Optimization of Production of Food Grade Gelatin from Bovine Hide Wastes

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Abstract

The optimum conditions for the production of food grade gelatin from the tannery bovine hide wastes are established. The process involves cutting the hide wastes into small pieces (1-2 cm^2), and washing them with water to remove the dirt. The washed pieces are placed in the liming tank containing 10% of lime in water with stirring for five weeks. After washing with water to remove the lime, the collagen is neutralized to pH 7 with hydrochloric acid. The extraction is carried out in four stages using hot water. The gelatin extracts are filtered, subjected to deionization and concentrated with vacuum evaporator up to 20-35 wt%. The gelatin is then dried by two types of dryers (tray dryer and spray dryer).

Box-Wilson method is adopted to obtain a relationship between the three variables (temperature, time and pH) and gelatin yield in the first stage of extraction and two variables (temperature and time) and the gelatin yield in the other third stages of extraction process. The experimental data were fitted to second order polynomial models for all stages.

The most favorable operating conditions for the extraction of gelatin are: -

The First Stage: Temperature = 64 ^oC , Time = 5 hr , pH = 7

The Second Stage: Temperature = 74 ^oC , Time = 3.72 hr , pH = 7

The third Stage: Temperature = 84 ^oC , Time = 3.69 hr , pH = 7

The fourth Stage: Temperature = 98 ^oC , Time = 3.83 hr , pH = 7

The overall yield of gelatin is 55%, under the previous condition. The quality of gelatin was checked against food grade specification at the Nutrition Research Institute of the Ministry of Health. The results are acceptable within the boundaries of the desired properties.

الخلاصة

يتناول البحث تحديد الظروف المثلى لإنتاج الجيلاتين بمواصفات غذائية من مخلفات جلود الأبقار التي تعتبر من النواتج العرضية للمدابغ. تمت عملية الإنتاج بتقطيع مخلفات الجلود بين (١-٢) سم^{٢}، ثم غسلت مخلفات القطع الجلدية بالماء ونقلت الى حوض المعالجة بمحلول النورة في الماء بتركيز (١٠ %). استمرت لمدة خمسة أسابيع مع التحريك المستمر. غسلت بعدها القطع بالماء لإزالة ما تبقى من النورة وتمت تعديل الدالة الحامضية الى حدود ٧ باستخدام حامض الهيدروكلوريك. أجريت أربع مراحل استخلاص عند الظروف المثلى لاستخلاص الجيلاتين من الكولاجين. رشح محلول الجيلاتين الناتج و أزيلت منه الأيونات ومن ثم ركز لما بين ٢٠ – ٣٥%وزنا باستخدام مبخر يعمل بالضغط المتخلخل. جفف المحلول باستخدام نوعين من اجهزة التجفيف، هما المجفف ذو الأطباق والمجفف المرذاذي الدوار.

استخدمت طريقة (Box-Wilson) لإيجاد علاقات رياضية تربط المتغيرات الثلاثة (درجة الحرارة و الزمن والدالة الحامضية) مع إنتاجية الجيلاتين في المرحلة الأولى من عملية الاستخلاص، وبين المتغيرين (درجة الحرارة والزمن) مع إنتاجية الجيلاتين في المراحل الثلاثة الأخرى من هذه العملية. تمت مطابقة النتائج العملية التي أمكن الحصول عليها بهذه الطريقة مع معادلات رياضية من الدرجة الثانية ولجميع المراحل. قمنا بدراسة ظروف التشغيل لمراحل الاستخلاص الأربعة بالتفصيل وذلك عن طريق أيجاد معادلة رياضية لكل مرحلة. وجد أن افضل ظروف تشغيلية للاستخلاص كما يأتي:

المرحلة الاولى: درجة الحرارة = ٦٣,٤٨ °م ، الزمن = ٥،٠٠ ساعة ، الدالة الحامضية = ٧،٠٢
المرحلة الثانية: درجة الحرارة = ٧٤،١١ °م ، الزمن = ٣،٧٢ ساعة ، الدالة الحامضية = ٧،٠٢
المرحلة الثالثة: درجة الحرارة = ٨٣،٨٦ °م ، الزمن = ٣،٦٩ ساعة ، الدالة الحامضية = ٧،٠٢
المرحلة الرابعة: درجة الحرارة = ٩٨،١٤ °م ، الزمن = ٣،٨٣ ساعة ، الدالة الحامضية = ٧،٠٢

بلغت إنتاجية الجيلاتين (٥٥ %) في الظروف التشغيلية المذكورة أعلاه.

