Review paper
UDC 577.388:542.949.41]:641.12

PROTEIN ACIDIC HYDROLYSIS FOR AMINO ACIDS ANALYSIS IN FOOD - PROGRESS OVER TIME: A SHORT REVIEW

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Abstract

Hydrolysis methods are used for liberate amino acids from protein substrate and quantitatively recover them in the hydrolysate. Due to a large number of factors (such as temperature, time, hydrolysis agent, additives) there is no hydrolysis method that can completely accomplish this task.

Hydrolysis can be performed by either chemical or enzymatic means, while chemical hydrolysis can be performed under either acidic or basic conditions. The earliest experiments on the acid hydrolysis of proteins were performed in 1820, while amino acid analysis was first developed in the early of 1950s by using 6M HCl acid hydrolysis in an oxygen-free environment at 110 ^{0}C for 22 hours to liberate amino acids from pure proteins. Since then, the majority of analysis methods use hydrolysis of the peptide bond in proteins using 6M HCl under vacuum and heating at 100 - 160 ^{0}C for 18 to 72 hours. The major challenge for the researchers was to help address the perceived weaknesses of the hydrolysis (long hydrolysis times, low yields, instability of some amino acids, etc.).

Acidic hydrolysis is the most important technique used to cleave the peptide bond in proteins. Although it was developed more than 60 years ago, acid hydrolysis in 6M HCl at 110 ^{0}C for 24 hours is still the most commonly used hydrolysis technique. Over the past decades, numerous articles have been published on acidic hydrolysis of proteins related to amino acids analysis, in which various hydrolysis agents have been used, hydrolysis time and temperature have been varied, in order to increase the amino acids recovery yield. Until now, no hydrolysis method can completely liberate all amino acids from a protein substrate and recover them with 100% yield.

***Key words**: Amino acids, Protein, Acidic hydrolysis.*

1. Introduction

The amino acids that occur naturally as constituents of proteins have an amino group (NH_2) and a carboxylic acid group (COOH) attached to the same carbon. They are called α-amino acids and have the general formula as shown in Figure 1.

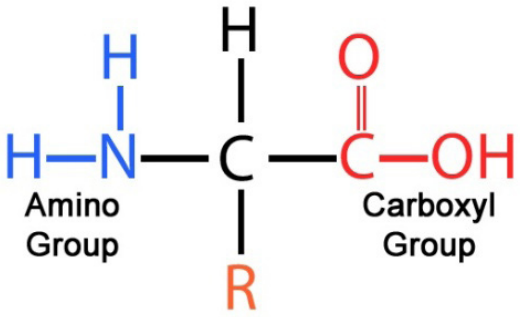
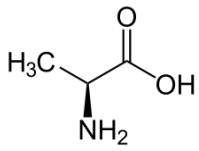
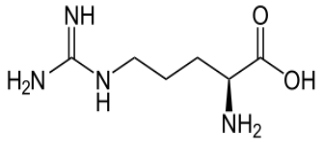
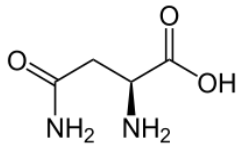
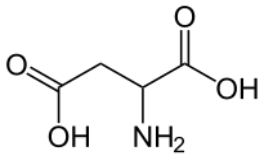
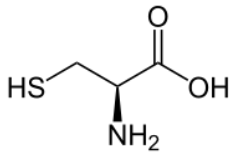
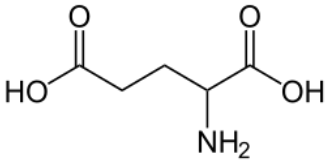
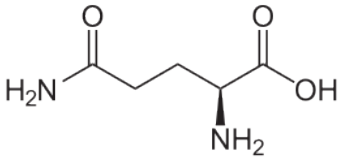
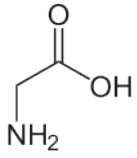
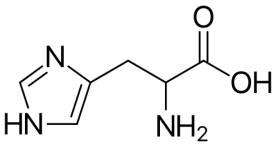
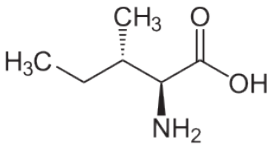


Figure 1. Amino acids general formula

The nature of the substituent R varies considerably. In some amino acids, R is a hydrocarbon group, whereas in others it possesses functional groups such as OH, SH, SCH_3, COOH, or NH_2.

