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Enzymatic Hydrolysis of Hairtail Surimi in an Ultra-High Pressure Bioreactor

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Abstract

Amino acids have been extracted from Hairtail surimi using enzymes in an ultra-high pressure bioreactor. The extraction efficiency of different enzymes including papain, trypsin, and proteases (acid, neutral, alkaline) also has been evaluated, and it has been discovered that neutral protease behaved the best. The amino acids were analyzed using automatic amino acid analyzer, and the enzymatic digestion conditions were optimized. For neutral protease, the optimal condition was 50˚C, 250 MPa, pH 7.0. Material to liquid ratio of enzyme is 6%. More than 29 amino acids were detected after 24 hours of hydrolysis; the enzymatic hydrolysis rate can reach 83.29%. The results show that enzymatic digestion under ultra-high-pressure provides a very promising approach to extract amino acids from Hairtail surimi.

Keywords

Enzymatic Hydrolysis, Ultra-High Pressure, Hairtail Surimi, Amino Acids

1. Introduction

Amino acids are used for a variety of applications in industry. The food industry is a major consumer of amino acids, in particular, glutamate, which is used as a flavor enhancer, and aspartame (aspartyl-phenylalanine-1-methyl ester) as a low-calorie artificial sweetener. Similar technology to that used for animal nutrition is employed in the human nutrition industry to alleviate symptoms of mineral deficiencies, such as anemia, by improving mineral absorption and reducing negative side effects from inorganic mineral supplementation.

Some amino acids derivatives are used in pharmaceutical industry. They include 5-HTP (5-hydroxytryptophan) used for experimental treatment of depression, L-DOPA (L-dihydroxyphenylalanine) for Parkinson's treatment, and eflornithine drug that inhibits ornithine decarboxylase and used in the treatment of sleeping sickness.

The earliest industrial production of amino acids in the world is the creation of the Japanese Ajinomoto company. First, "gluten" left by wheat flour to process starch is hydrolyzed with hydrochloric acid to obtain glutamate, and then neutralized by soda ash to obtain Sodium glutamate. Glutamate is the world's first industrially produced single amino acid product.

Since then, scientists have used protein hydrolysis to hydrolyze raw materials such as feathers, human hair, pig blood, meat chops and surimi into amino acids, but these amino acids are mostly "DL (Right-handed, Left-handed) mixed amino acids" and their resolution is very difficult until the specific catalysis of enzymes is used to obtain amino acids in a natural single configuration.

The industrial microbial fermentation process established in the 1960s brought the amino acid industry off. Since then, many common amino acid species (including glutamate, lysine, threonine, phenylalanine, etc.) can be produced by microbial fermentation, so that the yield is greatly increased and the cost is greatly reduced. But the fermentation process is a time-consuming and inefficient way.

Percy Williams Bridgman received a Nobel Prize in 1946 for advancing high pressure process area of physics by several magnitudes of pressure (400 MPa to 40,000 MPa). In the last several decades, the field of biology has taken Bridgman's advice, and hydrostatic pressure has become a robust physicochemical tool for the study of biological macromolecules [1].

Chemical bonding is likely to change under high pressure, when the P*V (Pressure, Volume) term in the free energy becomes comparable to the energies of typical chemical bonds, *i.e.* at around 100 GPa.

High-Pressure Processing has been used in many fields of food industry, such as fruit products [2], seafood (Teixeira *et al.*, 2013), vegetables [3], but it was often treated without exogenous enzyme, so the shape of fish needs a long time (1 or 2 months) to degrade into small fragments [4].

The aim of this work is to provide a specific and efficient treatment method for the exogenous enzyme hydrolysis of Hairtail surimi in an ultra-high pressure bioreactor.

The ultra-high pressure bioreactor utilizes biological tissue to hydrolysis by specific enzyme under ultra-high pressure conditions, producing favorable biological trait changes, and is regarded as a safe, green and effective biological treatment technology [5]. Using ultra-high pressure bioreactors, we extracted amino acids from meat emulsions under high pressure conditions for the first time to obtain higher extraction efficiency. We have found that ultra-high pressure conditions facilitate the enzymatic hydrolysis of meat emulsions, providing a new way to extract amino acids quickly and efficiently from meat emulsions.

