

Optimization of Production of Food Grade Gelatin from Bovine Hide Wastes

Mahmood M. Barbooti  **Shahrazad R. Raouf**
Firas H. K. Al-Hamdani,

Received on: 5/12/2005

Accepted on: 20/5/2007

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 cm²), 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 °C , Time = 5 hr , pH = 7

The Second Stage: Temperature = 74 °C , Time = 3.72 hr , pH = 7

The third Stage: Temperature = 84 °C , Time = 3.69 hr , pH = 7

The fourth Stage: Temperature = 98 °C , 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 للمدايح. تمت عملية الإنتاج بنقطة 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 لمرحل الاستخلاص الأربعة بالتفصيل وذلك عن طريق إيجاد 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 °C. 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

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

Grade Gelatin From Bovine Hide Wastes

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 cm². 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 (Mettmert 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 °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 °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. Optimization Of Production Of Food Grade Gelatin From Bovine Hide Wastes placed in a tray and heated at about 60 °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 °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 °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.

$$[\text{Gelatin}] \text{ (mg / ml)} = (0.0093 * \text{Absorbance} - 0.0005) * 1000 \dots\dots\dots(1)$$

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 °C). 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 °C 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):

$$(Y\% = (\text{gelatin produced} / \text{collagen input}) * 100\%).$$

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):

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

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):

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

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:

Optimization Of Production Of Food Grade Gelatin From Bovine Hide Wastes

$$\% \text{ Average absolute error} = \frac{\sum_{i=1}^n \left| \frac{\text{observed} - \text{predicted}}{\text{observed}} \right|}{\text{No.of data}} * 100 \dots\dots(4)$$

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

According to Table 4, the terms of interaction between the variables (X₁₁X₁₂), (X₁₁X₁₃) and (X₁₂X₁₃) are insignificant. Thus, the best form of the equation representing the first stage can be written as follows:

$$Y = 0.221037 + 0.020876 X_{11} + 0.032713 X_{12} + 0.017283 X_{13} -$$

$$0.005789 X_{11}^2 - 0.036133 X_{12}^2 - 0.031640 X_{13}^2 \dots\dots\dots(6)$$

Similarly, the terms of interaction between the variables (X₁₁X₁₂) 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:

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

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

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

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.

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 °C after 4.94 hrs. The yield then decreased to 23.15% with temperature increase to 65 °C. 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 °C and pH of 6.97. Further increase of temperature caused a decrease in yield down to 23.58% at 65 °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 °C, and there is some gelatin to be extracted when the temperature increases from a starting temperature

Optimization Of Production Of Food
Grade Gelatin From Bovine Hide
Wastes

of 55 °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 °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 °C. Meanwhile, at the temperature of 63 °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].

Optimization of 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:

$$\begin{aligned} X_{11} &= \text{temperature} = 64 \text{ }^\circ\text{C}; \\ X_{12} &= \text{time} = 5 \text{ hr}; \\ X_{13} &= \text{pH} = 7.02 \end{aligned}$$

Optimization Of Production Of Food
Grade Gelatin From Bovine Hide
Wastes

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 °C;
 X_{12} = Time = 3.72 hrs;
Maximum yield = 16.22%.

Third Stage:

X_{31} = Temperature = 84 °C;
 X_{32} = Time = 3.69 hrs;
Maximum yield = 8.65%.

Fourth Stage:

X_{41} = Temperature = 98 °C;
 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 Fourth Stage: Temperature = 98.14 °C;
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° 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

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° C,
Time = 5 hr, pH = 7

Second Stage: Temperature = 74° C;
Time = 3.72 hr; pH = 7

Third Stage: Temperature = 84 °C;
Time = 3.69 hr; pH = 7

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.

