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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Under the Supervision of

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Title of Thesis:

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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^{o}C to 43^{o}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

Signature:

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Table of Contents

List of Tables

List of Figures

Nomenclature

AA	Amino acid
Ala	Alanine
Arg	Arginine
Asp	Aspargine 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).

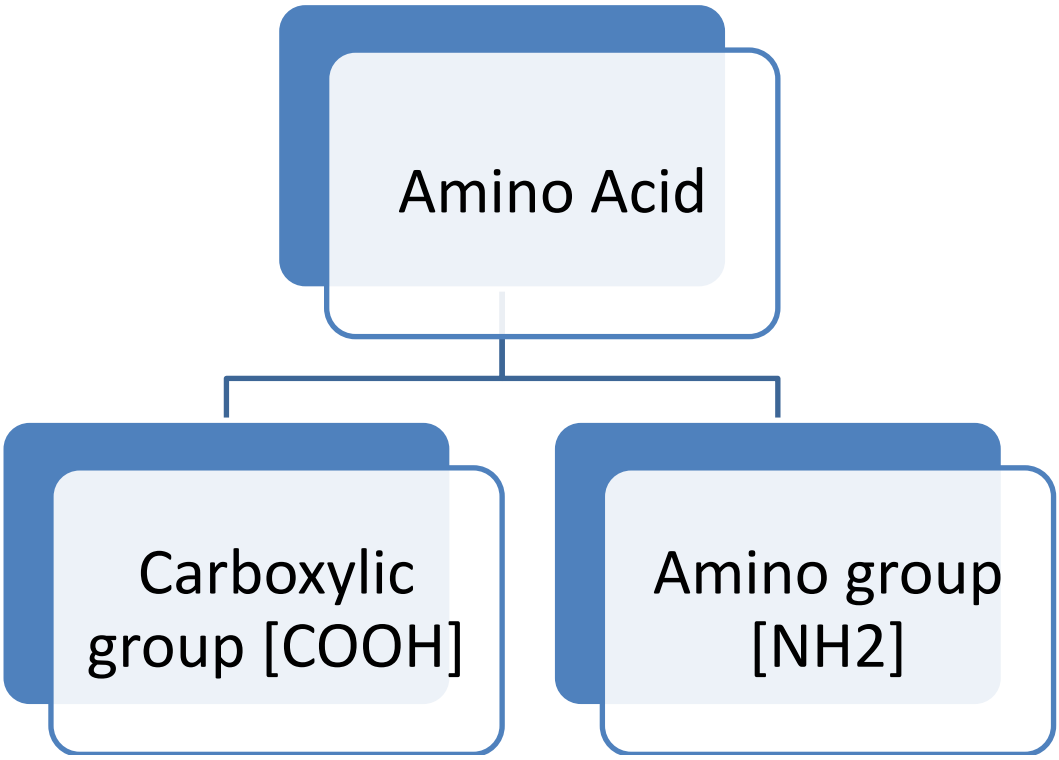

Figure 1.1: Amino acid composition

"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)

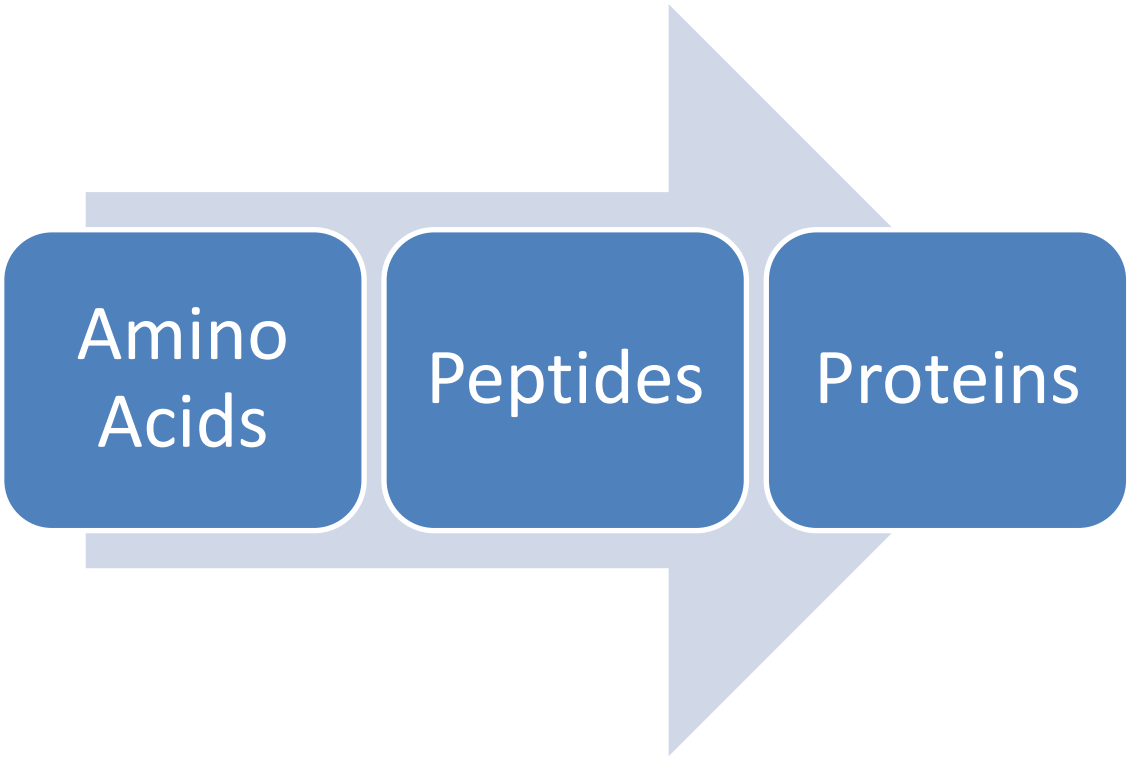

Figure 1.2: Amino acids, peptides and protein sequencing

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