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Enzymatic hydrolysis of insoluble silk sericin by Alcalase

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ABSTRACT

This study was undertaken to figure out the effects of hydrolysis conditions on the solubility of insoluble sericin, molecular weight distribution and thermal characteristics of hydrolysates in enzymatic hydrolysis by Alcalase 2.5 L. It was indicated that the optimum treatment temperature and pH for the insoluble sericin were 50°C and 11, respectively. When the insoluble sericin was hydrolyzed with a various treatment conditions, the solubility of all hydrolysates were represented above 85% at given conditions. As the enzyme concentration increased, the solubility increased roughly, but the solubility increasement ratio was less above 2% enzyme concentration. As the treatment time increased, the solubility was also increased. It was showed in the molecular weight distribution of hydrolysates treated various enzyme concentrations and treatment times that when enzyme concentrations were 0.5, 2, 3%, the peaks of the distribution curve were shifted to left side which meant low molecular weight and was distributed much quantity with treatment times. When enzyme concentration was 1% and treatment time was below 4hr., the peak was shifted to left side, but treatment time was 6 hr. the peak was shifted to right side. When enzyme concentration was 5% and treatment time was below 2 hr., the peaks were shifted to right side, but treatment time was above 4hr. the peak was shifted to left side. The number-average molecular weights were distributed from 300 to 800 and those were decreased when treatment time was up to 4 hr., but increased a little when treatment time was 6hr. It was showed in the DSC curves of hydrolysates treated with treatment time of 0.5, 1, 2, 4, 6 hr. fixed 1% o.w.s enzyme concentration and control that the endothermic peak was observed near at 200°C. The denaturation peak of the hydrolysates depending on treatment times had a tendency to shift to higher temperature. But, when the treatment time was 6 hr., the peak was shifted to lower temperature comparing another hydrolysates.

Key words : Insoluble sericin, molecular weight distribution, thermal characteristics, Enzyme hydrolysis, Alcalase 2.5 L.

INTRODUCTION

Silk is still used for high-quality fibre materials owing to its quiet sheen, characteristic handle and excellent drapability. Recently the studies for silk as a new application not textile materials are being progressed. As a result silk can be used in the production of foodstuffs, cosmetics, special chemicals and pharmaceuticals and the application of silk extends increasingly to many fields (Kaplan *et al.*, 1993). In practice it was indicated that the silk products obtained by acylation can be used in the fields of cosmetic material, hygiene, medicine manufacture and foodstuffs (Patent JP 6-256274). Rhee *et al.* (1997) also reported that the silk fibroin powder into the kimchi suppressed the growth of lactic acid bacteria, naturally possessed superior storage capability and also tasted better.

Silk sericin with fibroin is an essential constituent of cocoon filament. The degumming of silk, the process of eliminating sericin, has been carried out to improve a silk goods in their qualities and after degumming, that is handled a waste matter because it is difficult to separate only sericin. If it is possible that collection of sericin from degummed solutions, collected sericin can be used for a new application materials as itself but also played an important part in environmental hazard.

Recently the use of silk sericin are being progressed with a wide range. In practice, the freeze-dried products of obtaining from sericin solution through treating in high pressure and temperature(100 ~ 140°C) indicated the possibility that can be used as a natural nutritive protein (Patent JP 49-117712). The sericin hydrolysates which have the average molecular weight of 300-3,000 produced by ultrafiltration after enzymatic degumming exist

in the state of water-soluble materials and have the membrane formation ability. So it is possible to use as the effective components of hair and skin cosmetic materials (Patent JP 62-36308). Sericin is not homogeneous protein, and Komatsu (1985) has separated into four fractions, namely sericin I, II, III and IV in the descending order of solubility in their dissolution velocities. Four fractions are different in their amino acid compositions and in higher order structure. That study also reported that the conclusion about easily or hardly soluble fraction corresponded to the sericin having comparatively lower molecular weight and higher molecular weight.

For using of bio-material and foodstuff, it is expected the effect of nutritional quality depending on the amino acid content.

The main composition of silk sericin is serine (33.43 %), aspartic acid (16.71%), glycine (13.49%), threonine (9.74%) and alanine (5.97%) (Chopra and Gulrajani, 1993) and it is similar to constituent some amino acid compositions of natural moist factor (NMF) dissolved waters at a horny layer, so that offered a excellent moisture condition to skin (Yamamoto, 1995). Aspartic acid is releasing hangover and glycine is inhibiting cholesterol level in blood and alanine is accelerating alcoholic metabolism and improve the liver function and threonine is indicating essential amino acid that growth development in the young and nitrogen metabolic balance in adult (Bae, 1994).

