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Study the Effect of Hydrolysis Variables on the Production of Soya Proteins Hydrolysis

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Abstract

This study was conducted to determine the effects of concentration of hydrochloric acids, temperature, and time on the hydrolysis of soya proteins (defatted soya flour) by determining the value of total protein nitrogen concentration, and amino nitrogen concentration of protein, peptides, and amino acids, and then calculated the hydrolysis rate of proteins.

The variables of the conditions of hydrolysis process was achieved in this study with the following range value of tests parameter:

- Concentration of HCl solution ranged between 1-7 N,
- Hydrolysis temperature ranged between 35-95 °C, and
- The time of hydrolysis period ranged between 0.5-24 hr.

Experiments were designed according to the central composite rotatable design.

The practical study has shown the possibility of decreasing the negative effect of the acid on the biological characteristics of the protein; then affecting the possibility of using the product for biological purposes (for medical and microbiological laboratories) by:

- Decreasing the acid concentration used in the process of hydrolysis, firstly, and
- Decreasing the temperature of the hydrolysis process, secondly, and then
- Increasing the period of the time of hydrolysis process, thirdly.

Keywords : Acidic hydrolysis of soya proteins, acidic hydrolysis of soybeans proteins, acidic hydrolysis of vegetable proteins, proteins hydrolysis theory, methods of proteins hydrolysis.

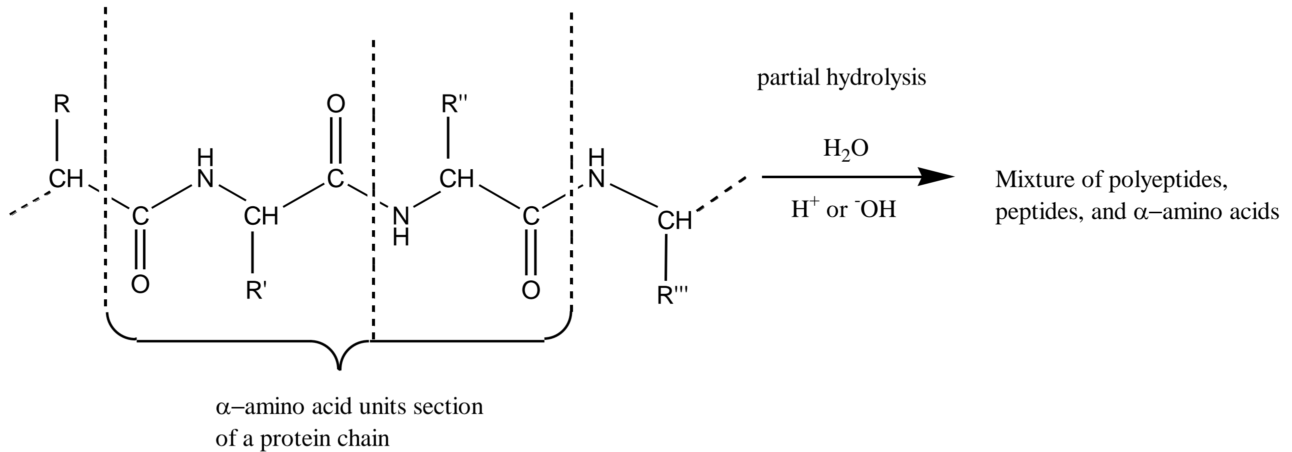
1. Introduction

The hydrolysis of proteins, which breaks them down to their constituent amino acids and peptides, can be achieved by the use of strong acids, strong bases or proteolytic enzymes, there are three main methods of hydrolysis of proteins[1].

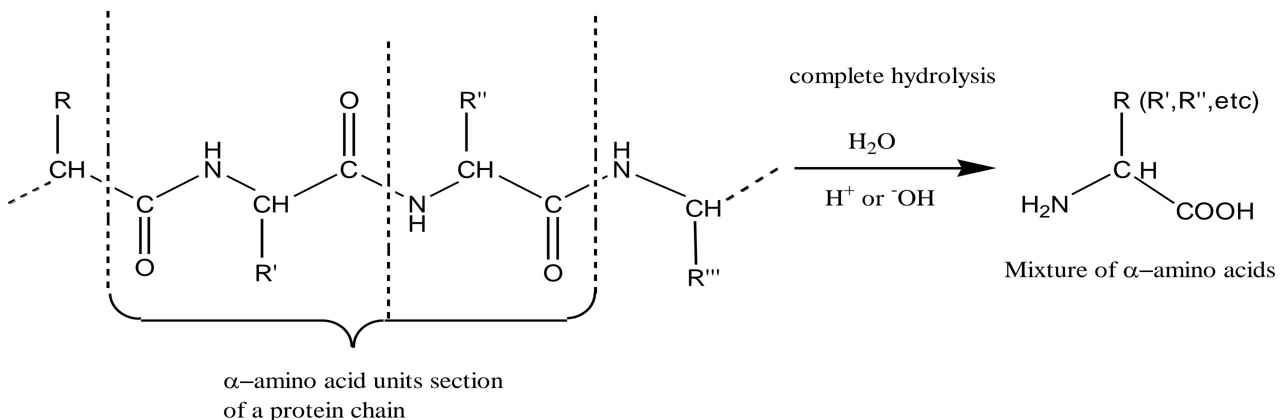
Hydrolysis with strong mineral acid or base (chemical catalysts of biological reactions) is nonspecific, attacking all peptides bonds, degrading proteins and polypeptides to low chain length peptides and amino acids[1], and also produces a large number of fragments[2]. In this process all peptide bonds are attacked and in theory, complete break down into component parts could be obtained[1]. Acid or base treatment of plant (soybean, corn) or animal (casein) proteins brings about desirable changes in flavor, texture, and solubility. Such treatments also destroy toxins and trypsin inhibitors and are used to prepare protein isolates[3]. One of the principals advantages of acid as compared with base hydrolysis is that the optical activity of the amino acids is not destroyed in the process[4], on the other hand, acid hydrolysis destroys tryptophan and partially destroys cystine, serine, and threonine. Asparagine and glutamine are converted to their acidic form[1][4], and a series of reaction may also take place between carbohydrates and amino acids (Maillard reaction) which gives rise to very dark - brown