2. Materials and Methods

2.1. Materials

All enzymes (papain, trypsin, and acid protease, neutral protease, alkaline protease) are bought from Nanning Pound Biotechnology Co., Ltd.

Fish meat of Hairtail is bought from local market.

Ultra-High Pressure Bioreactor (Lanzhou Kailande Technology Co., Ltd.)

S-433D Amino Acid Analyzer (German Sykam Scientific Instrument Co., Ltd.)

2.2. Methods

Selection of enzymes

Weigh 10 g of processed Hairtail surimi, Water content (%) of which is 73.4%, and add it to 20 mL of distilled water. In the solution, different kinds of enzymes (papain, trypsin, and proteases) are added by 3% or 6% (W/W). The sample is placed in an ultra-high pressure biological reactor. In the reactor, the pressure is controlled to 250 MPa at 50˚C. Next, the reaction was carried out for 24 h. By detecting the protein in different samples, the efficiency is taken to determine the amount of the optimal enzyme preparation added [6].

Selection of Material to liquid ratio

In order to improve the efficiency of the use of ultra-high pressure bioreactors, the liquid ratio is an important factor. Weighed Hairtail surimi 10 g, under the optimum temperature, pH and enzyme amount of various enzymes, Regulate different ratios of solid to liquid (3%, 6%) at 250 MPa. Under the pressure, the reaction time was 4 hr. By detecting proteins in different samples Extraction efficiency, determine the best ratio of material to liquid [6].

Enzymatic assay

Optimization of enzymatic conditions such as optimum T and optimum pH is the same as in previous literature [6].

Water content determination

The moisture content of samples is determined by reference to the national standard method [7].

Total nitrogen determination

The nitrogen content of samples is determined by reference to the national standard method [8].

Amino acids Analysis

First: Pipette 2.0 mL of enzyme hydrolyzate into a 10 mL centrifuge tube, add 6.0mL of water ethanol to the centrifuge tube, shake well, centrifuge in a centrifuge for 10 min, remove the protein from the hydrolyzate.

Second: Transfer the supernatant to a concentrating tube, concentrate to near-dry, add 0.02 mol/L HCL solution, re-dissolve and shake, shake it with 0.22 μm filter, and use it for measurement.

Evaluation of enzymatic hydrolysis rate

Since the products of enzymatic hydrolysis of Hairtail contained various

compositions of amino acids, it is difficult to calculate and evaluate the enzymatic hydrolysis rate by quantitative analysis of amino acids. In this study, the enzymatic hydrolysis rate is evaluated by comparison of the total nitrogen contents in both raw material and the liquid obtained after enzymatic hydrolyzing, which were measured by a standard quantitative analyzing method. Then the enzymatic hydrolysis rate can be calculated as below:

Enzymatic hydrolysis rate = total nitrogen content in the liquid obtained after enzymatic hydrolyzing/total nitrogen content in raw material.

3. Results

Water content of raw material

Water content in Hairtail is shown in Table 1

Total Nitrogen content of raw material

Nitrogen content in Hairtail is shown in Table 2

Comparison of enzymatic hydrolysis results of Hairtail under normal pressure and ultra-high pressure

It can be seen from Figure 1 that after 24 hours at 50˚C, the fish meat under 100 MPa high pressure gradually digested into a paste, and no deterioration occurred, while the Hairtail under normal pressure still maintained the shape of the fish, but it was obviously spoiled.

The ultra-high pressure enzymatic hydrolysis process can completely eliminate the influence of spoilage bacteria, so that the enzymatic hydrolysis process can be controlled completely, In accordance with the direction of the experimental design, the solid fish protein can finally be hydrolyzed into water-soluble liquid amino acids.

