



Cairo University

MODELLING OF PROTEIN SEPARATION FROM GELATIN WASTEWATER USING AMMONIUM SULFATE

By

Mahmoud Mohamed Mahmoud Mahmoud Abdel Ghaffar

A Thesis Submitted to the
Faculty of Engineering at Cairo University
in Partial Fulfillment of the
Requirements for the Degree of
MASTER OF SCIENCE
In
Chemical Engineering

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Title of Thesis:

**MODELLING OF PROTEIN SEPARATION FROM GELATIN
WASTEWATER USING AMMONIUM SULFATE**

Key Words:

Proteins; peptides; Amino acids; Ammonium Sulfate.

Summary:

Proteins are very important and have endless uses worldwide. The protein demand worldwide is increasing continuously. However, each day billions of gallons of wastewater are released domestically and industrially; the nitrogenous wastes are the worst; high percentage of nitrogenous waste in this water is proteinous wastes which contribute indeed to overall oxygen demand. Therefore, proteins, peptides and amino acids precipitation and separation from wastewater is a worth taken challenge.

The objective of our thesis is to precipitate proteins present in gelatin wastewater samples; moreover, the gelatin wastewater sample underwent citric acid hydrolysis at different durations followed by solvent precipitation using ammonium sulfate (salting out). The effect of different operating conditions was considered, analyzed and optimized. The considered time from 10 to 17 hrs, temperature from 23°C to 43°C, pH from 4 to 10 and ammonium sulfate concentration from 20% to 80%.

A fast, simple, economic and reliable method was conducted successfully to precipitate proteins present in gelatin wastewater samples achieving high precipitation efficiency.

Disclaimer

I hereby declare that this thesis is my own original work and that no part of it has been submitted for a degree qualification at any other university or institute.

I further declare that I have appropriately acknowledged all sources used and have cited them in the references section.

Name: Mahmoud Mohamed Mahmoud Mahmoud Abdel Ghaffar Date: 27/9/2018

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Table of Contents

DISCLAIMER	I
ACKNOWLEDGEMENTS	II
TABLE OF CONTENTS	III
LIST OF TABLES.....	V
LIST OF FIGURES.....	VI
NOMENCLATURE	VIII
ABSTRACT	IX
CHAPTER 1 :INTRODUCTION	1
1.1. DEFINITION AND HISTORY OF AMINO ACIDS AND PROTEINS	1
1.2. PROTEIN VITALITY AND PROTEINOUS CONTAMINATION.....	2
1.3. THESIS OBJECTIVE	5
1.4. ORGANIZATION OF THE THESIS	5
CHAPTER 2 :LITERATURE REVIEW	6
2.1. INTRODUCTION	6
2.2. STANDARD AMINO ACIDS IN GENETIC CODE.....	6
2.3. COMPOSITION OF SOME PROTEINS	10
2.4. AMINO ACIDS PRODUCTION TECHNIQUES	12
2.4.1. Protein hydrolysis method	12
2.4.2. Chemical synthesis method.....	12
2.4.3. Biological methods	13
2.5. PROTEINS SEPARATION TECHNIQUES	13
2.5.1. Separation of proteins according to size	16
2.5.2. Separation of proteins using chromatography	17
2.5.3. Separation of proteins using electrophoresis	21
2.5.4. Precipitation of proteins using solvent.....	26
2.6. TECHNICAL CONSIDERATIONS IN AMMONIUM SULFATE PROTEIN PRECIPITATION	28
2.6.1. Salting in and salting out agents	28
2.6.2. Ammonium sulfate priority.....	29
2.6.3. Ammonium sulfate methodology of precipitation	31
CHAPTER 3 : EXPERIMENTAL SET UP.....	35
3.1. INTRODUCTION	35
3.2. MATERIALS USED	35
3.2.1. Gelatin wastewater	35
3.2.2. Ammonium Sulfate (AS)	37
3.2.3. Chemicals used	37
3.3. EQUIPMENT USED	40

