

# ANTIOXIDANT ACTIVITY OF PORCINE LIVER PROTEIN HYDROLYSATES

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**Abstract** – In this study, porcine liver was hydrolyzed by alcalase, papain, pepsin, or *Monascus purpureus* microbial suspension (APLH, PaPLH, PePLH and MPLH, respectively), and analyzed. The result showed that yields of hydrolysates increased with hydrolysis time. The hydrolysate that hydrolyzed with alcalase had the highest yield, whereas the hydrolysate that hydrolyzed with papain had a higher degree of hydrolysis ( $P < 0.05$ ). The hydrolysate that hydrolyzed with *Monascus purpureus* microbial suspension had the highest 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and reducing power ( $P < 0.05$ ).

**Keyword** - Antioxidant activity, *Monascus purpureus*, Protein hydrolysates,

## I. INTRODUCTION

Porcine liver, which is a by-product during slaughtering, contains various nutrients. In addition to as edible by-products, utilization of this by-product has become a great concern. *Monascus* spp. which is food fungus, has been widely applied for making wines and other fermented food products, especially in many Asian areas [1]. In Taiwan, as regarded as a natural food additive source, *Monascus* spp. has also been approved as an edible natural colorant and allowed to be applied to foods. It has been reported the degradation of soybean protein by an acid proteinase from *Monascus anka* [2]. Some functional peptides have been reported to be isolated from Tofuyo fermented soybean food [3]. Additionally, some antioxidative peptides have been obtained from the various protein hydrolysates [4]. Therefore, the objective of this study was to evaluate the antioxidative properties of porcine liver hydrolysates which were hydrolyzed by *Monascus purpureus* microbial suspension, papain, pepsin or alcalase under various hydrolysis times.

## II. MATERIAL AND METHODS

*Monascus purpureus* CCRC 31499 was obtained from the Culture Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan. The methods described by [5] and [6] were utilized and described briefly as followed. *Monascus purpureus* was inoculated into a yeast glucose booth medium which containing 10% glucose and 0.8% yeast extract, the pH adjusted to 5.5, incubated at 35°C with 120 rpm shaking for 7 days. Then, the inoculums were homogenized with a sterile blender at high speed for 2 min, and filtered though No. 1 filter paper. The microbial suspension was prepared accordingly, and stored at 4°C for up to 3 days, and ready for use.

Homogenized porcine liver which mixed with water (1:2, w/v) thoroughly was heated at 95-100°C for 5 min to inactive indigenous enzymatic activities. After cooling, the mixtures with pH adjusted to the optimum conditions were added with enzymes accordingly (Table 1), and functioned at 37 or 50°C for 3, 6, or 12 hours. After heating at 95-100°C for 10 min to inactive enzymatic activities, the mixtures were centrifuged at 10,000 g for 10 min. Finally, the liver enzymatic hydrolysates were obtained after filtering the supernatant, followed by lyophilization and storage at -80°C until use.

Yields and degrees of hydrolysis of hydrolysates were determined according to the methods of Hsu [7]. DPPH radical scavenging activity was determined according to the methods described [8]. DPPH (2, 2-Diphenyl-1-picrylhydrazyl) scavenging (%) =  $[1 - (\text{absorbance sample} / \text{absorbance blank})] \times 100$  at a wave-length of 517 nm. Reducing power of samples was determined at a wave-length of 700 nm [9].

Data were analyzed using the ANOVA (Analysis of variance) of Statistical Analysis System's Procedures (SAS Institute Inc., Cary, NC) with a 5% level of significance. Means were separated using Scheffe's method.

Table 1 Optimum hydrolysis conditions of particular enzymes

Enzymes	Pepsin	Papain	Alcalase	<i>Monascus purpureus</i> microbial suspension
E/S	1:100	1:100	1:100	20:100
pH	3	6.5	8	5
Temperature	37°C	37°C	50°C	37°C

### III. RESULT

Figure 1 showed that yields of hydrolysates increased with hydrolysis time. This increase due to the longer hydrolysis time was more obviously for the hydrolysates hydrolyzed with alcalase or papain. A hydrolysate that hydrolyzed with alcalase for 12 hours had the highest yield of 20.9%, whereas a hydrolysate that hydrolyzed with *M. purpureus* microbial suspension for 3 hours had the lowest yield of 7.1%. Figure 1 illustrates that the degrees of hydrolysis increased with hydrolysis time. In this study, degree of hydrolysis of hydrolysates increased with hydrolysis time and agreed with Lakshman et al. [10]. Samples hydrolyze with papain had higher degrees of hydrolysis, followed by those hydrolyzed with pepsin or alcalase, and finally that samples hydrolyzed with *M. purpureus* microbial suspension. A hydrolysate that hydrolyzed with papain for 12 hours had the highest degree of hydrolysis (30.6%), whereas the sample hydrolyzed with *M. purpureus* microbial suspension for 3 hours had the lowest degree of hydrolysis (0.6%). This lower degree of hydrolysis was probably because the enzymes in this *M. purpureus* microbial suspension had lower enzyme activity. The hydrolysates that hydrolyzed with alcalase had higher yields but without higher degrees of hydrolysis, whereas the hydrolysates that hydrolyzed with papain or pepsin had higher yields with higher degrees of hydrolysis.

