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Production of valued materials from squid viscera by subcritical water hydrolysis

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Abstract: *Subcritical water hydrolysis was carried out to produce valued materials from squid viscera, the waste product of fish processing industries. The reaction temperatures for hydrolysis of raw and deoiled squid viscera were maintained from180 to 280°C for 5 min. The ratio of material to water for hydrolysis was 1:50. Most of the proteins from deoiled squid viscera were recovered at high temperature. The protein yield in raw squid viscera hydrolyzate decreased with the rise of temperature. The reducing sugar yield was higher at high temperature in subcritical water hydrolysis of both raw and deoiled squid viscera. The highest yield of amino acids in raw and deoiled squid viscera hydrolyzates were 233.25±3.25 and 533.78±4.13 mg g^{-1} at 180 and 280°C, respectively. Most amino acids attained highest yield at the reaction temperature range of 180-220°C and 260-280°C for raw and deoiled samples, respectively. The recovery of amino acids from deoiled squid viscera was about 1.5 times higher than that of raw squid viscera.*

Key words: *Subcritical water hydrolysis, Squid viscera, Amino acid, Valued materials*
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Introduction

Squid viscera like fish viscera are non-edible parts produced in large quantities in Korea by the fish processing industry as a by product. Since it was banned to dump these wastes into ocean by Landon treaty in 1996, the disposal has become a serious problem (Cheng *et al.*, 2008). These wastes contain a lot of protein, lipid and many kinds of biological active matter. But most of the wastes are not used as a source of earning. From environmental and economical point of view, it is appreciated to convert these wastes into useful materials. Some works have been carried out for isolation and identification of polyunsaturated fatty acids especially EPA and DHA, enzymes and other bioactive compounds from fish waste (Wu and Bechtel, 2008; Guerard *et al.*, 2002; Kang *et al.*, 2005; El-Beltagy *et al.*, 2004; Prasertsan *et al.*, 2001).

Recently the requirement of biomass (aquatic, livestock, bird *etc.*) products is enormously increasing around the world. Recognition of the limited biological resources and increasing environmental pollution have emphasized the need for better and more value-added utilization of the under-utilized fish and the by-products from the fishing industries. Traditionally, much of this material has been converted to powdered fish meal by a combined process of cooking, separation of soluble from insoluble, concentration of the soluble and dehydration of the insoluble (Ferreira and Hultinn, 1994). The hydrolysis of waste into value added products (proteins, amino acids, reducing sugar *etc.*) is an alternative and effective way. Current industrial hydrolysis methods of biomass waste include chemical (acid, alkali or catalytic) hydrolysis and enzymatic hydrolysis. But the chemical hydrolysis needs violent reaction conditions and often brings serious pollution of the environment. Enzymatic hydrolysis is expensive, and with long production cycle. Most of biomass waste is easily hydrolyzed in super- or subcritical water, which is structurally different from normal liquid water, and possesses some marvelous properties. Without any pollution, hydrolysis in super-or subcritical water is environment-friendly technology (Cheng *et al.*, 2008). Subcritical water is a promising medium for dissolution of biomass in water. The waste can be hydrolyzed into high value industrial raw material: amino acid, unsaturated fatty acid, oil and polysaccharide (Kang *et al.*, 2001; Yoshida and Tavakoli, 2004; Tavakoli and Yoshida, 2006; Toyoji, 2001) hydrogen and methane (Levin *et al*., 2007) and so on. The thermal protein hydrolysis is gaining in importance in economical as well as ecological aspects.

