OPTIMIZATION OF GELATIN EXTRACTION FROM LEATHER SCRAP WASTES USING DESIGN OF EXPERIMENT METHOD

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Title	Optimization of Gelatin Extraction from Leather Scrap Wastes using Design of Experiment Method
By	Ms. Kanruethai Charoensook
Field of Study	Petrochemical Engineering
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Abstract

The aim of this project is to find suitable conditions for gelatin extraction from leather scrap wastes. The alkali hydrolysis is a selected method to break down leather molecules. The total of 24 experiments was carried out randomly in laboratory scale by using multi-level factorial experimental design methodology to study three variables, i.e., concentration of calcium oxide (CaO) (4, 5 %wt.), temperature (30, 70 °C), and reaction time (6,9, and 12 hrs.). The regression analysis and response optimizer represented that maximum percent yield of gelatin could be achieved at 4.640 with hydrolysis condition at 4 wt% of CaO, room temperature (30 °C), and 12 hrs. of reaction time. The proximate analysis revealed that the average protein in a sample is 2.18%. In addition, the decolorization process of gelatin product was also studied. The black color from leather scrap waste was successful removed by Fenton reaction with ratio of Fe^{2+}:H_2O_2 at 3:50, pH of 3 for 6 hrs., and followed by adsorption of activated carbon. For the pilot scale experiment, 5.61 and 9.46 % yield were achieved by using CaO at 4 wt. %, room temperatures but extended reaction time to 18 and 24 hrs., respectively. The proximate analysis is revealed that the average protein in a sample is 22.73% and 52.34% respectively.

Keywords: Protein, Gelatin, Waste treatment, Extraction, Chrome-tanning leather waste.

เรื่อง	การหาสภาวะที่เหมาะสมที่สุดในสกัดเจลาตินจากเศษหนังฟอกโดยใช้วิธีการออกแบบการทดลอง
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บทคัดย่อ

วัตถุประสงค์ของโครงการนี้คือการหาสภาวะที่เหมาะสมในการสกัดเจลาตินจากเศษหนัง โดยใช้วิธีการไฮโดรไลซิสด้วยด่างเพื่อย่อยโมเลกุลของหนัง ทำการทดลองแบบสุ่มทั้งหมด 24 การทดลองในห้องปฏิบัติการ โดยการใช้วิธีการออกแบบการทดลองแบบแฟคเทอเรียลที่มีหลายระดับเพื่อศึกษาตัวแปร 3 ตัวได้แก่ ความเข้มข้นของแคลเซียมออกไซด์(CaO) (4 และ 5 % โดยน้ำหนัก) อุณหภูมิ (30 และ 70 °C) เวลาในการทำปฏิกิริยา (6, 9, และ 12 ชั่วโมง) ผลการวิเคราะห์การถดถอยและการหาค่าที่ดีที่สุดของผลตอบสนองพบว่า ร้อยละผลได้สูงสุดที่ทำได้คือ 4.64 โดยใช้สภาวะการไฮโดรไลซ์ ที่ CaO 4 % โดยน้ำหนัก อุณหภูมิห้อง (30 °C) และ เวลาในการทำปฏิกิริยา 12 ชั่วโมง ผลการวิเคราะห์ปริมาณโปรตีนด้วยวิธีประมาณพบว่าในตัวอย่างมีโปรตีนเฉลี่ย 2.18% นอกจากนี้ยังทำการศึกษากระบวนการกำจัดสีจากผลิตภัณฑ์เจลาติน ซึ่งพบว่าสามารถกำจัดสีดำที่มาจากเศษหนังได้โดยใช้ปฏิกิริยาเฟนตัน ที่ความเข้มข้นของ Fe^{2+} : H_2O_2 เท่ากับ 3:50 ที่พีเอชเท่ากับ 3 เป็นเวลา 6 ชั่วโมง ตามด้วยการดูดซับด้วยถ่านกัมมันต์ สำหรับการศึกษาในระดับกึ่งอุตสาหกรรมพบว่าได้ ร้อยละผลได้เท่ากับ 5.61 และ 9.46 เมื่อใช้ CaO 4 % โดยน้ำหนัก อุณหภูมิห้อง (30 °C) แต่เพิ่ม เวลาในการทำปฏิกิริยาเป็น 18 และ 24 ชั่วโมงตามลำดับ ผลการวิเคราะห์ปริมาณโปรตีนด้วยวิธีประมาณพบว่าในตัวอย่างมีโปรตีนเฉลี่ย 22.73 และ 52.34% ตามลำดับ

คำสำคัญ: โปรตีน, เจลลาติน, การกำจัดของเสีย, การสกัด, เศษหนังฟอกโครม

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CHAPTER 1
INTRODUCTION

1.1 Background

Leather industry is the one of important economic sector since leather product could be used to make various goods such as footwear, garment and furniture. The growth of world demand of leather products causes an increasing of leather wastes as well. The leather manufacturing process is divided into three main process, i.e., preparation of fresh hides, tanning and dyeing. During manufacturing process, various of chemicals such as alkali and sulfide are used to remove the remaining of hair and unwanted material in tanning process, chromium sulfate as a tanning agent, and pigment in dyeing process. Solid wastes from leather industry such as skin trimming, chrome shaving wastes and leather scrap wastes are consisted of proteins. However, the utilization of leather scraps is not easy since it is contaminated with heavy metal and pigment from manufacturing process. Therefore, the leather scrap wastes were mostly unutilized and sent to open dumping. However, open dumping of leather scraps wastes is unacceptable disposal method since chromium wastes could contaminated soil and water resources.

The alternative way to alleviate the leather scrap wastes is converting wastes to valuable materials. The treatment processes are divided into three steps, i.e., dechroming, extraction of protein, and application of extracted protein [1]. The dechroming step has been studied the previous work of separation of chromium from leather wastes [2]. This research will focus on the extraction protein. In addition, there are no available research on extraction of protein from leather waste in pilot scale. Therefore, this research will also plan to carry out in pilot scale.

1.2 Objectives

To find suitable conditions for proteins extraction from leather scrap wastes.

1.3 Scope of work

1) Find the effective conditions to extract gelatin from chrome tanning scraps in lab scale.

2) Find the effective condition to remove the dye bound from the extracted solution.

3) Perform an extraction of gelatin from chrome tanning scraps in pilot scale.

1.4 Expected outputs

The suitable conditions to extract gelatin from leather scraps.

CHAPTER II

THEORY AND LITERATURE REVIEW

In this chapter, the theories that relevant the research are presented, including leather manufacturing process, structure and components of leather scrap, hydrolysis reactions, design of experiments, Fenton oxidation process and literature review.

2.1 Leather Manufacturing Process [3].

As a matter of fact, animal skin is soft, flexible and tough when animal is alive but when the animal dies the skin will lose these characteristics. If it keeps wet, it rots and if it dries, it goes hard and brittle. The leather manufacturing process is conducted to retain the skins natural properties to stabilize its structure and prevent the putrefaction. There are three main processes in leather manufacturing industry, i.e., beam house process, tanning process, and finishing process.

2.1.1 Beam House Process

Beam house process is the preparation of animal hides before going to the tanning process. In this process, the hides are soaked in an alkali solution for breaking down the structure of the hair and removing the hair out as shown in figure 2.1. The hairless skin is then soaked in a sulfide solution to complete the removal of the hair and modify the properties of the skin proteins. The skin structures are opened and then treated with enzymes, further unwanted materials are removed. Then the skins are treated with acid solution to preserve them for at least two years.

Figure 2.1 Fresh hides are soaking in paddle [4].

