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The Optimum Conditions for Production of Soya Peptone by Acidic Hydrolysis of Soya Proteins

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Abstract

This study was carried out to obtain the optimum conditions necessary for the process of soya protein hydrolysis by using hydrochloric acid (as a chemical catalyst) instead of the papain enzyme (as a biological catalyst), for the production of soya peptone. These conditions are implemented to test the effect of the variables of the process of hydrolysis on the nature and quality of the product.

The production of soya peptone was studied for their importance in the process of preparing and producing the culture media used in medical and microbiological laboratories.

The process of production of soya peptone includes four main stages:

- Preparing the defatted soya flour, firstly.
- The soya protein hydrolysis, secondly.
- Purifying the product, thirdly , and then,
- Drying the product, finally.

By following the procedure of the present study, the optimum conditions for the process of soya proteins hydrolysis have been reached in present study are:

- Optimum concentration for the hydrochloric acid solution is 1N.
- Optimum hydrolysis process temperature ranged between 50-53°C.
- Optimum period of hydrolysis time ranged between 17.189-19.97 hr.

The productivity of soya peptone was 38.071%, by following the procedure and the optimum production conditions of the present study.

***Keyword*:** Soya Peptone, Soybean Peptone, Vegetable Peptone.

1. Introduction

Soya peptone is a vegetable peptone [(1)], It is obtained by the papain hydrolysis (enzymatic hydrolysate) of soya flour [(1-3)], prepared under controlled conditions especially for use in microbiological procedures [(2)].

It is recommended for use in media for the cultivation of a large variety of organisms, including fungi, and is also used in media for microbiological assay[(2)]. In addition to its nitrogen constituent, this peptone contains a high naturally occurring carbohydrate of the soybean [(2)].

Typical analysis of soya peptone produced by Oxoid laboratory is shown in Table (1).

Peptones are proteins partially digested; 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[(1)(4)], hydrolysis proteins yield metaproteins, proteoses, peptones, polypeptides, and finally the amino acids[(2)]. They are three types of peptones, these types are meat peptones (such as peptone bacteriological, tryptose, etc), vegetable peptone

(soya peptone), and casein and other milk derived peptones [2, 3].

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[3].

Table 1,
Average Analysis of Oxoid Soya Peptone [1, 3].

Typical Analysis (w/w%)	Oxoid Soya Peptones (1988)	Oxoid Soya Peptones (1995)
Moisture %	4.1	5.8
Ash%	7.8	13.5
Amino Nitrogen (AN%)	3.1	2.3
Total Nitrogen (TN%)	10.2	9.1
AN/TN%	30	25
pH of 2% solution (after autoclaving)	7	7.2
NaCl%	0.8	0.4

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 [3], and also produces a large number of fragments [5]. In this process all peptide bonds are attacked and in theory, complete break down into component parts could be obtained [3]. 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 [6]. 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[7], on the other hand, acid hydrolysis destroys tryptophan and partially destroys cystine, serine, and threonine. Asparagine and glutamine are converted to their acidic form[3, 7], 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 [6, 7].

Partial hydrolysis of protein with acids or bases produces mixture of α-amino acids, peptides, and polypeptides [8], as shown in the Figure 1 .

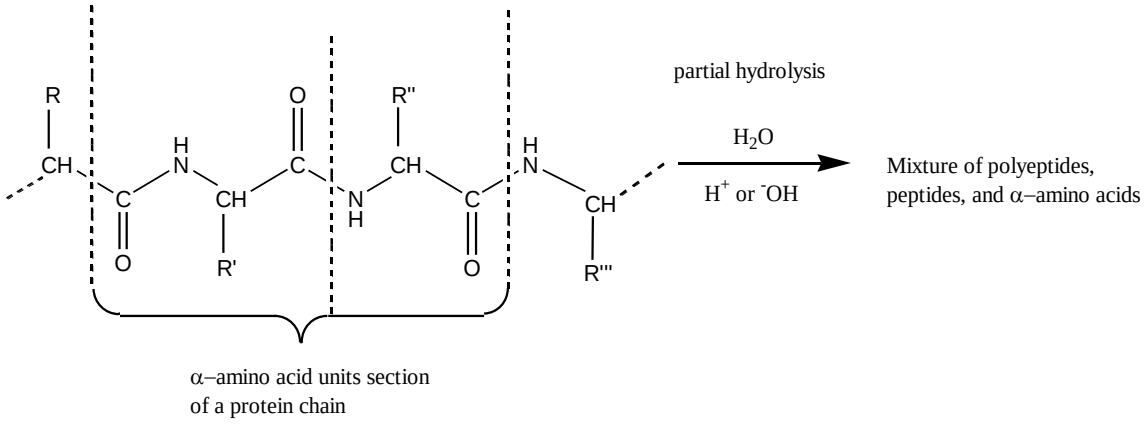

Fig.1. Diagram of Partial Hydrolysis of Protein with Acids or Bases

But complete hydrolysis of proteins with acids or bases, produces mixtures of α-amino acids as the principle products [8], as shown in the Figure 2.

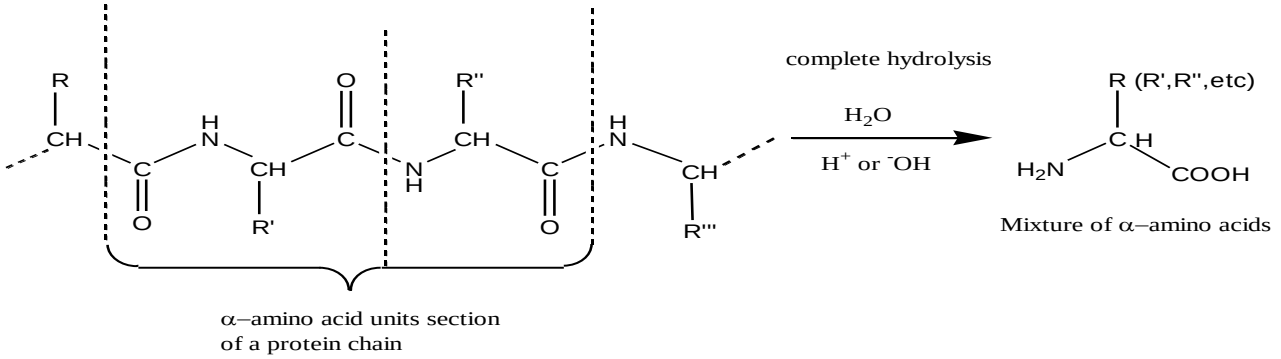

Fig.2. Diagram of Complete Hydrolysis of Protein with Acids or Bases

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[3].