Introduction

Gelatin is a purified protein derived from the selective partial hydrolysis of collagen. It is the major intracellular protein constituents of white tissue of cattle. Hides, bones, pigskins and fish skins are the principal commercial sources. A large production of the collagenous raw materials is obtained from hides and skin as by- product of tannery operations. It is a heterogeneous mixture of water - soluble protein of high average of molecular weights that is capable of forming a firm gel in an aqueous medium [1, 2].

Gelatin is nearly tasteless, odorless, colorless or slightly yellow, transparent, brittle, in sheets, flakes, or powder form, soluble in hot water, glycerol and acetic acid, and insoluble in organic solvents. Gelatin swells and absorbs 5-10 times its weight of water to form a gel aqueous solution between 30-35 ºC. At normal temperature and humidity it contains 9-12 % moisture. Gelatin is extremely heterogeneous and composed of polypeptides of many sizes. It is Depending on the material source, gelatin is either type-A or type-B. Type-A gelatin is produced from an acid process. It is mainly applied to pigskin, in which collagen molecule is young. Type-B gelatin is formed from an alkaline and acid process. It is mainly applied to cattle skin and bone, in which the triple-helix collagen molecule is odder, more densely cross - linked and complex. In both processes the raw material is first cut to a manageable size, then washed to cleanse and degrease. It then undergoes either type-A or type-B processes for further degreasing and demineralizing. In the extraction process, the pure collagen is mixed with clean warm water. By careful thermal control the gelatin is gradually and gently released in the form of liquor. In the following processes extraction, filtration, purification, concentration and drying [4, 5].

The physical properties of gelatin are influenced more by extraction conditions than by amino acid composition [6]. The advances in knowledge of the structure and properties of gelatin have contributed to some progress in understanding and improving the conversion of collagen to gelatin without loss of useful properties [7, 8].

Gelatin is used in the production of foods, pharmaceutical preparations and photographic products depending primarily on its gel forming ability. The ease of the reversible transition from gel to solution, viscosity of its water solution, and its effectiveness as

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classified as derived protein because it is obtained from collagen by hydrolytic action [3].

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protective colloid, have made gelatin to be used as a jelling agent stabilizer, emulsifier, thickener, forming agent, water binder, crystal growth modifier, adhesive binder and fining agent. Modern technological applications of gelatin depend on its high solubility in hot water, polyampholyte character, availability in a wide range of viscosities and thermally reversible gel formation [9, 10].

The aim of the present work is the preparation of gelatin from the tannery by-product (bovine hide wastes) by extraction of collagen with water in four stages. Two types of dryers are used.

Experimental

Materials and Chemicals:

Hide wastes were supplied from a local tannery as a by– product. The composition of the hides is shown in Table 1. They were washed and cut into small pieces about 1 to 2 cm^2. Lime was of industrial grade (purity of 99%). Hydrochloric acid, was of General Purpose reagent (32%) supplied from Hopkin and Williams.

Extraction Unit:

The extraction was carried out in a three – neck, 3-L Pyrex glass flask to which a condenser (vertical position), a thermometer and a mechanical stirrer were fitted. The flask was placed in a water bath (Memmert WB22 -

Germany). The overall set up of the The same set up was used for the evaporation of the extracts (Haake W19) and aided by vacuum (Fig. 2).

Procedure:

The hide-waste pieces were soaked in calcium hydroxide solution (10%) which stabilizes the pH at (12.5) with slow stirring (250 rpm) at 15-20 ^{o}C. The hide pieces were then washed thoroughly with water to remove the lime until the water became clear. The collagen obtained was immersed in distilled water twice its weight in the extraction flask. Extraction was performed in four stages. The time and temperature ranges of the extraction are specified in Table 2. The pH of the solution was maintained for the four stages at a value of 5-7.5 by adding hydrochloric acid. After each stage, the liquor was drawn off, and a new amount of water was added.

The combined gelatin extracts were filtered under partial vacuum (0.5 bar) over diatomaceous earth as a suitable filter aid. The filtrate was purified by passing through columns packed with cation and anion exchange resins. The purification conditions are given in Table 3. The purified liquor was concentrated by evaporation in a water bath kept at constant temperature.

Gelatin Drying was carried out by two methods: Tray Dryer and Spray Dryer. With tray drying, the concentrated liquor (25-35 wt%) was chilled in a refrigerated chamber at 4 ^{o}C for 15 h. It set to a firm gel as thin (2-4 mm) sheet. The chilled gel was extraction unit is shows in Fig. 1.

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placed in a tray and heated at about 60 ^{o}C for (20) hrs. The material left the dryer with moisture content of 10%. The gelatin was broken into small pieces.

