GLUCOSAMINE EXTRACTION FROM SILKWORM MOLT

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ABSTRACT

The objective of this study was to find the efficienct process of glucosamine extraction from hybrid silkworm molt. In principle, the main extractive process consisted of two steps. In the first step, silk worm molt was extracted to chitosan by deproteinisation, demineralisation, and deacetylation of chitin. In the second step, chitosan was depolymerised to glucosamine hydrochloride by hydrolysis reaction with variation of acid and base concentraion, temperature and reaction time. From the experiment of the first step, the suitable KOH concentration in deproteinisation was 4.0 % w/v at 80.0 °C for 1.5 hours while 1.0 M of HCl was the appropriate concentration in demineralisation at 45.0 °C and 1.5 hours. The appropriate deacetylation of chitin was to use 40% w/v KOH at 110.0 °C and 4.0 hours. The purity of glucosamine hydrochloride in depolymerisation was measured by HPLC (High Performance Liquid Chromatography) and compared the method of hydrolysis reaction to produce glucosamine by using HCl, *cellulase TV* enzyme and HCl followed by *cellulase TV.* enzyme as catalysts. The appropriate HCl concentration to hydrolyse chitosan was 10.7 M at 80.0 °C and 7.0 hours to reach 27.0% by crystal weight purity of glucosamine while the suitable ratio by weight in gram of *cellulase TV* enzyme to chitosan was 2:1 at 37.0 °C, pH 4.0 and 12.0 hours to obtain 76.2% by crystal weight purity of glucosamine. The hydrolization of chitosan by *cellulase TV* enzyme after HCl hydrolysis at the suitable condition was the most efficient to produce glucosamine hydrochloride by using the ratio by weight in gram of *cellulase TV* enzyme to chitosan at 2:1, 37.0 °C, pH 4.0 and 2.0 hours (total hydrolysis time was 9.0 hours) to reach 76.0% by crystal weight purity of glucosamine.

KEYWORDS : Chitosan / Glucosamine or Glucosamine Hydrochloride / Hydrolysis

1. INTRODUCTION

Glucosamine is the end product extracted from chitosan and it has been used to relieve arthritic pain in case of osteoarthritis in mammalian such as human, horse and dog many years ago [1,2]. The main source of chitosan could be extracted from crustacean shell insects, cell wall of yeast and mould [3]. Silk is the important exported product of Thailand. A lot of silkworm molt are left as the waste in the process of harvesting [4]. Penchouk (1983) reported that chitin is structure of silkworm molt[5]. Hence, the possibility to extract glucosamine from silkworm molt is an advantage not only for silk industries but also for drug industries. Glucosamine is mainly prepared in hydrolysis reaction by chemical and enzymatic method. Hitoshi et al. (2002) found that *cellulase TV* and *cellulase AC* are the most efficient enzymes to extract glucosamine from chitosan. HCl was also used to prepare glucosamine by hydrolysis of chitosan [6,7,8]. Thus, the aims of this study is to develop the method of extraction glucosamine from silkworm molt by using hydrolysis reaction of *cellulase TV* and HCl. This method will be useful for silk industry.

2. MATERIALS AND METHODS

Preparation of chitin10 g of silkworm molt was ground and deproteinized by 2, 3, 4, 5, 6, and 7 % w/v KOH solution. The reaction time variation in 0.5, 1.0, 1.5, 2.0, and 2.5 hours and the temperature variation in 60, 70, 80, and 90 °C were studied while stirring.

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The deproteinized molt was washed with distilled water until neutral (pH 7). HCl was used for demineralization at 0.65, 1.00, 1.30, 1.62, 2.00 and 2.30 M with time variation in 0.5, 1.0, 1.5, 2.0, and 2.5 hours and used different temperature at 25, 35, 45, and 55 °C while stirring. (Scheme 1)Preparation of chitosan (Scheme 1) :10 g of chitin was treated with 20, 30, 40, and 50% w/v KOH solution for 1.0, 2.0, 3.0 and 4.0 hours at temperature of 90, 100, 110, and 120 °C. The product was washed with distilled water until neutral then dry.