Table 1. Determination of Water content in Hairtail.

samples	Water content (%)
Hairtail surimi	73.4

Table 2. Determination of Nitrogen content in Hairtail.

samples	Nitrogen content (g/100 g)
Hairtail surimi	18.0

Figure 1. Enzymatic hydrolysis results of Hairtail under atmospheric and ultra-high pressure conditions.

Selection of enzymes and their proportion

The enzymatic hydrolysis temperature (T) is 50˚C; the enzymatic hydrolysis pressure (P) is 250 MPa; the enzymatic hydrolysis time (t) is 2 hr; the enzyme additional amount (A) is 6%.

It can be seen from Figure 2 that the best enzymatic hydrolysis effect is that the enzymatic hydrolysis rate is 22.75% under the experimental conditions of enzymatic hydrolysis pressure of 250 MPa, enzymatic hydrolysis time of 2 hr and addition of 6% neutral protease.

It can be seen from Table 3 that the best enzymatic hydrolysis effect is that the enzymatic hydrolysis rate is 86.64% under the experimental conditions of enzymatic hydrolysis pressure of 250 MPa, enzymatic hydrolysis time of 24 hr and addition of Neutral:Papain:Trypsin = 2:1:1 = 6%. But for economic reasons, we chose the addition ratio of neutral protease = 6% as the optimum.

Selection of Material to Liquid Ratio

It can be seen from Figure 3 that the best enzymatic hydrolysis effect is that the enzymatic hydrolysis rate is 33.5% under the experimental conditions of

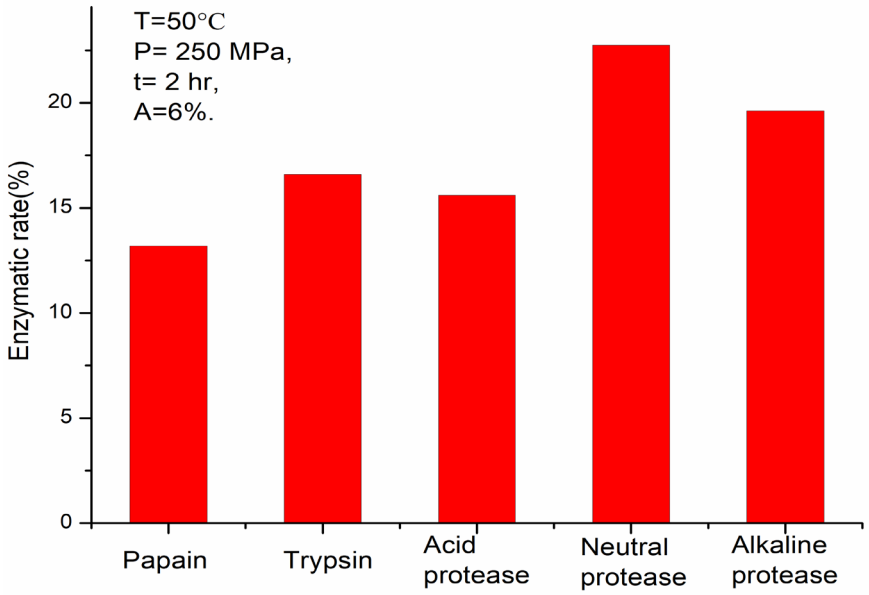

Figure 2. The enzymatic rate of different enzymes on Hairtail surimi.

Table 3. Nitrogen content and hydrolysis rate of Hairtail surimi after ultra-high pressure enzymatic hydrolysis with different proportion of enzymes.

Sample (Hairtail surimi)	Experimental condition	The addition ratio of Protease	Nitrogen content (g/100 g)	Enzymatic rate (%)
Proteolytic solution 1	The enzymatic hydrolysis pressure is 250 MPa, the enzymatic hydrolysis time is 24 h	the neutral protease is 6%	14.9	83.29
Proteolytic solution 2		Neutral:Papain:Trypsin = 1:1:1 = 3%	8.6	48.07
Proteolytic solution 3		Neutral:Papain:Trypsin = 1:1:1 = 6%	9.0	50.31
Proteolytic solution 4		Neutral:Papain:Trypsin = 2:1:1 = 3%	8.5	47.51
Proteolytic solution 5		Neutral:Papain:Trypsin = 2:1:1 = 6%	15.5	86.64

enzymatic hydrolysis pressure of 250 MPa, enzymatic hydrolysis time of 4 hr and addition of 6% neutral protease.