With spray drying, the concentrated gelatin solution was pumped at (3 bar) to a spinning disk atomizer system in which the solution to be dried is carefully fed onto a disk that spins at (15000 rpm). The atomized solution was then dried by flowing hot air at (75 ^{o}C) in the drying chamber. The dry gelatin was removed from the drying chamber in a stainless steel container at the bottom of the chamber. The final dry gelatin temperature was (55-60 ^{o}C).

Methods of Analysis:

The essential properties of the gelatin yield were determined by following some reference procedures. Moisture and ash contents were determined in accordance with a reference method [11]. The concentration of gelatin was measured by ultraviolet absorption spectrophotometric method at 280 nm as follows:

A calibration graph was prepared by measuring the absorbance of a series of standard solutions (0.1 % to 1% gelatin). The gelatin concentration in the extracts was determined by the equation of the standard curve.

[Gelatin] (mg / m1) = (0.0093 *Absorbance – 0.0005) * 1000 …………..(1)

However, some reference tests were performed on the gelatin according to the Food Chemical Codex requirements to determine the percentage of ash, lead, sulfur dioxide and microbial limit [12]. The tests were carried out at the Nutrition Research Institute, of the Ministry of Health, Baghdad.

Results And Discussion

Liming:

Alkali processing by using lime saturated solution has some advantages, which explain its general use. The solubility controls the total degree of alkalinity available of the raw material being cured; this solubility decreases with rise in temperature, thus providing an automatic control over processing operation, which took few weeks. Liming temperature did not exceed (20 ºC). The slurry was changed every few days to prevent the fogging of the gelatin emulsion. The slow stirring, however, avoids excessive loss of collagenous material.

Various impurities (e.g., elastin, mucins, mucopolysacchrides and albumin) are removed during liming. Ammonia is evolved during liming and this is probably due to hydrolysis of terminal amide group in the collagen [1, 3, 13].

According to Veis and Cohen [14], liming brings about a decrease in cohesion of the collagen fibers, together with a reorientation of the fibers. Inter-molecular bridges are

broken. These may be hydrogen bond or salt bridges between acid and basic side chains. The swelling, which occurs during liming probably, causes disruption of inter-chain in links of the hydrogen bond type [15].

Extraction Process:

The extraction of collagen with hot water causes denaturation (disruption of the helical conformation), additional hydrolysis and solubilization of the gelatin. Usually, the gelatin is extracted in stages of increasing time and temperature [5]. The high temperature is needed to exceed the shrinkage temperature of the collagen but not to damage the protein extensively [16].

Although temperatures as low as 40-45 ºC were employed by Zhang [17], it was gradually increased in succeeding stages until the last extraction occurs at the boiling point [18]. Meanwhile, the extraction had to be carried out at relatively low pH values to facilitate the conversion into gelatin by hot water [7, 8, 19, 20].

Analysis of Experimental Results:

The response of experimental work conducted according to Box-Wilson [21], is represented by the gelatin yield (Y):

(Y% = (gelatin produced / collagen input) * 100%).

It is fitted by a second–order polynomial mathematical model for each extraction stage. A second order

polynomial equation is employed in the range of the independent variables. For the first stage, three variables were considered. The general form of a second order polynomial is given in equation (2):

$$Y= B_{10}+B_{11}X_{11}+B_{12}X_{12}+B_{13}X_{13} +B_{14}X^2_{12}+B_{15}X^2_{12}+B_{16}X^2_{13}+ B_{17}X_{11}X_{12} + {}_{18}X_{11}X_{13} + B_{19}X_{12}X_{13} \ldots\ldots\ldots\ldots(2)$$

While for the other three stages, two variables, temperature and time, were used and the general form of second order polynomial is written as in equation (3):

$Y = B_i+B_{i1}X_{i1}+B_{i2}X_{i2}+ B_{i4}X^2_{i2}+B_{i5\ i2}X^2_{i1}+ B_i+B_{i1}X_{i1}+B_{i2}X_{i2}+B_{i2}X^2_{i1}+B_{i4}X^2_{i2}+B_{i5} X_{i1}X_{i2}$ (ith stage)……(3)

For postulating the best form of the models, the coded data are first fitted to equation (2), so that the regression analysis of central composite design can be applied to the approximation model to obtain the optimum conditions for the first stage. This procedure of calculation was repeated for the other three stages (at the optimum value of pH) found from first stage, and is the same in all stages with two variables; the coded data are fitted to equation (3).

