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Byproduct utilization for increased profitability, part 4

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Processing fish protein hydrolysates

A hydrolysate product gleaned from shrimp-processing waste may be used as a food additive for individuals who do not consume sufficient calcium because of lactose indigestion or intolerance.

The definition of waste or by-product in the seafood industry varies with fish and shellfish species and the harvesting and processing methods used. Generally, the main body muscle tissue is the main product in the processing industry. Heads, backbones, trimmings, skin, viscera and shells constitute what is generally thought of as by-product or waste.

Processes

Acid hydrolysis

Acid hydrolysis has been shown to result in complete protein hydrolysis with 6 molar (M) hydrogen chloride at 118 degrees C for 16 hours. Due to the extensive hydrolysis process, the solubility of the product is increased. However, the neutralization process produces a large quantity of salt, which makes the product unsuitable for food. Although the salt can be removed partially or completely using nanofiltration and ion-exchange resins, the product is limited to use as a flavor enhancer for human and pet food. While acid hydrolysis converts the proteins into individual amino acids and small-chain peptides, some of the essential amino acids, such as cystine, cysteine, methionine and tryptophan, are destroyed in the process.

Base hydrolysis

In a base hydrolysis, calcium, sodium and potassium are added to solubilize proteins that have been heated. The reaction is performed at a predetermined temperature, customarily in the range of 80 to 130 degrees C, for several hours until the desired degree of hydrolysis is obtained. Then the product is evaporated, pasteurized and spray dried. As stated in Part I of this series, the product has poor functional properties and poor nutritional qualities. These occur due to the elimination and addition processes that occur during processing.

Alkaline hydrolysis

The use of alkaline hydrolysis can potentially lead to the formation of undesirable compounds in food. The loss of cysteine, serine and threonine affects the disulphide bonds, and lysioalanine, ornithinoalanine, lanthionine and □-amino alanine, which are toxic substances, may be formed. Also, the products formed during alkaline hydrolysis have an inhibiting effect on proteolytic enzymes and reduce the hydrolysis rate.

Enzymatic hydrolysis

Enzymatic hydrolysis is the preferred method of hydrolysis, since it has less impact on the functional properties and nutritional value of the hydrolysate. Enzyme hydrolysis requires a relatively small amount of enzymes that can be easily inactivated, and the hydrolytic process can be performed under mild temperatures and pH conditions. Usually, there is an optimum combination of pH and temperature at which an enzyme is most active and a combination at which an enzyme can be deactivated.

Enzymatic hydrolysis can either be exogenous or endogenous. In endogenous hydrolysis, enzymes in the by-product, such as serine proteases, trypsin chymotripsin, thiol protease, pepsin, lysosomal proteases and catheptic enzymes, perform the hydrolytic action. This process results in free amino acids and small peptides.

When exogenous food-grade enzymes are used, the operator has the ability to choose an enzyme and the hydrolytic conditions to produce a product that possesses the desired functional properties. A list of commercial enzymes was contained in Part I of this series. From a technical and economic perspective, the microbial enzyme Alcalase operating at alkaline pH has been reported in the literature to be the most efficient.

Treatment of waste from salmon processing can yield a high-protein hydrolysate.

Optimization of hydrolysis

There are many variations in species and enzymatic and chemical hydrolytic conditions. The sample processes below should provide a starting place for developing one or more processes for the production of aquatic protein hydrolysates. Note that the degree of hydrolysis obtained through enzyme action is dependent upon many process variables.

Salmon

Heads of Atlantic salmon, *Salmo salar*, were treated with Alcalase under several process variables. The process pH was maintained constant with the addition of 4 M sodium hydroxide and allowed to continue for two hours. The highest yield of 71.0 percent was obtained with a process at 58 degrees C, an enzyme/substrate percentage of 8.0, degree of hydrolysis of 17.30 yield of 17.30 percent and a protein content of 78.86 percent. The highest protein yield (85.70 percent) was obtained with a temperature of 55 degrees C, an enzyme/substrate percentage of 7.0, degree of hydrolysis of 12.00, and yield of 57.6.

