

Behavior of Amino Acid Production from Hydrothermal Treatment of Fish-Derived Wastes

Kil-Yoon Kang and Byung-Soo Chun[†]

Institute of Seafood Science, Faculty of Food Science & Biotechnology, Pukyong National University, Busan 608-737, Korea
(Received 13 February 2004 • accepted 24 August 2004)

Abstract—The effect of operating parameters (temperature and time) and various reaction modes(batch, semi-batch and continuous flow) on the behavior of amino acid production from hydrothermal decomposition of fish-derived wastes was investigated. The amino acids obtained in batch experiments at 523 K were mainly alanine (Ala) and glycine (Gly) at maximum yield of 65 and 28 mg/g-dry fish, respectively. At a relatively lower temperature of 473 K, the relative concentration of high-molecular-weight amino acids such as aspartic acid (Asp) and serine (Ser) is significantly high, but decreases as temperature increases. It is likely that high-molecular-weight amino acids decompose faster than low-molecular ones. Semi-batch and continuous flow modes of reaction suppressed decomposition of amino acids into organic acids (or volatile materials) by continuously removing the products from the reaction zone as soon as they were formed. Thus, a large amount of high-molecular-weight amino acids such as Asp and Ser at these reaction modes was observed. This increases the yield of total amino acids at short reaction time and at temperature relatively higher than 523 K. It was also observed that the composition of the resulting products also depends on the modes of reaction.

Key words: Hydrolysis, Proteins, Amino Acids, Hydrothermal Reaction

INTRODUCTION

Reactions in high-temperature and high-pressure (HTHP) water have been gaining attention recently [Savage, 1999] due to potential for water to act as a reaction medium and catalyst under these conditions. Much research work has been done in this regard, and the technique was found useful especially for wastewater treatment [Goto et al., 1999] and solid wastes resources recovery [Shanableh, 2000]. Although these have not been widely used in industry, application to destruction of toxic wastes has shown to be practical in small scale [Shaw et al., 1991]. Some recent studies deal with the application of the technology to recover useful materials from various organic wastes, including plastics [Adschiri et al., 1997; Kim et al., 2002; Seo et al., 1999], cellulosic [Sasaki et al., 1998; Park et al., 2002] and proteinaceous (fish-derived) wastes [Yoshida et al., 1999; Kang et al., 2001; Daimon et al., 2001].

Regarding fish-derived wastes, large quantities of fish processed in industry per year are being put into wastes. Technologies that would treat these wastes, or even better, recover some useful organic materials before disposal are important. One possible valuable product that can be derived from fish wastes is amino acid obtained from hydrolysis of proteins. Yoshida et al. and our research group [Kang et al., 2001, 2004; Daimon et al., 2001] have studied sub-critical water hydrolysis to produce organic and amino acids from fish-derived wastes.

In the design of an applicable hydrothermal treatment process, information on the effects of various operating parameters is important. Among the parameters influencing hydrothermal reactions, temperature and time are usually the major concern of several research-

ers [Sasaki et al., 1998; Meyer et al., 1995]. These are the two most important parameters necessary for the technical and economic assessment of the process. To facilitate the design of an actual process, the behavior of products composition and yield on operating modes should also be studied.

The main purpose of this study is to investigate and analyze the behavior of amino acid production, with regards to composition and yield, from fish-derived wastes under various operating conditions (temperature and time) and reaction modes (batch, semi-batch and continuous flow). This information would be necessary in the design and economic evaluation of an applicable hydrothermal treatment process to recover amino acids from fish-derived wastes.

EXPERIMENTAL SECTION

1. Properties of Fish Wastes and Sample Preparation

A solid sample consisting of the entrails of “white croaker” was obtained from a local seafood processing company. Some of the characteristics of the entrails are shown in Table 1. Before the experiments, the entrails were homogenized for 5 min using a mixer (CQM-N1, Toshiba Corp.) at the maximum speed setting (4,500 rpm) and stored in a freezer at 252 K.