References

- 1- Hermen. F. and Norbest, M. ,
"Gelatin", Encyclopedia of
Polymer Science and
Technology, vol. 7, (1985), p.
488-511.
- 2- Austin, G.T " Shereve's
Chemical Process Industries ",
McGraw Hill, 5th. Ed. (1984).
- 3- Kirk, R. E. and Othmer, D.
E., "Gelatin, Encyclopedia of
Chemical Technology", Vol.
10, (1966), p.499-507.
- 4- Gelatin Manufactures Association
of Asia Pacific, " How is Gelatin
Made", GMAP, 2003.
- 5- Kirk, R. E. and Othmer, D. E.,
Food Ingredients ", New York:
Van Nostrand Reinhold, (1983).
- 6- Kirk, R. E. and Othmer,
D. E., "Gelatin", Encyclopedia
of Chemical Technology ", vol.
7, p. 145-153.
- 7- Egan, H, "Pearson
Chemical Analysis of Food",
Livingstone Churchill, N.Y.,
1981, p. 422.
- 8- "Food Chemical Codex "
4th Ed., Washington, DC,
National Academy Press,
1996,
([www.nap.edu/readingroom/b
ook/ fcc](http://www.nap.edu/readingroom/book/fcc))), [Internet 2003].
- 9- William, M., "The
Manufacture of Hide Glue and
Gelatin", J. Soc. Leather
Trades Chemists, vol. XXXIII.
No. 11, 1949, p. 407-421.
- 10- Veis , A. and Cohen , P.,
J. Amer. Chem. Soc., 76, 1954,
2476.
- 11- Gustawson, K. H. "The
Chemistry and Reactivity of
Collagen", Academic Press.,
Inc , New York ,1956.
- 12- USFAD, "Transmissible
Spongiform Encephalopathies
Advisory Committees",
Transcript of Meeting, April
23rd. 1997.
- 13- Gelatin Manufacture
Institute of America, (Gelatin
Information, News, History),
([http/www. gelatin-gmia.
Com.](http://www.gelatin-gmia.com))), [Internet 2003].
- 14- Armor, J., "Process for
Obtaining Gelatin", U.S Pat.
4176117, Nov. 27th 1970.
- 15- Hinterwarldner, R.,
"Technology of Gelatin
Manufacture", Edited by
Ward , A.G. and A. Courts,
1977.
- 16- Rowland, A. G. and
Burrows, D. J., "Enzyme
Method of Manufacturing
Gelatin", U.S. Pat. 6,100,381,
2000.
- 17- Davis. D.L., "Design and
Analysis of Industrial
Experiments", 2nd Ed., Oliver
and Boyd, London, 1963.
- 18- Herman, F. Norbest, M., "
Gelatin " "Encyclopedia of
Polymer Science and
Technology", vol. 7, 1976, p.
446-459.
- 19- Xuanyaliv, V.,
"Purification (Removal of
Metatic Ion), Decolorization
and Enhancement in
Transparency for Gelatin",
Canadian Pat. 1056512, Nov.,
27, 1992.

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, °C
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		Enter pH	Leave pH	Temp. °C	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
Coliforms	zero
Staphylococcus auras	zero
Moulds and Yeasts	zero