The coefficients of equations 2 and 3 are determined by using available statistic software. The percentage average absolute error and square error of estimation (SEE) are estimated by applying the following relationships:

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% Average absolute error

$$= \frac{\sum \left| \frac{observed - predicted}{observed} \right|}{No.of\ data} *100 \ldots\ldots.(4)$$

$$SEE. = \sum_{i=1}^{i=n} (Y_{obs.}-Y_{pred.})^2 \ldots\ldots(5)$$

According to Table 4, the terms of interaction between the variables $(X_{11}X_{12})$, $(X_{11}X_{13})$ and $(X_{12}X_{13})$ are insignificant. Thus, the best form of the equation representing the first stage can be written as follows:

$$Y = 0.221037 + 0.020876\ X_{11} + 0.032713\ X_{12} + 0.017283X_{13} – 0.005789\ X^2_{11}\ 0.036133\ X^2_{12} - 0.031640\ X^2_{13}\ldots\ldots(6)$$

Similarly, the terms of interaction between the variables $(X_{i1}X_{i2})$ for the other three stages were found insignificant. Thus, the best form of equation (3) for second, third and fourth stages can be written as follows:

$$Y= 0.11660 + 0.00868\ X_{21} + 0.00563\ X_{22}+ 0.00507\ X^2_{21} + 0.0507\ X^2_{22} \ldots\ldots(7)$$

$$Y= 0.069185 + 0.018195\ X_{31} + 0.007524\ X_{32} - 0.008586\ X^2_{31} – 0.006192\ X^2_{32} \ldots(8)$$

$$Y= 0.048561 + 0.009304\ X_{41} + 0.000572\ X_{42} - 0.007139\ X^2_{41} - 0.005597\ X^2_{42} \ldots(9)$$

Table 5 shows the correlation coefficients, average absolute error and SEE for the extraction stages.

These equations represent the best forms of the mathematical model that relates the yield with the three variables for the first stage and with two variables for the other stages in terms of coded level.

Optimization and Effect of Operation Variables on Yield

From these mathematical models, graphical figures of gelatin yield versus each variable can be constructed for each stage within the variables range used in forming the models. These figures describe at any stage the effect of each variable on the gelatin yield at different values of other variables. This is done to show the interaction between the variables.

For first stage, the yield increased with increasing temperature until it reached 24.12% at 62.5 ^oC after 4.94 hrs. The yield then decreased to 23.15% with temperature increase to 65 ^oC. On the other hand, after an extraction time of

5.0 hrs, the yield increased with increasing temperature and maximum of 24.03% at 62.5 ^{o}C and pH of 6.97. Further increase of temperature caused a decrease in yield down to 23.58% at 65 ^{o}C and pH of 6.97, as confirmed by some published results [5, 16].

Thus, the first extraction stage should be operated at a temperature between 63-64 ^{o}C, and there is some gelatin to be extracted when the temperature increases from a starting temperature

of 55 ^{o}C to the optimum value. This temperature range does not hydrolyze the gelatin found in extraction water.

The effects of extraction time on the percentage of gelatin yield at various temperatures and pH values are typically shown in Figs. 3 and 4, respectively. At constant PH value of 7.0, the yield increased with time up to a maximum value of 24.1% after 5 hrs of extraction. Further time caused a decrease in the yield because of the expected hydrolysis of gelatin. The highest yield-time curve was obtained at 62.8 ^{o}C. Maximum yield occurred after 4.5 hrs at moderate PH values. However, relatively high (7.5) and low (5.0) pH values reduced the yield due to the expected hydrolysis by longer extraction. Meanwhile, at moderate pH values (5.0-7.5), gelatin was less sensitive for prolonged extraction time and the yield was almost constant for period of 3.5 hr.

Fig. 5 shows the effect of pH value on the gelatin yield at various temperatures. After 4.94 hrs of extraction, the yield increased from 12.45% at pH of 5 up to 24.16% at pH 7. The yield then decreased to 23.9% at pH 7.5, the temperature of all above 3 steps was 64 ^{o}C. Meanwhile, at the temperature of 63 ^{o}C the yield increased from 10.8% at pH 5 to 24.08% at a pH of 7, then it decreased to 23.78% at pH 7.5. Thus, extraction at a pH close to 7 gives more stable gelatin.

The results are confirmed by Gelatin Manufacturers Institute of America [20]. They give the optimum value of pH between 5 and 7.5 for the preparation of gelatin by thermal extraction of collagen. The above results show that the prolonged cooking at the same temperature produced no further extractable gelatin but considerable hydrolysis occurred [5].

Optimizatioof Operating Variables:

The optimization procedure was applied to equation (6) to find the optimum operating conditions (temperature, time and pH) by:

a- differentiating equation (6) for three times, once with respect to X_{11} (temperature), X_{12} (time) and X_{13} (pH);

b- setting the resulting equations to zero;

c- solving these equations simultaneously to find the optimum values of variables (temperature, time and pH);

d- conducting a second differentiation to test for the sufficient conditions to ascertain that the optimum point is indeed a maximum point.