The names, abbreviations, structures and some properties (molecular weight, isoelectric point, solubility, melting point) of the important α-amino acids are shown in Table 1 and Table 2.

Table 1. Amino acids names, abbreviations, molecular weights and structures

Name	Abbreviations		Molecular weight (g/mole)	Structure
Alanine	Ala	A	89.1	
Arginine	Arg	R	174.2	
Asparagine	Asn	N	132.1	
Aspartic acid	Asp	D	133.1	
Cysteine	Cys	C	121.2	
Glutamic acid	Glu	E	147.1	
Glutamine	Gln	Q	146.2	
Glycine	Gly	G	75.1	
Histidine	His	H	155.2	
Isoleucine	Ile	I	131.2	

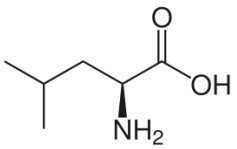
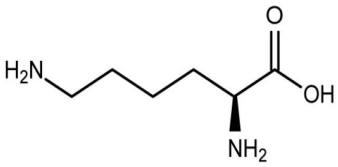
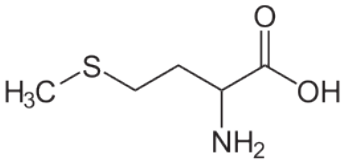
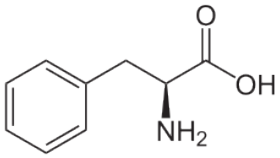
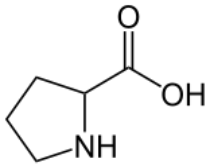
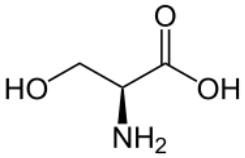
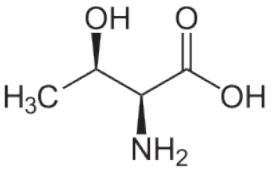
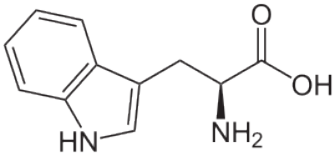
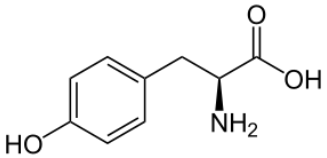
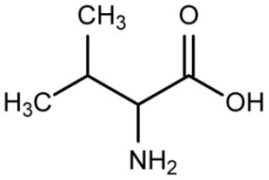
Leucine	Leu	L	131.2	
Lysine	Lys	K	146.2	
Methionine	Met	M	149.2	
Phenylalanine	Phe	F	165.2	
Proline	Pro	P	115.1	
Serine	Ser	S	105.1	
Threonine	Thr	T	119.1	
Tryptophan	Trp	W	204.2	
Tyrosine	Tyr	Y	181.2	
Valine	Val	V	117.1	

Table 2. Some of amino acids properties

Name	Isoelectric point (pI)	Solubility (*) (g/100g)	Melting point (°C)
Alanine	6.02	15.8	297
Arginine	10.76	Very sol.	230 - 244
Asparagine	5.41	2.4	236
Aspartic acid	2.98	0.4	269
Cysteine	5.02	Very sol.	258
Glutamic acid	3.22	0.7	247
Glutamine	5.70	3.16	184
Glycine	5.97	22.5	292
Histidine	7.59	4.0	287
Isoleucine	6.02	2.1	285
Leucine	5.98	2.4	337
Lysine	9.74	Very sol.	224
Methionine	5.06	3.0	283
Phenylalanine	5.48	2.7	283
Proline	6.30	154.5	220
Serine	5.68	4.3	228
Threonine	5.60	1.6	253
Tryptophan	5.88	1.1	283
Tyrosine	5.67	0.04	342
Valine	5.97	6.8	315
(*) Water solubility at isoelectric point of the L isomer in g/100 g at 20°C			

Protein hydrolysis is the breakdown of protein into smaller peptides and free amino acids (Figure 2).