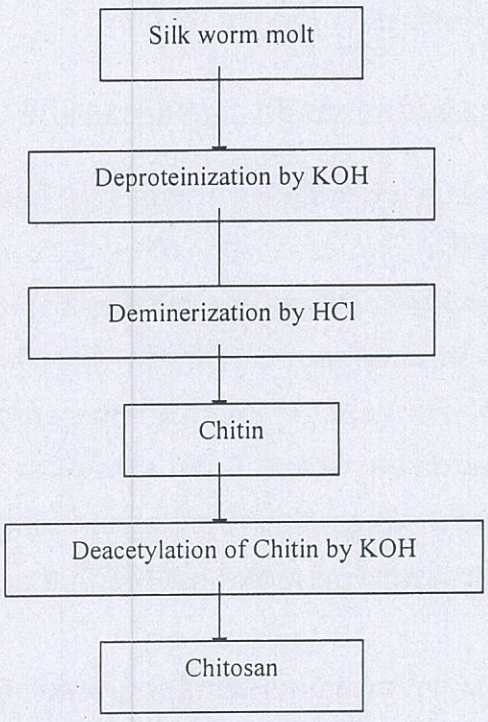
Preparation of glucosamine (Scheme 2) :Hydrolysis reaction was used to extract glucosamine from chitosan by comparing the percentage weight ratio of glucosamine to chitosan from hydrolysis reaction by HCl, *cellulase TV* and *cellulase TV* following HCl.

Hydrolyzed chitosan by HCl :12 M of HCl was used to find the suitable time and temperature for hydrolysation (10 g of chitosan) by variation time of 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 7.5, 8.0 and 8.5 hours at different temperature namely 70, 80, 90, and 100 °C. The appreciate time and temperature were used to investigate the best HCl concentration which varied from 4.9, 8.1, 9.7, 10.0, 10.4, 10.7, 11.0 and 11.3 M HCl.

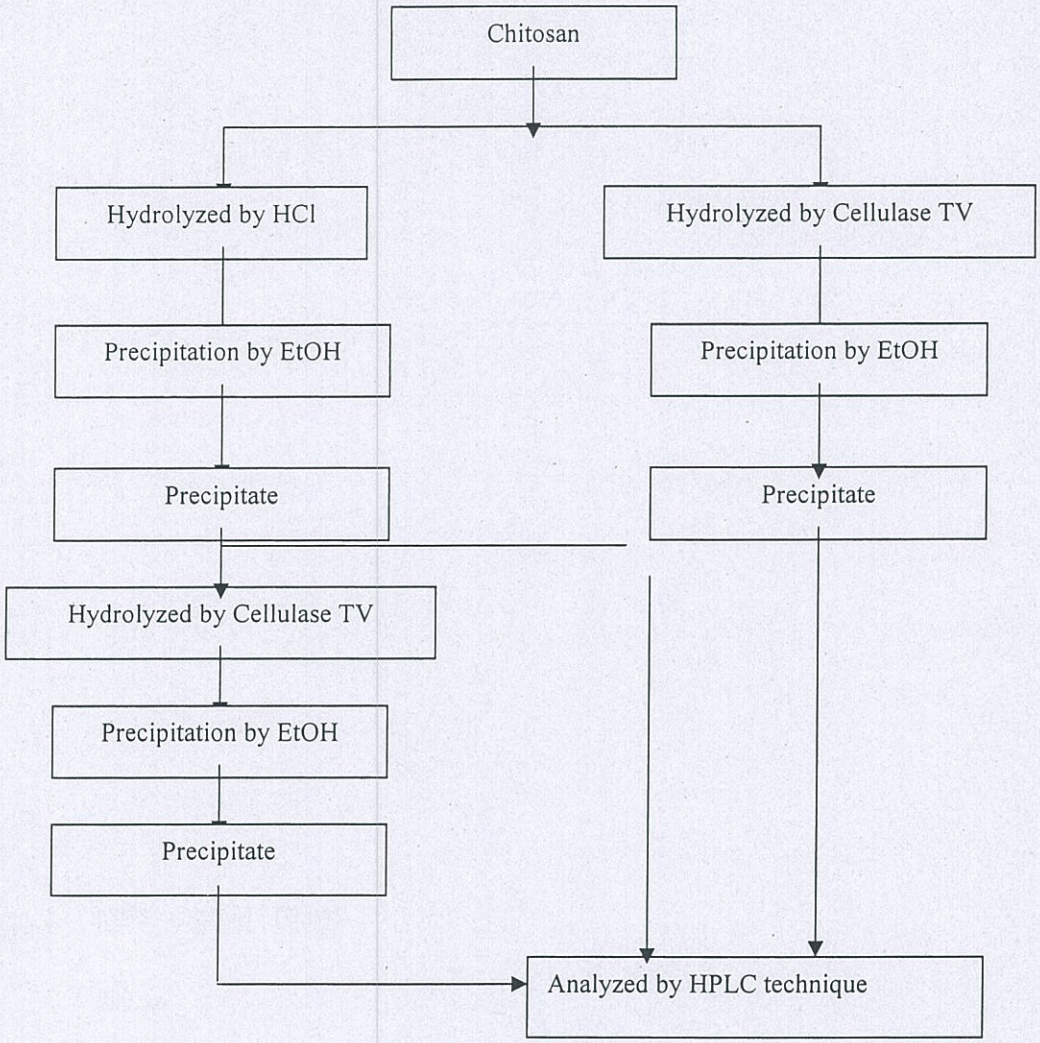
Hydrolyzed chitosan by *cellulase TV* :100 mg of chitosan was dissolved by 20 ml of acetate buffer solution and mixed with 20 ml enzyme solution at variation of weight ratio of enzyme to chitosan at 1:1, 2:1 and 3:1 at 37 °C and pH 4.0 for 10.0, 11.0, 12.0, 13.0, 14.0, 15.0, and 16.0 hours.

