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Research Article

Hydroxyl Radical Scavenging Activity of Peptide from Fish Intestine Protein by Hydrolysis with Complex Enzyme

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Abstract: Fish intestine is a byproduct of fish, which is often discarded and causes environmental pollution. In this study, the hydrolysates, with Hydroxyl-Radical-Scavenging Activity (HRSA), were prepared grass carp intestine protein by hydrolysis with complex enzyme. The hydrolytic process was monitored by HRSA and the hydrolysis conditions were optimized as follows: reaction time of 2 h, temperature 50°C, pH 6.5 and 12 mg complex enzyme 50 U/g protein. Under these conditions, maximum HRSA was obtained. The Protein Hydrolysates (PHs) were fractionated into four ranges on the basis of molecular weight using a gel column chromatography. Results indicate that PHs in the PH_3 induced the highest HRSA.

Keywords: Antioxidant activity, fish intestine, hydroxyl-radical-scavenging activity, peptide

INTRODUCTION

Fishes are rich in essential amino acids, eicosapentaenoic acid, 26 docosahexaenoic acid, vitamins, minerals and lecithin (Lall and Tibbetts, 2009). A large quantity of fishes is consumed in China each year. Consequently, also a large quantity of fish intestine is produced and often used as folder resources, leading to low economic efficiency. This has highlighted the need to develop value-added products from fish intestine.

Free radicals are unavoidably produced during oxidation process in all living organisms and excess free radicals can cause destructive effects on living tissues and foodstuffs (Wang *et al*., 2007). Among all free radicals, hydroxyl radical is considered to be the most reactive and can damage almost any compound in contact with in the living cells (Castro and Freeman, 2001). Therefore, there is a growing interest to identify antioxidative properties in many natural sources including some dietary protein compounds (Dong *et al*., 2008).

In recent years, the antioxidant activity of bioactive peptides from the hydrolysates of various proteins has attracted much attention. The peptides from swine, milk, eggs, mackerel protein, giant squid muscle, Alaska pollack frame protein and jumbo squid skin have been reported to show antioxidant activity (Ren *et al*., 2008a). In addition, aquatic products and by-products have also proven to be good sources of antioxidant peptides (Dong *et al*., 2008; Elavarasan *et al*., 2013; Li *et al*., 2006; Li *et al*., 2012; Ren *et al*., 2008a, b, 2010; Zhong *et al*., 2011).

So far, there is little information regarding the antioxidant effect of protein hydrolysates from the grass carp (*Ctenopharyngodon idellus*) intestine by enzymatic treatment. Thus, the objective of this study was to determine the antioxidant activity of the protein hydrolysate from grass carp intestine by complex enzyme. The antioxidative properties of grass carp intestine protein hydrolysate may lead to utilize fish by-producs as a natural antioxidant.

MATERIALS AND METHODS

Materials: The grass carp intestines were collected at the 1st canteen of Huaihai Institute of Technology, Lianyungang, China. The grass carp intestines were washed with tap water and minced in a MM 12 mincer (Shaoguan Food Machine Co., China). The minced material was frozen and stored at -20°C until use. The trypsin (1,200,000) and papain (1,000,000 U/g) were purchased from the Sinopharm Chemical Reagent Co., Ltd., (Shanghai, China). The complex enzyme was prepared by mixing the trypsin and papain at the ratio of 1:1 based on weight.

Hydrolyzing grass carp intestine protein with complex enzyme: The frozen minced grass carp intestine mince was thawed and mixed with deionized water to obtain a suspension of a concentration of 0.06% (w/v). The suspension was adjusted to the required pH (5, 5.5, 6, 6.5, 7 and 7.5, respectively) with

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0.01 M NaOH or 0.01 M HCl, heated in a water bath to the required temperatures (35, 40, 45, 50, 55 and 60°C, respectively) and then added different amount of the complex enzyme (3, 4, 5, 6, 7 and 8%, respectively). The hydrolysis reaction was carried out in a shaking incubator (New Brunswick Scientifics C 24, China). At the end of the hydrolysis period, the mixtures were heated in boiling water for 10 min to inactivate the proteases. The hydrolysates were centrifuged in a GL-21M refrigerated centrifuge (Xiangyi Instrument Co. Ltd., Changsha, China) at 4,000×g for 20 min. The supernatant obtained was fractionated using an ultra-filtration membrane bioreactor system (Millipore, USA) into four ranges of molecular weight as follows: PH_1: >10 kDa; PH_2: 10 to >5 kDa; PH_3: 5 to 1 kDa; PH_4: <1 kDa. The above mentioned different fractions were tested to evaluate their HRSA.