In our previous study, lipid was extracted from squid viscera for polyunsaturated fatty acids and also bioactive compounds by supercritical carbon dioxide (SC-CO_2) which is also environmentally friendly technology. Now a days, supercritical fluid extraction technology is used as an alternative for extraction of lipid and lipid soluble bioactive compounds to organic solvent extraction from different sources (Park *et al*., 2008; Temelli *et al*., 1995; Perakis *et al*., 2005, De Azevedo *et al*., 2008). However, after carrying out SC-CO_2 extraction, the squid viscera residues may also be used as a source of valuable materials. Therefore, the aim of this study was to produce the valued materials by subcritical water hydrolysis of the squid viscera residues obtained with SC-CO_2 extraction and also to compare them with that obtained from the production of raw squid viscera.

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Materials and Methods

The squid viscera samples were obtained from F & F Co., Busan, Korea. The visceral waste was washed and brought to the laboratory in iced condition. All reagents used in this study were of analytical or HPLC grade.

Sample preparation: The squid viscera samples were dried in a freeze-drier for about 72 hrs. The dried samples were crushed and sieved (700 μm) by mesh. These samples, called raw squid viscera, were then stored at -80°C. The stored samples were used for SC-CO_2 extraction of lipid and also for subcritical water hydrolysis (Uddin *et al.*, 2009).

Proximate composition: The moisture, ash and crude protein content were determined according to AOAC (1990) and lipid content was measured by conventional soxhlet extraction using hexane as solvent for 12 hrs. Non protein content was estimated by subtracting the sum of weight of moisture, ash, protein and lipid from total weight.

SC-CO_2 extraction: A laboratory scale supercritical fluid extraction unit was used for extracting oil from squid viscera. The SC-CO_2 extraction process has been described in details in our previous study (Uddin *et al.*, 2009).

Subcritical water hydrolysis: The subcritical hydrolysis was carried out in 80 ml of a batch reactor made of 276 Hastelloy with temperature control and stirring. The raw and deoiled material were suspended separately in distilled water at the material to water ratio of 1:50 and charged into the reactor. The reactor was then closed and heated by an electric heater which is previously heated to the desired temperature (180-280°C). The reaction time for each sample was 5 min. The short reaction time was considered to avoid the massive decomposition of amino acids into organic acids. The pressure in the reactor was estimated to be between 0.101 and 6.41 MPa based on saturated steam table for the temperature range studied. The reactor was then cooled immediately by immersing into cool water. The hydrolyzed sample from the reactor was collected and filtered. The liquid portion called hydrolyzate was analyzed for protein, amino acids and reducing sugars. All experiments were performed in duplicate.

Measurement of protein and reducing sugars: The protein content of the soluble product was analysed according to Lowry *et al.* (1951) using bovine serum albumin (BSA) as a standard. Reducing sugars content was analysed by dinitrosalicylic (DNS) acid method (Miller, 1959) using D-glucose as a standard. For each, 3 ml of the sample was added to 3 ml of DNS reagent. The mixture was heated in boiling water for 5 min. Then, 1 ml of 40% potassium sodium tartrate (Rochelle salt) solution was added to stablize the color, after which the mixture was cooled to room temperature in a water bath. The spectrophotometric reading was taken at 575 nm.

Analysis of amino acids: The protein concentration of the hydrolyzates of squid viscera obtained by subcritical water hydrolysis was made at 0.25 mg ml^{-1} by 0.02 N HCl. The sample was then filtered and loaded onto a Hitachi L-8900 amino acid auto analyzer for amino acid analysis.

Results and Discussion

Compositions of raw and deoiled squid viscera: The compositions of both raw and deoiled squid viscera are shown in Table 1. The squid viscera were dried in a freeze dryer for higher efficiency of SC-CO_2 extraction of lipid and lipid soluble bioactive materials. The data for SC-CO_2 extraction of lipid from squid viscera at different conditions are not shown. The highest yield of oil by SC-CO_2 extraction was approximately 34%, where as, it was about 39% by conventional organic solvent extraction. In raw material the protein content was approximately 45.76%. After deoiling by SC-CO_2, the protein content of squid viscera increased to 71.12%.