Enzymes commonly used are pepsin, papain, and pancreatin[3], as shown in the Figure 3.

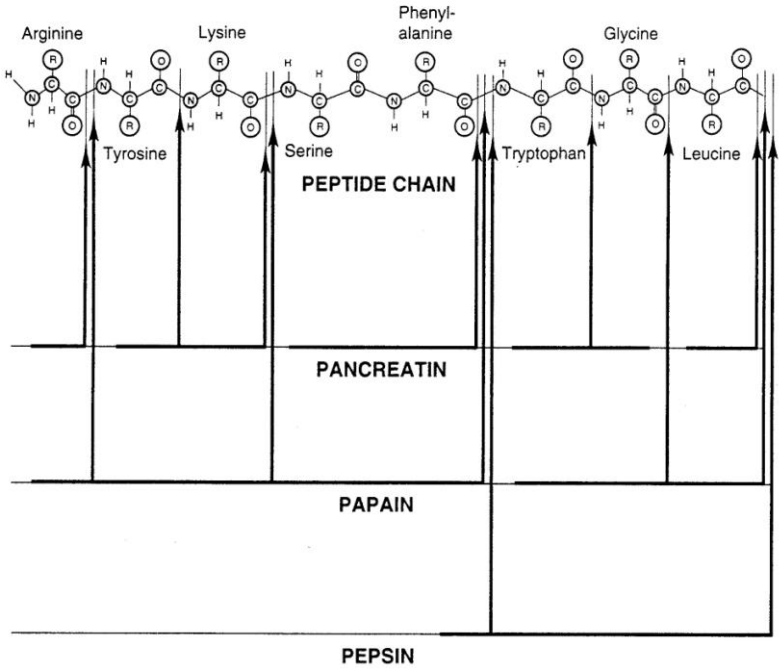

Fig3. Diagram of Enzymatic Action [3]

Peptone are proteins partially digested, 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[1].

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[4], hydrolysis proteins yield metaproteins, proteoses, peptones,

polypeptides, and finally the chemically simpler amino acids and their analogs[2].

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)[3].

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

Nageli is credited with earliest publications (1880-1882) describing the requirements of microorganisms for a protein component, which he called, as "peptone" [3]. Peptone prepared expressly for bacteriological purposes was first introduced by Difco laboratories in (1914) after many years of preliminary study.

This was followed several years later by proteose peptone, and in more recent years other peptones have been added to the group. All peptones are prepared today in the same manner as when first developed[2]. In (1924) Oxoid laboratories were developed the use of culture media. This was also the period when Oxoid laboratories increased investigation into enzymic and acid hydrolysis of meat and vegetable proteins to increase flavor and amino nitrogen content. This work eventually led to the familiar peptones [3].

BDH Laboratory (1985) produces only bacteriological peptone by peptic digest of muscles and animal tissue for general bacteriological purposes[9] . Difco laboratory (1964) produces soya peptone by enzymatic hydrolysate of soya flour[2], and Sigma laboratory (1988) produces it by enzymatic hydrolysate of soya flour with the typical analysis (total nitrogen (TN) 9.4%, amino nitrogen (AN) 1.8% w/w, AN/TN ratio 20%[10], and Oxoid laboratory (1988) produces soya peptone also by enzymatic (papain) hydrolysate of soya flour with the typical analysis (TN 10.2% (w/w), AN 3.1% (w/w), AN/TN ratio 30%)[1], but typical analysis of soya peptone produced by the same company in (1995) is (TN 9.1% (w/w), AN 2.3% (w/w), AN/TN ratio 25%)[3]. The United Stated Pharmacopoeia (USP 1990) depended only on soya peptone produced by papaic digest of soya flour [11]. But soya peptone prepared expressly for bacteriological purposes was first introduced by Ibn-Al Beitar Center [12] in (1993) by using acidic hydrolysate of soya flour after simple preliminary study. They are prepared soya peptone by simple procedures without any typical analysis of the final product[12].

The aim of present study is the determination of the optimum conditions for preparation of soya peptone, depends on the partial hydrolysis of defatted soya flour by using hydrochloric acid (acid hydrolysis) instead of papain (enzyme hydrolysis).

2. Manufacture of Peptones

The basic steps for preparation of peptone are:

1. Preparation of raw protein materials.
2. Hydrolysis of protein with enzymes or acids.
3. Separation unites to purification of hydrolysis product.
4. Drying the final product to powder form [3].

3. Materials and Methods

3.1Experimental Trials

After clarifying and studying the process of soya protein hydrolysis, by studies the relationship between concentration of acid (HCl) solution, temperature, and time of hydrolysis of soya proteins, and effect of these process variable on the rate of soya proteins hydrolysis , and then, studying the relationship between DH% and TN,AN and AN/TN% ratio of hydrolysized product of soya proteins, as described in a pervious study[13].The present study which was represented a second step for pervious study by studies the relationship between the rate of hydrolysis of soya proteins DH% and TN, AN, AN/TN% ratio of partially purified hydrolysized product of twenty tests, these texts were carried out previously on each run on the second stage according to Box Wilson experimental design of three variables as described before[13], to obtain the optimum conditions for production of soya peptone by acid hydrolysis of soya protein.

3.2 Experimental Work

Experimental work includes mainly three principal stages, which are:

First and second stage: were carried out previously as described before [13].The following section, clarifying brief for these two steps.

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).

Second Stage Box-Wilson composite rotatable design is a common type of statistical experiment especially applicable to optimization analysis[14],[15],[16],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.

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.

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

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 [13].

Third stage: which represents the aim of present study, that which represents the process of partial purifying stage of hydrolysized product, resulting from the second stage by centrifuging it at 8000 rpm at 20 C°[12] .At the end, two layers were distinguished within the centrifuged hydrolysized product, upper and lower, each differs in the contents, volume, and colour. The colour of the upper layer was brown while the lower was dark brown. Directly after centrifuging, the upper partially purified hydrolysized product is sucked by a pipette and then separated from the lower layer. The volume of the upper layer was modified to a constant volume for all tests by adding deionized water, and was used to determine TN, AN, and AN/TN% of partially purified hydrolysized product.