2.1.2 Tanning process

After the beam house process, the skins are transferred to tanning process, which chemicals usage to stabilize skin by replacing the collagen with complex ions. There are two type of the tanning process, i.e., chrome tanning process and vegetable tanning process.

1) Chrome tanning process

Chrome tanning process is a popular method for tanning process because of shorter the operating time, and lesser chemical usage than other method. Furthermore, the leather product is more durable enough to withstand warm and moist environment. Chrome tanning process is operated in large agitation tanks contain chrome salts, as a tanning agent as shown in figure 2.2. Typically, 70% of chromium added are reacted with skins and 30% are unreacted and disposed in wastewater. After the tanning process, Magnesium oxide are used to adjust the pH to 4.5, which is the proper condition for embedding chrome into the leather. After embedding chrome for certain period, the leather products are transferred to the neutralization process.

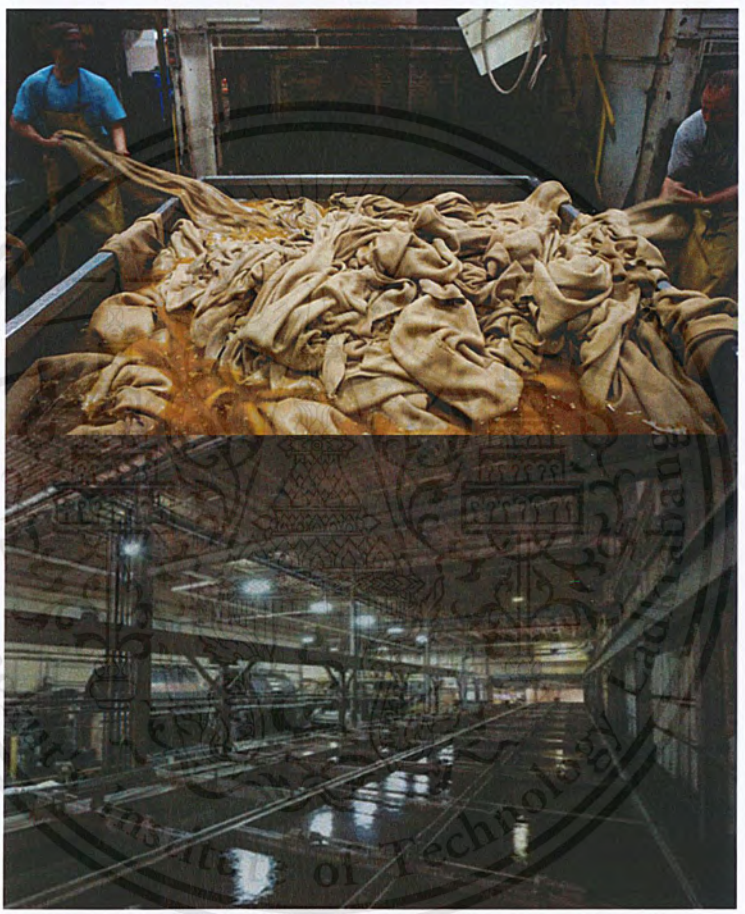


Figure 2.2 Chrome tanning process [5].

2) Vegetable tanning process

Vegetable tanning process is another type of tanning process. Tannins that extracted from various part of plants including woods, barks and leaves is used as tanning agent. Vegetable tanning process is operated in the series of wood agitation tanks as shown in figure 2.3. After tanning process, rinsing process using salicylic acid is necessary for removing of excess tanning agent, which is effect to the quality of leather. After that leather is dried and transferred to next step. Although the vegetable tanning process is better for environment, it is not proper for the industrial scale since it requires longer time and more expensive tanning agent than chrome tanning process.

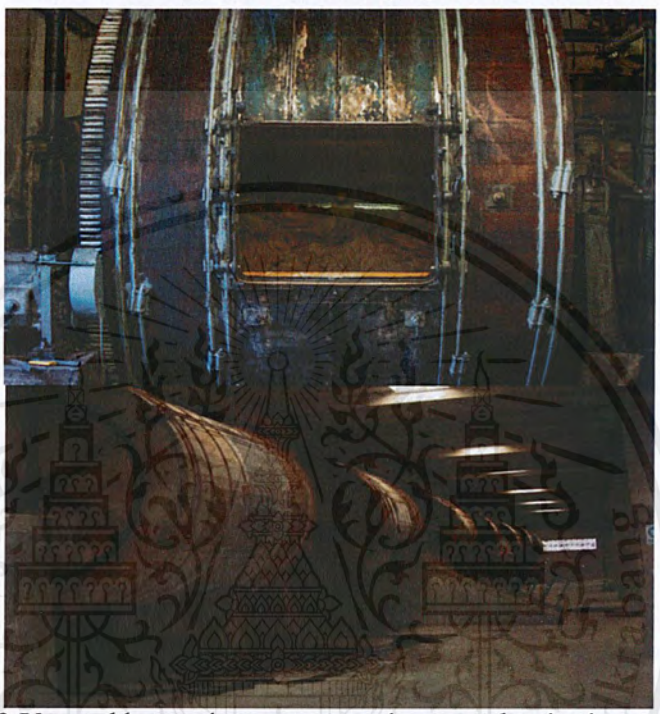


Figure 2.3 Vegetable tanning process using wood agitation tank [6].

2.1.3 Finishing Process

After tanning process leather is then neutralized by using alkali solution to remove unwanted acids. Then dyeing process is performing by using pigment or dye bound to stain leather for the desire color as shown in figure 2.4. Fat liquoring with reactive oils is then used for the fibrous structure and lubricate leather.

2.2 Structure and Components of Leather Scrap [3,8,9]

Fresh hides are composed of 60-65% water, 25-30% protein, and 5-10% fats. Therefore, proteins are most valuable component of leather scraps. However, those

protein are contaminated with chromium from manufacturing process and made difficulties to utilize protein from leather scrap.

Figure 2.4 Dyeing process to obtain a design color of leather [7].

2.2.1 Structure of Proteins

Proteins in leather are consisted of two forms; collagen and gelatin.

1) Collagen [3]

Collagen is the main structure of protein in connective tissues, skins and bones of animal, which is insoluble in water, weak-acid and weak-base. Most collagen is found in the form of fibrous tissue. The basic collagen structure consists of triple helix of peptide chains connected each other with hydrogen bonding. Inside peptide chains, there are number of amino acid connected each other with peptide boding which making a flexible structure as shown in figure 2.5 and 2.6. The amino acid component in collagen molecules are mainly consisted of 30% Glycine and 15% Arginine.

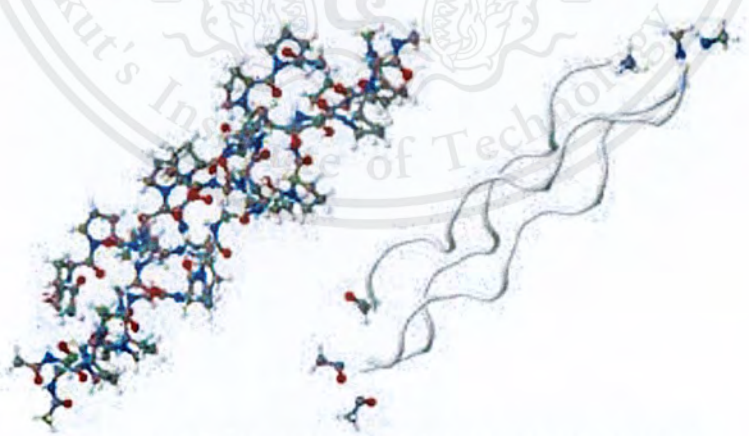


Figure 2.5. Structure of collagen triple helix [10].