For continuous flow experiments, liquid samples were prepared by mixing homogenized entrails with deionized water at a sample to water weight ratio of 1 : 20, and then centrifuged about 10 min

Table 1. Characteristics of the entrails used in this experiment

Elemental analysis (wt%)			Others (wt%)	Water content (wt%)
C	H	N		
44.9	6.6	10	38.5	78

[†]To whom correspondence should be addressed.

E-mail: bschun@pknu.ac.kr

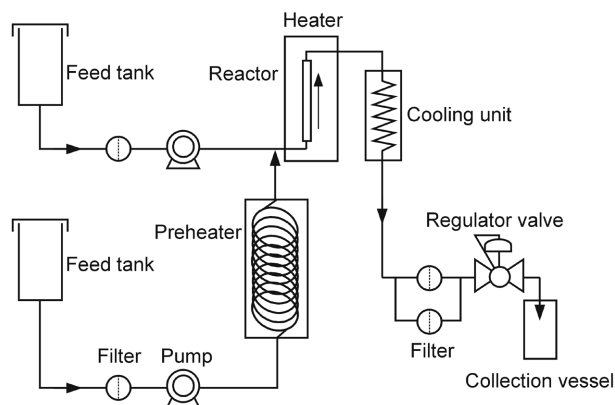


Fig. 1. Schematic diagram of continuous flow tubular reactor apparatus.

using a N-100F Centrifuge (Kokusan Corp.). The supernatant solution was used only in the experiments.

2. Experimental Apparatus and Methodology

The experimental apparatus and procedures for batch and semi-batch experiments have been described in detail elsewhere [Kang et al., 2001]. Briefly, in each run for batch experiments, waste fish entrails and deionized water were charged into the reactor at a weight ratio of 1 : 20. The reactor was immersed into the preheated molten salt bath. After the desired reaction time had elapsed, the reactor was plunged into a water bath to bring them quickly to room temperature, effectively ceasing any reactions occurring. For semi-batch experiments, about 2.5 g of solid sample was placed inside a capsule and introduced rapidly into the reactor at pre-existing hydrothermal conditions. Reaction products were then continuously collected at the sampling port.

For continuous flow experiments and investigation of reaction at short time, a continuous-flow tubular reactor (Organo Corporation) system shown in Fig. 1 was used. The apparatus consists of two pumps, a preheater, a reactor, a cooling unit and a regulator. The tubular reactor is made of Inconel 625 with an OD of 105 mm and ID of 68 mm. This could be operated at a maximum operating temperature and pressure of 673 K and 40 MPa, respectively. Sample and water were delivered at a constant flow rate. The apparatus was designed to mix two separate flows at a known temperature and fixed flow rates and to allow this mixture to react at isothermal conditions. The total flow rates can be varied from about 0.08 to 80 cm³/min. The reactor pressure was controlled by the regulator valve. The temperature of deionized water was brought to the desired level by a preheater, then mixed with the same amount of sample solution before entering the main reactor. To improve heat transfer between the heater and the fluid inside the reactor, the heater was coiled around stainless steel rods that were in direct contact with the Inconel reactor. Thermocouples were connected at the entrance, center and exit of the reactor. The residence time was adjusted manually by the flow rate of sample and water.

3. Analytical Methods

The amino and organic acids contents of the reaction products were determined with an amino acid analyzer (LC-10AD, Shimadzu Corp.) and an organic acid analyzer (LC-10A, Shimadzu Corp.), respectively. The amino acid analyzer is a combination of an ion

exclusion column (Shim-pack Amino-Na, Shimadzu Corp.) and post-column labeling methods with spectrofluorophotometer (RF-10A, Shimadzu Corp.). In sample preparation for amino acid analysis, filtration was done using ultra-filtration membrane (30,000 fractional molecular weight, Millipore Ultra Free C3) to maintain good performance of the chromatographic system. The quantities of 17 kinds of amino acids (presented here according to elution order) - namely, aspartic acid (Asp), threonine (Thr), serine (Ser), glutamine (Glu), proline (Pro), glycine (Gly), alanine (Ala), cystine (Cys), valine (Val), methionine (Met), isoleucine (Ileu), leucine (Leu), tyrosine (Tyr), phenylalanine (Phe), histidine (His), lysine (Lys) and arginine (Arg)- were determined in each analytical run.