3.3 Methods of Analysis

- Protein was determined by using the absolute method [17].
- Total protein nitrogen of samples was determined by using 5.71 factor to convert amount of protein to total protein nitrogen TN[18].
- Amino nitrogen was determined by using formaldehyde titration method [3, 19].
- Moisture was determined by using the air oven method[19, 20].
- Oil was determined by using intermitted extraction method [20].
- Ash was determined by using dry ashing method [19, 20].
- Fiber was determined by using the procedure of fertilisers and feeding stuffs regulations 1976 SI No. 840[20].
- Carbohydrate percentage was determined by subtracting all other components form 100 percent [20].
- Sodium chloride was determined by using the Volhard titration method on the ash residue[20].
- pH value was determined by using pH meter on an autoclaved 2%solution of final product[2,3].

4. Previewing the Results of Pervious Study

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).

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

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:

$$DH\% = 44.80638 + 13.33998\ X_1 + 11.94455\ X_2 + 10.64997\ X_3 + 0.1561251\ X_1^2 + 3.631374\ X_2^2 + 2.702626\ X_3^2 - 2.655856\ X_1X_2 - 2.8462\ X_1X_3 - 2.66819 X_2X_3 \quad \ldots(2)$$

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

Equation (2) has been figured to clarify the effect of each of (C), (T), and (t) on (DH %) by means of Figures from (4), (5), (6), and (7).