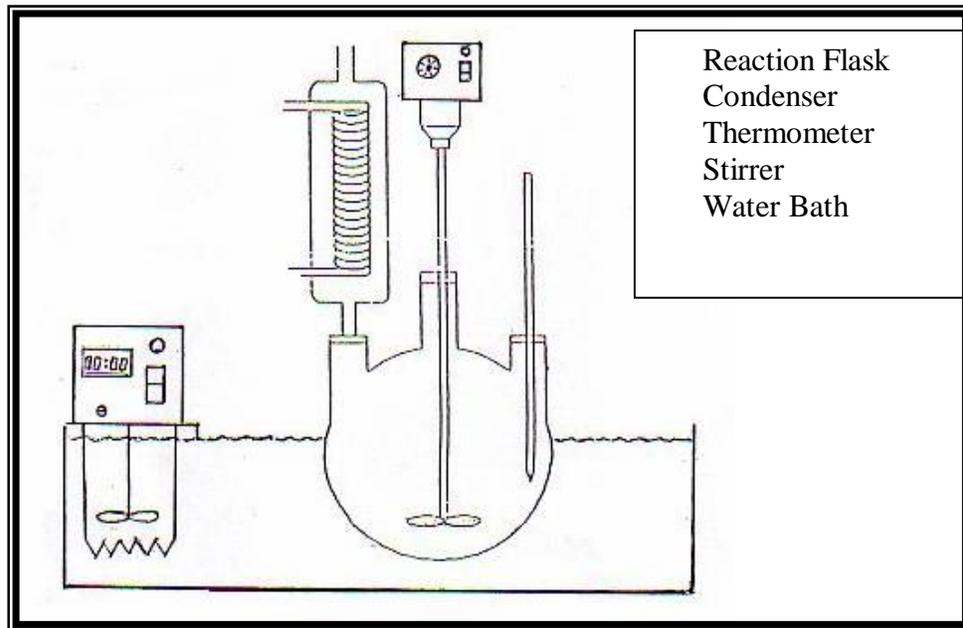


Fig.1: Schematic Diagram Of Extraction Unit.

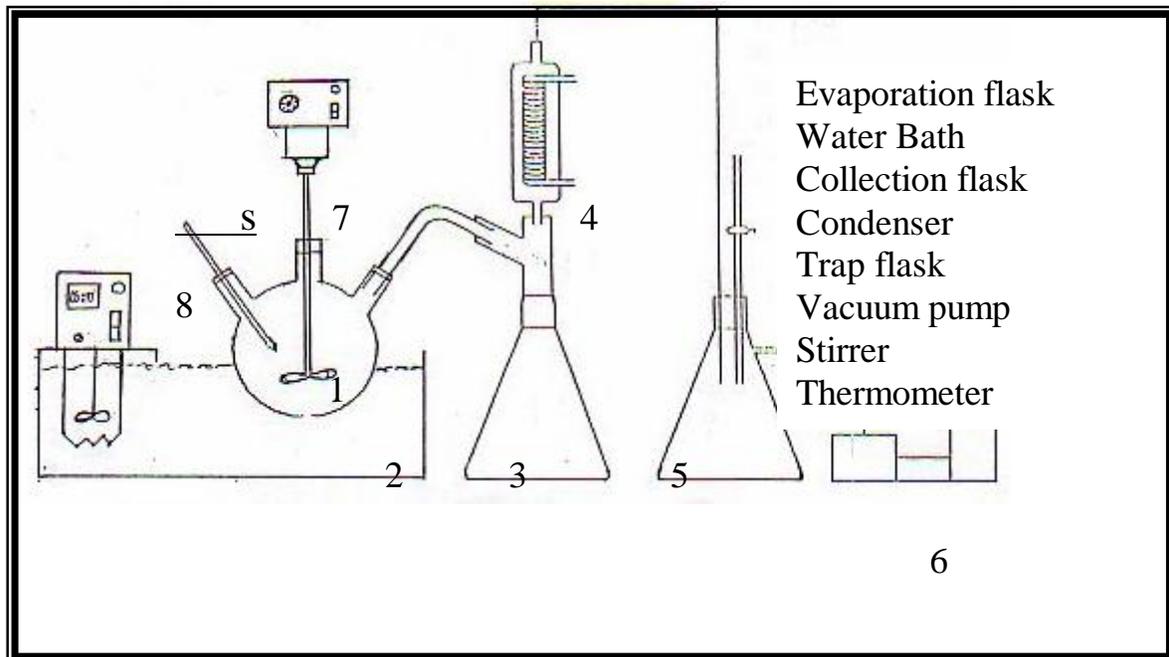


Fig.2: Schematic Diagram Of Evaporation Unit.