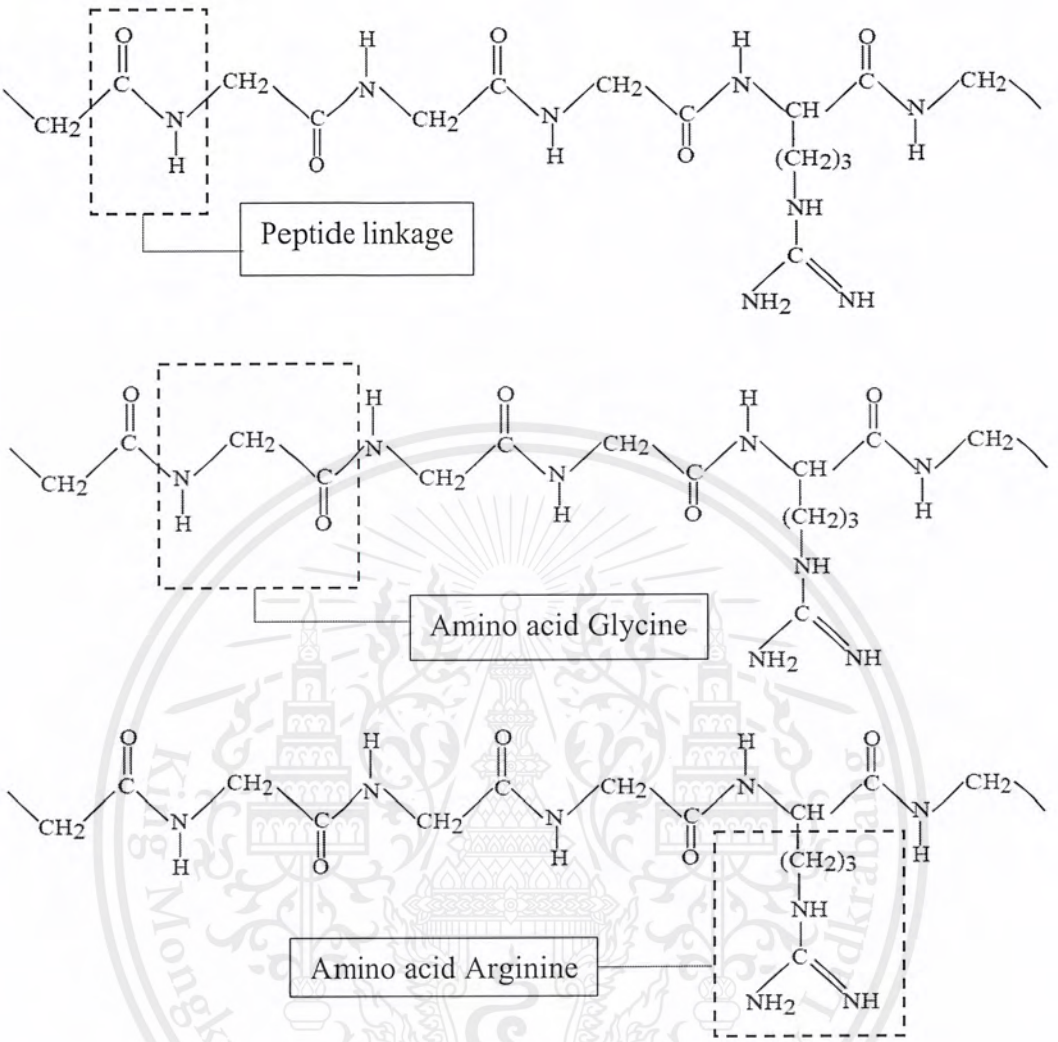


Figure 2.6. Fundamental structure of a peptide chains in collagen [3].

2) Gelatin

Gelatin is colloid protein that can be obtained by hydrolyzed collagen with suitable heat, acid or alkali solution. However, collagen hydrolysate is not the same thing as gelatin. In the hydrolyzed form, it has been processed more intensively to break up the proteins into smaller molecule. They both have the same amino acid and come from the same source but different chemical properties and react differently to liquids as shown in the table 2.1.

Table 2.1. comparison between gelatin and hydrolyzed collagen

	Gelatin	Hydrolyzed Collagen
Amino acid	18 amino acids	
Resource	connective tissue, skins and bones	
Composition	long chain of amino acid	short chain of collagen peptide
Taste	Flavorless	
Characteristic	brittle when dry and gels when mixed with liquid (solubility in hot liquid)	fine protein powder and does not gel when mixed with liquid (solubility in both hot and liquid)
Application	usually used in food ingredients	usually used in food ingredients, cosmetics and medicals

2.2.2 Chromium [11]

Chromium (Cr) is one of a heavy metal which is lustrous, brittle and hard. The most common oxidation states of chromium are trivalent (III) and hexavalent (VI). In the chrome tanning process, chromium (III) sulfate solution is used as a tanning agent because chromium (III) is the most stable form and has some characteristics which can stabilize skins structure and prevent the putrefaction by embedding a chrome in the leather.

Chrome tanning process is based on the cross-linkage of chromium ions with carboxyl groups in the collagen as shown in the figure 2.7.

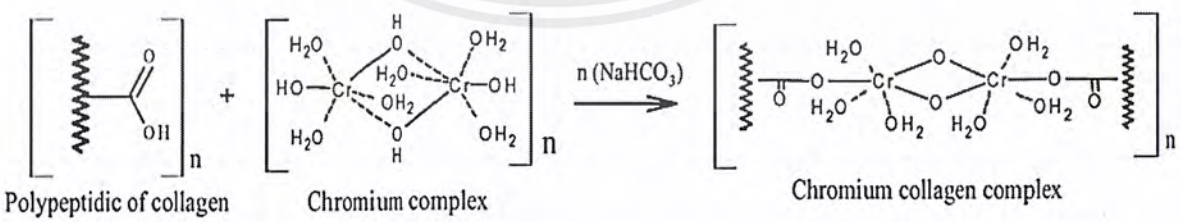


Figure 2.7 Chromium tanning agent with the free carboxyl groups in collagen [11].

2.3 Hydrolysis reaction. [11]

Hydrolysis reaction is used to breakdown chemical bonds of molecule by water. Hydrolysis reaction could be catalyzed by base, acid and enzymes.

Alkali hydrolysis or base hydrolysis reaction is a simple process which the complex molecules are broken down into their building blocks by insertion of ions of OH- between the molecule that link building blocks together as shown in the figure 2.8 through the olation and oxolation phenomena.

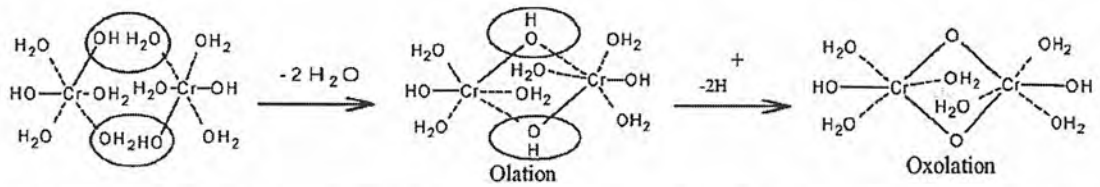


Figure 2.8 Olation and oxolation phenomena in alkali hydrolysis reaction [11].

As described by several researchers, the olation phenomena is observed with the increasing of the alkalinity of tanning medium. An acid discharge will take place with alkali while the oxygen-chrome coordinate bonding is transformed into covalent bonding (oxolation bridges).