The possibility of using aqueous precursors in a very energy efficient process that yields high performance with controlled variability in their properties is an attractive goal (Kaplan *et al.*, 1993). Tsukada *et al.* (1994) indicated the aqueous silk fibroin solutions represented a good starting material for the preparation of different kinds of silk-based materials, such as gel, powder, porous membranes and homogeneous membranes. The solubility is the amount of protein in the solution that dissolves and disperses thoroughly and the protein solubility profile is an excellent index of protein functionality, indicating potential applications. So in case of good solubility, the application of proteins can be broadly expanded (Hung and Zayas, 1992). It was also reported that degree of hydrolysis, distribution of the molecular weight and composition of the free amino acids in the hydrolysates effected significantly the water solubility of the silk powder (Chen *et al.*, 1991).

The peptides produced by proteolysis have smaller molecular sizes and less secondary structure than natural proteins and may be expected to having functional properties like high solubility, low viscosity and also significant changes in the foaming, gelation and emulsifying properties from those of nature proteins (Chobert *et al.*, 1988).

It generally considered that the Enzymatic modification is milder than the chemical modification and it has an advantage of potential stereochemical specificity, which permits controlled processes and fewer side reactions. Hydrolysis can alter functional properties and nutritional value of the proteins. The optimum degree of hydrolysis depends on the product required (Nakai and Modler, 1996).

In view of the foodstuffs application about the effect of enzymatic hydrolysis, the small peptides are suitable for the production of flavor hydrolysates, whereas limited proteolysis yielding a mixture of polypeptides is favored for the enhancement of foaming and emulsification (Nakai and Modler, 1996). Several investigators have reported the functional properties of other enzymatically hydrolyzed food proteins from different sources using various proteolytic enzymes (Chobert *et al.*, 1988; Deeslie and Cheryan, 1988; Quaglia and Orban, 1990; Mahmoud *et al.*, 1992). Protein hydrolysates can be used nutritional reinforcement instead of protein containing soup, fruit juice, refreshing drink and etc. The reason is that the amino acids and peptides produced by hydrolysis remain stable on condition of low pH or heating process (Kim *et al.*, 1995).

Hydrolysis can alter nutritional value of the proteins as well as functional properties. In reality, the nutritional value of the enzymatic protein hydrolysates was tested and noted enhancement of the nutritional value (Imondi and Stradley, 1974; Lalasidis *et al.*, 1978; Lacroix *et al.*, 1983; Boza *et al.*, 1995). Protein hydrolysates are also useful in the manufacture of clinical nutrition products that must be easily absorbed. Peptides may be absorbed slightly better and more quickly than amino acids or whole protein according to several human and animal studies (Matthews *et al.*, 1977; Grimble and Silk, 1989; Boza *et al.*, 1995). This is due to the fact that dipeptides and tripeptides enter the absorptive cells by a process

involving active transport and their uptake systems are independent of those of free amino acids (Matthews *et al.*, 1977). Enzymatic hydrolysate containing small peptides may be utilized with greater efficiency than a mixture of free amino acids during catabolic states. That is the dietary protein is absorbed in the form of oligopeptides as well as free amino acids, so this possibility has important clinical implications and deserves further attention (Imondi and Stradely, 1974; Lalasidis *et al.*, 1978; Grimble and Silk, 1989). Chen *et al.* (1995) and Kim (1999) investigated the effects of hydrolysis conditions on the solubility and the distribution of molecular weight of sericin as well as silk fibroin by enzymatic hydrolysis.

It was considered that the control of molecular weight was very important for the wide range of applications of silk sericin.

So, It was carried out in this study to know the effects of hydrolysis conditions on the solubility and molecular weight distribution and thermal characteristics of hydrolysates which was hydrolyzed with Alcalase 2.5 L.

MATERIALS AND METHODS

1. Materials

Cutted cocoons(commercial products) were degummed at 130°C, for 1 hr.(L.R. = 1:30). The degummed solution was adjusted to pH 4 by HCl solution and kept at 70°C for 1 hr. We can obtained the insoluble sericin by filteration with that solutions. The concentration of insoluble solution was adjusted to 2%(w/w) by adding distilled water, and the suspension stirred by Homogenizer (Top Misung) was stored in refrigerator. When it was necessary, we used the solution after stirring sufficiently. Following experimental reagents and chemicals used in this study were the first grade. The enzyme was commercial products.