decomposition products, called “humin” often toxic to the growth of microorganisms[4],[3].

Partial hydrolysis of protein with acids or bases produces mixture of α-amino acids, peptides, and polypeptides[5], as shown.



But complete hydrolysis of proteins with acids or bases, produces mixtures of α-amino acids as the principle products[5], as shown.



Another method for hydrolysis of proteins by using proteolytic enzymes (biological catalysts) acts on proteins under less severe conditions, they will function at much lower temperatures, and at normal pressure and are usually specific to the peptide bond they will attack(1). Enzymes commonly used are pepsin, papain, and pancreat(1), as shown in fig.(1).

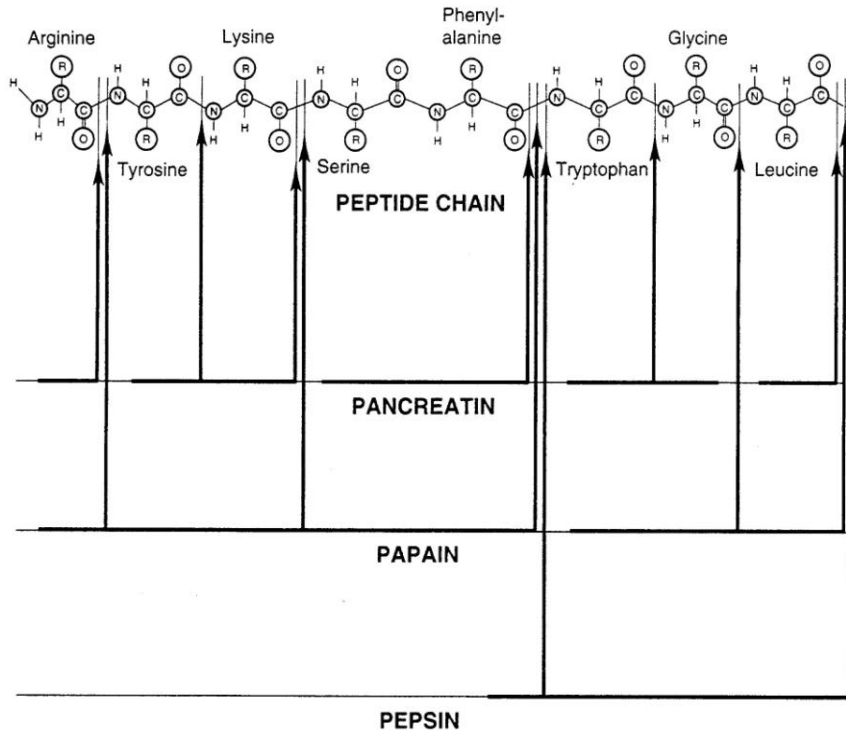


Fig.1. Diagram of Enzymatic Action[1].

The hydrolysis of proteins, they are prepared by the enzymic or acidic hydrolysis of proteinaceous material. These hydrolysates contain secondary protein derivatives such as polypeptides, dipeptides and amino acids. They provide a readily assimilable source of nitrogen which is water soluble, does not coagulate on heating, and is therefore particularly suitable for inclusion in microbiological culture media[6].

The hydrolysis of a protein molecule is a gradual process by which the gigantic molecule of protein is converted in to products of successively lower molecular weight[7], hydrolysis proteins yield metaproteins, proteoses, peptones, polypeptides, and finally the chemically simpler amino acids and their analogs[8].

The degree (rate) of hydrolysis of proteins (DH %) is measured by the number of peptide bonds cut, divided by the total number of peptone bonds, multiplied by a hundred and is calculated by the formula of Equation (1) [1].

$$DH\% = [(AN \text{ of hydrolysis protein} - AN \text{ of protein}) / TN \text{ of protein}] \times 100 \quad \ldots(1)$$