The results of optimization indicate that the optimum conditions are:

X_{11} = temperature = 64 ^{o}C;
X_{12} = time = 5 hr;
X_{13} = pH = 7.02

The maximum yield was equal to (24.28%)
The pH value is taken as constant (7.0) for the other three stages.
Similarly for the other stages the optimum conditions were estimated on basis of the experimental results to be:

Second stage:
X_{21} = Temperature = 74 ^oC;
X_{12} = Time = 3.72 hrs;
Maximum yield = 16.22%.
Third Stage:
X_{31} = Temperature = 84 ^oC;
X_{32} = Time = 3.69 hrs;
Maximum yield = 8.65%.
Fourth Stage:
X_{41} = Temperature = 98 ^oC;
X_{42} = Time = 3.83 hr;
Maximum yield = 5.85%.

Results of Optimum Experiments:

Following the evaluation of the optimum condition of the four stages, an experiment was carried out to produce gelatin under such conditions. The purpose was to show the applicability of the experimental conditions in giving gelatin of acceptable properties and acceptable yield. A run on 1500 gm of cattle hide wastes gave 338.4 gm collagen which was hydrolyzed, purified, concentrated and finally dried to yield 204.7 gm of gelatin (overall yield = 13.6%). This gelatin is characterized by a moisture content of 10% which agrees well with value for gelatin. The other analytical results are listed in Table 6.

Conclusions:

Gelatin can be successfully extracted from bovine hide wastes with hot water after a few days liming process. The optimum conditions of gelatin extraction process from bovine hide wastes are:

First Stage: Temperature = 64^{o}C,
Time = 5 hr, pH = 7

Second Stage: Temperature = 74^{o}C;
Time = 3.72 hr; pH = 7

Third Stage: Temperature = 84 ^oC;
Time = 3.69 hr; pH = 7

Fourth Stage: Temperature = 98.14 ^oC;
Time = 3.83 hr, pH = 7

The reduction of the size of the hide pieces and thorough soaking are important to increase the effective surface area between hide and lime solution and clean it. The temperature of the liming process is an important factor and should not exceed 20^o C, otherwise the resultant gelatin (which is sensitive to emulsions) tends to fog.
The partial hydrolysis of the cured collagen to give gelatin is a relatively slow reaction so that the extraction of the gelatin goes as quickly as it is formed. Thus, extraction must be performed by a series of extraction A larger scale experiment was run to extract gelatin from 1500 gm of cattle hide wastes. This amount gave 338.4 gm collagen which was hydrolyzed, purified, concentrated and finally dried to yield 204.7 gm of gelatin. This gelatin is characterized by a moisture content of 10% which agrees well with the reference value for gelatin.

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Table 1: Overall Composition Of Calfskin

Skin Constituents	**%**
Grease	1.0-10
Water	60-65
Protein	20-25
Carbohydrate	1.0
Mucopoly saccharide	0.5-1.0
Protein Constituents	**%**
Collagen	90-95
Globular Protein	4.0-6.0
Epidermis	0.5-1.0
Elastin	Small
Retiction	Small
Muscle	Very Small

Table 2: Extraction Conditions.

Stage	Time, h	Temperature Range, ^oC
First	1-6	55-65
Second	1-4	65-75
Third	1-4	75-85
Fourth	1-4	85-100

Table 3: Purification Conditions By Ion Exchange Resin.

Column		Resin	Condition			
Length cm	Diameter cm	Duolite	Enter pH	Leave pH	Temp. ^oC	Flow rate cm^3/ hr
80	3.0	A-20	4.78	4.0	60	1000
80	3.0	C-113	4.0	7.0	60	1000

Table 4: Analysis Of Variance For Orthogonal Variable For The First Stage,

Source of variation	Sum of squares	Degree of freedom	Mean square	Computed F	conclusion
X_{11}	60.872	1	60.872	5600	S
X_{12}	149.838	1	149.838	13784.045	S
X_{13}	41.7926	1	41.7926	3844.76	S
X^2_{11}	2218.598	1	2218.598	204102.81	S
X^2_{12}	1323.729	1	1323.729	121778.2	S
X^2_{13}	1442.2898	1	1442.2898	137685.35	S
$X_{11}X_{12}$	2.02607	1	0.0202607	2.976	NS
$X_{11}X_{13}$	0.0609005	1	0.0609005	4.6026	NS
$X_{12}X_{13}$	5.571122	1	0.0055712	0.522	NS
Error	0.1087	8	0.01087		

Table 5: Correlation Coefficients, Average Absolute Error And See For The Extraction Stages.

