INFORMATION ON DOCTORAL DISSERTATION

Title of thesis: Study on purification of digestive enzymes from visceral of Tra (*Pangasius hypophthalmus*) catfish

Sector: Food and Beverage Processing

Major code: 62540201

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1. DISSERTATION SUMMARY:

In Tra (*Pangasius hypophthalmus*) catfish processing industry, 30% are fillets, the rest are by-product and waste, in which, fish viscera, accounting for 5-6% of total mass, estimated approximately 100.000 tons per year, has wide biotechnological potential as a source of digestive enzymes. Therefore, the objectives of this thesis were to study on (1) separation and characterization of digestive enzymes from Tra catfish viscera, especially pancreatic protease and lipase (2) technology methods proposal for extraction, purification and highly valued enzyme products formation from waste materials. The issues were addressed in the thesis as follows:

- ***Issue 1:*** Investigate the influence of individual visceral organs of Tra catfish on the activities and properties of lipase, protease and amylase. Determine the optimal conditions for digestive enzyme extraction.
- ***Issue 2:*** Study on forming digestive enzyme products from hepatopancreas of tra catfish. Investigate 02 technology methods (1) membrane method by using combined micro-filtration (MF) with ultra-filtration (UF); (2) precipitation method by using agents such as solvent (ethanol, acetone, isopropanol) or ammonium sulfate.
- ***Issue 3:*** Study on purification of protease/ lipase from extracted enzyme solution. Protease/ lipase from the hepatopancreas of Tra catfish was purified by ammonium sulfate fractionation, followed by ion-exchange chromatography on DEAE-Cellulose and gel filtration Sephadex G-75.
- ***Issue 4:*** Determine some properties of purified protease/ lipase such as molecular weight, amino acid component, optimal pH, optimal temperature, kinetic properties…
- ***Issue 5:*** Study on applying enzyme products for food-processing or digestion aids. Determine enzymes performance in the hydrolysis of some proteins and vegetable oil.

2. CONTRIBUTIONS OF DISSERTATION:

Result 1: This is the first work in Viet Nam that researches intensively on digestive enzymes system from hepatopancreas of tra catfish, especially protease and lipase,

contributing to important science data for enzymes from viscera of Tra (*Pangasius hypophthalmus*) catfish.

- The protease showed a molecular weight of 31 KDa. The pH and temperature optima of purified protease were 8.5 and 55^0C respectively. This protease was stable at a pH range of 7.0-9.0. In temperatures from 35 to 55^0C, during 120 min, the enzyme decreased activity slowly. Enzyme activity was enhanced by Ca^{2+} but inhibited by heavy metals Zn^{2+}, Cu^{2+}. On substrate BSA, kinetic properties of protease purified from hepatopancreas of tra catfish were established with results of K_m= 897 mg/L and V_{max}= 15.7 mg/L.min.
- The pure lipase has a molecular weight of 57 Kda. The optimum pH and temperature of hepatopancreas lipase are at 8 and 50^0C, respectively. The lipase was stable at a pH range of 7.0-9.0 and in temperatures of 35-50°C. Lipase activity was stimulated by Ca^{2+} and inhibited by Zn^{2+}, Cd^{2+}. On substrate triolein, purified lipase has K_m= 1.381 mM and V_{max}= 0.063 mM/min. The enzyme was specific for the α-positions (1, 3) of triglyceride. In bile salt solution of 0.015M NaTC, lipase activity of the enzyme increased in 3.08 folds in comparison of sample without NaTC.

Result 2: The extraction/ purification methods of protease/ lipase from hepatopancreas of Tra catfish, are described in this study for the first time, contributing to diversification of enzymes from various materials. The hepatopancreas of Tra catfish contain a considerable amount of protease and lipase activity that can be used in different food-processing or digestion aids, thereby contributing to increasing the value of Tra catfish, which helps develop of the Tra catfish industry in Viet Nam.

3. APPLIED AND THEORETICAL IMPLICATIONS

- Results revealed that the hepatopancreas was the best source for three digestive enzymes, with the highest activities of lipase (674.02 $U.g^{-1}$ dry hepatopancreas), protease (84.28 $U.g^{-1}$ dry hepatopancreas) and amylase (419.69 $U.g^{-1}$ dry hepatopancreas), in comparison with intestine or mixed viscera. The optimal conditions for enzyme extraction were as follows: hepatopancreas and Tris-HCl buffer ratio- 1/2 (w/v); pH8; temperature 5^oC; extraction time is 1 hour.
- Lipase fraction from the hepatopancreas of Tra catfish was purified by membrane filtration. Crude extracted enzyme was diluted by water with a ratio of 1:3 (v/v), followed by a micro filtration (MF) sequencially with 1µm then 0.1µm membranes. After MF, the enzyme solution was purified by ultra filtration (UF) with 10 kDa membrane within 120 min at 6 psi. Results revealed that the specific activity of purified lipase was 52.7 U/mg protein. The purification was 2.07 times higher than the crude enzyme before UF with yield 90.6 %.
- Ethanol was a suitable precipitating agent for enzymes purification. The optimal ratio of ethanol and enzyme extract was 3/1 (v/v). The pancreatic protease recovery yield was

90.7% and the purity degree was 1.94. The pancreatic lipase recovery yield was 91% and the purity degree was 2.02. Moisture enzyme is dried at 40^0C within 3 hours to remove ethanol. Dry enzyme is produced with lipase activity 587.85 U/g, protease activity 49.26 U/g with yield 8.3% (w/w) based on fresh material.

- Protease or Lipase from the hepatopancreas of Tra catfish was purified by ammonium sulfate fractionation, followed by ion-exhange chromatography on DEAE Cellulose and gel filtration Sephadex G-75. The preparation was homogeneous on polyacrylamide disc gel electrophoresis. The specific activity of the purified protease (28.59 U/mg protein) was 22.41 times higher than that of the crude extract with yield 23.67%. The specific activity of the purified lipase (509,71 U/mg protein) was 37.95 times higher than that of the crude extract with yield 40.08%.
- Dry enzyme can be applied for producing peptone with ratio of Namin/Ntotal to be 20.97 and 22.15% on fish and beef substrate, respectively. Therefore, this enzyme product can be applied as a medical material for digestion aids, contributing to reducing the malnutrition problem for children.

4. FURTHER RESEARCH OF DISSERTATION

- Determine the methods for the clarity of molecular structure of the protease/ lipase from hepatopancreas of Tra (*Pangasius hypophthalmus*) catfish.
- Set up the industrial production for the lipase enzyme by membrane technology.
- Build up and develop more scales related to purified protease/ lipase from hepatopancreas of Tra catfish.
- Examine some safety standards when using this enzyme product as a medical material for digestion aids.

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