The organic acid analysis system consists of an ion exclusion column (Shim-Pack SCR-102H) and electroconductivity detector (CDD-6A). The objective of analysis was to know aliphatic carboxylic acids, hydroxycarboxylic acids, ketocarboxylic acids and other organic acids having dissociation constant (pKa) of 2 to 5 and carbon number of 5 or less.

TOC of the products was measured with a TOC analyzer (TOC-500, Shimadzu Corp.).

RESULTS AND DISCUSSION

1. Speculated Main Reaction Pathway for Hydrolysis of Proteins under Hydrothermal Conditions

Two main consecutive reactions are being considered for the recovery of amino acids by hydrolysis of proteins in fish-derived wastes. These are production of amino acids from hydrolysis of proteins and decomposition of amino acids to other products such as organic acids. Proteins decompose to amino acids via low-molecular peptides. The amino acids degrade to organic acids, resulting in complete decomposition to volatile carbon (carbon dioxide, etc.) and water under severe conditions or at long reaction time. In this regard, proper control of reaction parameters such as temperature and time is necessary in order to obtain high yield of amino acids.

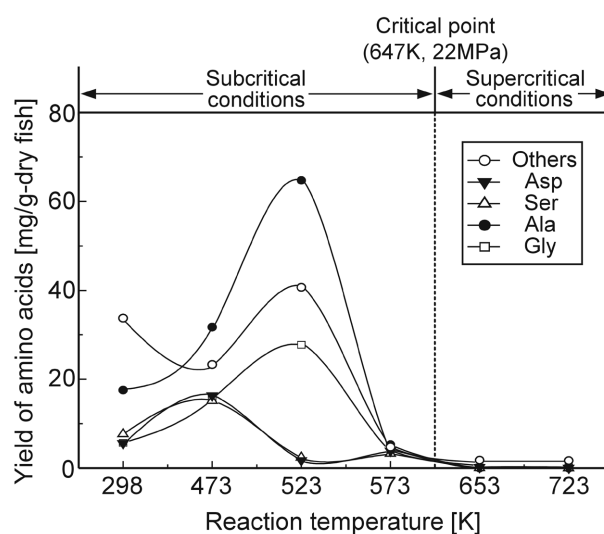


Fig. 2. Effect of reaction temperature at sub- and supercritical conditions on the yield of amino acids in batch experiments (reaction time=60 min).

2. Temperature-Dependence of Reaction

The effect of reaction temperature on amino acid production was studied in detail with a batch reactor [Kang et al., 2001]. A summary of the results in Fig. 2 shows that the maximum production of total amino acids was obtained at a reaction temperature of 523 K, the temperature at which the ion product of water is maximum under saturated vapor pressure. "Others" indicates the amount of other amino acids analyzed as described in the **Analytical Methods**. The amino acids obtained under this condition were mainly alanine and glycine (65 and 28 mg/g-dry fish, respectively).

At a relatively lower temperature of 473 K, the maximum yields of high-molecular-weight amino acids such as Asp and Ser are observed, but decrease as temperature increases. It can be postulated from the results that high-molecular-weight amino acids decompose faster than low-molecular ones. Thus, operation at this temperature would be sufficiently effective to obtain high-molecular-weight amino acids (Asp and Ser) because this condition would suppress decomposition of amino acids to other organic compounds. At temperatures higher than 523 K, the yield of total amino acids decreases as a result of rapid decomposition rate compared to production rate of amino acids.

