lysine	-	25.53	27.66	29.55	-	10.42	12.26	12.55	3.31	5.16	5.05	5.05
Isoleucine	22.83	23.58	24.12	22.95	6.60	7.28	8.27	7.08	5.29	7.29	7.41	7.67
Leucine	36.78	37.21	38.05	36.40	6.33	8.15	10.34	9.33	9.03	12.09	12.14	12.53
Phenylanine	24.66	24.56	25.61	24.53	5.24	5.35	6.19	5.32	6.08	7.95	7.87	8.40
Cystine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total	341	378	384	378	119	142	157	150	80	108	107	110

Note : ND is none detected

4. Conclusion

All of mushroom protein hydrolysates (MPHs) producing by papain enzyme with the different hydrolysis times exhibited the different physicochemical properties. Oyster mushroom protein hydrolysate showed the higher degree of hydrolysis, soluble protein content and free amino acid composition than that of Abalone and Shiitake mushrooms. The suitable timing to produce OMPH, AMPH and SHMPH with papain hydrolysis were 18, 18 and 24 hours, with the approximately highest of %DH of 46, 38 and 25 providing the highest total amino acid content of 384, 157 and 110 mg/100 ml and %yield of 55, 65 and 63, respectively (data not show in this paper). There is a possibility that the selected mushroom could be produced as natural flavor and seasoning products. However, further work could be done on flavor characteristics, sensory profile, and applications in food products.

Acknowledgements

The authors would like to thank Thailand Institute of Scientific and Technological Research (TISTR) for financial supporting.

References

Aaslyng, M.D., Martens, M., Poll, L., Nielsen, P.M., Flyge, H. and Larsen, L.M. 1998. Chemical and sensory characterization of hydrolyzed vegetable protein, a savory flavoring. Journal of Agricultural and Food Chemistry. 46: 481–489.

Alves, M.J., Ferreira, I.C.F.R., Dias, J., Teixeira, V., Martins, A. and Pintado, M. 2012. A review on antimicrobial activity of mushroom (*Basidiomycetes*) extracts and isolated compounds. Planta Medica. 78: 1707–1718.

AOAC. 2000. Official methods of analysis of AOAC International. 17th edition. Gaithersburg, MD, USA, Association of Analytical Communities.

BD Biosciences. 2009. Hydrolysis to hydrolysate. URL (http://www.bdbiosciences.com) (December 15th, 2016).

Chirinang, P. and Intarapichet, K. 2009. Amino acids and antioxidant properties of the oyster mushrooms, *Pleurotus ostreatus* and *Pleurotus sajor-caju*. Science Asia. 35: 326–331.

Hall, G.M. and Ahmad, N.H. 1992. Functional properties of fish protein hydrolysates. In G.M. Hall, (Ed.). Fish Processing Technology. Blackie Academic and Professional, New York.

Haslaniza, H., Maskat, M.Y., Wan Aida, W.M. and Mamot, S. 2010. The effects of enzyme concentration, temperature and incubation time on nitrogen content and degree of hydrolysis of protein precipitate from cockle (*Anadara granosa*) meat wash water. International Food Research Journal. 17: 147–152.

Howell, N.K. 1996. Chemical and enzymatic modification. In S. Nakai and H.W. Modler (Eds.). Food Proteins Properties and Characterization (pp. 235-280). VCH. New York.

Jamdar, S.N., Rajalakshmi, V. Pednekar, M.D., Juan, F., Yardi, V. and Sharma, A. 2010. Influence of degree of hydrolysis on functional properties, antioxidant activity and ACE inhibitory activity of peanut protein hydrolysate. Food Chemistry. 121: 178–184.

Palupi, N.W., Windrati, W.S. and Tamtarini. 2010. The effect of enzymatic hydrolysis on the properties of protein hydrolysate from paddy mushroom. Makara Journal of Technology. 14(2): 73–76.

Netto, F.M. and Galeazzi, M.A.M. 1998. Production and characterization of enzymatic hydrolysate from soy protein isolate. Food Science and Technology. 31: 624–631.

Ren, L., Perera, C. and Hemar, Y. 2012. Antitumor activity of mushroom polysaccharides: a review. Food and Function. 3: 1118–1130.

Shiau, C.Y. and Chai, T.J. 1999. Protein recovered from oyster wash water by ultrafiltration and their utilization as oyster sauce through fermentation. Journal of Marine Science and Technology. 7(2): 110–116.

Solina, M., Johnson, R.L. and Whitfield, F.B. 2007. Effects of soy protein isolate, acid-hydrolysed vegetable protein and glucose on the volatile components of extruded wheat starch. Food Chemistry. 104(4): 1522–1538.

Sujith, P.A. and Hymavathi, T.V. 2011. Recent developments with debittering of protein hydrolysates. Asian Journal of Food and Agro-Industry. 4(06): 365–381.

Thanasukhon, M. 2009. Production of enzymatic papain mushroom protein hydrolysate for flavoring agent. Thesis. Master of Science. Biochemical Technology, School of Bioresources and Technology. King Mongkut's University of Technology Thonburi.

Thanasukhon, M., Laohakunjit, N., Kerdchoechuen, O. and Bunjongsinsiri, P. 2009. Effect of mushroom protein hydrolysates by acid on physicochemical properties and volatile compounds. Agricultural Science Journal (Supplement). 40(3): 37–40.

Velíšek, J. and Cejpek, K. 2011. Pigments of higher fungi: a review. Czech Journal of Food Sciences. 29(2): 87–102.

Yanfang, Z., lijuan, W. and Wenyi, T. 2009. Biochemical changes in low-salt fermentation of solid- state soy sauce. African Journal of Biotechnology. 8(24): 7028–7034.

Yazawa, K. and Numata, K. 2009. Recent advances in chemoenzymatic peptide syntheses. Molecules. 19: 13755–13774.

Zarei, M., Ebrahimpour, A., Abdul-Hamid, A., Anwar, F., Bakar, F.A., Philip, R. and Saari, N. 2014. Identification and characterization of papain-generated antioxidant peptides from palm kernel cake proteins. Food Research International. 62: 726–734

Zhu, F., Du, B., Bian, Z. and Xu, B. 2015. Beta-glucans from edible and medicinal mushrooms: characteristics, physicochemical and biological activities. Journal of Food Composition and Analysis. 41: 165–173.