Acidic hydrolysis or acid hydrolysis reaction performed under a strong condition which is similar to the alkali hydrolysis reaction but ions of H+ will be inserted into their building block instead of the ions of OH-.

Enzymatic hydrolysis is considered as eco-friendly method for hydrolysis reaction. There are various kind of enzymes that used to break down the complex molecule like two previous methods.

2.4 Design of Experiments [12-13]

2.4.1 Principle of Design of Experiments (DoE)

Design of Experiments (DoE) is statistical method for planning, conducting, analyzing, and interpreting the controlled experiment to evaluate input variables that control output or responses variables. DoE can identify an understanding of interactions among the factors in the experiment. In addition, DoE can minimize the experiment error by improving the accuracy of the design or process variation. The processes of design of experiment are divided into 4 steps.

2.4.1.1 Planning step

Good planning can reduce the problems during the experiment and can improve the accuracy of experiment trial. There are 4 main steps in planning step which are;

1) Define the problem
2) Define the objective
3) Design an experiment
4) Statistical process control

2.4.1.2 Screening step

Typically, an experiment is beginning with a large number of potential factors and then the elimination of the ones with little effect on the response. Screening designs are experimental designs that are intended to identify the most important factors from a larger set of factors.

2.4.1.3 Optimization step

After the screening step, the important factors are obtained. The optimization step is used for determining the maximum value of the factor. However, the determined value is depended on the objective of experiment.

2.4.1.4 Verification step

Verification step is done by repeat an experiment for verify the optimum value to confirm the result of experiment.

2.4.2 Guideline for Designing an experiment

There are 7 main steps for designing an experiment.

2.4.2.1 Problem statement

Problem statement is the most important step which the objective of the research will be defined.

2.4.2.2 Choice of factors, levels and ranges

Factor is the input variable which can be arrange into 2 groups which are control variables (operating temperature, equipment used and operating time) and disturbance variables (atmospheric temperature, and humidity).

A number of level or a level of factor is a number of value of factor that can be change in a trial of experiment; for example, there are 3 varying values of operating time that are 6 hrs., 9 hrs., and 12 hrs., that meant there are 3 levels of factor (factor of operating time).

2.4.2.3 Choice of response variables

Response variable is the variable which needed to be controlled for the desired value.

2.4.2.4 Choice of experimental design

An experimental design depends on the number of factor in the experiment. There are several of experimental design i.e., Factorial design, Response surface design.

2.4.2.5 Performing an experiment

In the experiment, there are 3 concerning point i.e., the experiment has to perform randomly, the replication of experiment is needed, and reducing an error in the experiment.

2.4.2.6 Statistical analysis

There are several statistical analysis methods that could be used, such as the analysis of variance (ANOVA), mean plot and interaction plot method. Furthermore, there are several programs for statistical analysis such as Minitab program, Stata and JMP

2.4.2.7 Conclusion of the experiment result

In this step the experiment result will be analyzed for the confirmation of initial assumption of the experiment.

2.4.3 Type of the Design of Experiment

There are many design methods of DoE; for example, factorial design, screening design, response surface design, mixture design and taguchi design. However, only the full factorial design is used in this research. Therefore, only the full factorial design will be described.

2.4.3.1 Full factorial design

Full Factorial design is allowed for the study of the effects from several factors on a process and study of interactions between the factors. A full factorial experiment is the ideal experiment to have information on all the main factors and interaction terms. In full factorial design, the number of probability of the experiment trials is calculated from a^n where a is a number of level of each factor and n is a number of factor; for example, the experiment focus on 4 factors which each factor containing 3 level, the number of experiment is equal to 4^3.

2.5 Fenton Oxidation Process. [14]

Fenton oxidation process is the reaction between hydrogen peroxide (H_2O_2) and iron ions to form active oxygen which can oxidize organic or inorganic compounds. Fenton process is widely used for the removal of hazardous organics from wastewater. The Fenton mechanism is represented by following equation;

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + OH. \quad (1)$$

This equation is considered as Fenton reaction and indicated the oxidation of ferrous to ferric ions to decompose H_2O_2 into hydroxyl radicals. It is usually considered as the core of the Fenton chemistry. The generated ferric ions can be reduced by reaction with excess hydrogen peroxide to form ferrous ion and more radicals as shown in equation (2).

$$Fe^{3+} + H_2O_2 \longrightarrow Fe^{2+} + HO_2 + H^+ \quad (2)$$

Equation (2) is represented the Fenton-like reaction which take longer time than the Fenton reaction, and allow Fe^{2+} regeneration in an effective cyclic mechanism. In Fenton-like reaction, hydroperoxyl radicals (HO.) are generated. The hydroperoxyl radicals will attack organic contaminants, but less sensitive than hydroxyl radicals. It is indicated that the iron ion added is considered to be a catalyst.

2.6 Literature reviews

From relevant research, it was reported that there are many methods for the extraction of gelatin from the leather scraps waste. Wang and Fu et al. studied on the effect of phosphoric acid on the amount of dechroming and collagen hydrolysis. It was found that collagen hydrolysis was depend on the amount of dechroming agents, and the acid group was the main factor [15-16]. In the next few year, Rai et al. also developed the conditions of acid hydrolysis using the mixed solution of formic acid and propionic acid. It was observed that the collagens could be obtain at the room temperature [17-18]. On the other hand, Fang et al. treated the chromium-containing leather by using calcium oxide as the alkali reagent. The results indicated that the recovery efficiency of chromium (III) oxide and the collagen yield could be reach 75% and 66. 7% respectively. In addition, the coordination capacity of OH- and chromium will be the mainly consideration, which should be larger than that of the carboxyl groups of collagens, otherwise the dechroming efficiency will be highly reduced. Zhang et al. investigated the effects of enzyme species on the extraction efficiency of collagen

from the leather scrap waste. The results showed that the efficiency of extraction collagen and removal amount of chromium was depend on the enzyme selectivity [19]. The comparison of the three methods for protein extraction from leather wastes is summarized in table 2.2

Table 2.2 comparison between several techniques of hydrolysis reactions

Techniques	Advantages	Disadvantages
Acidic hydrolysis method	High collagen extraction rate and no pollution from toxic chromium (VI)	Collagen structure is damaged, and equipment is corrosive.
Alkali hydrolysis method	Simple operation, high recovery efficiency and easy to separate of collagen	Large consumption of lime
Enzymatic hydrolysis method	Environmental friendly, higher extraction efficiency of collagen	specific enzyme is required, temperature effects on the enzyme

The decolorization method for collagen/gelatin extracted from leather scrap is one of the concerning issues. In this study, Fenton process is studied. Sureyya et al (2003) [22-23] studied on the degradation of relative black color from synthetic wastewater using Fenton oxidation process. The study was performed in a determination optimum concentration of $FeSO_4$ and H_2O_2, pH and temperature for 100 mg/l of sample were observed as 3.0 and 40 °C, respectively, using 100 mg/l of $FeSO_4$ and 400 mg/l of H_2O_2 resulted in 99 % color removal. For 200 mg/l of sample, more than 99 % color removal were obtained using 225 mg/l of $FeSO_4$ and 1000 mg/l of H_2O_2 yielding 0.05 molar ratios at pH 3.0 and 40 °C. In addition, Sureyya et al (2005) [24] used Fenton oxidation process to decolorize and degrade some reactive dyes and raw textile finishing industry effluents containing mainly reactive dyes. The operational conditions for pH varied between 2.5 and 4.0 while temperature ranges from 30 °C to 50 °C. The concentrations of $FeSO_4$ and H_2O_2 varied from 200–600 mg/l of $FeSO_4$ and 300–1000 mg/l of H_2O_2 depending on the type of the dyes and their mixture in the process. Fenton oxidation process was highly effective for color removal (>99 %) for reactive dyes and (87-94 %) for textile finishing wastewater. Yu, et al. (2005) [25] investigated the removal of color, dye and dissolved organic carbon by Fenton using the synthetic dye wastewaters containing various dyes such as Reactive blue 19, Eriochrome Black T and Fast Green FCF. The results indicated that discoloration of dyes was very rapidly, but mineralization of dyes was insignificant based on the removal of dissolved organic carbon.