3.3.1.	pH meter	40
3.3.2.	Magnetic stirrer with heater	41
3.3.3.	Refrigerated micro-centrifuge.....	41
3.3.4.	Sensitive weight scale	41
3.3.5.	Spectrophotometer	42
3.3.6.	Other equipment	42
3.4.	ENGINEERING ASPECTS AFFECTING THE PROCESS	43
3.4.1.	pH	43
3.4.2.	Temperature	43
3.4.3.	Time	43
3.5.	METHODOLOGY	43
3.6.	GELATIN WASTEWATER CHARACTERISTICS	45
3.6.1.	Chemical oxygen demand (COD).....	45
3.6.2.	Bio-chemical oxygen demand (BOD).....	45
3.6.3.	Total suspended solids (TSS).....	46
3.6.4.	Density	47
3.7.	EXPERIMENTAL TECHNIQUE	47
CHAPTER 4 : RESULTS AND DISSCUSSION.....		49
4.1.	INTRODUCTION	49
4.2.	CHARACTERIZATION OF GELATIN WASTEWATER	49
4.3.	EFFECTS OF DIFFERENT OPERATING CONDITIONS ON PROTEIN PERCENTAGE RECOVERY	49
4.3.1.	Effect of hydrolysis time on protein percentage recovery	50
4.3.2.	Effect of precipitation time on protein percentage recovery ..	55
4.3.3.	The effect of temperature on protein percentage recovery	59
4.3.4.	Effect of pH on protein percentage recovery	62
4.4.	ECONOMICAL ASPECTS OF PROTEIN RECOVERY FROM GELATIN WASTEWATER USING SALTING-OUT APPROACH	67
4.4.1.	Proposed protein recovery process	67
4.4.2.	Salting Out Process Cost Estimation	68
CHAPTER 5 :CONCLUSION AND RECOMMENDATIONS.....		72
REFERENCES		73

List of Tables

Table 2.1: Twenty amino acids listed in genetic code.....	6
Table 2.2: Ammonium sulfate VS. ammonium phosphate	30
Table 2.3: AS saturated solution properties VS. Temperature	31
Table 2.4: P values at different temperatures	32
Table 2.5: AS calculated weights at 0°C	34
Table 3.1: The analysis of gelatin wastewater and tanneries wastewater	37
Table 3.2: Sodium Hydroxide Specifications.....	38
Table 3.3: Citric Acid Specifications	39
Table 3.4: Hydrochloric Acid Specifications	40
Table 3.5: Selected hydrolyzed samples	44
Table 3.6: Different operating conditions values affecting protein recovery efficiency	44
Table 3.7: Considered compositions of ammonium sulfate solution	44
Table 4.1: The analysis of gelatin wastewater and tanneries wastewater	49
Table 4.2: Effect of AS saturation percentage on protein percentage recovery for sample #1	50
Table 4.3: Effect of AS saturation percentage on protein percentage recovery for sample #2.....	51
Table 4.4: Effect of AS saturation percentage on protein percentage recovery at t = 15 min, pH =4 and T = 23°C	55
Table 4.5: Effect of AS saturation percentage on protein percentage recovery at t = 30 min, pH =4 and T = 23°C	56
Table 4.6: Effect of AS saturation percentage on protein percentage recovery at t = 60 min, pH =4 and T = 23°C	56
Table 4.7: Effect of AS saturation percentage on protein percentage recovery at T = 23°C, pH = 4 and t = 60 min.....	62
Table 4.8: Effect of AS saturation percentage on protein percentage recovery at T = 33°C, pH = 4 and t = 60 min.....	62
Table 4.9: Effect of AS saturation percentage on protein percentage recovery at T = 43°C, pH = 4 and t = 60 min.....	62
Table 4.10: Effect of AS saturation percentage on protein percentage recovery at pH = 4, t = 60 min and T = 23° C	65
Table 4.11: Effect of AS saturation percentage on protein percentage recovery at pH = 7, t = 60 min and T = 23° C	65
Table 4.12: Effect of AS saturation percentage on protein percentage recovery at pH = 10, t = 60 min and T = 23° C	65
Table 4.13: The best parameters values for salting out process	66
Table 4.14: Chemicals used in protein recovery process	67
Table 4.15: Equipment cost estimation in 1998	69
Table 4.16: Detailed total capital investment calculation	69
Table 4.17: Detailed direct production cost calculation	71

