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Influence of enzymatic hydrolysis conditions on the degree of hydrolysis and functional properties of protein hydrolysate obtained from Chinese sturgeon (*Acipenser sinensis*) by using papain enzyme

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ABSTRACT

In this study, protein hydrolysate was prepared from the muscles of Chinese sturgeon (*Acipenser sinensis*). The effects of different conditions on the degree of hydrolysis (DH) by using papain were investigated. The DH of 24.89% was attained under the optimum conditions including solid-to-liquid mixing ratio of 1:1, enzyme–substrate ratio of 3%, pH 6, temperature of 70 °C, and incubation time of 6 h. The yield of protein hydrolysate was 17.47%, in which the protein content was 79.67% and amino acid content was 96.35%. The molecular weight of peptides decreased with the progress in hydrolysis time. Protein hydrolysate solubility ranged between 86.57% and 98.74%, emulsifying activity index was 11.0–13.27 m^2/g, emulsion stability index was > 94% at different pH levels, water holding capacity was 1.93 g water/g protein, oil holding capacity was 2.59 g oil/g protein, and foam capacity was 76.67%. The obtained fish protein hydrolysate contains improved functional properties and has potential applications in food industries.

1. Introduction

Chinese sturgeons are considered as one of the largest anadromous fish species in the world. They are widely distributed along China's coastline and in the estuaries of the large rivers. Currently, the Yangtze river is the only river where Chinese sturgeons live [1]. Adult Chinese sturgeons range between 2 and 5 m in total length, and weigh between 200 and 500 kg, ranking them among the largest sturgeons in the world [2]. Among the different species of sturgeons, the Chinese sturgeon *Acipenser sinensis* has the fastest growth rate. It is a species of the protected fish and classified as a kind of migratory or semi-migratory fish species [3]. Recently, aquaculture of sturgeon has increased in many countries including China, which contributed more than 44,200 t in 2011 equivalent to about 86% of the world sturgeon meat production, and was statistically ranked in the second order in terms of the number of sturgeon farms until 2013 [4].

Protein hydrolysates are small fractions of peptides that contain several amino acids [5]. They can be obtained by using conventional chemical methods, strong chemicals, and solvents. These chemicals make the products unsuitable for use in food industry, while the use of enzymes to extract protein gives a better product in terms of nutritional value and functional properties [6]. Currently, the enzymatic hydrolysis technique of proteins is employed to recover the physiologically and nutritionally important peptides that result during the production of fish protein hydrolysates (FPHs). Several enzymes such as alcalase, papain, pepsin, flavourzyme, neutrase, protamex, trypsin, a-chymotrypsin, protease N, protease A, pancreatin, pronase, bromelain, cryotin F, orientase, validase, and thermolysin have been used to hydrolyze the fish proteins for the production of FPH [5]. Papain is a plant proteolytic enzyme and belongs to the cysteine proteinase family. It naturally exists in papaya (*Carica papaya L.*) but manufactured from the latex of raw papaya fruits. Papain breaks down proteins, which are made up of amino acids, known as polypeptides [7]. Enzymatic hydrolysis decreases the peptide size, thereby making FPH the most available amino acid source in human and animal food. FPH can be used as a protein source owing to their good functional properties [5]. Compared with autolytic hydrolysis, enzymatic hydrolysis presents better control conditions for the process in terms of the reaction speed and the high quality of the obtained products [8]. The use of enzymatic hydrolysis is often considered as an appropriate and useful method for improving the

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functional properties of proteins and maintaining their nutritional value [9]. The process depends on several factors including enzyme type, substrate, and hydrolysis conditions such as enzyme concentration, temperature, pH, and time. These factors cooperatively affect the enzyme activity and thus make the hydrolysis process more controllable [10,11]. To increase the solubility of proteins and improve the functional properties of fish proteins, enzymatic hydrolysis can be used because it is the most efficient method to achieve this purpose [9]. Proteins obtained by enzymatic hydrolysis with control of reaction conditions can be modified to enhance their quality and functional properties such as solubility, oil holding capacity (OHC) and water holding capacity (WHC), emulsification, foaming properties, and sensory properties [12,13]. The functional properties of FPH are affected by the substrate and enzyme used and the degree of hydrolysis (DH) [12,14]. FPHs can be used as healthy foods, functional foods, dietary supplement, and nutraceuticals, and they have potential applications for the prevention and the treatment of multiple conditions including gastrointestinal syndromes. In addition to the incorporation into different foods such as desserts, crackers, and products of cereals and meat, FPH can also be used as an excellent food source for some species of fish [5]. In the same context, the essential nutrients and bioactive peptides present in the FPH prepared from different fish species gained interest in food and pharmaceutical industries [15]. Recently, the enzymatic hydrolysis of proteins has attracted widespread attention owing to the high quality of the produced FPH, which can be used as a raw material for the production of bioactive peptides for the treatment of various diseases [16].

Two different fractions can be produced as a result of the enzymatic hydrolysis of fish proteins, namely, soluble and insoluble proteins. The insoluble part has a potential application in animal feed [12]. The soluble fraction of the hydrolyzed protein can be converted into a nutrient component and used as a nitrogen source for maintaining the growth of microorganisms [17]. The resulting powder from the dehydration of the soluble hydrolysate is known as FPH, which is considered stable during the storage period and possesses higher content of protein [8,12].