Enzyme (Alcalase 2.5 L, Novo, 2.5 AU/g)

NaOH (Sodium Hydroxide, Duksan pure chemical Co., Ltd)

HCl (Hydrochloric Acid, Duksan pure chemical Co., Ltd)

Filter paper (Whatman, 5C)

2. Methods

(1) The effects of pH on the solubility in enzymatic hydrolysis

Material (2% insoluble sericin solution) was diluted with 0.2% solution by adding distilled water. The pH of solutions was adjusted to 6, 7, 8, 9, 10, 11 and 12 with 1N HCl or 1N NaOH solution respectively and enzyme (5% on the weight of insoluble sericin(o.w.s)) was added, and hydrolysis was carried out at 60 for 2 hr. First hydrolyzed solution was precipitated with 1N HCl solution, and next was centrifuged (Beckman, J2-21) to remove turbid ma-terials. The ultraviolet absorption method was used to find optimum pH in enzymatic hydrolysis of insoluble sericin solution by UV-spectro photometer (Beckman, DU 650). The absorbance of supernatant were measured at 276 nm which represented maximum absorption wavelength, respectively. To figure out the degree of hydrolysis by only enzyme activity, the blank without enzyme were also treated at the same conditions.

(2) The effects of treatment temperatures on the solubility in enzymatic hydrolysis

Hydrolysis with 0.2% insoluble sericin solution was carried out at 30, 40, 50, 60 and 70°C, pH 11 for 2 hr. with enzyme (5% o.w.s), respectively. Supernatants were obtained as the same method above mentioned and measured as the ultraviolet absorption method and then the absorbance of samples were compared, respectively.

(3) The effects of treatment time and enzyme concentrations on the solubility in enzymatic hydrolysis

Hydrolysis with 2% insoluble sericin solution was carried out with enzyme concentrations of 0.5, 1, 2, 3, 5% o.w.s. and treatment times of 0.5, 1, 2, 4, 6 hr. at 50°C, pH 11, respectively. Hydrolyzed samples were immediately heated in boiling water bath (95°C) for 5 min. in order to inactivate enzyme. Hydrolyzed solution was adjusted to pH 4 by adding HCl solution and then filtered through filter paper. Therefore, the precipitate was washed in using distilled water and dried at room temperature.

The solubility was calculated as below :

$$\text{Solubility}(\%) = (W_i - W_p)/W_u \times 100\ (\%)$$

Where W_i is the weight of initial insoluble sericin and

W_p is the precipitated weight of insoluble sericin after hydrolysis.

(4) Analytical procedures

1) Preparation of analytical samples

The enzymatic hydrolysates obtained depending on conditions were filtered, and the precipitates were separated by centrifugation at 20,000 rpm. for 20 min. The soluble phases were freeze-dried (ISE, Bondiro) and used for analysis.

2) Gel Permeation Chromatography (GPC) analysis

The molecular weight distributions of the twenty five products were carried out by gel permeation chromatography (Viscoteck, USA) on the TOSOH G 3,000 SW-XL, in a column (7.8 mmID-30.0 cmL).

GPC runs were carried out under the following conditions.

Eluent : 0.2N $NaNO_3$

Sample concentration : 0.5% (w/v)

Column and injector compartment treatment : 35°C

Sample injection volumn : 100 microliter

The column eluent in GPC was continuously monitored by differential refractometer (RI detector), ultraviolet photometer, light scattering, diffractive pressure. To calibrate column retention in terms of specific molecular weights(M.W) and MW distributions, Pollutan Mw 5,900, Mw 11,800, Mw 22,800, Mw 47,300 (Showa Denko Standard Co. Ltd), PEG Mw 2,345, Mw 980, Mw 355 (American Polymer Standard Corp.) were used as standard reference materials.

3) Differential Scanning Calorimetry (DSC) analysis

Thermal analysis of hydrolysates by various teatment time, fixed enzyme concentration 1% o.w.s and insoluble sericin were carried out by DSC. DSC measurements were made with the universal V1 11A TA instruments calibrated over the range from 50 to 300°C at 10°C/min under a nitrogen gas purging.

Table 1. The sample I.D. of hydrolysates analyzed by GPC

Sample I.D.	Treatment conditions
T0.5	Treatment time of 30 min.
T1	Treatment time of 1 hr.
T2	Treatment time of 2 hr.
T4	Treatment time of 4 hr.
T6	Treatment time of 6 hr.

RESULTS AND DISCUSSION

1. The effects of enzymatic hydrolysis conditions on the solubility of insoluble sericin

Hydrolysis of peptide bonds change the functional properties of proteins. As the results of hydrolysis, the molecular weight of polypeptides decreases and the $-NH_3^+$, $-COO^-$ contents increase. It is well known that the solubility of proteins is mainly due to a reduction in the molecular weight and an increase in the number of polar groups (Damodaran *et al.*, 1997). It is apparent that the solubility of a protein or its hydrolysates are governed by its molecular size, but molecular size alone is not a sufficient criterion, even at the low molecular weights. The change in solubility could be due to specific changes in the distribution and arrangement of amino acids in the fractions obtained at longer operating times, resulting from changes in activity and specificity of the enzyme during reactor operating (Deeslie and Cheryan, 1988). Enzymes are generally used to modify proteins via hydrolysis of the peptide bonds to produce amino acids and peptides. Enzyme act on a limited number of substrates and will catalyze only one specific type of reaction. The specificity and catalytic activity of an enzyme depends on its chemical structure and molecular configuration (Nakai and Modler, 1996). It is very complex that the mechanism of enzymatic hydrolysis of insoluble protein substrates because the configuration of peptides bonds in protein and the affinity of enzyme with them are different each other. Archer *et al.*(1973) suggested that the kinetics of enzymatic hydrolysis of an insoluble substrate, enzymes adsorbed to an insoluble protein particle and, in a fast reaction, cleaved off polypeptide chains that were loosely bound to the surface, and acted on the more compacted core protein more slowly.