3. Effect of Reaction Time on Formation of Amino and Organic Acids

Further experiments were carried out to elucidate the effect of reaction time at temperatures of 523 and 653 K (the results are shown in Fig. 3). A dashed line indicating the amount of initial free amino acids, obtained by extraction at 298 K for 30 min, is also shown to serve as a reference. This amount is similar to the amount at 298 K in Fig. 2. At 523 K (4 MPa), the production of amino acids is evident from the increase in the yield of amino acids against reaction time. The yield at reaction time of 60 min is about twice the initial amount of amino acids. On the other hand, at 653 K (45 MPa), organic compounds decompose readily resulting in a decrease in the yield of amino acids, even lower than the amount of initial free amino acids. Based on the speculated main reaction pathway of hydrolysis of proteins, the significant decrease in the yield was due to decomposition of amino acid to organic acids. In this regard, the formation of low-molecular-weight carboxylic acids was also investigated, and the results at temperatures of 523 and 653 K are shown

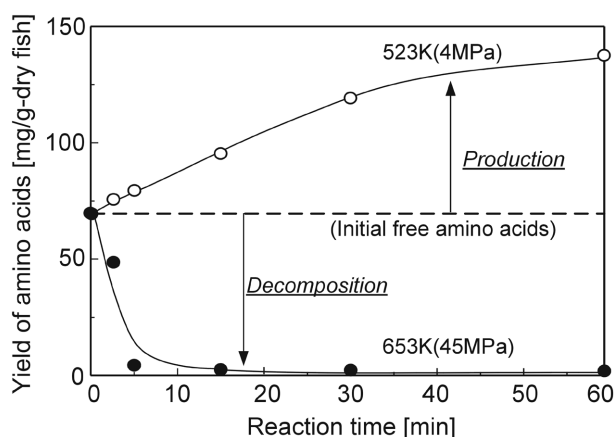


Fig. 3. Yield of total amino acids against reaction time at 523 K (4 MPa) and 653 K (45 MPa).

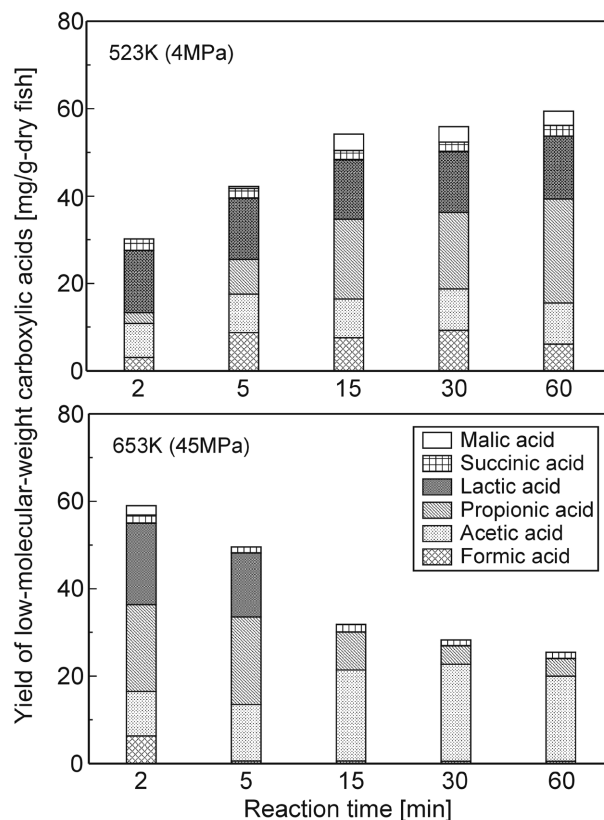


Fig. 4. Yield of low-molecular-weight carboxylic acids against reaction time at 523 K (4 MPa) and 653 K (45 MPa).