Selection of enzymatic hydrolysis time

The results from Figure 4 showed that the enzymatic hydrolysis rate continued to increase with the prolongation of the enzymatic hydrolysis time, and the enzymatic hydrolysis rate can reach 83% after the neutral protease was on the fish for 24 hours.

Amino acids Analysis

From Figure 3 and Table 4, we can find that the solid fish meat can be

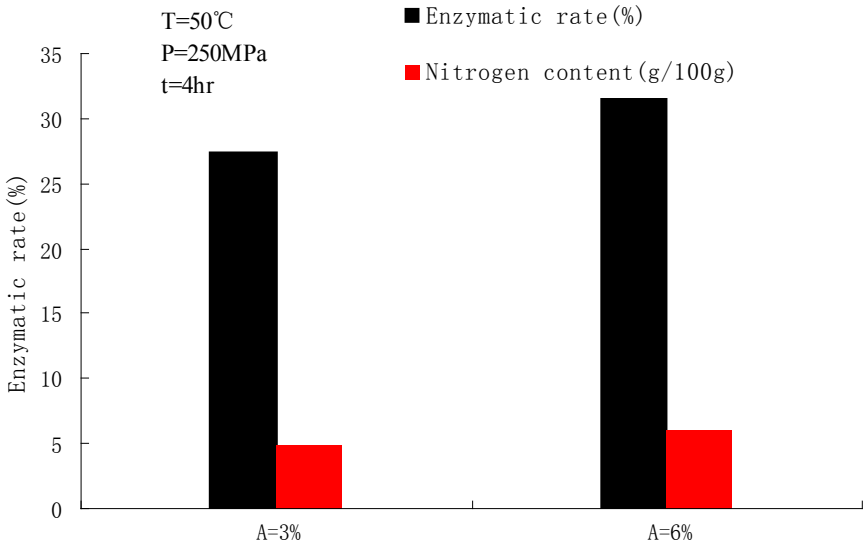

Figure 3. Effect of the amount of neutral protease (A) on the enzymatic hydrolysis rate.

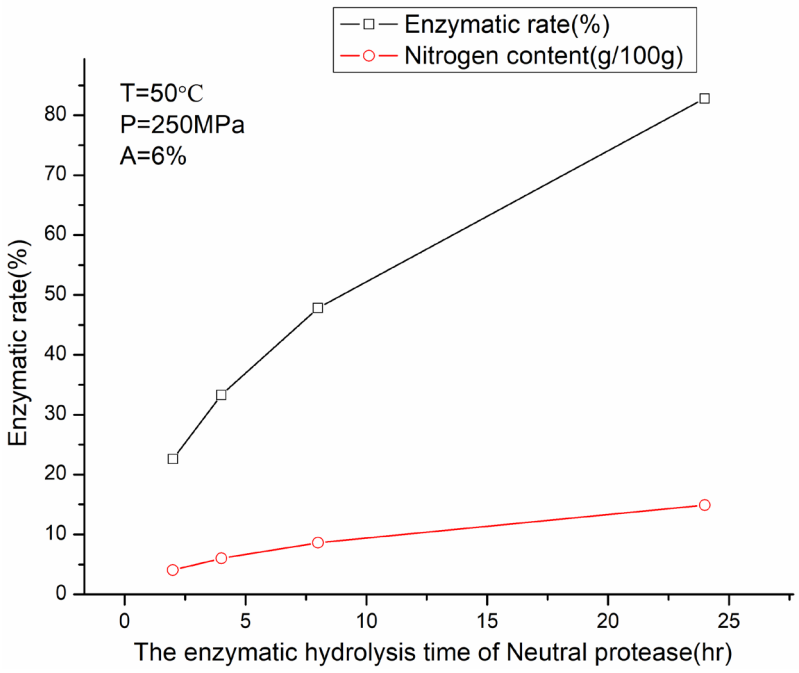

Figure 4. The relationship between enzymatic hydrolysis time and enzymatic hydrolysis rate.