The dominant environmental factors affecting enzyme action are pH and temperature. The effects of pH on enzymatic reactions are caused largely by the reversible ionization of substrate or amino acid residues of the enzyme, which can affect substrate binding or transformation to product directly or affect enzyme stability (Nagodawithana, 1993).

Fig. 1 shows the effects of pH in enzymatic treatment solution on the solubility of insoluble sericin. The ultraviolet absorption method was applied to solubility. It

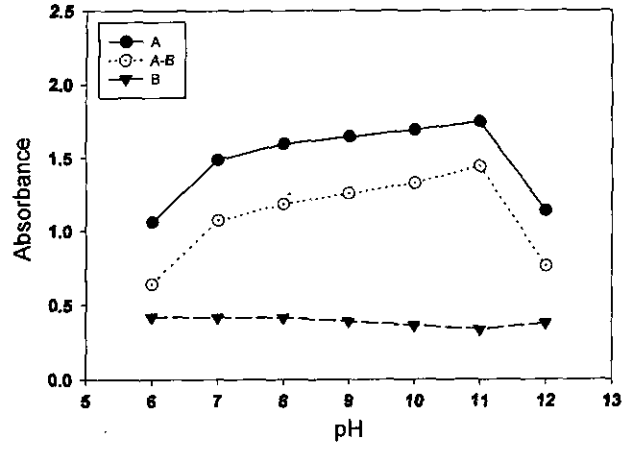

Fig. 1. The effect of pH on the solubility of insoluble sericin with Alcalase 2.5 L for 2 hr. at 60°C.
A : Absorbance of enzymatic hydrolysates on various pH
B : Absorbance of blank solutions on various pH
A-B : Absorbance of subtraction B from

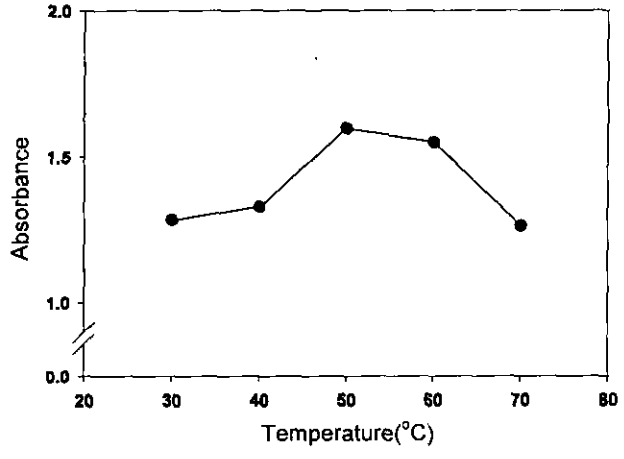

Fig. 2. The effect of treatment temperatures on the solubility of insoluble sericin with Alcalase 2.5 L for 2 hr. on pH 11.

was indicated that the absorbance was slightly increased from neutral range up to pH 11, but was rapid decreased above pH 11. These results suggest that the optimum pH is 11 in enzymatic hydrolysis using Alcalase 2.5 L.

Fig. 2 shows the effects of treatment temperature in enzyme hydrolysis on the solubility of insoluble sericin. Also the ultraviolet absorption method was applied. It was showed that the absorbance was increased depending on the elevating the treatment temperature up to 50°C, but decreased above that temperature. These results suggest that an appreciable inactivation of the enzyme occurs at the temperature above 50°C. That is due to denature enzyme and then lose the catalytic activity. Now then the hydrolysis was done at pH 11, at 50°C, which were considered to optimum pH and treatment temperature.