Hydrolyzed vegetable protein is widely utilized in food production, the production of protein hydrolysate from plant proteins has been done using microbial fermentation. This process however, is slow and takes from 4 to 6 months to accomplish. The use of mineral acids reduces the time required to accomplish hydrolysis. These products made by acid hydrolysis are available in European, American, and Japanese markets from1945[9]. The hydrolysis of protein samples as a function of time was studied by Hirs, Stein and Moore (1954) who suggested corrections necessary for incomplete hydrolysis or for destruction of amino acids[10]. The hydrolysis of protein samples with acid was studied by Pham and Rosario (1983), they studied the preparation of protein hydrolysate from defatted coconut and soybean meal by determination of the effects of temperature, time, and concentration of acids on the hydrolysis of coconut and soybean meal, using 6 N HCl, the complete hydrolysis of soybean meal was reached after 36 hr at 95 °C, while it took only 24 hr to complete the process when 18 N H_2SO_4 was used at the same temperature. Coconut protein exhibited some degree of resistance to hydrolysis, using 10 N HCl and 18N H_2SO_4 in two separate tests, it took 48 hr to complete hydrolysis at 95 °C [11],[12] . Husein (1985) studied the hydrolysis of protein from soya grits and dehydrated alfalfa flour by HCl hydrolysis. Protein was added with 33% HCl in a 1:1 ratio by weight, the conditions of protein hydrolysis was

in a reactor with 130 °C, 2.5 atm , for 4 hr [13].On the other hand, Al-Aaragi (1994) treated sample of soybean flour with 6 N HCl at 110 °C in an evacuated tube for 24 hr, to determine the amino acid composition of soya proteins , above conditions, it is necessary to complete hydrolysis of soybean protein according to Toffer procedure for analysis[14]. Davies (1995), studied the conversion of protein to free amino acid form by treatment the protein with high concentration of acid (5M HCl) and high temperature (105°C-103°C),and he studied the effect of these variables on the production of free amino acids[15].

2. Materials and Methods

2.1 Experimental Design and Experimental Trials

Box-Wilson composite rotatable design is a common type of statistical experiment especially applicable to optimization analysis(16),(17),(18), therefore, experiments were designed according to the central composite rotatable design (Box-Wilson composite rotatable design), to can be achieved the purpose of this study .

The study was devoted to test the effect of process variables on the rate of hydrolysis of soya protein; the experimental work was designed for the above purpose in the following experimental operating ranges:

Variable1: Concentration of HCl solution ranging between 1-7 N.

Variable2: Operating temperature of hydrolysis ranging between 35-95 °C.

Variable3: Duration time of specimen ranging between 0.5-24 hr.

Response function was rate of hydrolysis DH%. The center composite rotatable design of three variables was used.

A preliminary step is the setup of the relationships between coded levels, and the corresponding real levels, as following:

$$X_1 = \frac{C-4}{1.732} \quad \ldots(2)$$

$$X_2 = \frac{T-65}{17.321} \quad \ldots(3)$$

$$X_3 = \frac{t-12.25}{6.784} \quad \ldots(4)$$

The working ranges of coded and corresponding real variables are listed in Table (1).

Table 1,
Working Range of the Coded and Corresponding Real Variables.

Coded Level	Concentration of HCl Solution in N	Operating Temperature in°C	Duration Time in hr
-1.732	1	35	0.5
-1	2.268	47.679	5.466
0	4	65	12.25
+1	5.732	82.321	19.034
+1.732	7	95	24

According to experimental design of the three variables there are twenty experiments (there are fifteen tests, and five tests are added at the center), were carried out in the sequence as listed in Table (2), where the coded values of +1.732,-1.732,0 represent the maximum, minimum and average values, respectively.

According to the central composite rotatable design of the experimental work, it explains the relationship between concentration of HCl solution, temperature, and time of hydrolysis of soya proteins, and effect of these variables on the hydrolysis rate of soya proteins, as well as, it studies the relationship between the rate of hydrolysis of soya proteins and TN, AN, AN/TN% ratio of hydrolysized product of soya proteins.

Table 2,
Sequence of Experiments According to Central Composite Design.

Exp. No.	Coded Variable			Real Variable		
	X_1	X_2	X_3	C (N)	T (°C)	t (hr)
1	-1	-1	-1	2.268	47.679	5.466
2	+1	-1	-1	5.732	47.679	5.466
3	-1	+1	-1	2.268	82.321	5.466
4	-1	-1	+1	2.268	47.679	19.034
5	+1	+1	-1	5.732	82.321	5.466
6	+1	-1	+1	5.732	47.679	19.034
7	-1	+1	+1	2.268	82.321	19.034
8	+1	+1	+1	5.732	82.321	19.034
9	-1.732	0	0	1	65	12.25
10	+1.732	0	0	7	65	12.25
11	0	-1.732	0	4	35	12.25
12	0	+1.732	0	4	95	12.25
13	0	0	-1.732	4	65	0.5
14	0	0	+1.732	4	65	24
15	0	0	0	4	65	12.25
16	0	0	0	4	65	12.25
17	0	0	0	4	65	12.25
18	0	0	0	4	65	12.25
19	0	0	0	4	65	12.25
20	0	0	0	4	65	12.25

2.2 Experimental Work

Experimental work includes mainly two principal stages, which are:

First stage: Includes necessary steps for preparation of defatted soya flour from dehulled and defatted soybeans, by using whole mature seed of Lee class of soybean provided by IPA Center for Agricultural Researches (Iraq).

At the beginning, moisture must be removed from the seeds of soybeans to facilitate the following processes of removing the hulls and of grinding. The main purpose of removing the hulls is to get rid of the soil attached to them. Besides that, their compositions contain a high degree percent of fibers. Accordingly, this will affect the quality of the product.