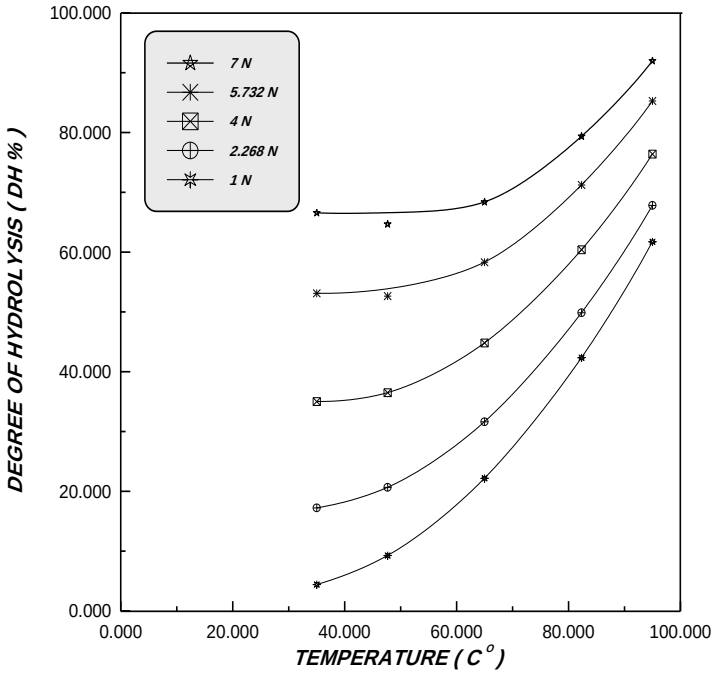

Fig. 4. Degree of Hydrolysis as a Function of Temperature (C°) at 12.25 hr

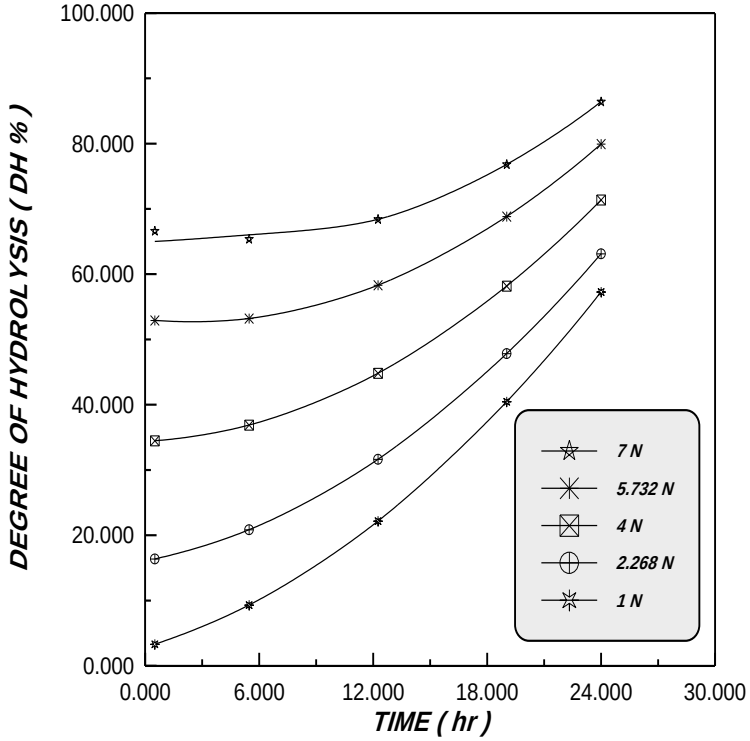

Fig. 5. Degree of Hydrolysis as a Function of Time (hr) at 65C°

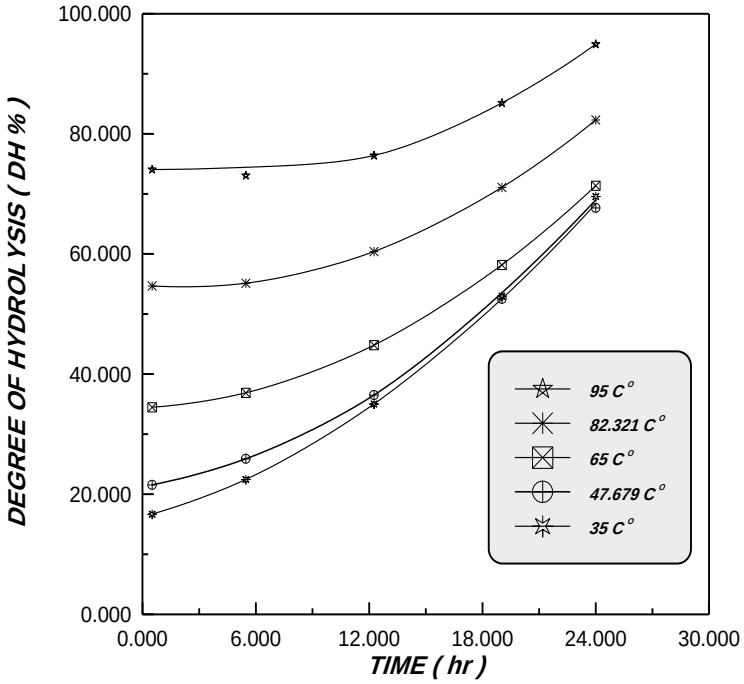

Fig. 6. Degree of Hydrolysis as a Function of Time (hr) at 4 N

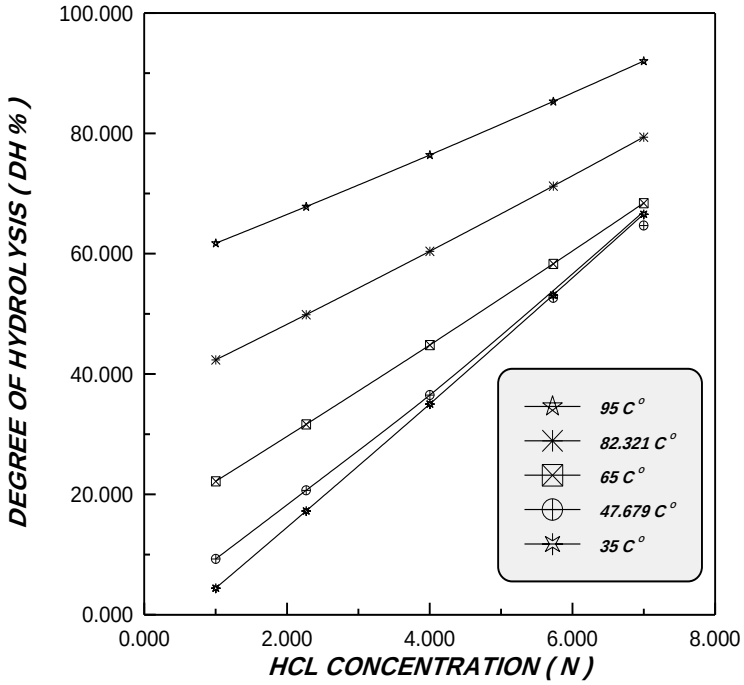

Fig. 7. Degree of Hydrolysis as a Function of HCL Concentration (N) at 12.25 hr

5. Results and Discussion of Present Study

Third stage involves studying the process of partial purifying of the hydrolysized product resulting from the second stage (mainly proteinous materials), in addition to other materials constituting the hydrolysized product. These materials have somehow long series that cause dark colour at the bottom of the tube after the process of centrifuging.

The processes of limiting the value of TN and AN is carried out, and the percentage of TN/AN% is calculated. Results obtained from the processes of analyzing the partially purified hydrolysized product are shown in Table (5).

Table 5,
Analysis of Partially Purified Hydrolysized Product.

Exp. No.	Partially Purified Hydrolysized Product		
	TN gm/100ml	AN gm/100ml	AN/TN%
1	0.24566	0.03203	13.038
2	0.41437	0.10502	25.344
3	0.47088	0.11293	23.983
4	0.36115	0.06436	17.821
5	0.57427	0.21067	36.685
6	.60647	0.23664	39.019
7	0.64339	0.22897	35.588
8	0.77142	0.54485	70.629
9	0.27458	0.0413	15.041
10	0.69386	0.35948	51.809
11	0.33415	0.06255	18.719
12	0.74354	0.35338	47.527
13	0.38424	0.07235	18.829
14	0.65844	0.3386	51.425
15	0.65797	0.22836	34.707
16	0.60226	0.23371	38.805
17	0.65376	0.25737	39.368
18	0.61855	0.25768	41.659
19	0.64137	0.24553	38.282
20	0.59875	0.22596	37.739

This stage represents the process of partial purifying stage and not complete purifying stage of hydrolysized product, resulting from the second stage by centrifuging it. This is due to many reasons: primarily, the process of purifying by the centrifuging is not 100% efficient in separating the long protein series, and also hydrolysis with acid is nonspecific [(3),(5),(6),(7)].

The effect of DH% on the concentration of TN, AN and the percentage of AN/TN% of the partially purified protein product is shown in Figures (8), (9), and (10).The results predicted from Figures (8),(9), and (10) are concluded in Table (6).

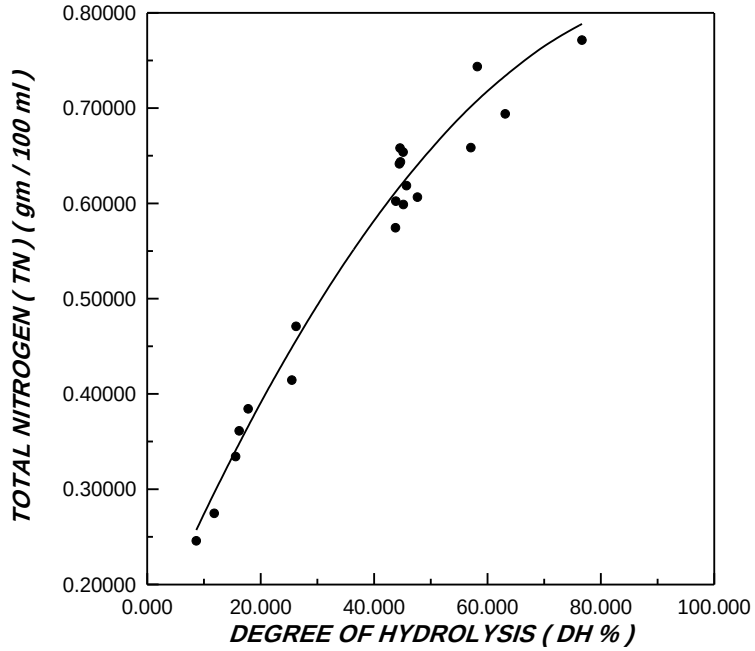

Fig.8. TN Concentration of Partially Purified Hydrolysized Product as a Function of DH%.

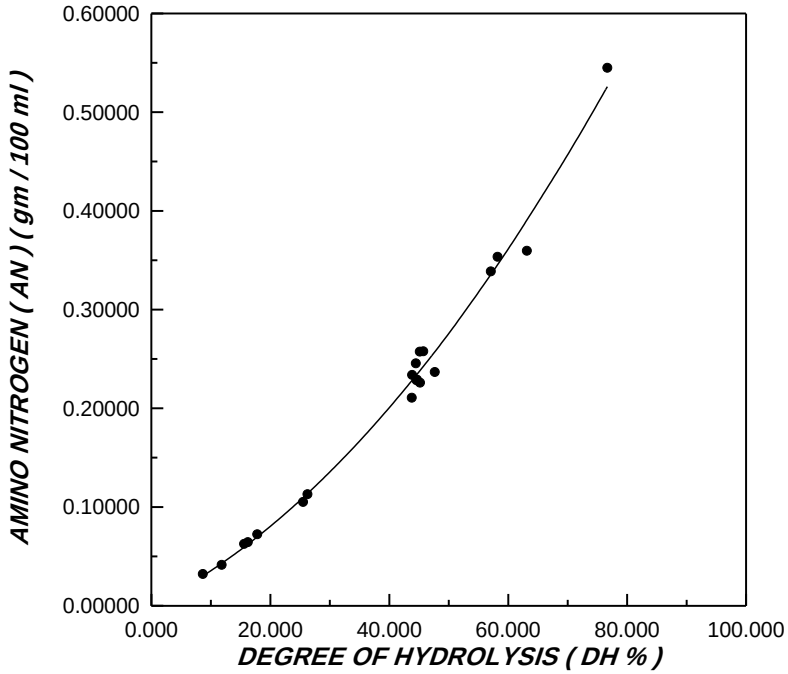

Fig.9. AN Concentration of Partially Purified Hydrolysized Product as a Function of DH%.