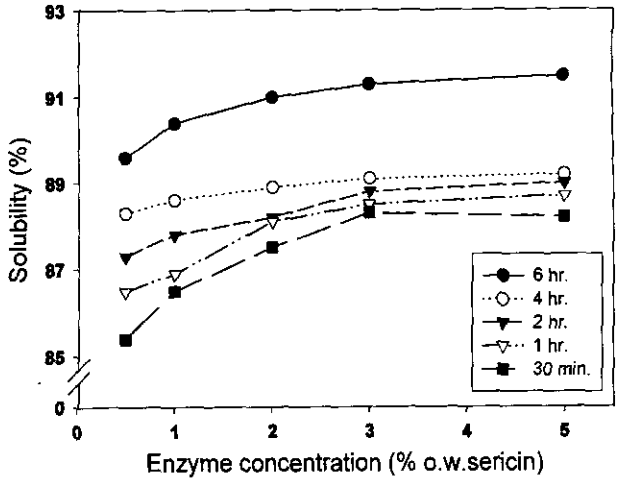

Fig. 3. The effects of treatment time and enzyme concentration on the solubility of insoluble sericin with Alcalase 2.5 L on pH 11 at 50°C.

Fig. 3 shows the solubility of hydrolysates depending on the enzyme concentrations and treatment times at 50 °C, pH 11. Practically, it was represented that the hydrolysates had high solubility as above 85% regardless of enzyme concentrations and treatment times. As the enzyme concentration increased, the solubility roughly increased, but the solubility increasement ratio is slight above 2% enzyme concentration. As comparing the solubility between the treatment time of 6 hours and below 4 hours, the difference of solubility was admitted regardless enzyme concentrations.

It could be explained from the schematic model for enzymatic hydrolysis of insoluble sericin as shown in Fig. 4. At the first stage, the soluble sericins are continued to be made from the insoluble sericin particles by hydrolysis with treatment times, and the second stage, hydrolysis was also continued from the soluble sericin already made. The size of soluble sericin particles made from first stage hydrolysis should be not uniform and big more or less. The size of soluble sericin made from second stage hydrolysis should be more uniform than that made from first stage and the size of insoluble sericin is going on small. Accordingly, as the treatment time is increasing, the solubility is also increasing. As going on the hydrolysis, insoluble sericin can be converted into a water soluble form by breaking down sericin itself and accordingly, the solubility could be increased abruptly from that time.

As shown in Fig. 3, the solubility increasement ratio less up to treatment time 4 hr. but, much from 4 hr. to

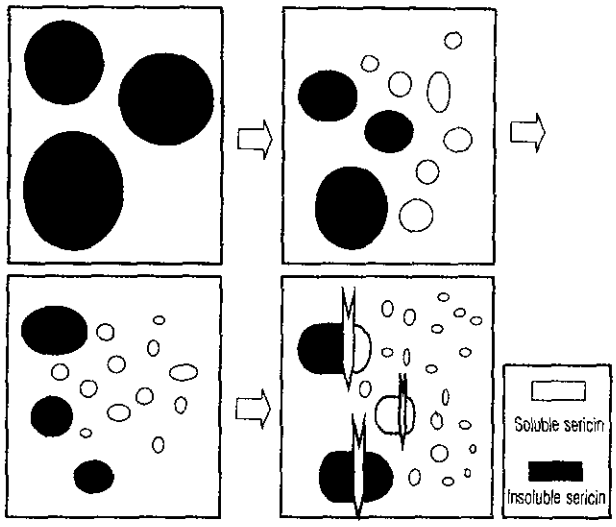

Fig. 4. The schematic model for enzymatic hydrolysis of insoluble sericin.

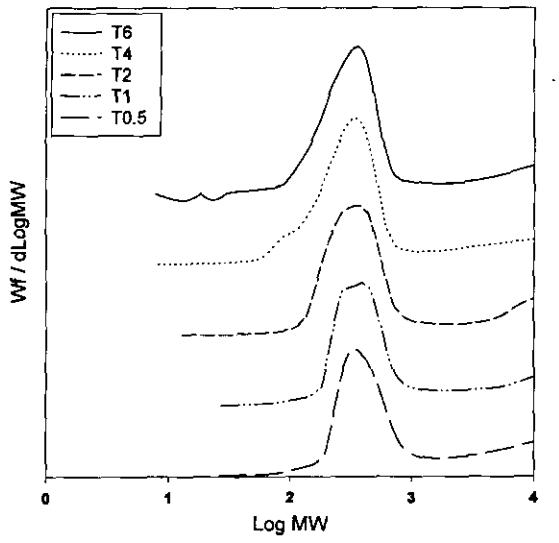

Fig. 5. Molecular weight distribution on peptide fractions of the hydrolysates by various treatment time, fixed enzyme concentration 0.5% o.w.s analyzed by GPC.

6 hr. That results could be explained that breaking down the insoluble sericin itself was happened at the treatment time between 4 hr. and 6 hr. So, it is considered that the treatment time is more effective than enzyme concentration to solubility. This is the reason for more effective that the solubility should be effected by the treatment time, because Alcalase 2.5 L is endoactivty type enzyme and the substrate is insoluble. In general, obtaining the same degree of hydrolysis the more treatment time is demanded in insoluble substrate than soluble because of structural hindrance.