Protein yield in hydrolyzates: The amount of protein content in squid viscera hydrolyzates at different temperatures are shown in Table 2. It was found that the hydrolyzate of deoiled squid viscera contained more protein than that of raw material hydrolyzate. The hydrophobic content within the raw materials made them less accessible to water. The protein yield increased with the increase in temperature in subcritical water hydrolysis of deoiled squid viscera. On the other hand, the protein yield decreased with the temperature in raw squid viscera hydrolyzates. Watchararuji *et al.* (2008) reported that the protein yield in subcritical water hydrolysis of soybean was found to decrease when temperature increased from 200 to 220°C. The highest protein yields in raw and deoiled squid viscera hydrolyzates was 340.55±6.25 and 660.58±2.94 mg g^{-1} at 180 and 280°C, respectively. On comparing with the crude protein of squid viscera, this result suggested that almost all protein would be recovered in the hydrolysis of deoiled material sample. Similar results were found in subcritical water hydrolysis of rice bran and soybean meal (Watchararuji *et al.,* 2008). In general, at ambient temperature, protein normally has low solubility in water due to

Table - 1: Proximate compositions of raw and deoiled squid viscera

Composition (%)	Raw squid viscera (Freeze dried)	Deoiled squid viscera (SC-CO_2 extracted residues)
Moisture	3.5	2.51
Ash	3.15	5.57
Protein	45.76	71.12
Lipid	39.24	5.05
Non protein	8.35	15.75

Table - 2: Protein yield from raw and deoiled squid viscera by subcritical water hydrolysis at different temperatures

Composition (%)	Raw squid viscera hydrolyzate (mg g^{-1})	Deoiled squid viscera hydrolyzate (mg g^{-1})
180	340.55±6.25	439.35±3.12
200	324.25±4.89	434.25±5.79
220	303.12±2.55	460.21±1.69
240	302.22±5.66	468.56±2.19
260	296.22±1.76	616.5±3.55
280	275.76±5.22	660.58±2.94

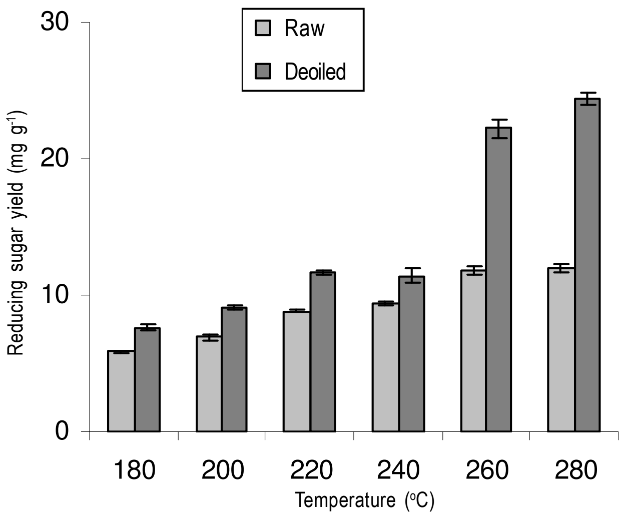

Fig. 1: Reducing sugar yield by subcritical water hydrolysis of raw and deoiled squid viscera at different temperatures

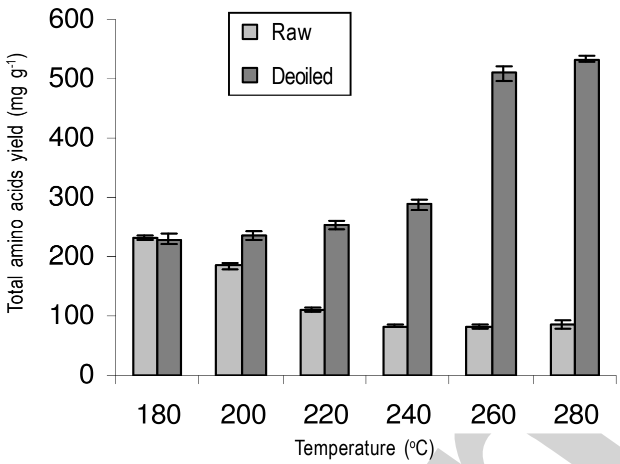

Fig. 2: Total amino acid yield by subcritical water hydrolysis of raw and deoiled squid viscera at different temperatures

