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In vitro and *in vivo* anti-oxidation and anti-fatigue effect of monkfish liver hydrolysate

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ABSTRACT

Monkfish liver, which is rich in protein, has high nutritious and medicinal value. Moreover, in recent studies, properties of bioactive peptides hydrolysates that make potential ingredients of health functional foods. In this study, monkfish liver hydrolysates (MLH) produced commercially enzyme. The *in vivo* and *in vitro* antioxidant activity and anti-fatigue activity of MLH were determined. The test of climbing endurance of mice were performance after one and half months of MLH administration in the anti-fatigue activity. After six weeks of MLH administration, mice were then sacrificed and assessed for biochemical parameters, SOD activity and MDA content in hepatic and kidney homogenates. Results showed that MLH contained the amino acids and peptides, which were expected to contribute to its antioxidant and anti-fatigue activities. The climbing period of experimental groups was increased compared with that of Control group. And execution with MLH could significantly increase SOD activity in hepatic and kidney. MDA contents of hepatic and kidney homogenate in the Aging Model had a higher level. MLH could significantly alleviate fatigue of the mice and had an anti-oxidative effect on aging mice. Conclusively, monkfish liver hydrolysates may be a beneficial ingredient to use in functional foods, indicating that monkfish liver is valuable for further study.

1. Introduction

The world generates plenty of by-productions in the fish industry, and one of the most quantities of waste is monkfish. Nowadays, Seven specials of monkfish found in the worldwide (Farina et al., 2008). The special of monkfish is *Lophius litulon* in the East China and Yellow Seas, and have become an important commercially fish (Jin, Zhang, & Xue, 2010; Yoneda et al., 2002). Monkfish is an important fish commodity, and also represent a globally important food fish occupying a high trophic degree in the marine food chain (Freeman et al., 2011). Nevertheless , The amount of by-products produced during monkfish processing can be as high as 60% of the total weight of the fish, and 10% of the waste is livers that can process into sashimi. The component of monkfish liver showed in the Table S1. However , monkfish livers are easy perishable products, which rapidly degraded after fishing. Thus, fresh monkfish liver cannot deliver to a longer distance and is directly discarded as processing waste. The other side , monkfish are composed of many useful protein , which can be converted into hydrolysate peptide. Environmental pollution, economic concerns and legal standard regarding the disposal of processing wastes cause to increased research in the exploitation and understand valuable products, such as bioactive peptides from these wastes (Wilson, Hayes, & Carney, 2011). Recently, several studies have reported on the using by-products from fish processing to convert into hydrolysate for the recovery of various valuable products (Zhong, Ma, Lin, & Luo, 2011, Rustad, Storro, & Slizyte, 2011, Nazeer & Kulandai, 2012, Kannan, Hettiarachchy, Marshall, Raghavan, & Kristinsson, 2011, Herpandi, Rosma, & Nadiah, 2011, Hathwar, Bijinu, Rai, & Narayan, 2011, Mendis, Rajapakse, & Kim, 2005). Several researchers have reported on hydrolysis peptides which hydrolyzed from tuna liver have various physiological functionalities such as antioxidant activities, anti-ACE activities (Ahn, Lee, & Je, 2010; Je, Lee, Lee, & Ahn, 2009). Peptides also had significant effects on the anti-fatigue of mice, which hydrolyzed from some difference resources such as milk, jellyfish and fish (Bøgwald, Dalmo, Leifson, Stenberg, & Glldberg, 1996; Ding et al., 2011; Pan, Guo, & Jiang, 2011; Park, Kim, Ahn, & Je, 2016; Ren, Zhao, Wang, Cui, & You, 2011; Zhang et al., 2016).

Hydrolysates are nutrition supplements and can absorb easily without competition from amino acids. They also take advantage of amino acids, proteins and glucose (van Loon, Saris, Kruijshoop, &

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Wagenmakers, 2000). Bioactive peptides effects a positive physiological influence on the body, which are hydrolysates with specific amino acid sequences (Choi, Sabikhi, Hassan, & Anand, 2012). According to 'radical theory', intense exercise can cause an imbalance in the body's oxidation system and its antioxidation system, thus cause the accumulation of reactive O_2^-(Harman, 1956). Therefore, bioactive peptides may be useful in postponing physical fatigue.

However, no studies on the *in vivo* anti-fatigue and antioxidation effection of hydrolysate from monkfish liver have yet reported. This paper researched on (as shown in Fig. S1) the effects of monkfish liver on anti-fatigue and anti-oxidation, and aimed to provide an experimental basis for further study of monkfish liver. Besides, it also may be the medicinal value.

2. Materials and methods

2.1. Materials

Institute of Cancer Research mice (18–22 g) were obtained from the Center of Laboratory Animal of Zhejiang Province. Monkfish (*L. litulon*) purchased from Ningbo Port and rinsed the monkfish livers by DI water then frozen at −20 °C until hydrolysate. The Qingchunbao Yong Zhen Tablets and Spirulina were purchased from Chiatai Qingchunbao Pharmaceutical Co., Ltd. And Green-A Co., Ltd. Kunming respectively. Enzyme (Protamex, 3.6×10^5U/g) purchased from Guangxi Pangbo Biological Engineering Company. Other reagents purchased from Nanjing Jiancheng Bioengineering Institute, such as Malonaldehyde (MDA), Superoxide dismutase (SOD), and other reagents used in this study were of analytical grade.