2. The molecular weight distribution of enzymatic hydrolysates by GPC

It is difficult to define the molecular size quantitatively, as there are many size parameters that can be used to describe molecules or particles of difficult shapes and conformations. Gel permeation chromatography normally is used as an analytical procedure for separating small molecules by their difference in size and to obtain molecular weight average or information on the molecular weight distribution of polymers (Yau *et al.*, 1979). The number-average molecular weight is has been correlated with a number of profile of performance property dependence on molecule-structure parameters for typical parameter and is highly sensitive to the presence of a small number fraction of low molecular weight macromolecular (Rabek, 1980).

The molecular weight distributions of hydrolysates treated fixed enzyme concentrations and various treatment times on optimum pH and temperature were showed from Fig. 5 to 9.

Fig. 5 showed the results of analysis by GPC on hydrolysates treated with enzyme(concentration, 0.5% o.w.s.) for different treatment times. The left side area fixed on the basis on peak was larger in treated hydrolysate than non-treated and with treatment time. It meant that the hydrolysate having low molecular weight was much formed with treatment time. And it also meant that the degree of hydrolysis increased with treatment time and consequently the molecular weight of polypeptides decreased.

Fig. 6 showed the molecular distributions of hydrolysates treated with 1% enzyme concentration for various treatment times. Generally, the left side area fixed on the basis on peak was larger with treatment time. When treatment time was below 4 hr., the peak was shifted to left side, but treatment time was 6 hr. the peak was shifted to right side.

Fig. 7 showed the results of molecular weight distributions of hydrolysates treated with 2% enzyme concentration at various treatment times. It was indicated that not only the peak but also curve were shifted to left side definitely with treatment time. It meant that the peptides having low molecular weight were formed much more.

It was indicated at Fig. 8 that as increasing with the treatment time, relatively, the slope of right side on the

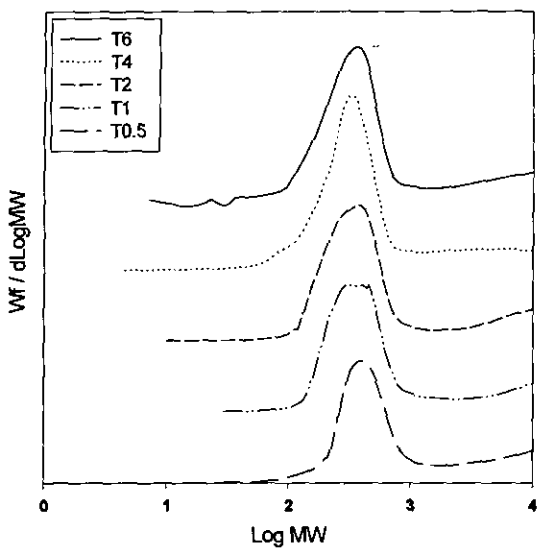

Fig. 6. Molecular weight distribution on peptide fractions of the hydrolysates by various treatment time, fixed enzyme concentration 1% o.w.s analyzed by GPC.

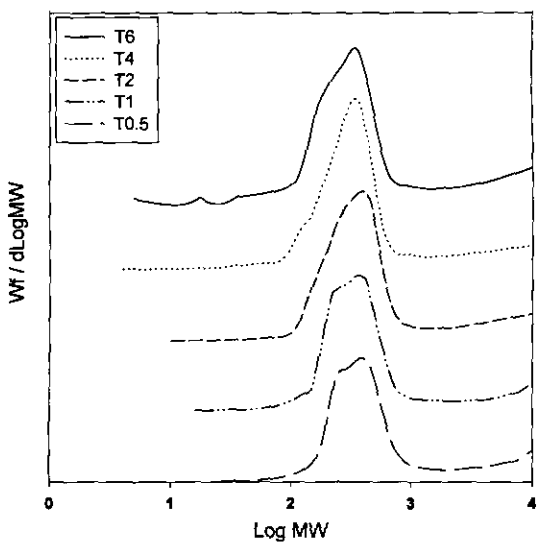

Fig. 8. Molecular weight distribution on peptide fractions of the hydrolysates by various treatment time, fixed enzyme concentration 3% analyzed by GPC.

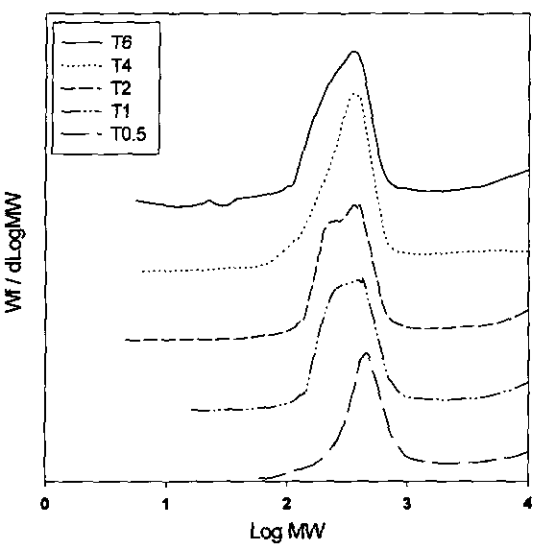

Fig. 7. Molecular weight distribution on peptide fractions of the hydrolysates by various treatment time, fixed enzyme concentration 2% o.w.s analyzed by GPC.