CHAPTER III
RESEARCH METHODOLOGY

From the literature reviews, all experiments were carried out in laboratory scale. However, this research aims to find suitable condition to extract protein from leather scraps in laboratory and pilot scale. Thus, the experiments were broken down to three steps, i.e., laboratory experiment, decolorization of protein extracted, and pilot scale experiment.

3.1 Laboratory experiment

The objective of this experiment is to find a suitable condition for gelatin extraction.

3.1.1 Materials and Chemicals

1) Chrome tanning leather scraps
2) Calcium oxide (CaO)
3) Sulfuric acid (H_2SO_4)
4) Phenolphthalein
5) Deionized water
6) Activated carbon
7) Acetone

3.1.2 Equipment and Apparatus

1) Beaker
2) Cylinder
3) Volumetric flask
4) Temperature-controlled magnetic stirrer
5) Magnetic bar
6) Stirring rod
7) Balance
8) Oven
9) pH probe
10) Vacuum filter set
11) Filter paper
12) Vacuum oven

The experiments were carried out based on literature review for factors that would affect the effectiveness of protein extraction process. The three factors that that were considered are concentration of CaO, operating temperature, and reaction time. Based on factorial design of experiments, there were total of 24 experiments for vary 2 levels of CaO concentration at 4 and 5 %, 2 levels of temperature at 30 and 70 °C, and 3 levels of reaction time at 6, 9 and 12 hrs with 2 replicates. According to randomization requirement of factorial design method, the 24 experiments will be run in random order as shown in table 3.1. The standard order, run order, temperature, reaction time, and concentration of CaO that will be used for each experiment are also shown in the same table

3.1.3 Procedure

1) Perform experiment according to RunOrder in Table 3.1. Starting with RunOrder#1, read the listed conditions for the experiment, i.e.,

concentration of Cao (wt %), Temperature(°C), and duration for experiment(hrs).

2) Add amount of CaO according to listed concentration of CaO into 200 mL beaker contains 100 mL water.
3) Stir solution with Temperature-controlled magnetic stirrer until become well-mixed. If required, heat the solution until reach the listed temperature that shown in Table 3.1.
4) Measure pH of solution.
5) Add 10 g of chopped chrome tanning leather scraps in the same beaker.
6) Keep the mixture to be controlled at temperature and time as listed in Table 3.1 by using oven or ambient room.
7) Measure pH of mixture again.
8) Neutralize the mixture by titration using H_2SO_4
9) Filter sludge from the mixture using vacuum filter.
10) Decolorized the extracted solution with steps that described in section 3.2.
11) Filter the mixture using vacuum filter again
12) Evaporate the solution for obtaining dry powder of proteins by using vacuum oven
13) Weight the proteins and calculate %yields.
14) Repeat step 1-12 by changing concentration of CaO, Temp, and time according to RunOrder#2 and keep doing until RunOrder#24 are done.

Table 3.1 Order of experiment.

StdOrder	RunOrder	Temperature (°C)	Time(hrs)	Concentration (wt%)
4	1	30	9	5
21	2	70	9	4
18	3	30	12	5
6	4	30	12	5
23	5	70	12	4
20	6	70	6	5
12	7	70	12	5
13	8	30	6	4
22	9	70	9	5
7	10	70	6	4
5	11	30	12	4
16	12	30	9	5
15	13	30	9	4
11	14	70	12	4
8	15	70	6	5
17	16	30	12	4
24	17	70	12	5
3	18	30	9	4
2	19	30	6	5
19	20	70	6	4
9	21	70	9	4
10	22	70	9	5
14	23	30	6	5
1	24	30	6	4

3.2 Decolorization of gelatin extract

Since color of gelation solution that extracted from leather scrap is black, decolorization process is required to produce a clear solution. The four techniques were chosen to find a suitable method to remove black color from solution, i.e., Ozonation, Fenton's reaction, adsorption by activated carbon, and combined of Fenton's reaction and adsorption by activated carbon.

3.2.1 Materials and Chemicals

1) Hydrogen peroxide (H_2O_2)
2) Ferrous sulfate ($FeSO_4$)
3) Sodium hydroxide (NaOH)
4) Deionized water
5) Activated carbon

3.2.2 Equipment and Apparatus

1) Beaker
2) Volumetric flask
3) Erlenmeyer flask
4) One-Hole rubber stopper
5) Balance
6) pH probe
7) Indicator paper
8) Stirring rod
9) Cylinder
10) Filter paper
11) Vacuum filter set
12) Silicone tube
13) Air bubble stone
14) Ozone generator (Model: Ozzon OZ-735)

Figure 3.1 Ozone generator (Model: Ozzon OZ-735)

3.2.3 Ozonation

1) Pour filtrate solution into an Erlenmeyer flask.
2) Place a 500 ml Erlenmeyer flask on a table and hold the neck of flask with clamp and stand.
3) Insert rubber gas tube from the ozone generator to one-hole rubber stopper and connect the other end with air bubble stone.
4) Seal an Erlenmeyer flask with a rubber stopper.
5) Turn on a valve to supply air to ozone generator and switch on an ozone generator and adjust the flow rate of air and generated ozone to 1600 cc/min and 300 mg/hr., respectively.
6) Allow ozone to react with dye in a solution until color of solution is clear or not changing.

3.2.4 Fenton's reaction

1) Pour filtrate solution into a beaker.
2) Measure pH of the filtrate solution.

3) Adjust pH to 3
4) Prepare the mixture for Fenton reaction with ratio of Fe^{2+}:H_2O_2 at 3:50
5) Mix the 50 ml of prepared solution with filtrate solution and leave to react for 6 hrs.
6) Filter the mixture using vacuum filter

3.2.5 Adsorption by activated carbon

1) Add activated carbon 5 g into a beaker of filtrate solution
2) Stir solution with magnetic stirrer and wait until color of solution is clear or not changing.

3.2.6 Fenton's reaction + Adsorption by activated carbon

1) Pour filtrate solution into a beaker.
2) Measure pH of the filtrate solution.
3) Adjust pH to 3
4) Prepare the mixture for Fenton reaction with ratio of Fe^{2+}:H_2O_2 at 3:50
5) Mix the 50 ml of prepared solution with filtrate solution and leave to react for 6 hrs.
6) Filter the mixture using vacuum filter
7) Neutralize the solution by titration using NaOH
8) Add activated carbon and wait until color of solution is clear or not changing.
9) Filter the mixture using vacuum filter again

3.3 Pilot scale experiment

The objective of this experiment is to scale up the gelatin extraction method for pilot scale after the suitable condition is reached.

3.3.1 Materials and Chemicals

1) Chrome tanning leather scraps
2) Calcium oxide (CaO)
3) Sulfuric acid (H_2SO_4)
4) Phenolphthalein
5) Deionized water
6) Activated carbon
7) Acetone

3.3.2 Equipment and Apparatus

1) Reactor (3L)
2) Agitator blade
3) Volumetric flask
4) Balance
5) pH probe
6) Vacuum filter set
7) Filter paper
8) Vacuum oven

3.3.3 Procedure

1) Add CaO 40 g into reactor contains 1 L water.
2) Stir solution until become well-mixed
3) Measure pH of solution.