List of Figures

Figure 1.1: Amino acid composition	1
Figure 1.2: Amino acids, peptides and protein sequencing.....	2
Figure 1.3: Protein importance and vitality	3
Figure 1.4: Proteinous waste cycle	4
Figure 1.5: Dual benefits for protein separation from wastewater	4
Figure 2.1: Casein AAs composition.....	11
Figure 2.2: Gelatin AAs composition.....	11
Figure 2.3: Soybean AAs composition.....	12
Figure 2.4: Protein separation techniques	14
Figure 2.5: Protein separation techniques subdivision	15
Figure 2.6: Proteins separation by dialysis	16
Figure 2.7: Proteins separation through ion exchange chromatography	18
Figure 2.8: Proteins separation through affinity chromatography.....	19
Figure 2.9: Proteins separation through size exclusion chromatography.....	20
Figure 2.10: Proteins separation by gel electrophoresis	22
Figure 2.11: Proteins separation by Iso-electric focusing	23
Figure 2.12: Proteins separation by Iso-electric focusing	24
Figure 2.13: Proteins separation by two dimensional electrophoresis	25
Figure 2.14: Ammonium sulfate protein precipitation	26
Figure 2.15: Salting in and salting out.....	29
Figure 2.16: Ammonium sulfate priority reasons.....	29
Figure 2.17: Hofmeister Series for Anions and Cations.....	30
Figure 2.18: AS weight online calculator interface.....	33
Figure 3.1: Google maps screenshot for the Amin for Gelatin Factory	36
Figure 3.2: pH meter.....	40
Figure 3.3: Magnetic stirrer with hot plate	41
Figure 3.4: Refrigerated micro-centrifuge.....	41
Figure 3.5: Sensitive weight scale	42
Figure 3.6: Bio-systems spectrophotometer	42
Figure 3.7: UV spectrophotometer for COD measurement.....	45
Figure 3.8: BOD oxygen meter	46
Figure 3.9: TSS portable hand meter.....	46
Figure 3.10: Densitometer used to measure liquid density	47
Figure 4.1: The effect of the hydrolysis time on protein percentage recovery at pH =4, t= 60 min and T = 23° C	52
Figure 4.2: Protein percentage recovery at the five selected AS Saturation Percentages for sample #1	53
Figure 4.3: Protein percentage recovery at the five selected AS Saturation Percentages for sample #2	53
Figure 4.4: Comparison between protein percentage recoveries at both hydrolysis times	54
Figure 4.5: Effect of time on protein percentage recovery at pH =4 and T = 23°C	55
Figure 4.6: Protein percentage recovery at t = 15 min, pH =4 and T = 23°C.....	57
Figure 4.7: Protein percentage recovery at t = 30 min, pH =4 and T = 23°C.....	57
Figure 4.8: Protein percentage recovery at t = 60 min, pH =4 and T = 23°C.....	58
Figure 4.9: Protein percentage recoveries at three different contact times	58

Figure 4.10: Effect of temperature on protein percentage recovery at pH =4 and t = 60 min.....	59
Figure 4.11: Protein precipitation recovery at T = 23° C, pH =4 and t = 60 min	60
Figure 4.12: Protein precipitation recovery at T = 33° C, pH =4 and t = 60 min.....	60
Figure 4.13: Protein precipitation recovery at T = 43° C, pH =4 and t = 60 min.....	61
Figure 4.14: protein percentage recoveries at three different temperatures	61
Figure 4.15: Effect of pH on protein percentage recovery at t=60 min and T= 23° C ...	63
Figure 4.16: Protein precipitation recovery at pH 4, t = 60 min and T = 23° C	63
Figure 4.17: Protein precipitation recovery at pH = 7, t = 60 min and T = 23° C.....	64
Figure 4.18: Protein precipitation recovery at pH = 10, t = 60 min and T = 23° C.....	64
Figure 4.19: Protein percentage recoveries at three different pH values	66
Figure 4.20: Process Flow Diagram for protein recovery process	68
Figure 4.21: Gantt chart for protein recovery process.....	68

Nomenclature

AA	Amino acid
Ala	Alanine
Arg	Arginine
Asp	Asparagine or Aspartic acid
AS	Ammonium sulfate
Cys	Cysteine
Glu	Glutamine or Glutamic acid
Gly	Glycine
His	Histidine
Ile	Isoleucine
KDa	Kilo Dalton
Leu	Leucine
Lys	Lysine
Met	Methionine
Phe	Phenylalanine
Pro	Proline
Ser	Serine
Thr	Threonine
Trp	Tryptophan
Tyr	Tyrosine
Val	Valine
Rpm	Revolution per minute
TSS	total suspended solids
Time	T
Protein concentration	C
Protein percentage recovery	W%
Temperature	T

Abstract

Proteins, peptides and amino acids are very important and have endless uses worldwide. The protein demand worldwide is increasing continuously. However, each day billions of gallons of wastewater are released domestically and industrially; the nitrogenous wastes are the worst; high percentage of nitrogenous waste in this water is proteinous wastes which contribute indeed to overall oxygen demand. Therefore, proteins, peptides and amino acids precipitation and separation from wastewater is a worth taken challenge.