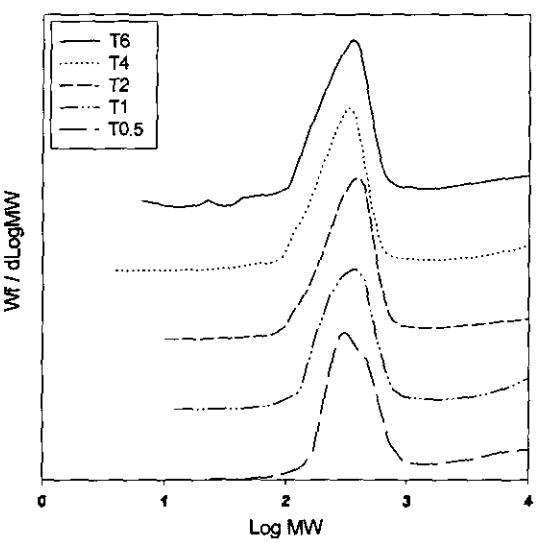

Fig. 9. Molecular weight distribution on peptide fractions of the hydrolysates by various treatment time, fixed enzyme concentration 5% analyzed by GPC.

basis of peak was steep much more, while that of left side was gradual. It was also considered that these results were due to becoming the low molecular weight peptides with treatment times.

Fig. 9 showed the molecular weight distribution of hydrolysates treated with various treatment times and fixed 5% o.w.s enzyme concentration. There were a little different from another GPC results. When enzyme concentration was 5% and treatment time was below 2 hr., the peak was shifted to right side, but treatment time was above 4 hr. the peak was shifted to left side. Because the substrate used this experiment was insoluble particle, the hydrolysis was not so simple.

In the reaction of hydrolysis, inhibitors which lower the catalytic activity of hydrolysis is related to the particle size of substrate, product inhibition and structure of substrate (Kim, *et al*, 1994). Accordingly, it is needed to more study in relation to the substrate structure in hydrolysis.

Fig. 10 showed the number average molecular weight

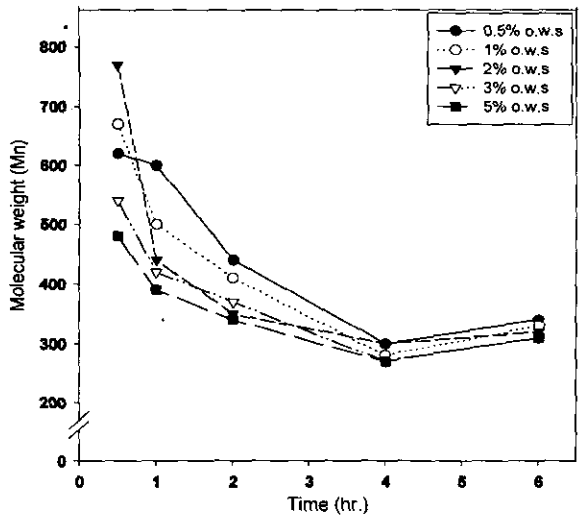

Fig. 10. Number average molecular weight of hydrolysates by various treatment times and enzyme concentrations analyzed by GPC.

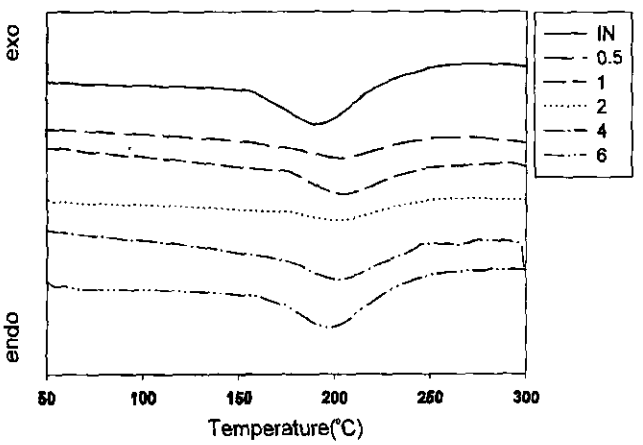

Fig. 11. DSC curves of hydrolysates treated with treatment time of 0.5, 1, 2, 4, 6 hr. fixed 1% o.w.s enzyme concentration and control of insoluble sericin powder.

of hydrolysates treated with various treatment times and enzyme concentrations. Entirely, the number average molecular weight distributed from 300 to 800, and it was extremely decreased up to treatment time, 4h r. but increased a little above 6 hr.