4) Add 100 g of small chopped of chrome tanning leather scraps into a reactor and allow hydrolyzation to continue for desired peroid.
5) Filter sludge from the mixture using vacuum filter set.
6) Measure pH of mixture.
7) Neutralize the mixture by titration using H_2SO_4.
8) Decolorized solution with Fenton process and follow by activated carbon.
9) Filter the mixture using vacuum filter again.
10) Evaporate the solution for obtaining dry powder of proteins by using vacuum oven.
11) Weight the proteins and calculate %yields.

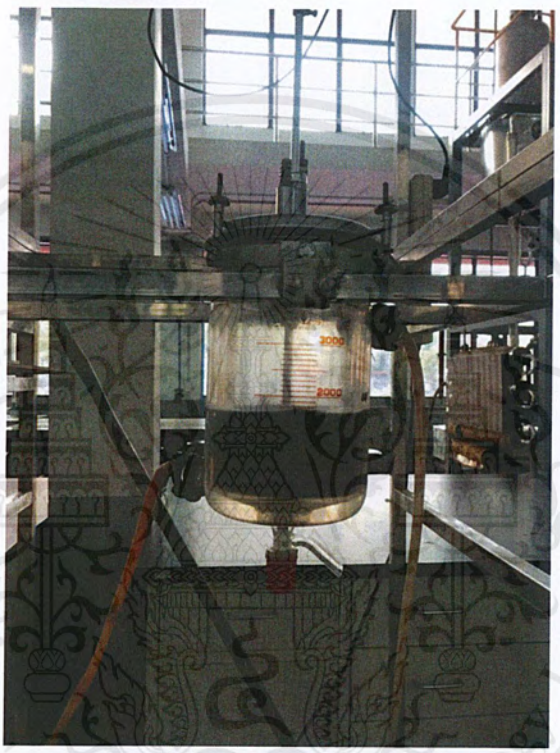


Figure 3.2 3 l Reactor with an agitator blade.

CHAPTER IV
RESULTS AND DISCUSSION

In this chapter, the results from the experiment are described and discussed into 3 parts, i.e., laboratory experiment, decolorization of protein extracted, and pilot scale experiment.

4.1 Laboratory experiment

4.1.1 Statistical analysis on effect of parameters on yield of gelatin.

In this research, the alkali hydrolysis is a selected method to break down leather molecules. The total of 24 experiments was carried out randomly in laboratory scale by using multi-level factorial experimental design methodology to study three variables, i.e., concentration of calcium oxide (CaO) (4, 5 %wt.), temperature (30, 70 °C), and reaction time (6,9, and 12 hrs.). The results are shown in Table 4.1.

The Pareto chart which shows effect of three variables on % yield of gelatin is shown in Figure 4.1. This result reveal that only one main effect(temp) and three-way interactions(temp*time*conc) are statistical significant on yield while other main effects (time, and conc), two-way interaction (temp*time, temp*conc, time*conc) are statistically insignificant. The Analysis of Variances(ANOVA) as shown in Figure 4.2, is also confirmed that temperature and three-way interaction (temp*time*conc) are only variables that statistical significant on percent yield from p value that less than 0.05.

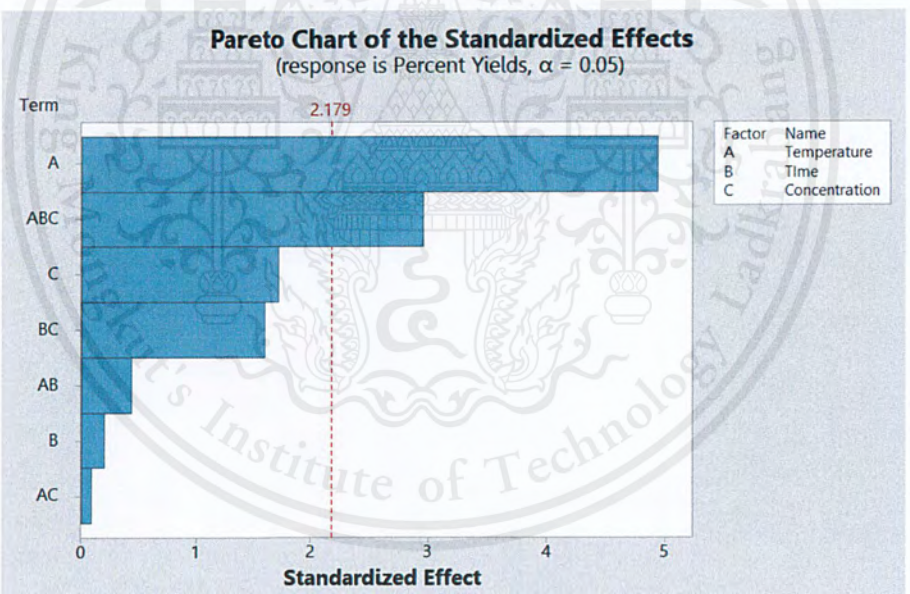


Figure 4.1 The Pareto Chart of the standardized effect.

Table 4.1 the results from laboratory experiment with various condition.

RunOrder	Temperature (°C)	Time (hrs)	Concentration (wt%)	Weight of leather (g)	pH before neutralization	pH after neutralization	Weight of gelatin (g)	Percent yield
1	30	9	5	10.03	12.46	6.04	0.31	3.09
2	70	9	4	10.03	12.02	6.45	0.34	3.39
3	30	12	5	10.00	12.39	6.17	0.40	4.00
4	30	12	5	10.02	12.33	6.40	0.30	2.99
5	70	12	4	10.03	12.09	6.78	0.14	1.40
6	70	6	5	10.05	12.09	6.61	0.10	1.00
7	70	12	5	10.00	12.14	6.69	0.30	3.00
8	30	6	4	10.02	12.29	6.39	0.30	2.99
9	70	9	5	10.01	12.06	6.38	0.16	1.60
10	70	6	4	10.02	12.42	6.61	0.33	3.29
11	30	12	4	10.02	12.38	6.42	0.53	5.29
12	30	9	5	10.01	12.46	6.01	0.40	4.00
13	30	9	4	10.02	12.36	6.34	0.41	4.09
14	70	12	4	10.01	12.04	6.17	0.21	2.10
15	70	6	5	10.02	12.03	6.58	0.31	3.09
16	30	12	4	10.03	12.35	6.46	0.40	3.99
17	70	12	5	10.02	12.06	6.52	0.37	3.69
18	30	9	4	10.00	12.43	6.03	0.43	4.30
19	30	6	5	10.03	12.31	6.77	0.36	3.59
20	70	6	4	10.01	11.95	6.6	0.32	3.20
21	70	9	4	10.02	12.07	6.40	0.35	3.49
22	70	9	5	10.05	12.03	6.47	0.16	1.59
23	30	6	5	10.00	12.24	6.08	0.40	4.00
24	30	6	4	10.02	12.37	6.05	0.39	3.89

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	11	19.4499	1.7682	4.22	0.010
Linear	4	11.6410	2.9103	6.94	0.004
Temperature	1	10.2443	10.2443	24.43	0.000
TIme	2	0.1455	0.0728	0.17	0.843
Concentration	1	1.2513	1.2513	2.98	0.110
2-Way Interactions	5	2.3033	0.4607	1.10	0.410
Temperature*TIme	2	0.3436	0.1718	0.41	0.673
Temperature*Concentration	1	0.0033	0.0033	0.01	0.931
TIme*Concentration	2	1.9565	0.9783	2.33	0.139
3-Way Interactions	2	5.5056	2.7528	6.57	0.012
Temperature*TIme*Concentration	2	5.5056	2.7528	6.57	0.012
Error	12	5.0314	0.4193		
Total	23	24.4813			

Figure 4.2 The Analysis of Variance(ANOVA) of variables.