Red salmon

Oncorhynchus nerka heads were treated with several enzymes (Alcalase, Flavour-yme, Palatase, Neurtase, Protex and G.C. 106) under reaction durations of 25, 50 and 75 minutes. The hydrolysis was performed at 50 degrees C, and 0.5 g of enzyme was added at 100 g/head of head mince protein. The greatest degree of hydrolysis – from 6.4 to 16.7 percent – was obtained with the 75-minute digestion. Oil yield ranged from 4.9 percent with G.C. 106 to 8.5 percent with Palatase. Protein hydrolysis powders contained 62.3 to 64.8 percent protein with high levels of amino acids. There was no statistical difference ($P > 0.05$) among the six enzymes used.

Yellowfin tuna

The optimum conditions to reach the highest degree of hydrolysis of visceral waste proteins of yellowfin tuna, *Thunnus albacares,* were 60.4 degrees C for 90.24 minutes and protease activity with 2.4 L Alcalase of 70.22 AU/kg protein. The resulting protein had a relatively high 72.34 percent protein content and low 1.43 percent lipid content.

The chemical content of the hydrolysate indicated that it fulfills adult human nutritional requirements except for methionine. Lysine and methionine were the first and second limiting amino acids. In addition, the protein efficiency ratio of the visceral hydrolysate was 2.85 to 5.35.

Pacific halibut
By-products of Pacific halibut, *Hippoglossus stenolepus*; and arrowtooth flounder, *Atheresthes stomias*; were evaluated as replacements for fishmeal in diets for *Litopenaeus vannamei* shrimp. One of the hydrolysates was produced by phosphoric acid under pH 3.8 and left in the liquid state. The other hydrolysate was produced under the same conditions and subsequently neutralized to pH 6.5 with a 50 percent solution of sodium hydroxide and drum dried.

The feeding study indicated that both hydrolysates could replace 50 percent of the menhaden meal in the shrimp diets since the performance of the shrimp was equivalent to that of the shrimp given the control diet.

Pacific whiting
Whiting, *Merluccius productus*, tissue was used to produce hydrolysates with 10, 15 and 20 percent degrees of hydrolysis using Alcalase. Liquid enzyme was added at 1.0, 1.5 and 3.0 mL to 99.0, 98.5 and 97.0 g of muscle homogenate, which contained 8 percent protein at pH 8.0 and 50 degrees C, and characterized at pH 4.0, 7.0 and 10.0 according to their solubility, emulsifying and foaming properties.

Proteins recovered in soluble fractions increased proportionally with the hydrolytic process, yielding 48.6, 58.6 and 67.8 percent of total protein after 10, 15 and 20 percent degrees of hydrolysis, respectively. Freeze-dried hydrolysates presented almost 100 percent solubility at the different pH values evaluated.

Persian sturgeon
When hydrolysis of Persian sturgeon, *Acipenser persicus*, visceral protein were performed under several pH and temperature conditions, Alcalase-hydrolyzed fish protein had the highest degree of hydrolysis, 50.13 percent, while Trypsin resulted in only 14.21 percent hydrolysis. The greatest chemical hydrolysis, 68.87 percent, was related to pH 3.3 at 85 degrees C. The highest protein recovery (83.64 percent) and protein content (73.34) were related to enzymatic hydrolysis by Alcalase.

Microbial enzymes could produce fish hydrolysates with a higher degree of hydrolysis when compared to animal enzymes. Also, it appeared that chemical hydrolysis at lower pH and higher temperature resulted in greater protein recovery and degree of hydrolysis.

Grass carp
Protein hydrolysate was produced from the skin of grass carp, *Ctenopharyngodon idella*, using Alcalase. The optimum values for temperature, pH, enzyme:substrate ratio, and time of hydrolysis were found to be 59.74 degrees C, 8.25, 1.70 and 83.83 minutes, respectively. The freeze-dried hydrolysate product was highly water-soluble with good water-holding, oil-binding and emulsifying properties.