Proteins, peptides and amino acids can be separated according to size through dialysis, ultra and nano filtration; then, they can be separated according to chromatography through size exclusion chromatography, affinity chromatography and ion-exchange chromatography; also, they can be separated through electrophoresis through gel electrophoresis, Iso-electric focusing and two dimensional electrophoresis; as well as through solvent precipitation using metal salts, organic solvents and organic acids.

In this thesis, all these precipitation and separation technologies are explained as well as comprehending the related previous work done regarding these technologies within the last 18 years through a literature survey.

The objective of the present work is to precipitate proteins present in gelatin samples; moreover, the gelatin sample underwent citric acid hydrolysis at different durations (10 – 17 hrs) followed by solvent precipitation using ammonium sulfate (salting out). The effect of different operating conditions was considered, analyzed and optimized.

A fast, simple, economic and reliable method was conducted successfully to precipitate proteins present in gelatin samples achieving high precipitation efficiency.

CHAPTER 1 :INTRODUCTION

1.1. Definition and History of Amino Acids and Proteins

AAs are simple organic compounds containing carboxylic group and amino group as illustrated in figure 1.1. There are about 500 types of amino acids, twenty of which are in the genetic code. They are divided into several groups according to their size, hydrophobicity, hydrophylicity and functional group (Berg, Tymoczko, & Stryer, 2002).

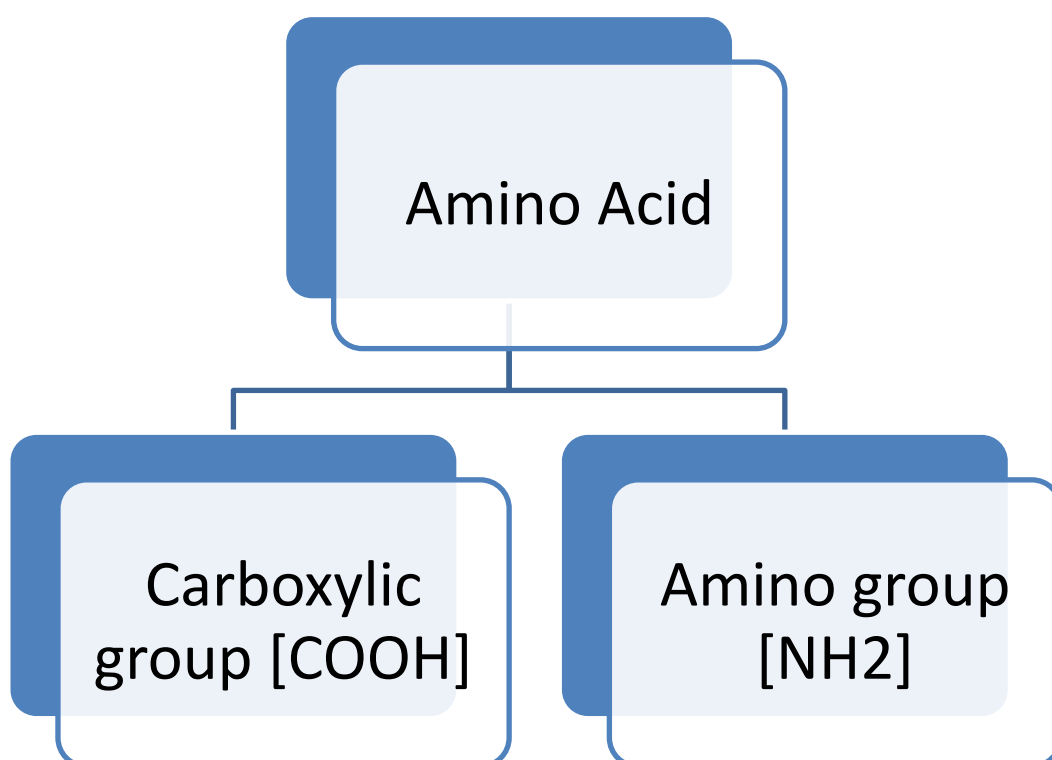


Figure 1.1: Amino acid composition

“Amino acids are the structural units that make up proteins. They join together to form short polymer chains called peptides or longer chains called either polypeptides or proteins” as shown in figure 1.2. (Mohanty, Jayasri, & Elumalai, 2012)