These tendencies could be also explained from the schematic model for enzymatic hydrolysis of insoluble sericin as shown in Fig. 4. The degree of hydrolysis and solubility were increased with increasing treatment time as shown in Fig. 3. It was evident that the more treatment time made the more solubility. But, in case of average molecular weight, that concept was not clear. The average molecular weight should be determined by both quantities of soluble sericin made from first stage hydrolysis and second stage. It is suggested that the molecular weight of soluble sericin is not determined by the solubility but the balance of two quantities. As these reasons, it is possible that the average molecular weight of hydrolyzed sericin at the treatment time 6 hr, is larger than that at 4 hr.

Enzymatic hydrolysis will provides the possibility of controlling the degree of hydrolysis in substrate. Using suitable enzyme concentrations and times, it will be permitted the production of hydrolysates with required molecular weight fractions. So, that can find the various applications.

3. The thermal characteristics of enzymatic hydrolysates by DSC

It was reported that the transition from random coil to β-structure and crystallization of silk sericin is observed around 200°C by DSC (Hojo *et al.*, 1980). Fig. 11 showed the DSC curves of hydrolysates treated with treatment time of 0.5, 1, 2, 4, 6 hr. fixed 1% o.w.s enzyme concentration and control of insoluble sericin powder. The endothermic peak was observed around 200°C, so that peak was regarded as the thermal denaturation. The denaturation peak of the hydrolysates depending on treatment times had a tendency to shift to higher temperature. But, when the treatment time was 6 hr., the peak was shifted to lower temperature comparing another hydrolysates. Considering the results of Fig. 6, the denaturation peak of hydrolysates were shifted to higher temperature as the peak of the distribution curve was shifted to left side which meant low molecular weight. Considering the results of Fig. 10, the endothermic peak of hydrolysates were also shifted to higher temperature as the number average molecular weight of hydrolysates lowered. It could be explained from those results as following. Generally, it is suggested that micro Brown motion needs lower thermal energy than macro Brown motion. Therefore, macro Brown motion runs above temperature beginning the micro Brown motion. However, with a view to molecular size on the basis of molecular weight, the large molecular weight whose molecular chain is liable to fracture is easily carried on micro Brown motion. With a view point of above, micro Brown motion was hard to carry on because enzymatic treatment lowered the molecular weight. So, the thermal denaturation peak had a tendency to shift to higher temperature.

적 요

불용성 세리신을 Alcalase 2.5 L 효소로 가수분해함에 있어 최적 처리 pH와 온도를 구하였고, 그 조건에서 처리 시간과 효소농도에 따른 용해도의 변화와 GPC에 의한 분자량 분포의 변화, DSC를 통한 열적 거동을 알아본 결과 다음과 같은 결론을 얻었다.

1. 불용성 세리신에 대한 최적 처리 pH와 처리온도는각각 11, 50°C일 때로 나타 났다.

2. 처리조건에 따른 가수분해물의 용해도 변화를 보면, 효소의 농도와 처리시간에 관계없이 모두 85% 이상으로 높게 나타났고, 효소의 처리농도가 증가함에 따라 전체적으로 용해도는 증가하는 경향을 보였으며, 효소의 양이 세리신의 2%까지는 현저하게 증가하였지만, 그 이상의 농도에서는 완만하게 증가되었 다.

3. 효소농도와 처리시간에 따른 가수분해물의 GPC에 의한 분자량 분포에서는, 효소농도가 0.5, 2, 3%인 경우는 처리시간이 경과함에 따라 peak가 저분자 쪽으로 shift 되었고, peak를 기준으로 좌측에 많이 분포되는 것을 확인할 수 있었고, 효소농도가 1%인 경우는 처리시간 4시간까지는 peak가 좌측으로 shift되다가 그 이상에서는 우측으로 약간 shift되는 경향을 나타냈으며, 효소농도 5%인 경우는 처리시간이 2시간일 때까지는 peak가 우측으로 shift 되다가 그 이상에서는 좌측으로 shift되는 경향을 나타냈다.

4. 효소 처리시간과 농도에 따른 가수분해물의 수평균 분자량은 300~800 사이에 분포하고 있었으며, 처리시간이 4시간까지는 대체적으로 감소하는 경향을 보이다가 그 이후에는 다소 증가하는 경향을 나타냈다.

5. DSC를 통한 열분석 결과에서는, 열변성 흡열 peak가 200°C 부근에서 나타났고, 처리시간이 4시간까지는 가수분해물의 열변성 peak가 고온측으로 shift되는 경향을 보이다가 그 이상의 처리 시간에서는 저온측으로 shift되는 경향을 보였다.

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