Marine swimming crabs
Researchers examined the conditions for enzyme hydrolysis of processing waste from marine crabs, *Portunus tritberculatus* and *P. sanguinolentus*, to produce a crab flavor. The optimum hydrolysis conditions for neutrase were identified as: a material:water ratio of 1:3, 4.0 percent enzyme, pH 6.5, 50 degrees C temperature and five hours of treatment.

Results showed the hydrolysis conditions required for mixed enzymes were a material:water ratio of 1:3, 3.5 percent enzyme, pH 6.0, 55 degrees C and seven hours, and a ratio of mixed enzymes (neutrase:flavorase:papain) of 1:2:1. The mixed enzyme hydrolysate contained 17 free amino acids with a total amino acid content of 51.75 mg/g and a content of essential amino acids of 25.42 mg/g. After spray drying, the hydrolysate, a powder, had a yield of 10.44 percent.

Processing waste from another marine crab species, *Chionoecetes opilio*, hydrolyzed with 5 normal hydrochloric acid produced yields from a 12-hour hydrolysis that varied from 28 to 31 percent. Yields increased marginally to 29 to 32 percent with 24 hours of hydrolysis, but decreased with further hydrolysis. Depending on hydrolysis time, approximately 42 to 44 percent of the total amino acids were essential amino acids, principally leucine, arginine, valine and threonine. The remaining non-essential amino acid fraction was composed mainly of glutamic acid, aspactic acid and glycine.

Marine shrimp
Shrimp processing by-products were hydrolyzed with various enzymes at optimal temperatures: Flavourzyme, 50 degrees C, Protamex, 50 degrees; Alcalase, 55 degrees; pepsin, 37 degrees; and trypsin, 40 degrees. The enzyme:substrate ratio was 1:1,000 (w/v). The pH levels of the protein solution were adjusted to optimal values (Flavourzyme, 7.0; Protamex, 6.5;

Alcalase, 8.0; pepsin, 2.0; and trypsin 8.2) before hydrolysis was initiated and adjusted to the optimal value every 15 minutes during hydrolysis with 1 M sodium hydroxide or 1 M hydrogen chloride. After hydrolysis for six hours, the pH of the solution was adjusted to 7.0, and the solution was heated at 95 degrees C for 10 minutes to inactivate the enzyme.

Among the digests, trypsin showed the most potent calcium-binding activity (0.294 mmol/g-protein) and highest degree of hydrolysis (18.4 percent). The hydrolysate was fractionated, and the lowest molecular weight fraction showed the greatest calcium-binding activity of 2.70 mmol/g-protein. The peptide was responsible for higher calcium-binding properties and may be a natural functional additive in the food industry for individuals who do not consume sufficient calcium because of lactose indigestion or intolerance.

Shrimp
Processing wastes from *Crangon crangon* shrimp were produced from shells preliminarily demineralized with 10 percent hydrogen chloride solution at a ratio of 1:20, at 20 degrees C for 30 minutes. The proteolytic enzyme Alcalase was used at 55 percent C and pH 8.5. The recovered protein hydrolysate contained, on a dry weight basis, 64.3 percent protein, 6.2 percent lipids and 23.4 percent sodium chloride. At pH 4.0, it had a minimum solubility and 81.7 percent of total nitrogen in the product. The protein efficiency ratio value of the obtained product was 2.99 as compared with 2.64 for hydrolysates from capelin and 2.81 for beef longissimus dorsi muscle protein.

Mussels
Muscle meat from *Perna perna* was subjected to enzymatic hydrolysis using Protamex under temperatures of 46 to 64 degrees C, enzyme:substrate ratios of 0.48 to 5.52 percent, and pH values of 6.7 to 8.3. Optimum conditions for hydrolysis were pH 6.5, 51 degrees C and enzyme:substrate ratio of 4.5 percent. Under these conditions, hydrolysis of 26.5 percent and protein recovery of 65.0 percent were obtained.

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