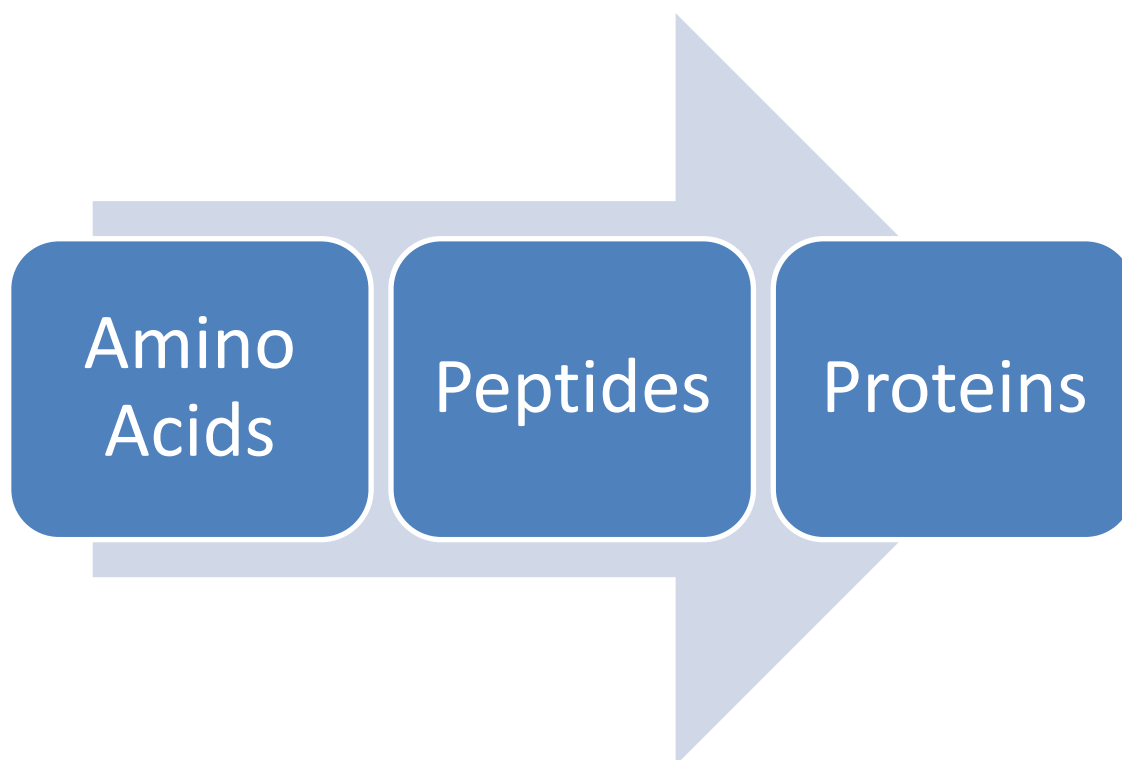


Figure 1.2: Amino acids, peptides and protein sequencing

Almost two hundred years ago, protein was identified as a primary material; however, during the last few decades, proteins and peptides were discovered in the brain, heart, skin and many other tissues and organs. (Wieland & Bodanszky, 1991)

In 1806, the French chemists Louis Nicolas Vauquelin and Pierre Jean Robiquet discovered the first amino acid Asp. In 1810, Cys was discovered and later in 1820, Gly and Leu were discovered. (Mohanty, Jayasri et al, 2012)

Amino acids are considered amphoteric compounds which can act as an acid or a base; moreover, they are considered as ampholytes which are amphoteric compounds which exist mostly as zwitterions (molecules that can be positively charged or negatively charged depending on the pH). (McNaught and Wilkinson, 1997)

1.2. Protein Vitality and Proteinous Contamination

Proteins, peptides and AAs are vital for every living organism; they are present in skin, hair, muscles, tendons and bone; they hold together to provide the organism's body its structure and regulate the body chemistry through hormones and enzymes; they affect the transport of oxygen and other vital substances. (Reucsh, 2013)

Proteins are necessary components in the diet of all animals and humans; they help animals and humans in surviving and fighting disease through immune-globulins and white blood cells; moreover, all antibiotics and vaccines are protein based. (Reucsh, 2013)

The worldwide annual consumption of amino acids was 3.3 million tons in 2005 (Drauz et al., 2007) and increased to 6.19 million tons in 2013. (Grand View Research, 2015) The human diet protein demand is expected to double in 2050 as the population