The purpose of the next step is to remove the oil completely from the soya flour. At the same time the quality of the removed oil must be reserved from any destruction that may take place because of the conditions of the process of extraction, to avoid the destruction of soybeans fat , the process of fat removal from the soybeans flour has been done in three stage .

After the process of removing the fat, the soybeans flour is re-grinded to the size of 100 mesh which represents the stage of excessive grinding. Then, the defatted soya flour will be ready for the next stage.

Defatted soya flour was prepared by drying the beans at 68°C for 8 hr, and dehulling them. The dehulled beans were ground to pass through a 60 mesh sieve (first milling process)[11]. Oil was extracted with hexane in three stages[19], and then the solvent was removed from flour[19]. The sample flour obtained after milling were passed through a 100 mesh sieve (second milling process), packed in plastic bags , and stored at 10°C[11].

Second stage: According to the central composite rotatable design, Table (2), twenty trials were done on each run, the analysis was done for each trial, using the following procedure:

Hydrochloric acid solution of each concentration was added to sample of defatted soya flour in a glass beaker, and stirred by a mechanical stirrer (the ratio of defatted soya flour to HCl solution was 1:3 (w:v)) [20]. The temperature of hydrolysize was controlled in the water bath, also the glass beaker was covered by using silver paper. After finishing each trial, the hydrolysized product was cooled directly to 25 °C, and then it was neutralized by sodium hydroxide solution. The volume of neutralized hydrolyzed product was modified to a constant volume for all trials by adding deionized water, and then it was filtered through Whatman filter paper No.541, filtration was carried out under vacuum [11],[12]. After that, the filtrate volume was modified to a constant volume for all trials, after that, the final filtrate volume was used to determine TN, AN, and AN/TN%.

2.3 Methods of Analysis

- Protein was determined by using the absolute method[21].
- Total protein nitrogen of samples was determined by using 5.71 factor to convert amount of protein to total protein nitrogen TN[19].
- Amino nitrogen was determined by using formaldehyde titration method[1],[22].
- Moisture was determined by using the air oven method[22],[23].
- Oil was determined by using intermitted extraction method[23].
- Ash was determined by using dry ashing method[22],[23].
- Fiber was determined by using the procedure of fertilisers and feeding stuffs regulations 1976 SI No. 840[23].
- Carbohydrate percentage was determined by subtracting all other components form 100% percent [23].

3. Results and Discussion

First stage: The main purpose of the procedure followed in this stage is to prepare the defatted soya flour to be ready for carrying out the laboratory experiments.

After finishing this stage, the defatted soya flour will be ready for the next stage. The chemical analysis of soybeans during the stages of preparing the defatted soya flour, can be shown in Table (3).

Table 3,
Chemical Analysis of Soybeans During the Stages of Preparing the Defatted Soya Flour.

Chemical Compositions	The Degree Percent of the Chemical Compositions (w/w%)					
	Soybeans	Dried Soybeans	Dehulled Soybeans	Defatted Soybeans		
				First Stage	Second Stage	Third Stage
Moisture	6.856	2.321	2.352	2.887	3.001	3.048
Protein	35.412	37.136	39.932	49.011	50.941	51.733
Oil	20.235	21.22	22.811	5.262	1.531	-
Ash	5.634	5.908	5.862	7.195	7.478	7.594
Fiber	5.375	5.637	1.544	1.896	1.97	2.001
Carbohydrate	26.488	27.778	27.499	33.749	35.079	35.624

Second stage: The results of the carried out experiments according to the experimental design of Box Wilson are shown in Table(4).

To find out the relation between the DH% and the effect of the following variables on the process of protein hydrolysis: concentration of acid, temperature and the duration of the process of hydrolysis, the conditions of each experiment were fed into a computer program (statistical program). These conditions were entered according to the significance of coded variable and the degree of hydrolysis, which were prepared according to the experimental design shown in Table (4). This process is done to get the coefficients of the polynomial equation of the second order. The resultant equation is as follows:

$DH\% = 44.80638 + 13.33998\ X_1 + 11.94455\ X_2 + 10.64997\ X_3 + 0.1561251\ X_1^2 + 3.631374\ X_2^2 +$

$2.702626\ X_3^2 - 2.655856\ X_1X_2 - 2.8462\ X_1X_3 - 2.66819\ X_2X_3$ …(5)

Average error = 3.584888%
Correlation coefficient = 0.9945404
Standard deviation = 1.892087

Equation (5) has been figured to clarify the effect of each of (C), (T), and (t) on (DH%) by means of Figures (2), (3), (4) and (5).

Table 4,
Results of Experiments Planned According to Composite Rotatable Design.