Table 4. Analysis of Amino Acid Composition in Hydrolysate of Hairtail surimi.

No.	Amino acids	Unit	Measured value (μg/ml)
1	Glu	μg/m	902.75
2	Gly	μg/m	141.23
3	Ala	μg/m	360
4	Val	μg/m	264.51
5	Met	μg/m	596.34
6	Tyr	μg/m	531.35
7	Phe	μg/m	883.56
8	*β*-Ala	μg/m	554.64
9	*β*-Aminoisobutyric acid	μg/m	593.03
10	*γ*-Aminobutyric acid	μg/m	150.07
11	Try	μg/m	169.6
12	Leu	μg/m	1432.83
13	Lys	μg/m	1237.35
14	Arg	μg/m	1584.43
15	Phosphoserine	μg/m	39.64
16	Taurine	μg/m	68.97
17	Phosphaglycolamine	μg/m	5.89
18	Urea	μg/m	23.51
19	Asp	μg/m	106.4
20	Thr	μg/m	211.51
21	Ser	μg/m	195.83
22	Asn	μg/m	278.97
23	Alpha amino adipic acid	μg/m	125.91
24	Citrulline	μg/m	118.59
25	Cys	μg/m	81.67
26	His	μg/m	97.95
27	1-Methylhistidine	μg/m	46.13
28	Carnosine	μg/m	26.06
29	Ornithine	μg/m	124.9

hydrolyzed into water-soluble amino acids by using a single neutral protease under ultra-high pressure conditions, If the enzymatic hydrolysis time last for 24 hours, the enzymatic hydrolysis rate can reach more than 83% and the enzymatic hydrolysate detects up to 29 kinds of free amino acids. From which, we can find several important amino acids such as GABA, Taurine, Leu etc.

4. Discussion

The results showed that the enzymatic hydrolysis under ultra-high pressure condition can obtain as high as 82.8% of enzymatic hydrolysis rate for hairtail meat. In this study, only a single sort of enzyme was used in the experiment. A

further research on this subject should be focused on using complex enzymes to optimize the enzymes formula technologically and economically, and optimize other enzymatic hydrolysis conditions such as temperature, pressure and time as well.

A previous research analyzed amino acids contained in hairtail meat [9], only fourteen different kinds of amino acids were detected. In this study, up to twenty nine different kinds of amino acids were detected. This implicates that the enzymatic hydrolysis under ultra-high pressure condition can hydrolyze hairtail meat more sufficiently and the amino acids were destructed less during the enzymatic hydrolyzing process. Therefore, a lot more amino acids can be detected in the products from enzymatic hydrolysis under ultra-high pressure condition.

5. Conclusions

1) The ultra-high pressure enzymatic hydrolysis process can completely eliminate the influence of spoilage bacteria, so that the enzymatic hydrolysis process can be simply controlled, and finally the solid fish meat can be hydrolyzed into water-soluble liquid amino acids.

2) Using a single neutral protease, enzymatic hydrolysis rate of hairtail protein under ultra-high pressure conditions can reach up to 83%.

3) The enzymatic hydrolysis of the fish meat under the ultra-high pressure environment is very sufficient, and the various amino acids in the enzymatic hydrolysis process are not destroyed, and the enzymatic hydrolysate detects up to 29 kinds of free amino acids.

Acknowledgements

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Novelty Statement

It is the first enzymatic hydrolysis experiment of Hairtail surimi in an ultra-high pressure environment of 250 MPa by using a single neutral protease. We found that the enzymatic hydrolysis of the fish meat in the ultra-high pressure environment is sufficient, and the amino acid lost during the enzymatic hydrolysis process is small. We can detect up to 29 kinds of free amino acids from the enzymatic hydrolysate.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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