BIOCHEMICAL PROPERTIES OF MYOFIBRILLAR PROTEIN HYDROLYSATES ISOLATED FROM CATFISH (*Pangasius sutchi*)

Leila Najafian[1], Mehdi Nadalian[1], Abdul Salam Babji[1]

[1]School of Chemical Sciences and Food Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia

Abstract – The effects of different proteolytic treatments on the biochemical properties of protein hydrolysates from patin, a freshwater fish, were investigated. The myofibrillar protein isolate was digested using papain and Flavourzyme® 500L to produce protein hydrolysates (MPH) with varying properties. The effect of hydrolysis time (30, 60, 90, 120 min) with concentration of 1% (v/w substrate); pH = 7.0 was studied to determine the degree of hydrolysis (DH), amino acid composition, peptide content. Results showed that the highest DH of both papain and Flavourzyme were 89.17% and 74.17% after 120 min incubation respectively. The major amino acids of both hydrolysates were Glu, Asp and Lys. The amino acid composition of the papain hydrolysate differed from the Flavourzyme hydrolysate. Content of essential amino acids of papain and Flavourzyme hydrolysates were 49.01% and 47.84% respectively. The peptide content of myofibrillar protein hydrolysates were significantly increases by the increase in time of incubation ($p < 0.05$). These results suggest that protein hydrolysates derived from patin have functional properties which can be further explored.

Key Words: Enzymatic hydrolysate, Degree of hydrolysis, Amino acid composition, Peptide content.

I. INTRODUCTION

Fish is known to contain certain polyunsaturated fatty acids that can regulate prostaglandin synthesis and hence induce wound healing [1]. ω-3 and ω-6 polyunsaturated fatty acids composition may very among species of fish, even among fresh water and marine fish [2]. Many studies have demonstrated that functional properties of protein can be improved by enzymatic hydrolysis under controlled conditions [3]. Hydrolysis potentially influences the molecular size, hydrophobicity and polar groups of the hydrolysate [4]. Protein hydrolysate has an excellent solubility at high degree of hydrolysis [5]. In the present study, myofibrillar protein from freshwater fish, *P. sutchi*, was used as substrate for two proteases like using papain and Flavourzyme 500L to measure degree of hydrolysis and the compositions of the fish protein hydrolysis.

II. MATERIALS AND METHODS

Pangasius sutchi, were bought from a nearby farm. Flavourzyme 500L, an exopeptidase and endoprotease complex from *Aspergillus. oryzae* was obtained from Novo Nordisk A/S (Copenhagen, Denmark), and Papain from papaya latex and other chemicals of analytical grade were purchased from Sigma (Sigma–Aldrich). After extraction of myofibrillar protein by using alkali solubilisation method as described in Hultin and Kelleher [6], optimal pH and temperature for each enzyme was adjusted; papain (pH 7; 60 °C) and Flavoyrzyme 500 L (pH 7; 50 °C) and incubated with these enzymes for 30, 60, 90 and 120 min. The ratio of each enzyme to protein isolate was 1:100 (w/w). The enzymatic hydrolysis was stopped by boiling for 5 min. The hydrolysate was centrifuged at 3000 × g for 20 min and the supernatant was lyophilised. The degree of hydrolysis (DH) was estimated as described by Hoyle & Merritt [7]. Acid hydrolysis, performic acid and alkaline hydrolysis were performed using Waters AccQ.Tag amino acid analyzer (Waters Corporation, Irland). The peptide contents of hydrolysates were measured by the method of Church, Swaisgood, Porter, and Catignani [8] with some modifications using o-phthaldialdehyde (OPA) spectrophoto- metric assay.

III. RESULTS AND DISCUSSION

Figure 1 presented the effect of hydrolysis time on degree of hydrolysis of myofibrillar protein hydrolysates with Papain (MP) and Flavourzyme (MF) enzymes. Degree of hydrolysis differed ($P < 0.05$) depending on the use of papain or flavourzyme, and reaction time. Myofibrillar protein showed higher rate of hydrolysis when treated with papain than of Flavourzyme. The rate of enzymatic hydrolysis was not increased after 90 min significantly. It could be explained by a decreased in the concentration of peptide bonds available for hydrolysis or the inhibition of the enzyme by the products formed at high degree of hydrolysis.