Exp. No.	Hydrolysized Product			
	TN gm/100ml	AN gm/100ml	AN/TN %	DH %
1	0.647	0.10613	16.403	8.629
2	0.76967	0.28075	36.477	25.494
3	0.74497	0.2884	38.713	26.233
4	0.70441	0.18455	26.199	16.203
5	0.79686	0.46992	58.971	43.763
6	0.88121	0.51	57.875	47.634
7	0.79826	0.47918	60.028	44.658
8	0.99922	0.81068	81.131	76.673
9	0.61249	0.13901	22.696	11.805
10	0.99257	0.67056	67.558	63.141
11	0.65014	0.17812	27.397	15.582
12	0.87897	0.61963	70.495	58.222
13	0.65997	0.201	30.456	17.792
14	0.85299	0.6078	71.255	57.08
15	0.81341	0.47839	58.813	44.581
16	0.88961	0.47053	52.892	43.822
17	0.88111	0.48393	54.923	45.117
18	0.78502	0.49002	62.421	45.705
19	0.87397	0.47712	54.592	44.459
20	0.79628	0.48432	60.823	45.154

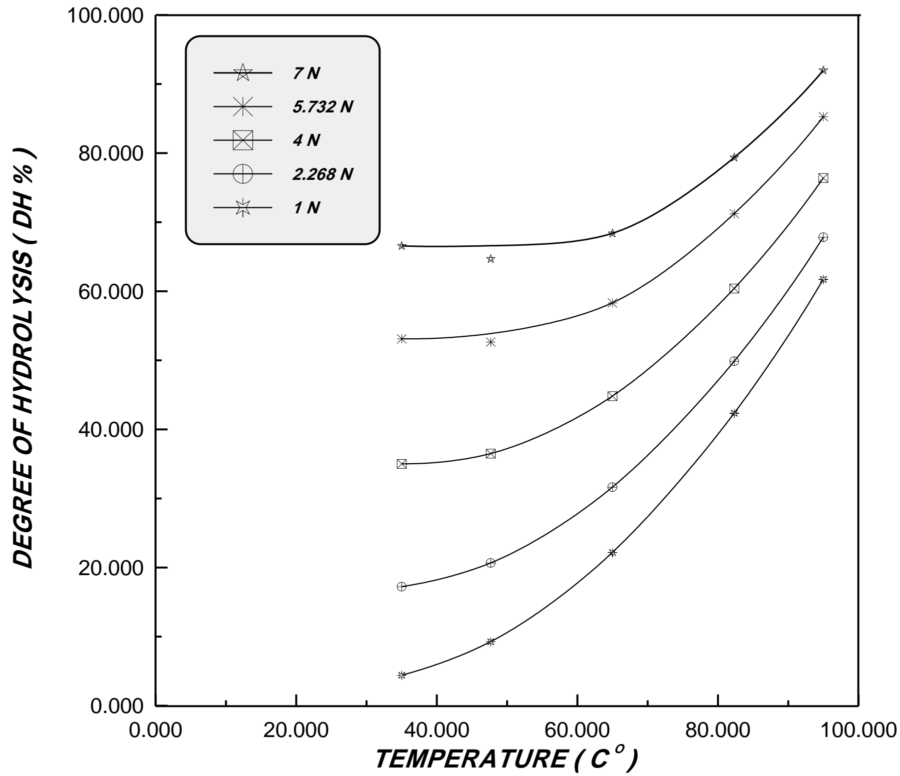


Fig. 2. Degree of Hydrolysis as a Function of Temperature (°C) at 12.25 hr.

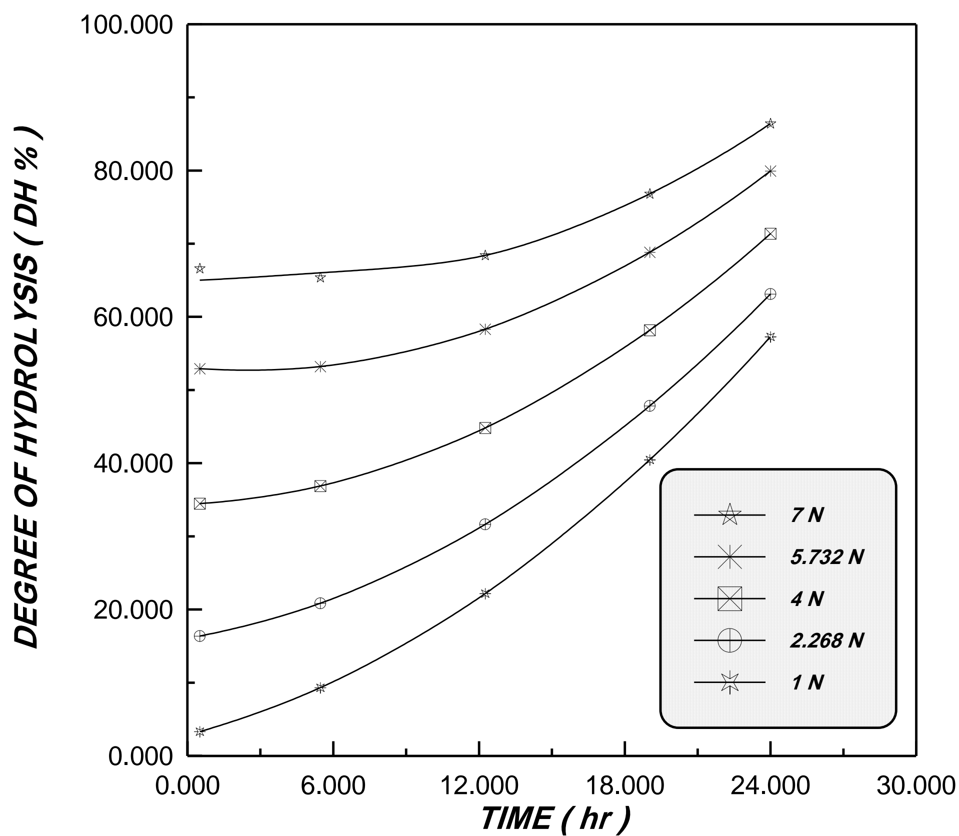


Fig. 3. Degree of Hydrolysis as a Function of Time (hr) at 65 °C.