When considered main effects plot on percent yield as shown in Figure 4.3, those graphs revealed that temperature has higher effects on yield than time and concentration. When increasing temperature, yield is decreasing significantly. While increasing hydrolysis time show slightly increasing of percent yield. The increasing concentration of CaO cause decreasing on percent yield but as not large as temperature.

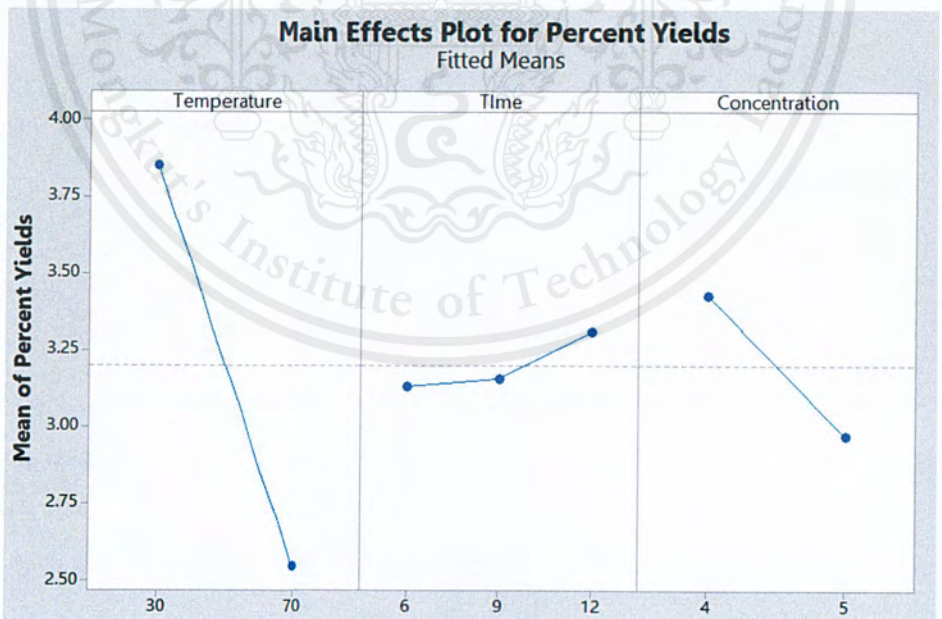


Figure 4.3 Main effects plot for percent yields.

4.1.2 The optimum condition for gelatin extraction

From regression analysis and response optimizer, result revealed that maximum percent yield of gelatin could be achieved at 4.640 with hydrolysis condition at 4 wt % of CaO, room temperature (30 °C), and 12 hrs. of reaction time as shown in Figure 4.4. The proximate analysis to find % of protein in gelatin is shown in Table 4.2. The average protein in a sample is 2.18%.

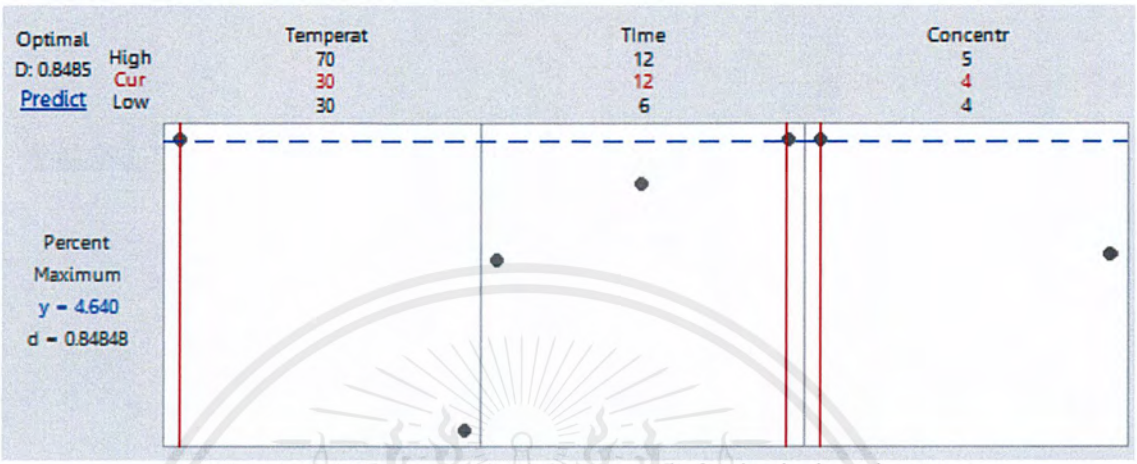


Figure 4.4 Response Optimizer result for hydrolyzed extract.

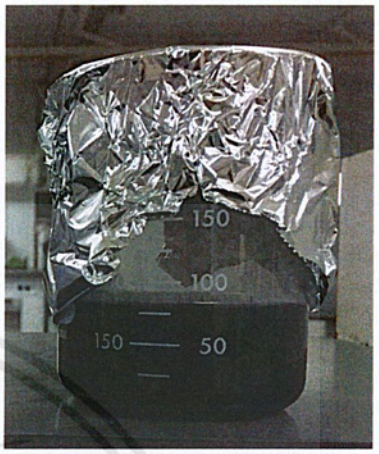
Table 4.2 the result of crude protein analysis using Kjeldahl method.

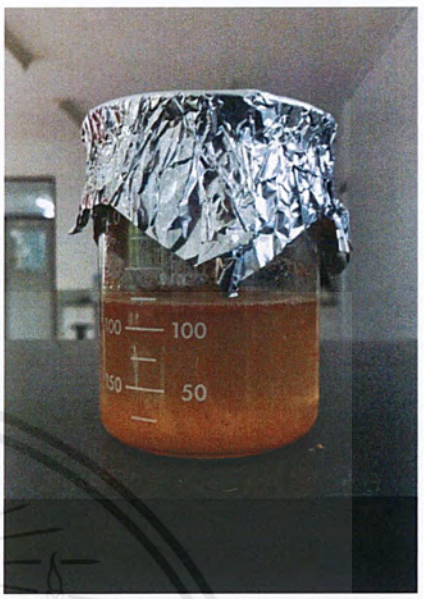
Sample	Sample #	% of protein in sample	Average
Hydrolyzed product	1	2.09	2.18
	2	2.26	

4.2 Decolorization of gelatin extract

There are four techniques were chosen to find a suitable method to remove black color from solution, i.e., Ozonation, Fenton's reaction, adsorption by activated carbon, and combined of Fenton's reaction and adsorption by activated carbon. The results from those four methods are presented in Table 4.2.

Table 4.3 the results of decolorization method of gelatin extracted.

Method	Color of solution after treatment	Picture of solution
Activated Carbon	Black	
Ozonation	Black	

Method	Color of solution after treatment	Picture of solution
Fenton's Method	orange	
Fenton's Method + Activated Carbon	Clear	

From literature review, Azo dye is the most widely used for synthetic color. it contains an aromatic compound structure with one or more azo group (-N=N-) that has the properties of high stability and resistance to degradation. The adsorption by activated carbon and ozonation method are not applicable to remove black color from gelatin solution. The Fenton's reaction with Fe^{2+}:H_2O_2 at 3:50 mM, pH of 3 for 6 hrs.is strong enough to remove black color. However, color of Fe^{3+} left orange color in a solution. The effective method to make clear solution is treated solution with Fenton's reaction, followed by adsorption by activated carbon.