To the best of our knowledge, this is the first study that deals with the preparation of FPH of Chinese sturgeon from Yangtze River by using the papain enzyme. The current study aimed to investigate the effects of solid–liquid (S/L, w/v) ratio, enzyme–substrate (E/S, w/w) ratio, pH, hydrolysis temperature, and incubation time on the DH and subsequently evaluate the functional properties of the obtained product.

2. Materials and methods

2.1. Materials

2.1.1. Samples

Chinese sturgeon (*Acipenser sinensis*) was obtained from the Yangtze River by Hua Da Aquatic Products Science and Technology Industry Co., Ltd. The fish was transported directly to the laboratory. It was then cleaned, and the viscera were removed and kept frozen at −20 °C until use. Before the enzymatic hydrolysis process, the samples were transferred to the refrigerator at 4 °C for 12 h.

2.1.2. Enzyme and chemicals

Enzyme papain (Enzyme activity/1000 casein, pH 6.0, 40 °C: ≥6000/(USP-U/mg)) was purchased from Ourchem Co., Ltd, Guangdong Province, China. The enzyme was directly stored at 4 °C. All chemicals and reagents used in the experiments were of high purity and analytical grade.

2.2. Methods

2.2.1. Proximate chemical composition

The chemical compositions of sturgeon fish samples and FPH obtained by enzymatic hydrolysis were determined according to the AOAC method [18]. The moisture content was examined by the evaporation method under 105 °C until an unchanged weight was recorded. Crude protein content was determined by the Kjeldahl method (N × 6.25). Fat content was estimated by the Soxhlet method with petroleum ether. The total ash content was determined using the incinerator at 550 °C until the sample was completely turned to ash. Carbohydrate content was estimated by subtracting the total contents of moisture, protein, fat, and ash from 100%. All experiments were performed in triplicate.

2.2.2. Amino acid analysis

Amino acids were determined according to the AOAC method [18] with some modifications. Three hundred milligrams of the solid sample were digested with 8 mL of 6 M HCl at 110 °C for 22 h under nitrogen atmosphere. After cooling, 4.8 mL of 10 M NaOH was added, the volume was made up to 25 mL with distilled water, then filtered through two layers of filter paper No. 40, and finally centrifuged at 10,000*g* for 10 min. Amino acids were analyzed by using the reverse-phase high-performance liquid chromatography (Agilent 1100 HPLC; Agilent Ltd., Palo Alto, CA, USA). Each sample (1 µL) was injected into a Zorbax, 80 A C-18 column (column size: 4.0 × 250 mm, 5 µm particle size; Agilent, USA) at 40 °C with detection at 338 nm. The mobile phase A was 7.35 mM/L of sodium acetate/triethylamine/tetrahydrofuran (500:0.12:2.5, v/v/v), adjusted to pH 7.2 using acetic acid, while the mobile phase B (pH 7.2) was 7.35 mM/L of sodium acetate/methanol/acetonitrile (1:2:2, v/v/v). The amino acid composition was expressed as grams of amino acids per 100 g of protein.

2.2.3. Fatty acid profile analysis

Total lipids were extracted from the Chinese sturgeon muscles (1.0 g) with chloroform:methanol (2:1, v/v). Fatty acid methyl esters (FAMEs) were prepared according to the method of Chalamaiah, Jyothirmayi, Bhaskarachary, Vajreswari, Hemalatha, and Kumar [15]. FAMEs were analyzed by using a gas chromatography (GC-14B, Shimadzu, Tokyo, Japan), equipped with a flame ionization detector and a fused-silica capillary column (PEG–20 M, 30 m × 0.32 mm × 0.5 mm). The column was initially held at 100 °C for 4 min, followed by temperature programming to 180 °C at the rate of 15 °C/min, then held at 180 °C for 4 min, and increased to 215 °C at the rate of 4 °C/min. The temperatures of injector and detector were set at 250 °C. GC peaks were identified by comparing their retention times with those of the reference standards and expressed as percentages. The analysis was carried out in triplicate, and mean values were reported.

2.2.4. Preparation of protein hydrolysates

To obtain the optimal enzymatic hydrolysis conditions of Chinese sturgeon muscles by using papain, a single-factor test and multiple experiments (S/L, E/S, pH, temperature, and time) were applied (Table 1). Protein hydrolysate was prepared according to the method by Ovissipour, Abedian, Motamedzadegan, Rasco, Safari, and Shahiri [19] with some modifications. The minced substrate was mixed with 25 mM

Table 1
The parameters and their levels used to obtain the optimum hydrolysis conditions of sturgeon muscle by using papain enzyme.

Factors	Units	Symbol	Levels								
			1	2	3	4	5	6	7	8	9
Solid/Liquid ratio		S/L	1:1	1:2	1:3	1:4	–	–	–	–	–
Enzyme/ Substrate ratio	%	E/S	0.5	1	2	3	4	5	–	–	–
pH	pH	pH	5.5	6	6.5	7	–	–	–	–	–
Temperature	°C	T	35	40	45	50	55	60	65	70	75
Time	hour	t	0.25	0.5	1	2	3	4	6	8	–