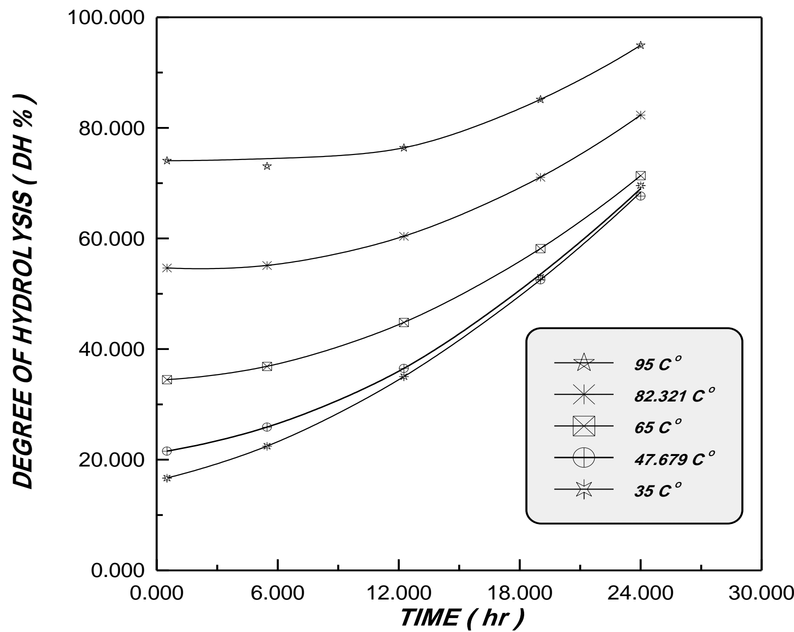


Fig. 4. Degree of Hydrolysis as a Function of Time (hr) at 4 N.

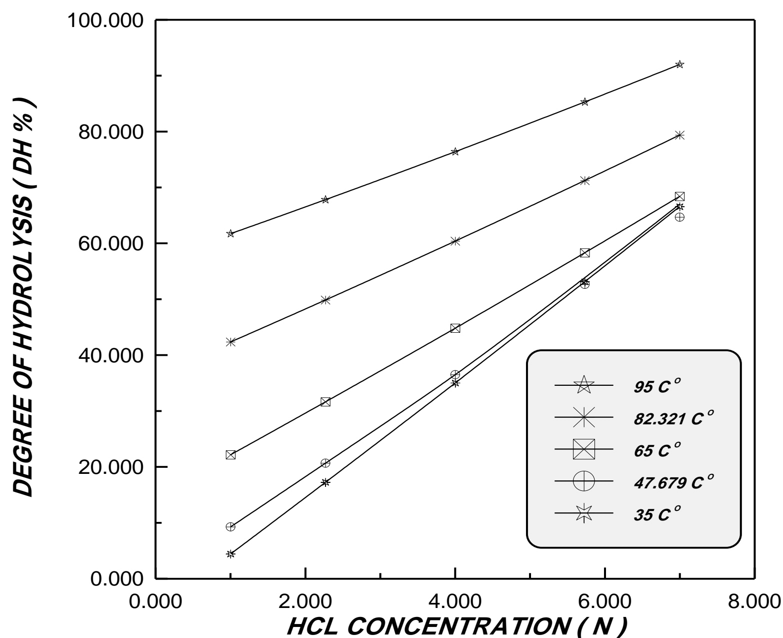


Fig. 5. Degree of Hydrolysis as a Function of HCL Concentration (N) at 12.25 hr.

3.1 Effect of Acid on Protein Hydrolysis

1. Breaking down some of the amino acids, completely and breaking down some other amino-acids partially.
2. Converting some of the amino acids to the acidic formula.
3. Improving the reactions between the carbohydrates and proteins (Malliard reaction) that leads to making poisonous materials.
4. NaCl salt will result (as chemical material in the final product) from the reaction of HCl and NaOH when neutralizing the hydrolyzed product.

The first and second effects occur under all the condition of the process of hydrolysis since the acid is a catalyst for this process. From AL–Aaraji result [(10)] about Lee class of soybeans, the degree percent of amino acids that break down completely and partially and amino acids that are

converted into the acidic formula , which have a very little degree percent in comparison with other amino acids that constitute of soybean protein. Therefore, the side-effects of the first and second effects can be neglected.

With regard to the third effect of treating the protein by the acid, this reaction can not be prevented assertively, even in the lowest conditions, yet the occurrence of this reaction could decreased by depending on the intensity of the conditions.

The fourth effect of using the acid HCl in the process of hydrolysis is producing NaCl , a case which can not be prevented, but may be reduced as much as possible by using low concentrations of the acid. However, less amounts of salt obtained when neutralizing the hydrolysizd product.