2.2. Preparation of the monkfish liver hydrolysates (MLH)

The 10 g of monkfish liver was homogenized, and then added 100 mL of PB buffer solution. The homogenate was digested with 2.5% (w/v) Protamex at 50 °C for 1–5 h.

After the hydrolysis, in order to inactivate the enzyme, the solutions were put in a boiling water bath for 10 min. The hydrolysates centrifuged at 4000 rpm for 10 min to remove the unhydrolyzed residue. Finally, the monkfish liver hydrolysates were obtained and stored at −25 °C until use (Ding et al., 2011; Je et al., 2009).

2.3. Determination of the degree of hydrolysis

The degree of hydrolysis (DH) of MLH determined following to the TNBS method of Adler (Adler-Nissen, 1979). The measurements of hydrolysates were in triplicates. The TNBS reagent consisted of 0.1% (w/v) TNBS in water. DH values were calculated by the formula (1) (Spellman, McEvoy, O'cuinn, & FitzGerald, 2003):

$$DH\% = \left(\frac{AN_{ML} - AN_{MLH}}{Nml}\right) \times 100 \quad (1)$$

where: AN_{mL} – the amino nitrogen content of the monkfish liver, mg g^{-1} protein; AN_{MLH} – the amino nitrogen content of the monkfish liver hydrolysates, mg g^{-1} protein; Nml – the nitrogen content of the monkfish liver, mg g^{-1} protein.

2.4. DPPH radical scavenging assay

In order to investigate the different degree of hydrolysis of MLH leading to difference scavenging capability, we design five different times of hydrolysis groups (1, 2, 3, 4, 5 h).

The scavenging effect of hydrolytic samples was estimated according to the method described previously (Liao, Wu, Lai, Lin, Liou & Chan, 2012). 0.5 mL of hydrolysate solution mixed with 1.5 mL of 100 μM DPPH in ethanol. These mixture solutions stayed in the dark environment for 30 min in room temperature. Then the solution measured by Spectrophotometer at 517 nm. The inhibitory ratio of DPPH was calculated by the Eq. (2) (Chaouch, Hafsa, Rihouey, Le Cerf, & Majdoub, 2016):

$$\text{DPPH scavenging effect}(\%) = \left(1 - \frac{Abs_s}{Abs_b}\right) \times 100 \quad (2)$$

where Abs_s is the absorbance value of the mixture hydrolysate solution, Abs_b is the absorbance value of the blank.

2.5. SDS-PAGE

The MLH obtained with different DHs were subjected to SDS-PAGE. Those MLH samples were dissolved in DI water to catch the concentration of 1 mg protein per mL. Then mixed 50 μL of a diluted solution and 100 μL of a sample buffer. After electrophoresis separation, the gel stained with the solution containing 0.05% Coomassie Brilliant Blue. Then, destaining was carried out with a solution of 7% (V/V) ethanol and 7% (V/V) acetic acid. Protein standards (Bio-Rad, Hercules, CA) with a range of molecular weight from 66.4 to 14.3 kDa (Chen et al., 2015).

2.6. Amino acid analysis

The amino acid profile of MHL determined according to the method of Ding et al. (Ding et al., 2011). The amino acid composition determined by HPLC (Agilent, 1200, USA). The total amino acids determined contain acid hydrolysis and alkaline hydrolysis. The amino acid standards were conducted the same conditions with the hydrolysate samples.

2.7. In vivo anti-oxidative experimental

Sixty ICR mice divided into five groups (each group with six female and six male mice): control group (C), aging model group (A), low dose group (L), high-dose group (H) and positive group (P). The control group was only feed distilled water (10 mL kg^{-1} d^{-1}), the other groups were administered D-galactose (1000 mg/kg d^{-1}). The aging model group was feed with DI water (10 mL kg^{-1} d^{-1}), the experimental group were fed hydrolysate of 5 mL kg^{-1} d^{-1} (L) and 10 mL kg^{-1} d^{-1} (H), and the positive were feed Qingchunbao Yong Zhen Tablets (4 g kg^{-1} d^{-1}). After six weeks had administered, all mice were detected such as MDA, SOD (Ding et al., 2011).

2.8. Anti-fatigue experimental

There are four mice groups (each group with six female and six male mice): the first group designated as a control group (Control), which was feed with DI water of 10 mL kg^{-1} body weight day. The second group was low dose group (L) was administered with MLH hydrolysate of 5 mL kg^{-1} per day. The 3rd group was high dose group (H) which was fed hydrolysate of 10 mL kg^{-1} per day. The fourth group was positive group (P) which was fed spirulina of 10 mg/kg body weight day. After six weeks conducted, anti-fatigue test will operate.

Rotarod test: the mice placed in the rotarod instrument (Fly de, Guangzhou, China) and received the Rotarod test (Fig. S3). The mice were standing on the rod while it is rotating at its low speed (4 rpm). The rotational speed of the rod then was increased to 40 rpm. To adapt the Rotarod instrument, the mice were practiced and the training time was 30 min for two days. Then fasted overnight, the mice feed hydrolysate, 30 min later the test started at the speed of 40 rpm. Everyday these 30 min test conducted for a week and average number of time to estimate the index of anti-fatigue effect (Lu et al., 2008).