Hydrolyzed chitosan by *cellulase TV* following HCl :100 mg oligomer of chitosan from the hydrolysis reaction by 10.7 M HCl solution was hydrolyzed by enzyme with the same process mention above but varied the time for 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 hours.

The end product of hydrolysis reaction was purified by ethanol and the purity of glucosamine was determined by HPLC using Asahi pack amino column with flow rate of mobile phase 0.1 ml/min, IR detector and mobile phase was acetonitrile/water (70:30 by volume).



Scheme 1 Methods for Preparation of Chitin and Chitosan



Scheme 2 Methods for Glucosamine preparation

3. RESULTS AND DISCUSSION

In chitin preparation processes, 4.0% w/v KOH at 80.0 °C for 1.5 hours was the suitable conditions for deproteinization while 1.0 M of HCl at 45.0 °C for 1.5 hours was found to be the best condition for demineralization.

In the process of chitosan preparation, the appropriate condition to deacetyl of chitin was 40%w/v KOH at 110 °C for 4.0 hours.

The results of chitosan hydrolysis to obtain glucosamine by 12 M HCl are shown in fig. 1 and table 1. The quantity of cystal obtained from the HCl hydrolysis at 70 °C was the lowest weight compared to those of other temperature levels. However, the temperature at 80 °C was the most efficiency to get similar amount of crystal comparing with hydrolysis at 90 and 100 °C. Moreover, the result in table 1 showed that 7 hours of reaction time is the shortest time to reach the glucosamine percentage similar from longer reaction time (7.5 and 8.0 hours).

Table 1. Percent by weight of glucosamine in crystal from HCl hydrolysis measured by HPLC with variation of temperature and time.