3.2 Effect of Temperature

Temperature greatly affects on the process of protein hydrolysis. By increasing the temperature, protein hydrolysis degree increases. This effect follows the effect of acid concentration on protein hydrolysis. The direct effect lies in increasing the rate of the protein molecules digest reaction, whereas the indirect effect causes increasing in the acidic efficiency.

On the other hand, the negative effect of temperature on the process of protein hydrolysis appears directly through its help in the occurrence of Malliard reaction at high degree accompanied by high temperature.

3.3 Effect of Time

Importance of time effect on the process of protein hydrolysis comes after the effect of both the concentration of the acid and temperature. Time is considered nearly to be the only factor that has a positive effect on the protein hydrolysis. It also retains its biological characteristics in comparison with the effect of both the concentration of the acid and temperature when digesting the protein by the acid. Therefore, if the product of protein digest process is wanted to be used for biological purposes, it is necessary to make use of time factor before that of the concentration of acid and temperature. This is because the hydrolysized product has good biological characteristic in spite of the occurrence of Malliard reaction with somehow little degree.

3.4 Studying the Mathematical Relationships and Figures

The relationship between each of TN, AN, and TN/AN% for the hydrolysized product with the degree percent of protein hydrolysis DH% are shown in the Figures (6), (7), and (8).The results predicted from Figures (6), (7) and (8), are concluded in Table (5).

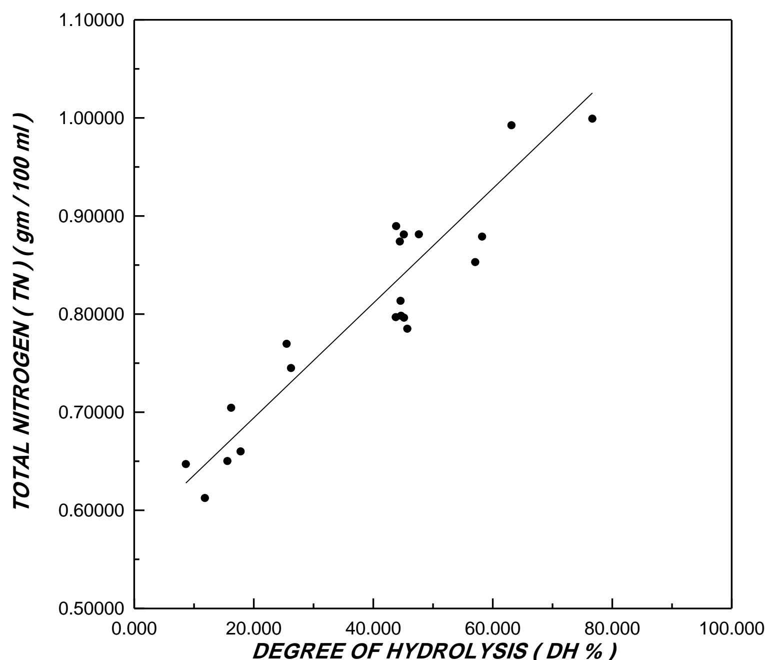


Fig. 6. TN Concentration of Hydrolysized Product as a Function of DH%.

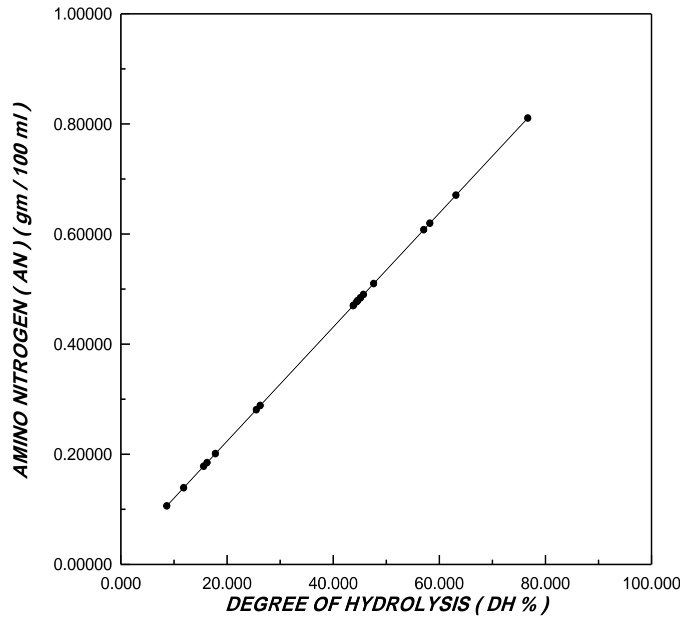


Fig. 7. AN Concentration of Hydrolysized Product as a Function of DH%.