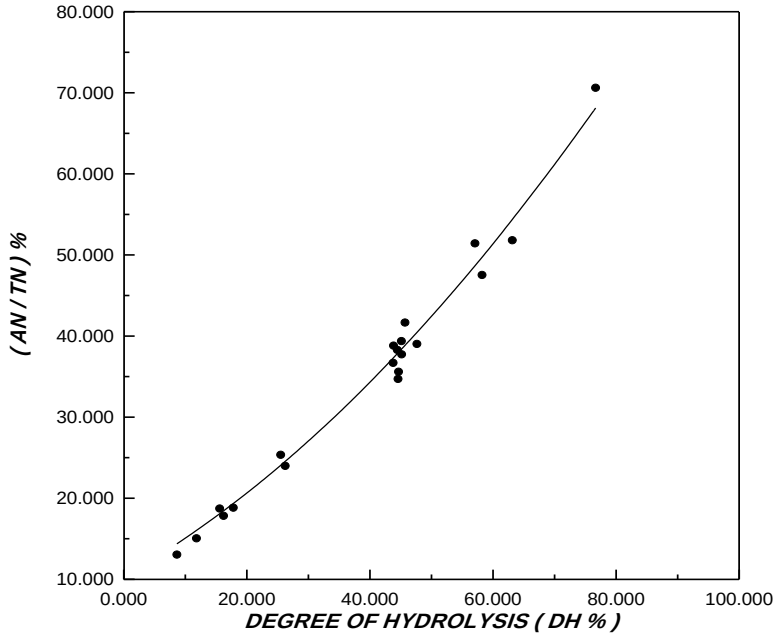

Fig.10. AN/TN% Concentration of Partially Purified Hydrolysized Product as a Function of DH%.

Table 6,
Equations Concluded from Figures (1), (2), and (3).

No. of Fig.	Equation	Average Error %	Kind of Relationship	Extent of Application
8	TN(gm/100ml)=0.1438015+0.0137331(DH%)-6.946058E-5 $(DH\%)^2$ ……(3)	4.485	Non-linear Relationship	$8.629 \leq DH\% \leq 76.673$
9	AN(gm/100ml)=5.451805E-4+2.988322E-3(DH%)+5.036919E-5$(DH\%)^2$ ……(4)	4.497	Non-linear Relationship	$8.629 \leq DH\% \leq 76.673$
10	AN/TN%=10.34685+0.4300127 (DH%)+4.221502E-3 $(DH)^2$ ……(5)	4.369	Non-linear Relationship	$8.629 \leq DH\% \leq 76.673$

From the relationship connecting TN of the partially purified hydrilysized product with DH% which shown in Equation (3) and Figure (8), it could be noticed that whenever the protein hydrolysis degree increases, the TN of the partially purified hydrolysized product increases. At the same time concentration of the protein materials removed from the hydrolysized product by means of centrifuging is reduced. Contrary to this case, whenever the protein hydrolysis degree comes closer to zero.

Regarding to the relationship between AN of the partially purified hydrolysized product and DH% which shown in Equation (4), and Figure(9), whenever the protein hydrolysis degree increases, AN value of the partially purified hydrolysized product came closer to the TN value of the same product, due to the increase in the number of broken peptide bonds with the increase of the hydrolysis degree. The opposite behavior happened when the hydrolysis degree is low.

To find out and clarify the indirect effect of the system variables studied before[13] (concentration of the acid, temperature, and time of the hydrolysis) on the concentration of TN and AN of the partially purified hydrolysized product, are shown in Figures (11) to (16).

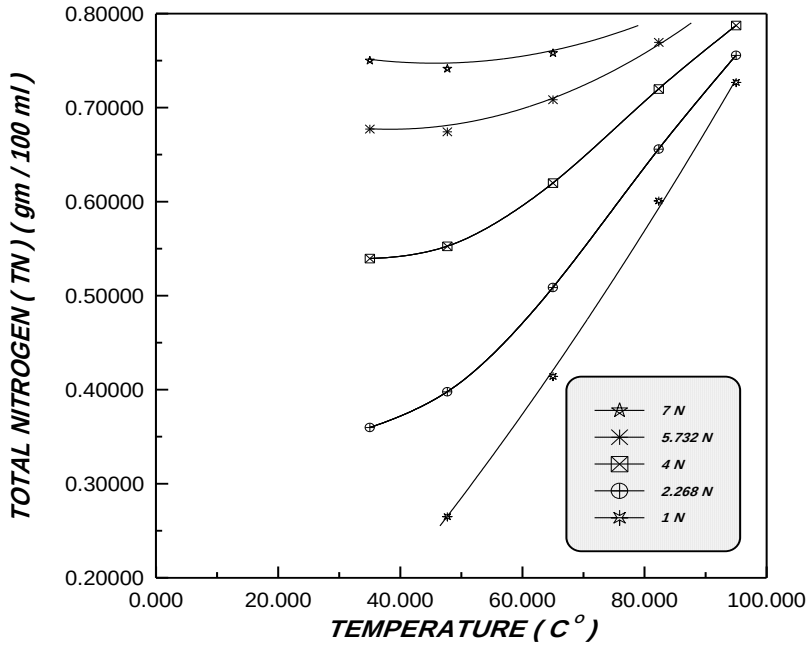

Fig.11. TN Concentration of Partially Purified Hydrolysized Product as a Function of Hydrolysis Temperature (C°) at 12.25 hr.