Stage No.	Correlation coefficient (R)	Average absolute error, %	$SEE = \sum_{i=1}^{i=n} (Y_{obs.}-Y_{pred.})^2$
First	0.988746	5.498	0.1087
second	0.984015	1.254	0.3158
Third	0.997393	0.285	0.2459
Fourth	0.977121	0.435	0.4848

Table 6: Chemical And Microbiological Analysis Of The Gelatin Produced At The Optimum Conditions.

Chemical Test	
Parameter	Test Value
Pb	1.2 ppm (N.V.not more than 1.5 ppm)
SO_2	Nil
Ash	0.6%(N.V.Max.3.0%)
Microbiological Test	
Parameter	Test Value
Total Bacterial Count	80 / gm
Colifroms	zero
Staphylococcus auras	zero
Moulds and Yeasts	zero

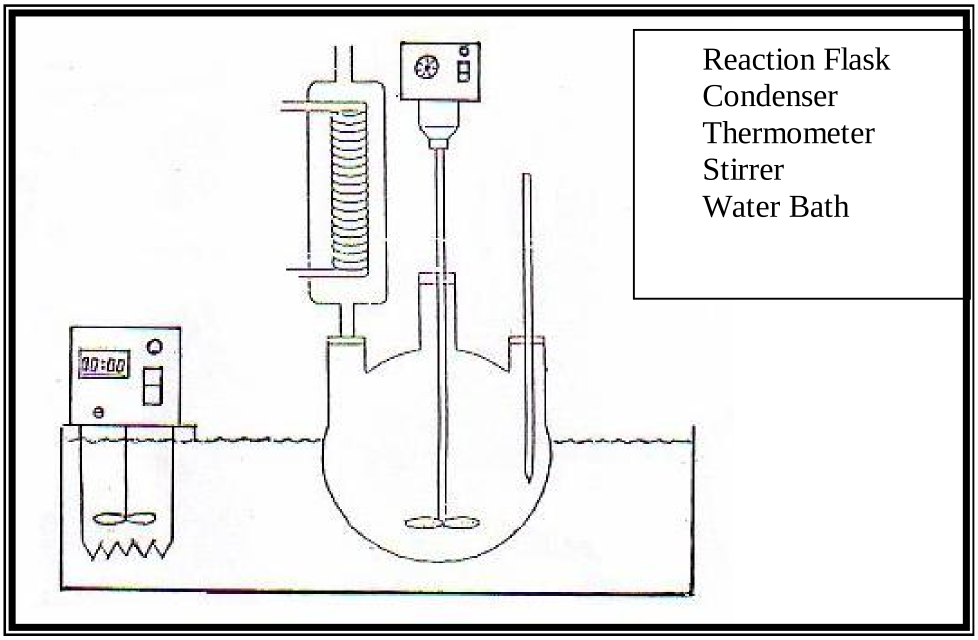

Fig.1: Schematic Diagram Of Extraction Unit.