The result showed that samples hydrolyzed with *M. purpureus* microbial suspension or pepsin had higher DPPH scavenging activities than those hydrolyzed with papain or alcalase (Fig 2a). The DPPH scavenging activities of protein hydrolysates that hydrolyzed by *M. purpureus* microbial suspension, pepsin, or alcalase increased with hydrolysis time, whereas the DPPH scavenging activities of sample that hydrolyzed with papain decreased. Reducing power of porcine liver hydrolysates that hydrolyzed with *M. purpureus* microbial suspension was higher than that of hydrolysates that hydrolyzed with other enzymes (Fig. 2b). Reducing power of the hydrolysate that

hydrolyzed with papain decreased after hydrolysis for 6 and 12 hours. The DPPH scavenging activities of retorted gelatin hydrolysates from cobia skin increased with hydrolysis time, up to its maximal value at 2 h and then decreased at 3 h [11]. Antioxidative properties, including DPPH scavenging and reducing power, of loach protein hydrolysates increased with the degree of hydrolysis between 18 and 23%; however, these antioxidative properties were further decreased when the degree of hydrolysis was elevated up to 33% [12]. In the same study, this pattern of increase and then decrease was also coincided with the total antioxidant amino acid contents of the hydrolysates along with the degree of hydrolysis. It might be due to the non-specific activity of papain which could cleavage different sites of hydrolysates [13].

### IV. CONCLUSION

Yield and degree of hydrolysis of porcine liver hydrolysates increased with time. Samples that hydrolyzed with *M. purpureus* microbial suspension had higher DPPH scavenging activity and reducing power even though it had comparatively lower yield. How to increase its yield should be addressed in the future.

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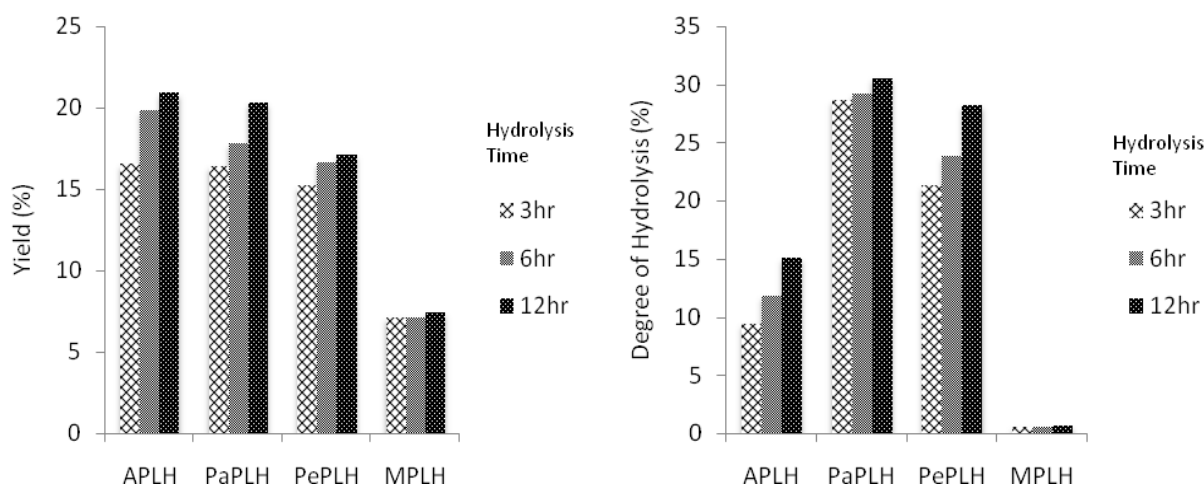


Figure 1 (a) Yield and (b) degree of hydrolysis of porcine liver hydrolysates

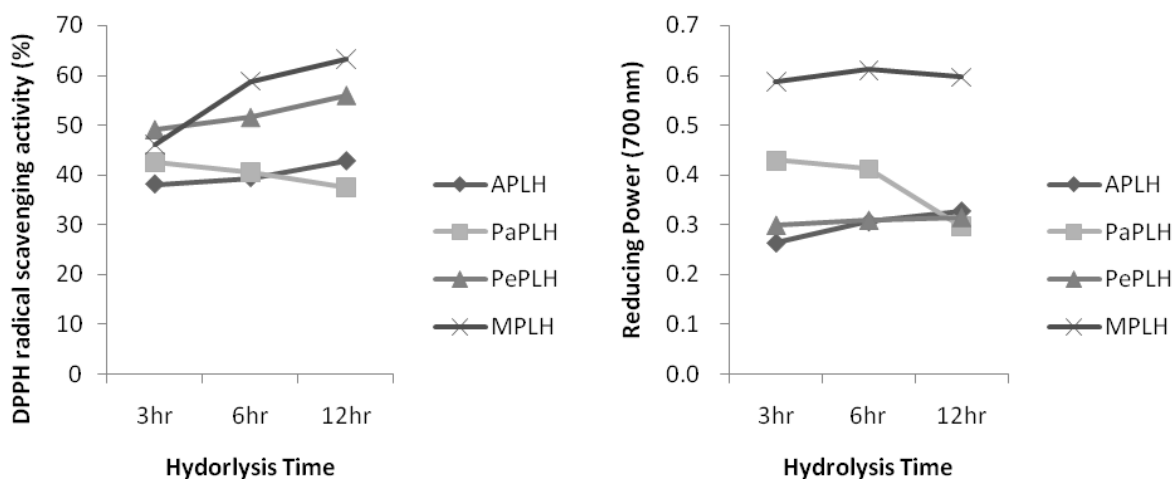


Figure 2 (a) DPPH scavenging activity and (b) reducing power of porcine liver hydrolysates

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