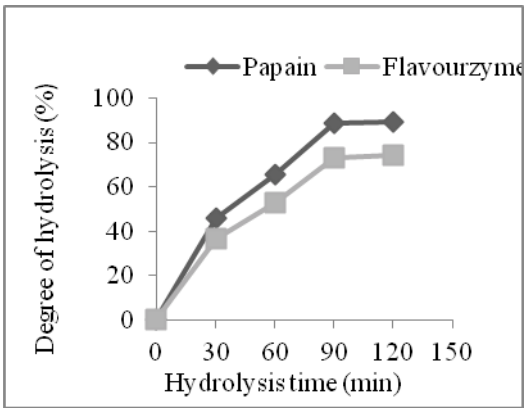

Figure 1: Effect time of hydrolysis on Degree of hydrolysis of myofibrillar protein hydrolysates

The amino acid compositions of the patin MPHs and unhyrolysed patin myofibrillar protein are shown in Table 1. Although the major amino acids of both hydrolysates were Glu, Asp and Lys, the amino acid composition of the papain hydrolysate differed from the Flavourzyme hydrolysate. Based on the total amino acids, the essential amino acids comprised 49.01%, 48.04% and 47.82% of the papain-MPH, the Flavourzyme-MPH and UPMP, respectively, providing an excellent source of these valuable nutrients.

The peptide content of myofibrillar protein and its hydrolysates were significantly increased by the increase in time of incubation ($P < 0.5$) .The peptide content of myofibrillar protein and its hydrolysates were increased from 85.4 to 299.1 mg/g (Table 2).

Table 1 Amino acid composition of myofibrillar protein of *P. sutchi* and its hydrolysates (%)[a]

AA%	UPMP[b]	Papain-MPH	Flavourzyme-MPH
Asp	10.17 ± 0.16	10.22 ± 0.17	10.15 ± 0.12
Ser	4.18 ± 0.11	4.24 ± 0.18	4.14± 0.15
Glu	15.99 ± 0.22	16.12 ± 0.30	18.02 ± 0.24
Gly	3.56 ± 0.14	3.57 ± 0.17	3.30 ± 0.12[c]
His*	2.37 ± 0.16	2.61 ± 0.20	2.26 ± 0.12[c]
Arg	4.88 ± 0.26	4.80 ± 0.18	4.49 ± 0.25[bc]
Thr*	6.26 ± 0.82	6.49 ± 0.18	6.26 ± 0.15
Ala	5.36 ± 0.88	5.48 ± 0.26	5.52 ± 0.23
Pro	2.99 ± 0.64	3.05 ± 0.12	2.49 ± 0.17
Tyr	3.49 ± 0.28	2.79 ± 0.13	2.44 ± 0.17
Val*	4.77 ± 0.02	4.73 ± 0.14	4.68 ± 0.19
Met*	5.43 ± 0.57	5.40± 0.23	5.68 ± 0.11
Lys*	9.34 ± 0.66	9.9 ± 0.11	10.37 ± 0.13
Ile*	4.32 ± 0.42	4.33 ± 0.11	4.26 ± 0.19
Leu*	7.86 ± 0.27	7.88 ± 0.18	7.84 ± 0.11
Phe*	3.63 ± 0.29	3.26 ± 0.11	2.87 ± 0.12
Cys	1.56 ± 0.15	1.60 ± 0.17	1.49 ± 0.12
Trp*	3.84 ± 0.17	4.41 ± 0.10	3.84 ± 0.18
TEAA[c]	47.82	49.01	48.04

[a] Values are reported as the mean ± SD, experiments were performed in triplicate.
[b] UPMP: Unhydrolyzed Patin Myofibrillar Protein.
[c] Total essential amino acids.
* Essential amino acid.

Table 2. Effect of hydrolysis time on degree of hydrolysis and peptide content of myofibrillar protein hydrolysates

Samples	Times of incubation (min)	DH (%)	Peptide content (mg/g)
Myofibrillar protein		-	85.4 ± 1.47
Papain-MPH	30	46.16 ± 1.41	245.2 ± 0.98
	60	65.83 ± 1.28	261.4 ± 1.14
	90	88.53 ± 0.25	279.2 ± 1.47
	120	89.17 ± 0.31	299.1 ± 1.71
Flavourzyme-MPH	30	36.53 ± 0.70	188.0 ± 1.02
	60	52.80 ± 1.36	196.8 ± 1.22
	90	72.80 ± 0.86	210.2 ± 0.98
	120	74.16 ± 0.53	224.2 ± 1.47

Values are reported as the mean ± SD, experiments were performed in triplicate.

IV. CONCLUSION

The protein hydrolysate derived from freshwater fish patin may potentially serve as a good source of desirable quality peptides and amino acids. The peptide content increases with higher degree of hydrolysis. In this study, protein hydrolysis by Papain had the highest peptide content.

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