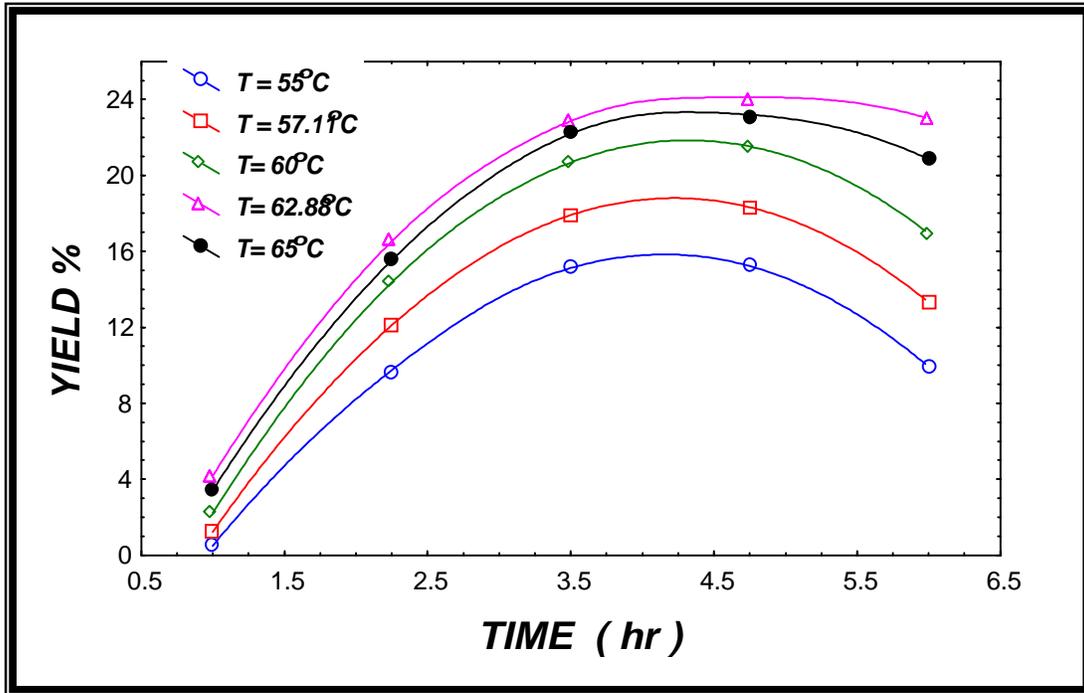


Fig. 3: Effect Of Time On The Yield Of Gelatin At Different Temperatures, And Ph =7.0