strong aggregation through hydrophobic interactions. However the solubility of protein in water increased at higher temperature. Moreover, the protein yield increased at elevated temperature was due to the increased rate of hydrolysis activity caused by the increase in dissociation constant or ion product constant of water.

Reducing sugar yields: The reducing sugar content in raw and deoiled squid viscera hydrolyzates are shown in Fig. 1. Carbohydrate which reacts with hydronium and hydroxide ions produces reducing sugars. The amount of reducing sugar in both raw and deoiled squid viscera were increased with increasing temperature. The decomposition of reducing sugar was not favoured over its production within this temperature range (180-280°C) for short reaction time. Similar results were found in subcritical water hydrolysis of rice bran and soybean meal (Watchararuji *et al.,* 2008). The reducing sugar yield in hydrolyzate of deoiled viscera was higher than that of raw viscera hydrolyzate. This result also agreed with high content of non-protein substances in deoiled squid viscera (Table 1).

Amino acid yields: Amino acids play an important physiological role in all life-forms. Amino acids are relatively tasteless. Nonetheless, they contribute to the flavor of foods. Amino acids and protein hydrolyzates are therefore useful additives in food industry (Rogalinski *et al*., 2005). In this study, short reaction time had been used for subcritical water hydrolysis. The short reaction time decreased the decomposition of amino acids (Kang *et al*., 2001). Low ratio of material to water was used considering higher efficiency of subcritical water hydrolysis for amino acids yield. Lamoolphak *et al*. (2008) obtained highest amino acids yield by subcritical water hydrolysis at similar ratio of material to water. The total amino acids yield in raw and deoiled squid viscera hydrolyzates are shown in Fig. 2. It was found that the total amino acid yield of deoiled hydrolyzates were higher than that of raw material hydrolyzates. This result agreed with the high protein yield of deoiled squid viscera hydrolyzates comparing to raw material hydrolyzates. For deoiled squid viscera, the amino acids yield was increased with the rise temperature. The highest yield of amino acids in deoiled squid hydrolyzate was 533.78$\pm$4.13 mg g^{-1} at 280°C. Cheng *et al*. (2008)