4.3 Pilot scale experiment

After obtained the suitable condition from the laboratory experiment, the pilot scale experiment was performed by using condition at 4 wt % of CaO, room temperature (30 °C). However, the reaction time was extended to 18 and 24 hrs. since the imperfect mixing in a large-scale production. The results from pilot scale production are shown in Table 4.4. The % yield at reaction time of 18 hrs yields slightly larger value than that of laboratory scale. However, when double reaction time, % yield is jumped to 9.46. The proximate analysis to find % of protein in samples is shown in Table 4.5 The average protein from the experiment 1 and 2 is 22.73 and 52.34 respectively. The experiment 3, the duplicated of experiment 2, was carried out to recheck the result of protein analysis. The % yield of this experiment is 9.77 and the proximate analysis is shown the average protein in a sample is 64.92. The percentage of protein in pilot scale of two duplicated experiments are much larger than that of laboratory scale. The further analysis or more experiments may be required to explain the result.

Table 4.4 the results from pilot experiment.

Experiment	Temperature (°C)	Time (hrs)	Concentration (wt%)	Weight of leather (g)	Weight of gelatin (g)	Percent Yield
1	30	18	4	100.06	5.61	5.61
2	30	24	4	100.00	9.46	9.46
3	30	24	4	100.03	9.77	9.77

Table 4.5 the result of crude protein analysis using Kjeldahl method.

Sample	Experiment	% of protein in sample	Average
Hydrolyzed product	1	24.21	22.73
		21.24	
	2	62.38	52.34
		42.09	
	3	65.44	64.92
		64.40	

CHAPTER V
CONCLUSION

5.1 Conclusion

This research aims to find an optimized condition to extract gelatin from the leather scrap wastes. There are parameters i.e., concentration of calcium oxide (CaO), operating temperature, and reaction time that considered. The total of 24 experiments were performed in laboratory scale by using design of experiment method to analyze effect of main parameters, interaction between parameters on % yield of gelatin. In addition, the decolorization of gelatin solution, and pilot scale experiment were also performed. The conclusion from three parts experiments are shown below.

1. The statistical analysis of 24 experiments revealed that temperature is the most significant parameter on % yield. When temperature is increased, yield is decreased significantly. While increasing hydrolysis time, percent yield is slightly increased. The increasing concentration of CaO causes decreasing on percent yield but as not large as temperature. The Analysis of Variances(ANOVA) is represented that temperature and three-way interaction (temp*time*conc) are only variables that statistical significant on percent yield from p value that less than 0.05.
2. The regression analysis and response optimizer represented that maximum percent yield of gelatin could be achieved at 4.640 with hydrolysis condition at 4 wt% of CaO, room temperature (30 °C), and 12 hrs. of reaction time. The proximate analysis revealed that the average protein in a sample is 2.18%.
3. The effective method to decolorize the extracted solution is treated solution by Fenton's reaction with Fe^{2+}:H_2O_2 at 3:50 mM, pH of 3 for 6 hrs., followed by adsorption with activated carbon.

For the pilot scale experiment, 5.61 and 9.46 % yield were achieved by using CaO at 4 wt. %, room temperatures but extended reaction time to 18 and 24 hrs., respectively. The proximate analysis is revealed that the average protein in a sample is 22.73% and 52.34% respectively. Moreover, 9.77% yield was achieved from the duplicated of experiment with reaction time 24 hrs. The proximate analysis is represented that the average protein in sample is 64.92%. The result confirmed that the reaction time parameter is also effect to the protein extracted. Therefore, the further analysis or more experiments may be required to explain the discrepancy of laboratory and pilot scale experiments.

5.2 Suggestions

1. The results on yield and % protein of the pilot scale experiments is significant different from the results of laboratory experiment. Therefore, this research requires more the experiment trials and further analysis of protein.
2. For production scale production, the feasibility study of investment is required for Rate of Return on investment.

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Appendix A
Picture of the Experiment

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Picture of the Experiment

Figure A1 the leather scrap waste from OTOP leather industry.

Figure A2 the physical characteristic of leather scraps residual after hydrolyzed with the optimum condition in laboratory experiment.

Figure A3 the physical characteristic of leather scraps residual after hydrolyzed with the optimum condition for 24 hrs. reaction time in pilot scale experiment.

Figure A4 extract gelatin with the optimum condition in laboratory experiment.

Figure A5 extract gelatin with optimum condition for 24 hrs. in pilot scale experiment.

Appendix B
Crude Protein Analysis

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Crude Protein Analysis [23]

Crude protein analysis is the method to determine the amount of nitrogen in the protein. Principally, protein is consisted of nitrogen about 16 percent. The Determination of crude protein content method is called **Kjeldahl method** which can be performed by the Digestion of organic matter with sulfuric acid in the presence of a catalyst, Rendering the reaction product alkaline then distillation and titration of the liberated ammonia. Finally, Calculation of the nitrogen content, Multiplication of the result by the conventional factor 6.25 to obtain the crude protein content.

There are three main steps for crude protein analysis which are following;

1) Digestion Step

The sample to be analyzed is digested by heating it in the presence of sulfuric acid which is an oxidizing agent. Anhydrous sodium sulfate and a catalyst, such as copper, selenium, titanium, or mercury are used to speed up the reaction. Digestion will convert nitrogen in the sample into ammonia, and other organic matter to CO_2 and H_2O. Ammonia gas is not liberated in an acid solution because the ammonia is in the form of the ammonium ion (NH^{4+}) which binds to the sulfate ion (SO_4^{2-}) and thus remains in solution following this equation;

$$H_2SO_4 \longrightarrow H_2O + SO_2 + O$$

$$CH_2(NH_2)COOH + 3O \longrightarrow 2CO_2 + H_2O + NH_3$$

$$2NH_3 + H_2SO_4 \longrightarrow (NH_4)_2SO_4$$

2) Distillation Step

When the solution from the digestion step is cooled down, the solution will be diluted with distilled water and then mix with NaOH solution to remove the ammonia and to be trapped in standard sulfuric acid with the known exact concentration following this equation;

$$(NH_4)_2SO_4 + 2NaOH \longrightarrow Na_2SO_4 + 2NH_3 + 2H_2O$$

$$NH_3 + H_2O \longrightarrow NH_4OH$$

After that, the solution is titrated with standard sodium hydroxide. The alkaline solution is reacted with the residual of the standard acid from ammonia trapped. Then, the values of titrant used are calculated to determine the amount of nitrogen or crude protein. which called "back titrate".

In addition, there are other similar methods but different of weak acids used, for example, determine the amount of nitrogen or crude protein by using boric acid for the ammonia trapping following by ammonia direct titration with standard sulfuric acid. Despite the two methods is equally effective, trapping ammonia using sulfuric acid is cheaper than others. However, trapping ammonia using boric acid is use the less of time.

Figure B1 Crude protein analysis instrument.

3) Crude Protein Calculation

$$\% \text{ Crude Protein} = \frac{1.4\ (V2-V1)N \times 6.25}{W} = \frac{1.4\ (V)N \times 6.25}{W}$$

where; V = Volume of sulfuric acid used

V2-V1 = Volume of ammonia; V1 is generally equal to 0

N = Normal concentration of H_2SO_4

W = Weight of the sample

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