in Fig. 4. Even at a relatively lower temperature of 523 K, low-molecular-weight carboxylic acids are formed, obtaining a total of about 60 mg/g-dry fish after 60 min. The products were mostly formic, acetic, propionic and succinic acids. The yield of acetic acid gradually increases with increasing temperature and time, while amount of other organic acids decreases. It should be noted that acetic acid is stable at high temperature, and is difficult to degrade even in the presence of oxidants [Meyer et al., 1995]. The decreasing amount of other organic acids indicates further decomposition into volatile compounds such as CO_2 , CO and H_2O at longer reaction time.

Based on the results, proper control of reaction time along with temperature is necessary in order to get high yield of amino acids. The main concern is to enhance the hydrolysis of proteins and effectively suppress the decomposition of amino acids. One possible solution is a process that would allow removal of amino acid as soon as it is formed. This process would shift the reaction towards production of amino acid (the intermediate product) while suppressing its decomposition to other compounds such as organic acids. For solid materials such as fish-derived wastes, a process that would treat solid samples at stable reaction conditions needs further consideration.

4. Process Improvement - On the Idea of Continuous Removal of Products from the Reaction Zone

To address the problems encountered with the batch operation, experiments were conducted in a semi-batch reactor with rapid sample injection. The experimental apparatus and procedures were described in detail elsewhere [Kang et al., 2001]. In brief, water was

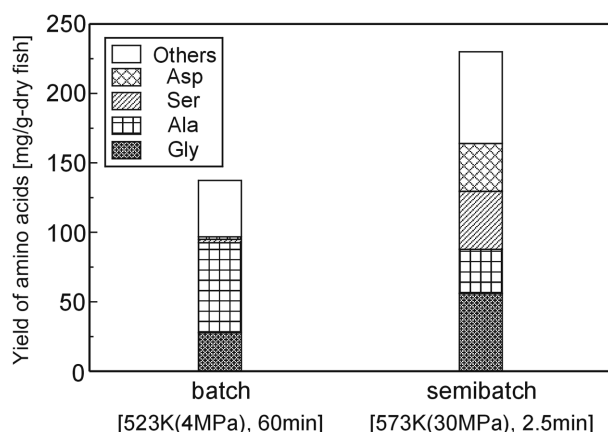


Fig. 5. Comparison of maximum yield and composition of amino acids in batch and semi-batch experiments.

allowed to flow through the reactor at constant flow rate. After reaching the desired operating conditions, sample was introduced rapidly to the reactor. After dropping the capsule containing about 2.5 g sample into the reactor, the first 96 cm³ water inside the pipeline downstream the reactor to the sampling port was removed. Then, sampling was done continuously for 2.5 min. The collected samples were analyzed for amino acids, organic acids and total organic carbon (TOC). The TOC of the first 96 cm³ water was negligible, indicating the absence of any organic compounds. The reaction temperature was varied at 523, 573 and 673 K, while the pressure was held constant at 30 MPa in all experiments.

In the operation of a semi-batch reactor, further decomposition of amino acids is suppressed resulting into high yield because the products are withdrawn right away from the reaction zone. This operation is more advantageous compared to the batch reactor because the effect of equilibrium and the presence of byproducts are minimized. The maximum yield under the tested conditions was obtained at 573 K and is shown in Fig. 5 in comparison with the maximum yield obtained in batch experiments.

Recovery of high-molecular-weight amino acids such as Asp and Ser was higher in semi-batch compared to batch operation, resulting in high yield of total amino acids. This agrees with the result of batch experiments in Fig. 2 that Asp and Ser could be obtained in great amount at mild condition. Moreover, in a semi-batch reactor system, further decomposition of these amino acids could be avoided because the products are continuously being withdrawn as soon as they are formed. To obtain high yield of amino acids, the results suggest operation of the system at short reaction time and relatively high temperature compared to the optimum temperature obtained from the batch reactor operation.