Condition		Percent of glucosamine in crystal
Temperature (°C)	Reaction time (h)	
80	6.0	24.36
	7.0	26.98
	7.5	26.99
	8.0	27.01
90	7.0	26.96
	7.5	27.05
100	7.0	26.97
	7.5	27.00

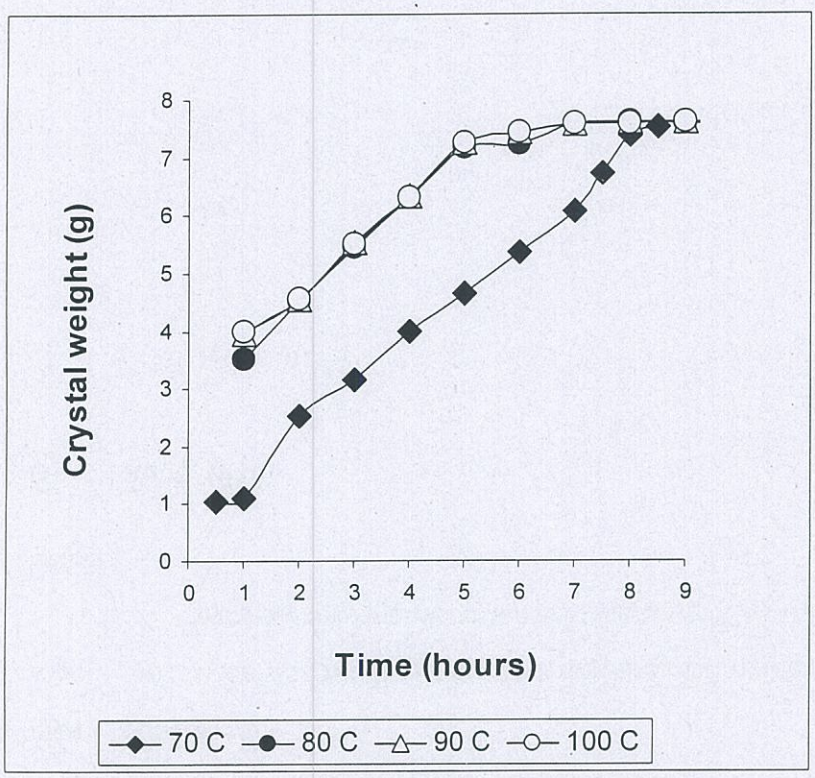


Figure 1. Relationship between the crystal weight of glucosamine and hydrolysation time of HCl. Chitosan was hydrolyzed by conc.HCl with variation of temperature.

From Fig. 2, when the HCl concentration was increased from 4.9 to 10.7 M, the glucosamine crystal weight were increased whereas the weight was constant after the HCl concentration increase from 10.7 M upward. The percentage by weight of glucosamine crystal obtained from each of HCl concentration were shown in table 2, it showed that the appropriate HCl concentration of 10.7 M with 7.0 hour reaction time resulted in 27.00 percent by weight of glucosamine crystal.

Table 2. Percentage of glucosamine in crystal from various concentration of HCl hydrolysis measured by HPLC with variation of HCl concentration.

Condition		Percent by weight of glucosamine crystal
HCl concentration (M)	Reaction time (h)	
10.4	7.0	25.88
10.7		27.00
11.0		26.96
11.3		27.14

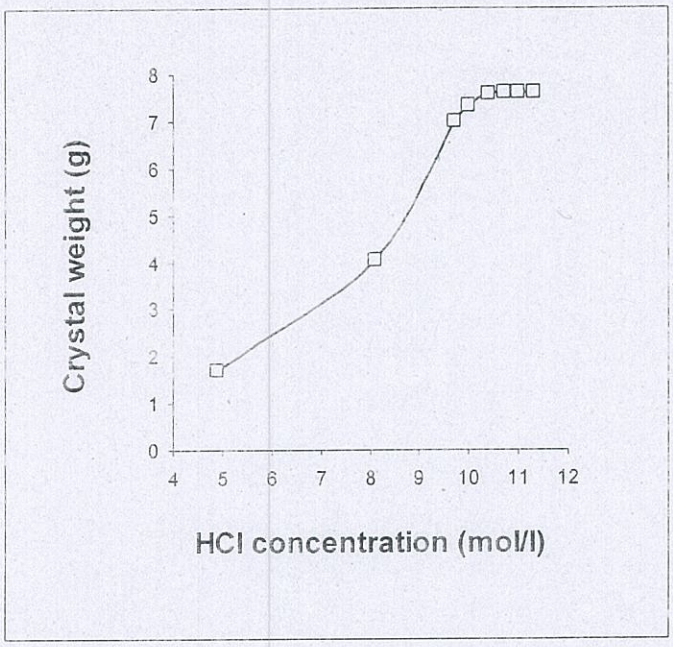


Figure 2. Relationship between crystal weight and HCl concentration. Chitosan was hydrolyzed with variation of HCl concentration.