Analytical methods: Hydroxyl radicals were generated by an iron-catalyzed Fenton Haber-Weiss reaction and the hydroxyl radicals generated were rapidly made to react with nitrone spin trap 5, 5-dimethyl-1-pyrroline-N-oxide. The resultant DMPO-OH adducts could be detectable with an Electron Spin Resonance (ESR) spectrometer. The frozen peptide solution was thawed. The peptide solution (20 μL) was mixed with DMPO (0.3 mol/L, 20 μL), $FeSO_4$ (10 mmol/L, 20 μL) and H_2O_2 (10 mmol/L, 20 μL) in a phosphate buffer solution (pH 7.4) and then transferred into a 100-μL quartz capillary tube. After 2.5 min, the ESR spectrum was recorded using an ESR spectrometer. Experimental conditions for this procedure were as follows: Magnetic field, 336.5±5 mT; power, 1 mW; modulation frequency, 9.41 GHz; amplitude, 1×200; and sweep time, 4 min. HRSA was calculated according to the following equation:

$$HRSA = \frac{1-H}{H_0} \times 100\%$$

where, H and H_0 are relative peak height of radical signals with and without sample, respectively (Pan *et al*., 2012).

RESULTS AND DISCUSSION

Effect of time on hydrolysis of grass carp intestine protein: Hydrolysis of grass carp intestine protein by using the complex enzyme was carried out for 2.5 h. As shown in Fig. 1, HRSA showed a sharp increase with time up to 1 h into the reaction, a slower increase over 1 to 1.5 h, reaching the maximum at 1.5 h, followed by a sharp decrease over 1.5 to 2.5 h. Therefore, the optimum reaction time was thus ascertained to be 1.5 h. This could be attributed to the fact that amino acids or peptides with too low degree of polymerization have low antioxidant activity. It is interesting to note that at the beginning of hydrolysis (reaction time: 0 h), the grass carp intestine slurry already has some HRSA,

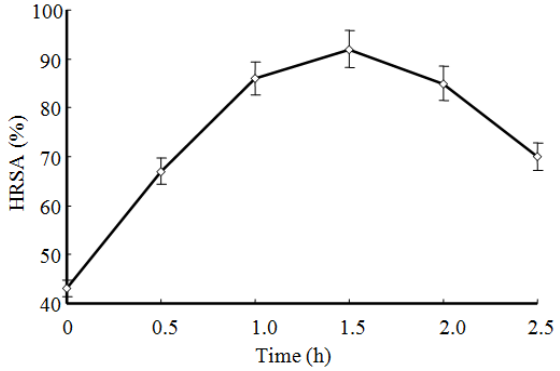

Fig. 1: Effect of time on hydrolysis of grass carp intestine protein by using the complex enzyme

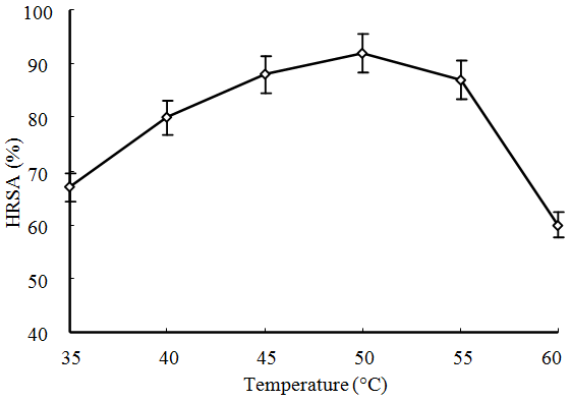

Fig. 2: Effect of pH on hydrolysis of grass carp intestine protein by using the complex enzyme

indicating that the grass carp intestine itself contains some quantities of natural materials that have HRSA.