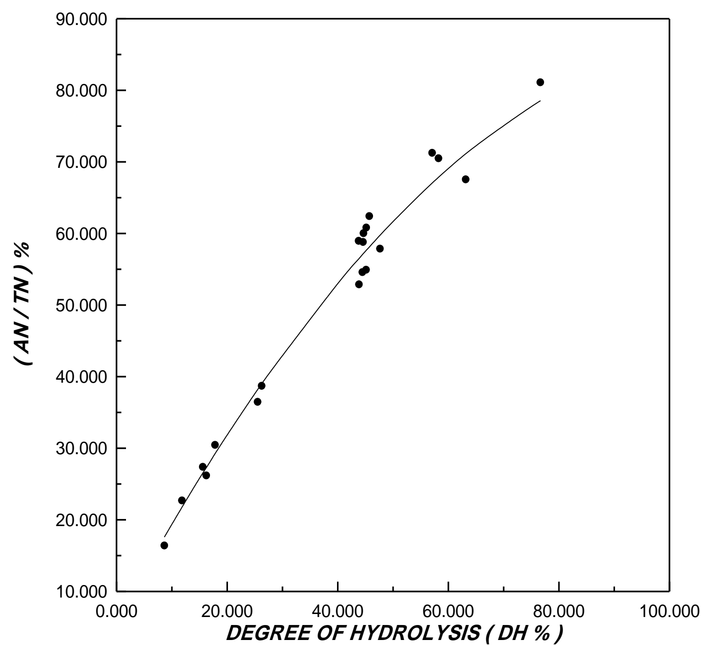


Fig. 8. AN/TN% of Hydrolysized Product as a Function of DH%.

Table 5,
Equations Concluded from Figures (6), (7), and (8).

No. of Fig.	Equation	Average Error %	Kind of Relationship	The Extent of Application
6	TN(gm/100ml)=0.5773207511+ 5.844792489E-3 (DH%) …(6)	4.412	Linear Relationship	$0 \leq DH\% \leq 100$
7	AN(gm/100ml)=0.01678+ 0.0103543(DH%) … (7)	0.81	Linear Relationship	$0 \leq DH\% \leq 100$
8	AN/TN%=5.653779+1.437379 (DH%)-6.345795E-3(DH%)^2 …(8)	4.506	Non linear Relationship	$8.629 \leq DH\% \leq 76.673$

The linear relationship between TN and DH% shown in Equation (6) and Figure (6) gives values approaching the real values of TN when the protein hydrolysis degree is low; whereas the inaccuracy of the TN values (obtained from this equation) increases in comparison with the real values of TN wherever the protein hydrolysis degree increases, because of the increase in the weight of the protein resulting from the process of digest (from the water molecule which replaces each broken bond of peptides bonds, consists of proteins molecule).

With regard to Equation (7), a new formula is reformulated from the linear Equation (1) where the protein hydrolysis degree has been calculated after limiting the AN . Hence, all the points resulted from the above equation lie on one straight line as shown in Figure (7).

Equation (8) clarifies relationship a non-linear between DH% and AN/TN% as shown in Figure (8), which, in its turn, is correct only at DH% extent of application ranging between 8.629% and 76.673%, it is concluded that whenever the protein hydrolysis degree increases, the AN/TN% value also increases. This approached to 100% with the increasing the protein hydrolysis degree to100%.

4. Conclusions

- The side effect of the acid on the protein can be decreased by dealing with low acid concentrations in the first place and then temperature.
- A middle degree of protein dissolving can be obtained under low conditions with regard to acid concentration and temperature but in a long time, whereas, the same dissolving can be get but within a short time by increasing the acid concentration and temperature. Yet, the difference between these two cases lies in that, in the first case the rate of the Malliard reaction between the carbohydrates and proteins is less. The opposite occurs with the second case. Therefore, the product of the first case will be suitable in biological uses, in contrary with that of the second case.

5. Abbreviations

DH% degree of protein hydrolysis

TN concentration of total protein nitrogen

AN concentration of amino nitrogen of protein, peptides, and amino acids

C concentration of hydrochloric acid solution in normality (N)

T operation temperature in °C

t duration time of specimen in hr

X_1 concentration coded variable

X_2 temperature coded variable

X_3 time coded variable

6. Acknowledgments

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دراسة تأثير متغيرات عملية التمييء على إنتاج بروتين الصويا المتميئ

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الخلاصة

تم أنجاز هذه الدراسة لأجل تحديد تأثير كل من تركيز محلول حامض الهيدروكلوريك، درجة الحرارة، والزمن على عملية تمييء بروتين الصويا (طحين الصويا مزال الدهن) بواسطة تحديد قيمة كل من تركيز النايتروجين الكلي للبروتين، و تركيز الامينو نايتروجين للبروتين والسلاسل الببتيدية والأحماض الامينية، وعند أذن يتم حساب درجة تمييء البروتين .

متغيرات ظروف عملية التميئ أنجزت في هذه الدراسة مع القيم المتباينة التالية للمتغيرات المختبرة :

- تركيز محلول HCl يتراوح بين 1-7 ع،
- درجة حرارة التميئ تتراوح بين 35-95 مo، و
- زمن فترة عملية التميئ يتراوح بين 0.5-24 ساعة .

تم تصميم التجارب باستخدام طريقة (Central Composite Rotatable Design).

بينت الدراسة العملية إلى إمكانية التقليل من تأثير الحامض السلبي على المواصفات الحيوية للبروتين عند التمييء الحامضي، وبالتالي إمكانية استخدام المنتج للإغراض البايولوجية (للاستعمال في المختبرات التحليلية الطبية والمايكروبايولوجية) بواسطة :

- تقليل تركيز الحامض المستخدم في عملية التمييء، أولا، و
- تقليل درجة حرارة التمييء، ثانيا، و
- زيادة الزمن اللازم لفترة عملية التمييء، ثالثا .