The results of glucosamine production from the hydrolysis of chitosan by *cellulase TV* was shown in table 3 and fig. 3. The crystal weight obtained from using enzyme to chitosan by weight of 2:1 and 3:1 were significantly different from 1:1. So, table 3 shows the percentage of glucosamine crystal obtain from using *cellulase TV* hydrolysation measured by HPLC with variation of the weight ratio by gram of chitosan to glucosamine and time. The 12 hour reaction time was the shortest time to get the weight percentage of glucosamine similar from longer reaction time .

Table 3. Percent of glucosamine in crystal from using *cellulase TV* hydrolysation measured by HPLC with variation of temperature and time.

Condition		Percent of glucosamine in crystal
Weight ratio of enzyme to chitosan	Reaction time (h)	
2:1	11.0	72.76
	12.0	76.16
	13.0	75.85
	14.0	76.18
3:1	11.0	73.82
	12.0	75.68
	13.0	75.86

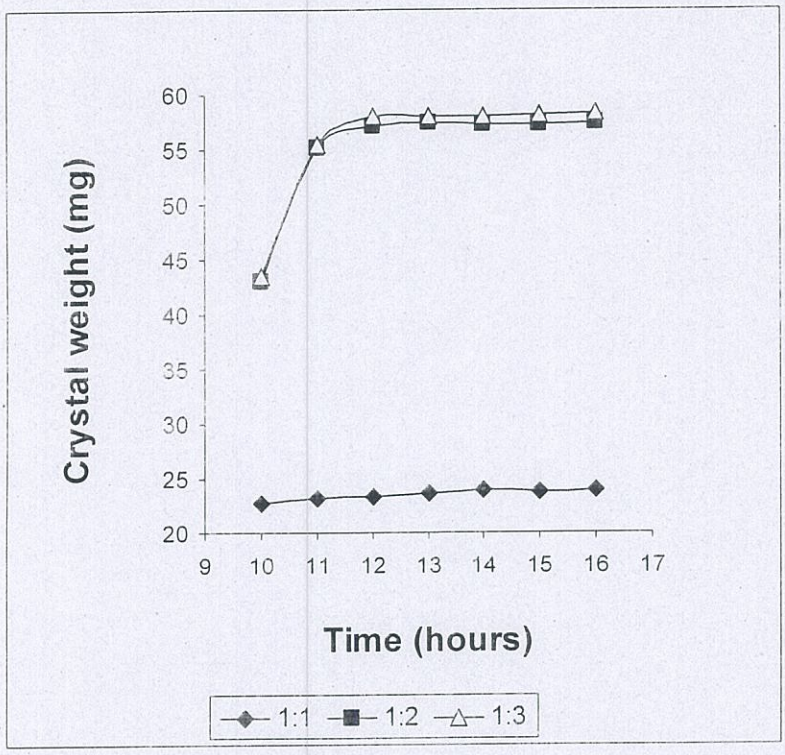


Figure 3. Relationship between the crystal weight of glucosamine crystal and the hydrolysation time ofreaction of enzyme. Chitosan was hydrolyzed by *cellulase TV* at 37 °C, pH 4 with weight ratio of enzyme to chitosan at 1:1, 1:2 and 1:3.

The hydrolysis results of chitosan oligomer under HCl and followed by enzyme *cellulase TV* were shown in table 4 and Fig.4. Weight ratio of enzyme to chitosan at 1:1 was less efficient to get the same amount percentage of glucosamine. However, if the time for hydrolysis was longer, the same amounts of glucosamine were obtained comparing with those using 2:1 and 3:1 wieght ratio of enzyme to chitosan. Figure 4 showed that the highest and stable weight of crystal was obtained when 2:1 and 3:1 weight ratio were used for hydrolysis within 2 hours. Using 2:1 weight ratio within 2 hours was more efficient by the reason of time and enzymatic cost.