5. Continuous Treatment of Liquid Samples in Short Reaction Time

It should be noted that it is quite difficult to get an accurate result using a batch reactor for reaction time shorter than 5 min due to the transition in temperature and pressure prior to reaching the desired reaction conditions. To further investigate the behavior in short reaction time, continuous flow experiments were conducted utilizing liquid samples and employing the continuous flow tubular reactor apparatus. The solution was allowed to react at reaction times shorter

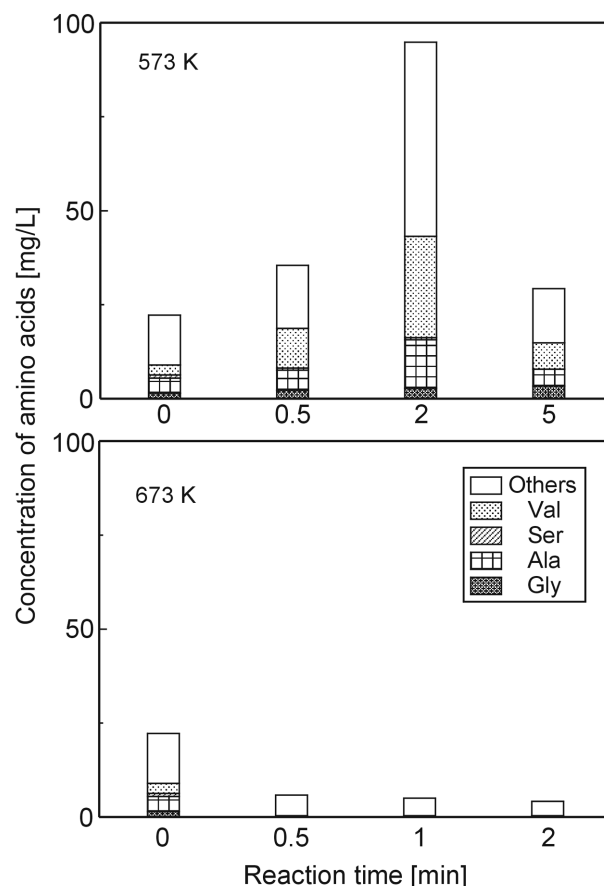


Fig. 6. Concentration of amino acids in the products obtained from continuous flow experiments at 573 and 673 K (P=30 MPa).

than 5 min and reaction temperatures of 573 and 673 K. The effect of reaction time (residence time in the reactor) on the concentration of amino acids of 573 and 673 K is shown in Fig. 6. The concentration of amino acids at reaction time of 0 min indicates the free amino acids in the original solution. At 673 K, the total concentration of amino acids decreased significantly at any reaction time because the degradation of amino acids is fast at high temperature. The highest amount of amino acids was obtained at 573 K and reaction time of 2 min. At 5 min, the total concentration of amino acids significantly decreased since most amino acids, except Gly, could have been degraded after 5 min. Asp and Ser were not detected in the products, presumably due to decomposition at these temperatures. Besides, only a small amount of Ser and no Asp was obtained in the acid hydrolysis of the sample.

The results agree with that of the semi-batch experiments; this suggests operation of the system in short reaction time and relatively higher temperature conditions to obtain high yield of amino acids. Operating conditions should be controlled depending on the reaction mode and desired amino acid (i.e., low temperature for high-molecular-weight amino acids and high temperature for low-molecular ones). In short reaction time of less than 3 min, a relatively higher temperature is preferred using the semi-batch or continuous flow mode and compared to the batch mode. Thus, the composition of the resulting products should also be considered.