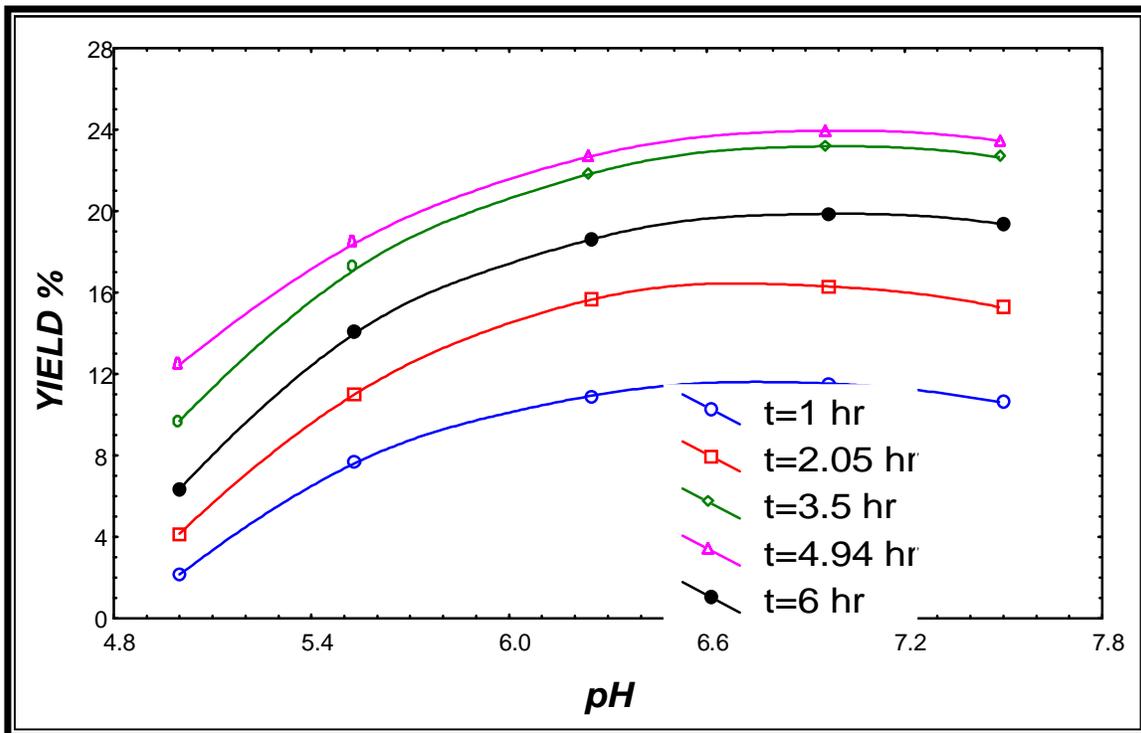


Fig.4: Effect Of Time On The Yield Of Gelatin At Different Ph, And At 64 °c

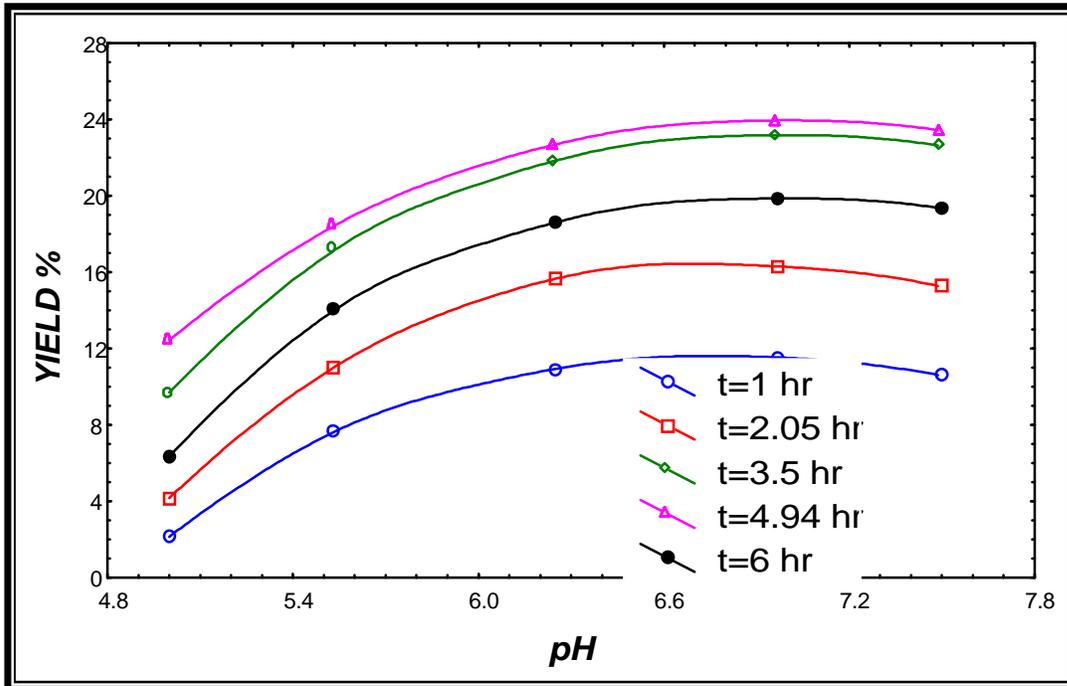


Fig. 5: Effect Of Ph On The Percentage Yield Of Gelatin At Different Times And At 64 °c.