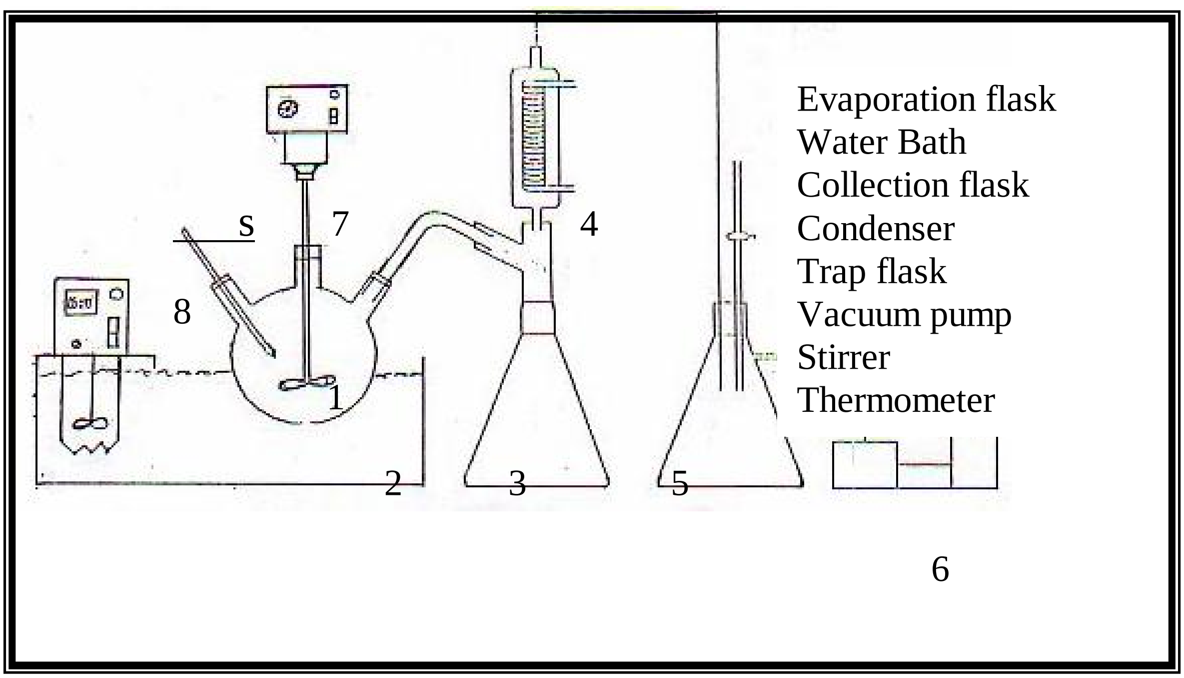

Fig.2: Schematic Diagram Of Evaporation Unit.

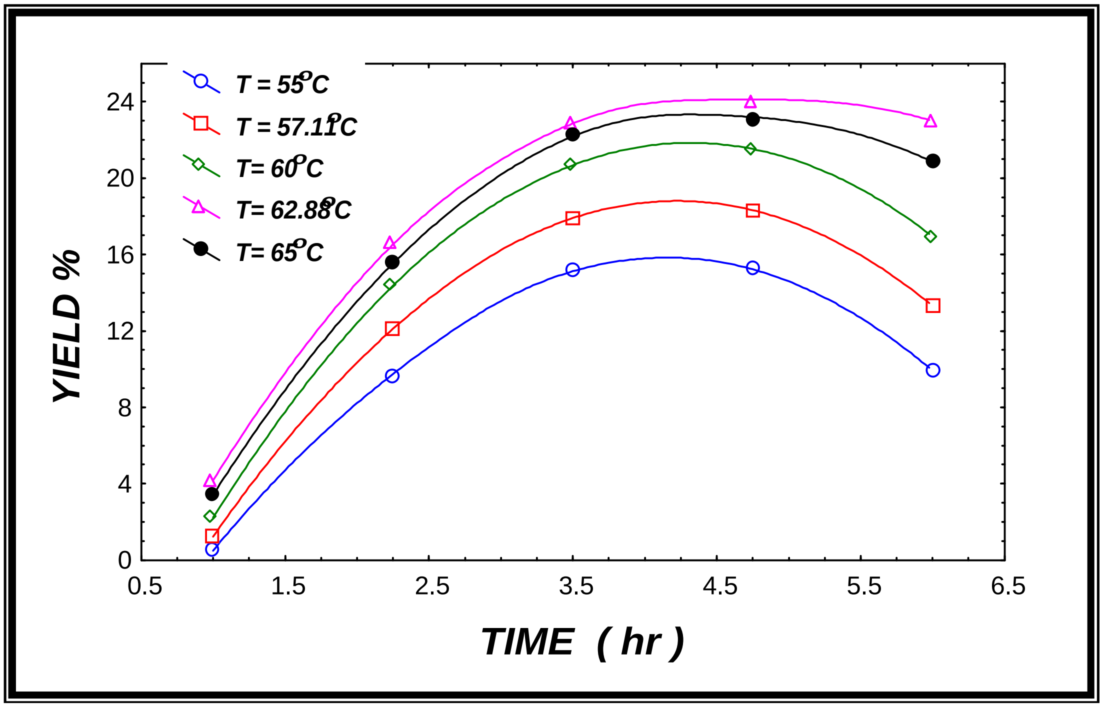

Fig. 3: Effect Of Time On The Yield Of Gelatin At Different Temperatures, And Ph =7.0