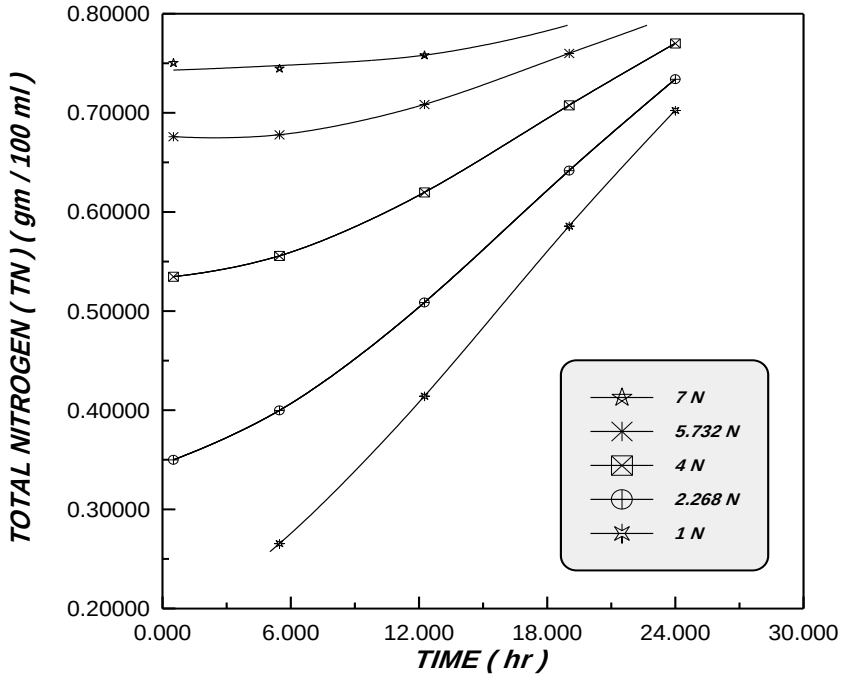

Fig.12. TN Concentration of Partially Purified Hydrolysized Product as a Function of Hydrolysis time (hr) at 65 C°.

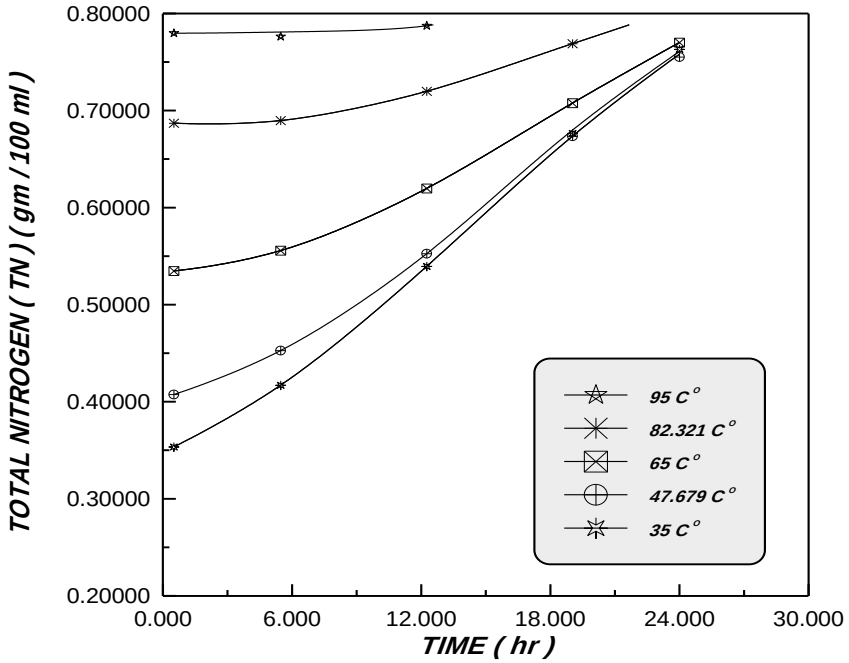

Fig.13. TN Concentration of Partially Purified Hydrolysized Product as a Function of Hydrolysis time (hr) at 4N.

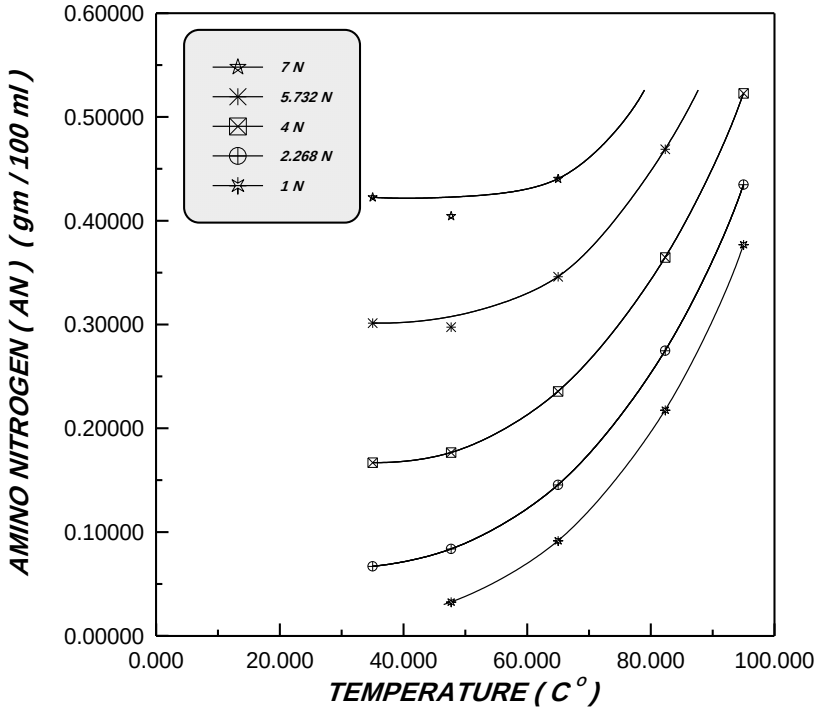

Fig.14. AN Concentration of Partially Purified Hydrolysized Product as a Function of Hydrolysis Temperature (C°) at 12.25 hr.