Effect of pH, temperature and amount of the enzyme complex on hydrolysis of grass carp intestine protein: The pH, temperature of the reaction mixture and the amount of the enzyme complex used can play pivotal roles in the hydrolysis of grass carp intestine protein; therefore, the effect of pH, temperature and quantity of the complex enzyme on hydrolysis of grass carp intestine protein was investigated. The optimum hydrolytic conditions were found to be pH 6.5 (Fig. 2) at a temperature of 50°C (Fig. 3), with 6% of enzyme complex in the reaction mixture (Fig. 4). It is worth noting that excessive hydrolysis of sea cucumber decreased HRSA, for example, when too much enzyme complex was added. This could also be ascribed to the fact that amino acids or peptides with too low degree of polymerization have low antioxidant activity. This result was consistent with the observations by Pan *et al*. (2012).

HRSA of the peptides: The fractioned peptides showed variable HRSA (data not shown) and the

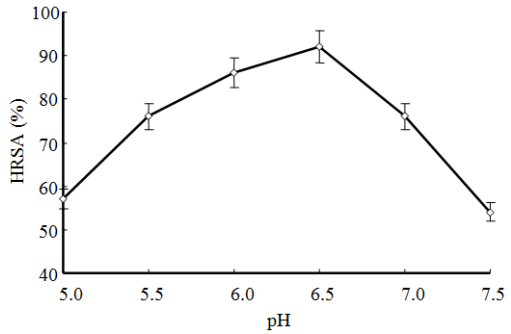

Fig. 3: Effect of temperature on hydrolysis of grass carp intestine protein by using the complex enzyme

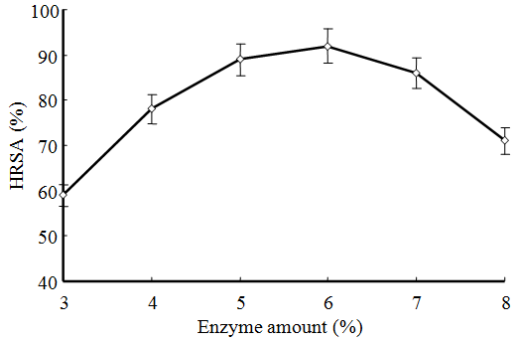

Fig. 4: Effect of complex enzyme amount on hydrolysis of grass carp intestine protein

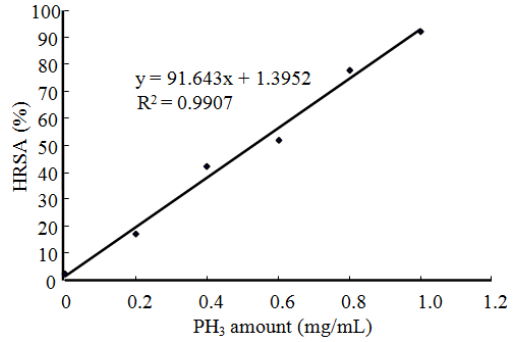

Fig. 5: The Hydroxyl Radical Scavenging Activity (HRSA) of the PH_3

fractions can be classified on the basis of HRSA as: PH_3>PH_4>PH_2>PH_1, indicating that peptides with too high or too low degree of polymerization had lower HRSA. The effect of concentration of PH_3 on HRSA is shown in Fig. 5. The results were subjected to best-fit linear regression and the coefficients were calculated, producing a fitted equation for predicting HRSA (Y) as follows:

$$Y = 91.643 \times x + 1.3952$$

where, x is the PH_3 concentration. The regression coefficient was 0.9907 for this reaction. In general, a regression model having an R^2 value >0.9 is considered to indicate a very high correlation (Haaland, 1989). The HRSA of the PH_3 reached 92.03% at a concentration of 1.0 mg/mL, thus indicating that the PH_3 has a very high HRSA.

CONCLUSION

The grass carp intestine protein can be effectively hydrolysed by the complex enzyme to yield peptides with antioxidant activity. The maximum antioxidant activity of peptides can be obtained under the optimum conditions of pH 6.5, temperature 50°C, enzyme amount 0.6% (w/v) and reaction time 1.5 h. The hydrolysates were centrifuged and fractioned. The PH_3 has a noticeable effect on the scavenging free radicals.

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