6. Comparison of Amino Acid Composition Obtained from

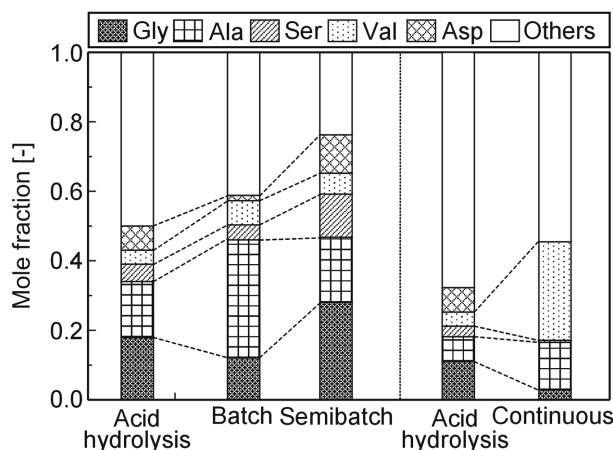


Fig. 7. Comparison of amino acid composition of products obtained from various reaction modes at 573 K (time=2 min, P=30 MPa, Note: the batch reactor was under saturated vapor pressure of 9 MPa).

Various Reaction Modes

Fig. 7 shows the comparison of amino acid composition of products obtained from various reaction modes at 573 K and reaction time of 2 min. The operating pressure was 30 MPa, except for the batch reactor that was under saturated vapor pressure of 9 MPa. The compositions of amino acids obtained by acid hydrolysis of the sample used in the experiment are also shown to serve as reference. The results of acid hydrolysis of the samples vary due to differences in sample preparations for batch (or semibatch) and continuous flow experiments. The acid hydrolysis of sample for continuous experiments contains high-molecular-weight amino acids that could have been dissolved easily in water.

At batch mode, low-molecular-weight amino acids such as Gly and Ala were dominant. In semi-batch and continuous flow operation, production of high-molecular-weight amino acids was enhanced. As discussed previously, this could be due to continuous removal of products from the reaction zone as soon as they are formed, thus inhibiting their decomposition into organic acids or volatile materials.

The combined fractions of Gly and Ala are almost the same for both batch and semi-batch experiments. However, Ala is dominant compared to Gly in batch than semibatch mode of reaction and vice versa. It should also be considered that it takes about 7 min for the batch reactor to reach the desired temperature of 573 K and the results presented here were taken during the transition period. Even if it so, the figure shows that the results of semi-batch operation are comparable to batch mode at short time. The continuous operation favors formation of high-molecular-weight amino acids compared to batch and semi-batch modes of operation. This behavior cannot be clearly explained and merits further investigation.

CONCLUSIONS

Various reaction modes (semi-batch, batch and continuous) are applied for reaction temperature and time effects study on production of amino acids in hydrothermal reaction. The following conclusions were obtained.

At 523 K, simple amino acids (Ala and Gly) were mainly obtained

at maximum yield of 65 and 28 mg/g-dry fish, respectively. At relatively lower temperature of 473 K, the yield of high-molecular-weight amino acids such as Asp and Ser is high, but decreases as temperature increases. It can be postulated from the results that high-molecular-weight amino acids decompose faster than low-molecular-ones. Thus, operation at 473 K would be sufficiently effective to obtain high-molecular-weight amino acids (Asp and Ser).

In semi-batch and continuous flow modes of reaction, recovery of high-molecular-weight amino acids at short reaction time and relatively higher temperature than 523 K increases the yield of total amino acids. The system suppresses decomposition of amino acids into organic acids or volatile materials by continuously removing the products from the reaction zone as soon as they are formed. It was also found that the composition of the resulting products also depends on reaction modes.

This result suggests that proper control of reaction temperature is necessary in order to obtain maximum yield of specific amino acids in the range of 474-573 K. Therefore, a hydrothermal process for production of amino acids from fish wastes should be operated at short reaction time. This is advantageous considering the problems (energy and capital cost) associated with the operation of a hydrothermal process.

ACKNOWLEDGMENT

This research was supported in part by 2001 Korea Sea Grant Program from Ministry of Marine Affairs and Fisheries.

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