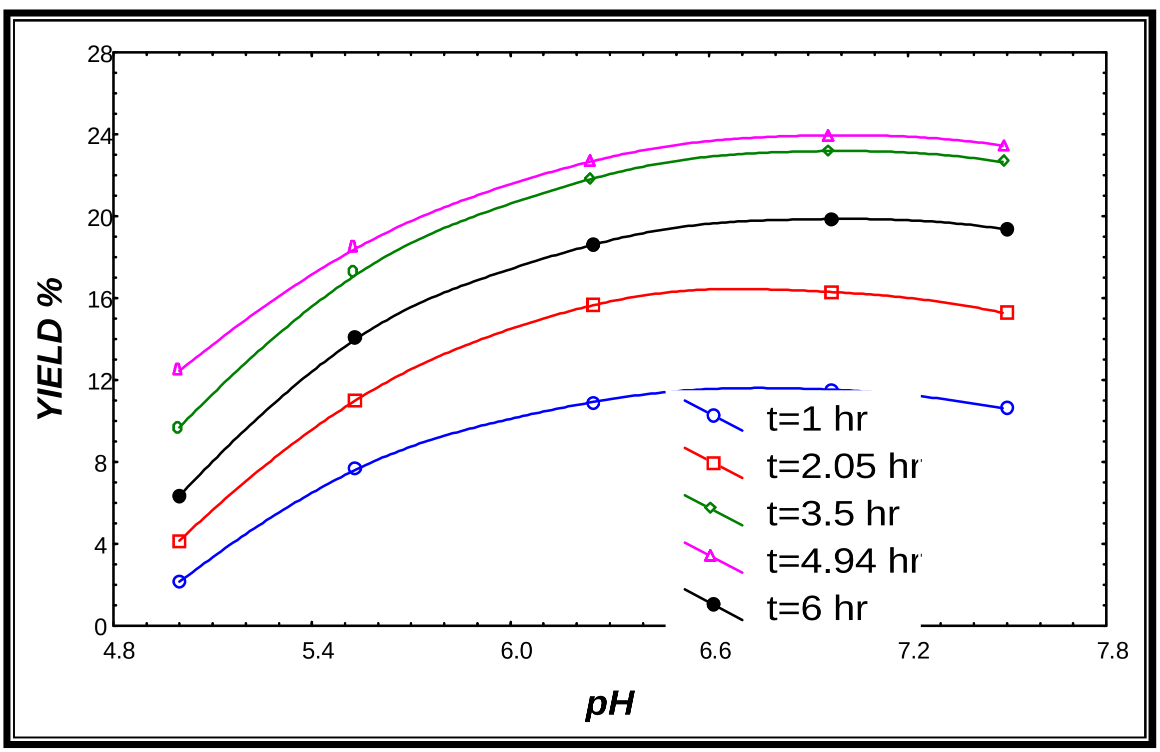

Fig.4: Effect Of Time On The Yield Of Gelatin At Different Ph, And At 64 ^{O}c

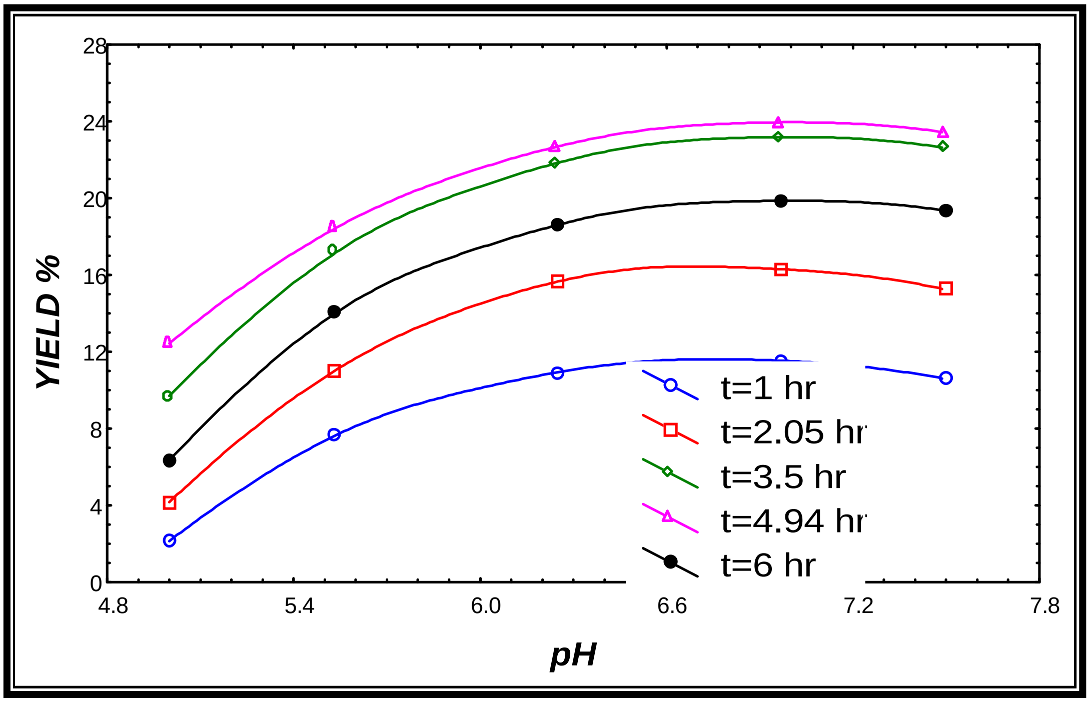

Fig. 5: Effect Of Ph On The Percentage Yield Of Gelatin At Different Times And At 64 ^{O}c.