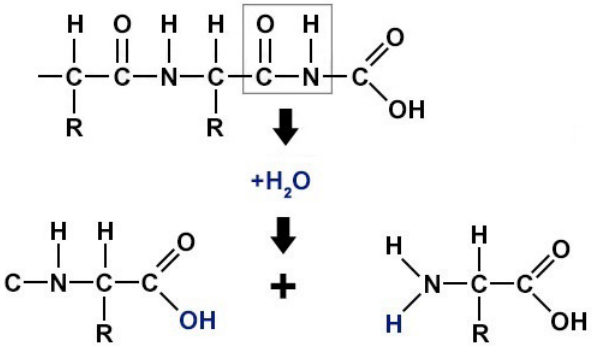


Figure 2. Protein hydrolysis reaction

The aim of a protein hydrolysis method is the quantitative recovery of the amino acids. Being a complex process, hydrolysis is influenced by several factors such as time, temperature, hydrolysis agent or different additives. This is the reason for which no hydrolysis method can completely liberate all amino acids from a protein substrate and recover them.

Because of different types of samples that are usually analyzed in a laboratory the choice of the right hydrolysis method depends of each particularly analysis [1].

Hydrolysis of polypeptides/proteins can be accomplished by 2 main ways: chemical or enzymatic. Chemical hydrolysis can be performed under either acidic or basic conditions.

The aim of this study is the progress recorded for acidic hydrolysis techniques over time highlighting the stability of amino acids residues during acidic hydrolysis conditions.

2. Protein hydrolysis

The earliest experiments on the acid hydrolysis of proteins were performed by Henri Braconnot in 1820, in which gelatin, wool and muscle fibers were hydrolyzed with concentrated sulphuric acid [1]. Amino acid analysis was first developed by Moore [2, 3] in the early of 1950s; he used 6M HCl acid hydrolysis in an oxygen-free environment at 110 °C for 22 hours to liberate amino acids from pure proteins. The same method was then used and several papers are reported [4 - 6]. The majority of analysis methods use hydrolysis of the peptide bond in proteins using 6M Hydrochloric acid under vacuum and heating at 100 - 160 °C for 18 to 72 hours [7]. This fact has remained largely unchanged [8].

The major challenge for the research community was to help address the perceived weaknesses of the hydrolysis steps such as long hydrolysis times, low yields and the instability of some of the amino acids [9].

Amino acids are chemically diverse group of compounds and only few of them (aspartic acid, glutamic acid, proline, glycine, alanine, leucine, phenylalanine, histidine and arginine) can be quantitatively determined during acid hydrolysis [10]. The other amino acids may undergo transformations during hydrolysis.

Asparagine and glutamine suffer deamination reactions during acid hydrolysis being converted in aspartic acid and glutamic acid. Therefore, they will be found in aspartic acid and glutamic acid obtained values. Over time, several methods for asparagine and glutamine analysis were proposed: esterification-reduction of carboxylic groups [11], carbodiimide modification to the free carboxylic groups [12], enzymatic hydrolysis [13] or conversion of the amide to the amine [14]. The rationale behind the carbodiimide - nucleophite approach, as presented by Carraway, [12], is shown in the Figure 3.