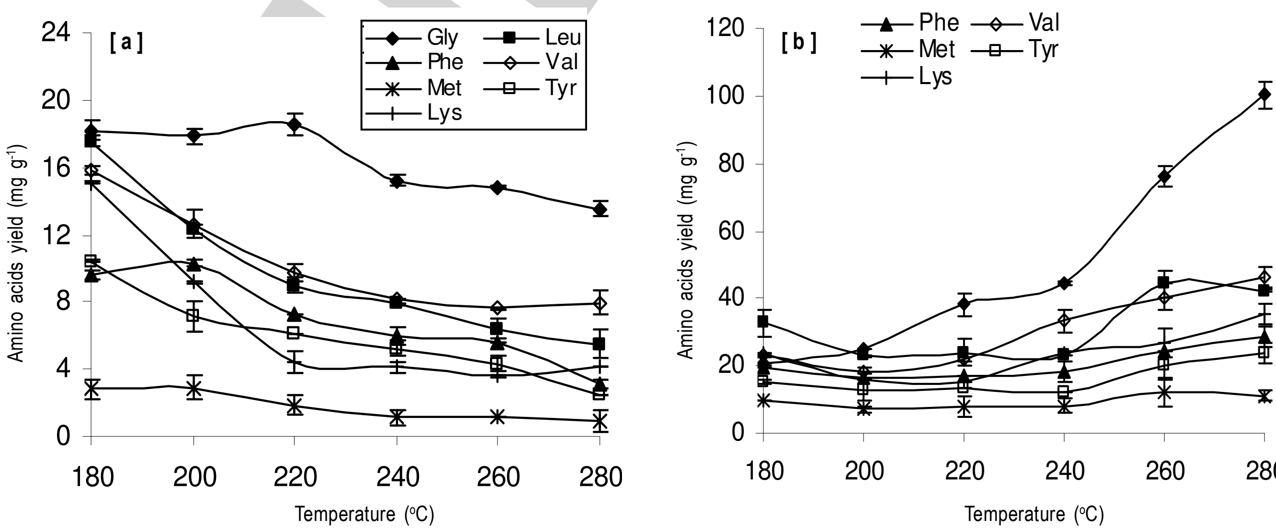

Fig. 3: Individual amino acids yield by subcritical water hydrolysis at different temperatures (a) Raw squid viscera (b) Deoiled squid viscera

Fig. 4: Recovery of amino acids from raw and deoiled squid viscera by subcritical water hydrolysis

also reported that the yield of amino acids increases with rise in temperature to a certain degree.

In raw squid viscera hydrolyzates, the opposite result was found in which the total amino acid yield was decreased with the increasing temperature. The highest amino acid yield in raw squid viscera hydrolyzates was 233.25±3.25 mg g^{-1} raw squid viscera at 180°C. The total amino acid yield in raw squid viscera hydrolyzate was low at high temperature. Since the amino acids yield in deoiled material was higher at high temperature, the oil present in the raw material may have interfered the breakdown of peptide of protein by subcritical water hydrolysis at high temperature. This was also in agreement with low protein yield in raw squid viscera hydrolyzates at high temperature. For short reaction time at high temperature, oil may form on complex with protein that may decrease the protein hydrolysis by subcritical water. On the other hand, high temperature causes the decomposition of amino acids into organic acids or other products. Kang and Chun (2004) also observed a significant decrease in the amino acid production from a hydrothermal process of fish-derived wastes due to the decomposition of amino acids into organic acids or volatile materials. In this study, the amino acids yield in raw squid viscera hydrolyzates was found more dominant to decomposition at low temperature.

The yield of some individual amino acids from raw and deoiled squid viscera by subcritical water hydrolysis at different temperatures are given in Fig. 3. For raw squid viscera hydrolyzates, the highest yield obtained within 180-220°C. On the other hand, for deoiled material it was found that the highest yield of amino acids obtained within the temperature of 260-280°C. Cheng *et al*. (2008) reported that most of amino acids reach maximum yield values at the reaction temperature range of 200-290°C. Some other works have been carried out in which the thermal degradation of amino acids occurs at temperature above 250-300°C, depending on the raw protein and corresponding contact time (Daimon *et al*., 2001; Yoshida *et al*., 1999; Quitain *et al*., 2001; Yoshida *et al*., 2003).

The highest recovery of amino acids from raw and deoiled squid viscera by subcritical water hydrolysis were about 51 and 76%, respectively (Fig. 4). The efficiency of subcritical water hydrolysis for amino acids yielding was significantly higher in deoiled material than raw material.

This study focused on the production of valued materials, especially amino acids, from squid viscera using subcritical water hydrolysis. Most of proteins from deoiled squid viscera were recovered in subcritical water hydrolysis at high temperature. In terms of protein hydrolysis, the highest amino acids yield from raw and deoiled squid viscera hydrolyzates were found at low and high temperature, respectively. The recovery of the amino acids from raw material hydrolyzate was lower than that of deoiled material hydrolyzate. Subcritical water hydrolysis was more effective for amino acid recovery from deoiled material than non deoiled material in short reaction time. Therefore, subcritical water hydrolysis may be a useful method for production of valued materials from squid viscera. This can act as source of food additives for living beings as well as a means to save the environment from pollution.

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