Table 4. Percentage of glucosamine in crystal from HCl followed by *cellulase TV* hydrolysation measured by HPLC with variation of temperature and time.

Condition		Percent of glucosamine in crystal
Weight ratio of enzyme to chitosan	Reaction time (h)	
2:1	1.0	75.70
	2.0	76.06
	3.0	75.91
3:1	2.0	76.08
	3.0	76.08

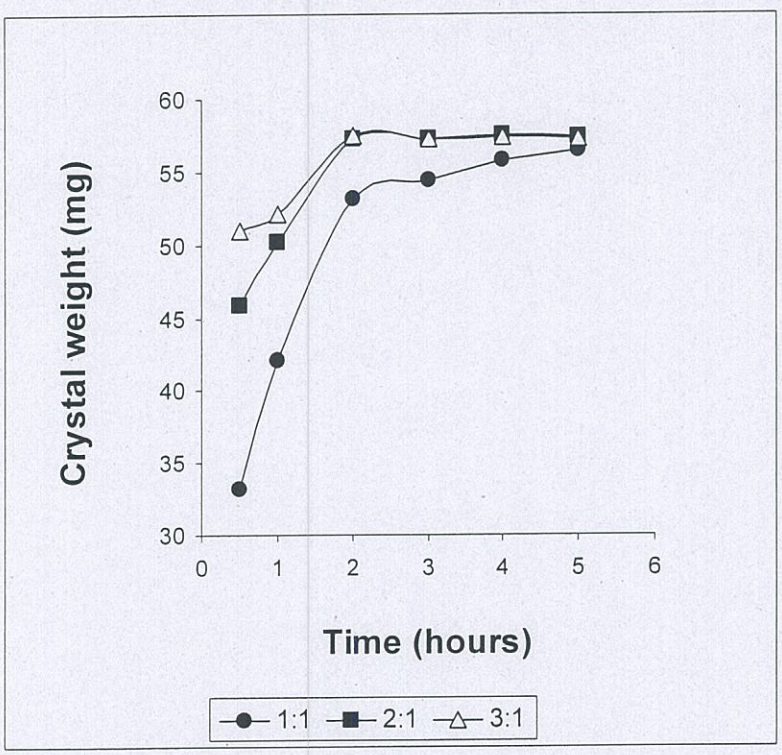


Figure 4. Relationship between the weight of glucosamine crystal and the hydrolysation time of HCl followed by enzyme. Chitosan was hydrolyzed by *cellulase TV* at 37 °C, pH 4 with weight ratio of enzyme to chitosan oligomer at 1:1, 1:2 and 1:3.

4. CONCLUSION

The different method of chitosan hydrolysis gave the different percentage of glucosamine. The suitable HCl concentration for chitosan hydrolysis was 10.7 M at 80.0 °C for 7 hours giving 27.0 %w/w glucosamine, while the suitable weight ratio of *cellulase TV* to chitosan was 2:1 at 37.0 °C, pH 4.0 for 12.0 hours giving 76.2%w/w glucosamine. The most efficiency to save time with high purity of glucosamine was reached when cellulase TV following HCl was used for hydrolysation at weight ratio of *cellulase TV* to chitosan 2:1, 37 °C, pH 4 for 2 hours giving 76.0% glucosamine. However, the best method for extraction depends on the need of customer who need to save time, economy or amount of glucosamine. The hydrolysis by HCl was the low cost but get a little amount of glucosamine. On the other hand, the hydrolysation by *cellulase TV* whether following HCl or not gave the high percentage of glucosamine but expensive. There will save more time when *cellulase TV* was used following HCl. Nevertheless, the cost for enzyme hydrolysis could be reduced especially in glucosamine producing industry to produce large amount of glucosamine with low cost of enzyme which could extracted from *Trichroderma Viride* (mold name) instead of import from aboard.

5. ACKNOWLEDGMENT

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