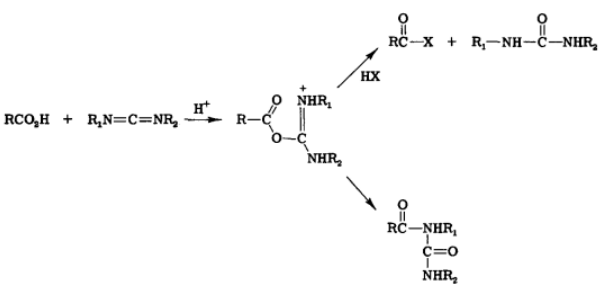


Figure 3. Carbodiimide modification to the free carboxylic groups [12]

Serine and threonine are two amino acids which are partially destroyed during acid hydrolysis, for them being reported losses of 5 to 15% [15, 16]. Several approaches were used in order to increase quantitative yields, such as hydrolysis for less than 24 hours [17], or multiple hydrolysis times and developing of correction factors in order to correct hydrolytic losses.

Cysteine and methionine also suffer transformation during acid hydrolysis, as cysteine can be destroyed while methionine can be oxidized to methionine sulfoxide and methionine sulfone. This is directly related to uncomplete removal of oxygen from the hydrolysis tubes. Often, for determining cysteine and methionine, they are oxidized to cysteic acid and methionine sulfone using performic acid prior acid hydrolysis, then unreacted performic acid is reduced to formic acid using sodium metabisulfite or HBr. The oxidized derivatives mentioned are generally stable during acid hydrolysis [18], but significant losses of cysteic acid during analysis were also reported [16]. Lot of works related to cysteine and methionine analysis were reported [19 - 29]. A detailed discussion regarding analysis of cysteine, methionine and methionine sulfoxide has been provided by Rutherfurd and Moughan, [25].

Valine and isoleucine contain peptide bonds with very hydrophobic residues are quite difficult to cleave with acid, thus, when using acid hydrolysis in 6M HCl for 24 hours at 110 ^{0}C, the obtained quantities are often low. In order to increase the yields of these amino acids hydrolysis times are increased to 72 hours [30].

Tyrosine is an amino acid that can undergo halogenation during acid hydrolysis using HCl. To prevent this, phenol is often added to the 6M HCl [31]. Also, the presence of iron and copper ions can reduce significant tyrosine recoveries, but this can be overcome by using constant-boiling HCl. High fat content can also reduce tyrosine yields, but this can be overcome by defatting samples prior to acid hydrolysis [32].

Lysine is stable under standard acid hydrolysis conditions, and in pure proteins and foods thermally untreated can be readily determined using HCl hydrolysis. Problems appear when foods are processed, particularly heat processed, because of the side-chain amino group that can react with other compounds such as reducing sugars producing Maillard products [33]. Some of the Maillard products are acid labile and will revert back to lysine [34], and different other compounds [35], which will overestimate lysine amount. There have been several methods developed to determine reactive lysine, including the fluorodinitrobenzene method [36], trinitrobenzenesulfonic acid method [37], sodium borohydride method [38], furosine method [39], dye-binding method [40], ninhydrin-reactive lysine method [41], o-phthaldialdehyde-reactive lysine method [42], guanidine method [43 - 45].

Tryptophan is an amino acid that can be destroyed during acid hydrolysis. Different methods for increasing tryptophan recovery after acid hydrolysis were developed by adding thiols [46], by adding tryptamine [47], by reduction of tryptophan to dihydrotryptophan using pyridineborane prior hydrolysis [48] or by using p-toluensulfonic acid [49]. Despite the numerous methods developed, the most commonly used approach is based on alkaline hydrolysis.

3. Conclusions

- Acidic hydrolysis is the most important technique used to cleave the peptide bond in proteins. Although it was developed more than 60 years ago, acid hydrolysis in 6M HCl at 110 ^{0}C for 24 hours is still the most commonly used hydrolysis technique. Over the past decades, numerous articles have been published on acidic hydrolysis of proteins related to amino acids analysis, in which various hydrolysis agents have been used, hydrolysis time and temperature have been varied, in order to increase the amino acids recovery yield.

- Until now, no hydrolysis method can completely liberate all amino acids from a protein substrate and recover them with 100 % yield.

Acknowledgement

This work was performed through the Core Program, carried out with the support of the Ministry of Research and Innovation, the project no PN 16460106.

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