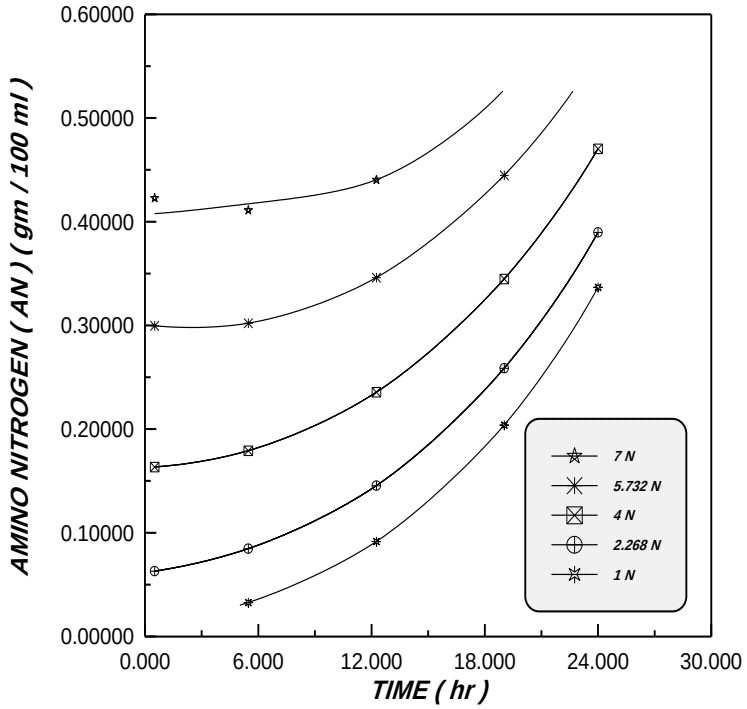

Fig.15. AN Concentration of Partially Purified Hydrolysized Product as a Function of Hydrolysis Time (hr) at 65 C°.

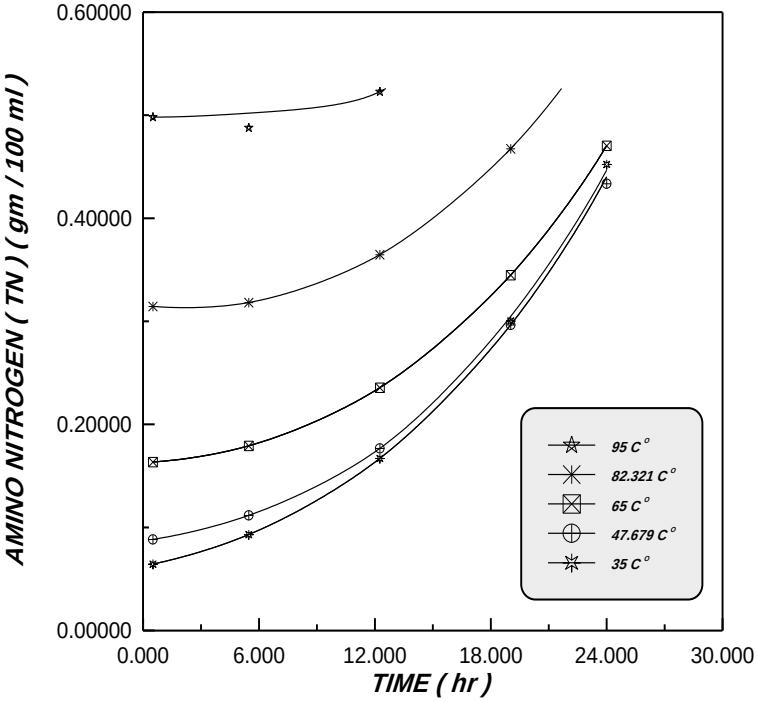

Fig.16. AN Concentration of Partially Purified Hydrolysized Product as a Function of Hydrolysis Time (hr) at 4N.

The following Table (7) clarifies the effect of concentration of each TN, AN and AN/TN% of the hydrolysized product before and after the process of partial purifying which has been carried out with the protein hydrolysis degree.

Table 7,
Comparison Between TN, AN and AN/TN% of the Hydrolysized Product Before and After the Process of Partial Purifying.

Hydrolysis Degree of Soya Protein	The Hydrolysized Product Before the Process of Partial Purifying			The Hydrolysized Product After the Process of Partial Purifying		
	TN gm/100ml	AN gm/100ml	AN/TN%	TN gm/100ml	AN gm/100ml	AN/TN%
0	0.57732	0.01678	-	-	-	-
8.629	0.62776	0.10613	17.584	0.25713	0.03008	14.372
76.673	1.02546	0.81068	78.557	0.78842	0.52578	68.134
100	1.1618	1.05221	-	-	-	-

5.1 Limiting the Appropriate Degree of Soya Protein Hydrolysis

The compositions of soya peptone product by Oxoid company have been limited with regard to TN, AN and AN/TN%. Accordingly, the characteristic of the soya peptone produced by this company at the years 1988 and 1995 (as shown in Table (8)) were depended as maximum and minimum ranges of the soya peptone produced by acidic hydrolysis of soya protein in the present study.

Table 8,
A Comparison between the Characteristic of Soya Peptone Analysis Produced by Oxoid Company for 1995 and 1988.

Analysis	Soya Peptone Analysis of 1995 (w/w%)	Soya Peptone Analysis of 1988 (w/w%)
TN	9.1	10.2
AN	2.3	3.1
AN/TN%	25	30

Now, the soya peptone analysis in Table (8) converted, from weight percentage formula (w/w%) to (gm/100ml) formula, that has been adopted in the laboratory for this study, Table (9) clarifies this operation.

Table 9,
Characteristics of the Soya Peptone Produced by Oxoid Company for 1995 and 1988 in gm/100ml.

Analysis	Soya Peptone for 1995		Soya Peptone for 1988	
	(w/w%)	gm/100ml	(w/w%)	gm/100ml
TN	9.1	0.47177	10.2	0.52879
AN	2.3	0.11924	3.1	0.16071
AN/TN%	25	25	30	30

The values in Table (9) were used as a characteristics range of the soya peptone produced by the acidic hydrolysis of the soya protein which studied in the present study.

The extent range of the characteristics of the soya peptone with regard to TN, AN, AN/TN% which shown in Table (9) are plotted in Figures (8),(9) and (10), the extent within which the degree of protein hydrolysis DH% ranges is limited, is shown in Table (10).

Table 10,
Soya Protein Hydrolysis Degree Limited by Using the Acid.

Analysis	Hydrolysis Degree	
	1995	1988
TN (gm/100ml)	27.787	33.818
AN (gm/100ml)	27.226	34.053
AN/TN %	27.365	34.757

Hence, it can be concluded that the best extent within which the soya protein hydrolysis degree values (provided that the product is a soya peptone that have characteristics ranging between the characteristics of the soya peptone produced by Oxoid company for the year 1988 and 1995) ranges between 27% and 34%.

5.2 Optimum Conditions for Soya Peptone Production

By using a computer program at a BASIC language , has been formulated, to find out the optimum conditions for producing the soya peptone This program gives the optimum extent for the time of soya protein hydrolysis at degree ranges between 27% and 34% by using Equation (2) (concluded from the pervious study[13]), as shown below, which shows the effect of the variables of the process (the concentration of the acid (N), temperature (°C) and the time of hydrolysis (hr)) on the soya protein hydrolysis degree .

$$DH\% = 44.80638 + 13.33998\ X_1 + 11.94455\ X_2 + 10.64997\ X_3 + 0.1561251\ X_1^2 + 3.631374 X_2^2 + 2.702626\ X_3^2 - 2.655856\ X_1X_2 - 2.8462\ X_1X_3 - 2.66819\ X_2X_3 \quad \ldots(2)$$

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

To determine the optimum conditions for the process of hydrolysis, 1N of HCl has been chosen in this process for the following reasons:

1. Decreasing the intensity of the occurrence of side-reactions.
2. Decreasing the amount of salt NaCl.
3. Decreasing the opportunities of destruction of protein and then its loss of the biological characteristics.

With regard to temperature, the best extent within which the optimum temperature of the process of the soya protein hydrolysis range is from 50 to 53 °C has been chosen for the following reasons:

1. Decreasing the intensity of the occurrence of side-reactions.
2. Decreasing the opportunity increasing the effectiveness of the acid.
3. Preventing changes in the nature of the protein itself at low temperatures.

After performing this computer program, the optimum time necessary for soya protein hydrolysis was obtained : from 17.189 to 19.97hr when dealing with the following conditions of hydrolysis: 1N of HCl at 50 to 53 °C, to reach a hydrolysis degree ranges from 27% to 34% for the soya protein.

6. Conclusions

- The process of hydrolysizing the soya protein by using the papain enzyme is not economic when produced locally. For this, enzyme is extracted from a special fruit found only in equatorial places.
- Whenever the protein hydrolysis degree increase, TN and AN of the hydrolysized product of centrifuging come closer to TN of this product before centrifuging and to TN of the soya protein before the acidic treatment. This is due to the low concentrations of the separated proteinous materials during the processes of filtering and centrifuging.
- According to the characteristic of soya peptone produced by Oxoid company 1988 and 1995,the appropriate hydrolysis degree is 27-34%.
- The optimum conditions necessary to get this degree are:
  - ❖ Concentration of HCl: 1 N

- Temperature: 50-53 °C
- Time: 17.189-19.97 hr

- The productivity of soya peptone which prepared at the optimum conditions limited in the present work was 38.071%.

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

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7. References

[1] "The Oxoid Manual", United Kingdom, (1988).
[2] "Difco Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures", 9th ed., Detroit 1, Michigan, U.S.A., (1964).
[3] Bridson, E.Y., "The Oxoid Manual", 7th ed., United Kingdom,(1995).
[4] Brewster, R. Q., and W. E. Mcewen, "Organic Chemistry", 3rd ed., (1958).
[5] Weininger, S. J., Stermitz, "Organic Chemistry", International Edition, (1984).
[6] Friedman, M., and R. Liardon, J. Agric. Food Chem., 33, 666, (1985).
[7] Haurowitz, F., "Biochemistry", An Introductory Textbook, John Wiley & Sons, Inc., New York, (1955).
[8] Hendrickson, J. B., D. J. Cram, and G. S. Hammond, "Organic Chemistry", 3rd ed., International Student Edition, McGraw-Hill Kogakusha, Ltd., (1970).
[9] "BDH Chemicals Ltd", laboratory chemicals & biochemicals, Manual, England, (1984/1985).
[10] "Sigma Chemical Company", Manual, U.S.A, (1988).
[11] "The United States Pharmacopoeia (USP XX II)", January (1990).
[12] Hossain, N, Report, Ibn Al-Beitar Center (Ministry of Industrial and Minerals-Iraq), (1993).
[13] AL-Bahri, M. B. A. G, "Preparation of Soya Peptone and Potato Extract for Use in Production of Some Culture Media for Medical Laboratories", M.Sc. Thesis, University of Technology , (2001).
[14] Cochran, W. G., and G. M. Cox, "Experimental Design", John Wiley and Sons, Inc., New York, (1957).
[15] Montgomery, D. C., "Design and Analysis of Experiments", John Wily and Sons, New York, (1976).
[16] Perry's, R. H., and C. H. Chilton, "Chemical Engineers Handbook", McGraw-Hill, 5th ed., (1973).
[17] Whitaler, J. R., and P. E. Granum, Anal. Biochem., 109, 156, (1980).
[18] Apostolatos, G., J. Fd. Technol., 19, 639, (1984).
[19] "AOAC-Official Methods of Analysis", 12th ed., Association of Official Agricultural Chemists, Washington D. C. (1975).
[20] Lees, R., "Food Analysis: Analytical and Quality Control Methods for the Food Manufacturer, and Buyer", 3rd ed.,Leonard Hill Book, (1975).

الظروف المثلى لإنتاج ببتون الصويا بواسطة التميئ ألحامضي لبروتين الصويا

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الخلاصة

تم انجاز هذه الدراسة لأجل إيجاد الظروف المثلى الضرورية اللازمة لعملية تميئ بروتين الصويا باستخدام حامض الهيدروكلوريك (كعامل مساعد كيميائي) بدلا من الإنزيم البابين (كعامل مساعد حيوي) لإنتاج ببتون الصويا. هذه الظروف قد اختيرت باختبار تأثير متغيرات عملية التميئ على طبيعة وجودة المادة المنتجة.

تم دراسة إنتاج ببتون الصويا لأهميته في عملية تحضير وإنتاج الأوساط الزرعية المستخدمة في مختبرات التحليل الطبي و المايكروبايولوجي.

إن عملية إنتاج ببتون الصويا تتضمن أربع مراحل رئيسية :

- إعداد طحين الصويا مزال الدهن، أولا.
- عملية تميئ بروتين الصويا،ثانيا.
- تنقية الناتج، ثالثا.
- وأخيرا تجفيف الناتج النهائي.

بأتباع خطوات العمل الموضحة في الدراسة الحالية،أمكن التوصل إلى الظروف المثلى اللازمة لعملية تميئ بروتين الصويا، و ألمبينه في أدناه :

- أفضل تركيز لمحلول حامض الهيدروكلوريك أللازم لعملية تميئ بروتين الصويا هو 1 ع.
- أفضل درجة حرارة لعملية تميى بروتين الصويا تتراوح بين 50 –53 °م .
- أفضل فترة زمنية لتميئ بروتين الصويا تتراوح بين 17.189 – 19.97 ساعة.

إذ بلغت الإنتاجية لببتون الصويا بإتباع خطوات العمل و الظروف المثلى للإنتاج الموضحة في الدراسة الحالية 38.071 %.