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 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

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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.

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 collagenuous raw materials is obtained from hides and skin as by-product of tanner 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 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 (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 °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 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.

\[
\text{Gelatin (mg / ml)} = (0.0093 \times \text{Absorbance} - 0.0005) \times 1000
\]

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):

\[ Y\% = \frac{\text{gelatin produced}}{\text{collagen input}} \times 100\% \]

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):

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

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_i1X_{i1} + B_i2X_{i2} + B_i3X_{i1}^2 + B_i4X_{i2}^2 + B_i5X_{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:

\[
\% \text{ Average absolute error} = \frac{\sum |\text{observed} - \text{predicted}|}{\text{observed}} \times 100 \quad \text{(4)}
\]

\[
\text{SEE} = \sum_{i=1}^{n} (Y_{\text{obs}} - Y_{\text{pred}})^2 \quad \text{(5)}
\]

According to Table 4, the terms of interaction between the variables (X_{i1}X_{i2}), (X_{i1}X_{i3}) and (X_{i2}X_{i3}) are insignificant. Thus, the best form of the equation representing the first stage can be written as follows:

\[
Y = 0.221037 + 0.020876X_{i1} + 0.032713X_{i2} + 0.017283X_{i3} - 0.005789X_{i1}^2 - 0.036133X_{i2}^2 - 0.031640X_{i1}X_{i2} \quad \text{(6)}
\]

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:

\[
Y = 0.11660 + 0.00868X_{i1} + 0.00563X_{i2} + 0.00507X_{i3} - 0.006192X_{i1}^2 - 0.008586X_{i2}^2 - 0.006192X_{i1}X_{i2} \quad \text{(7)}
\]

\[
Y = 0.069185 + 0.018195X_{i1} + 0.007248X_{i2} - 0.007524X_{i3} - 0.006192X_{i1}^2 - 0.007139X_{i2}^2 - 0.005597X_{i1}X_{i2} \quad \text{(8)}
\]

\[
Y = 0.048561 + 0.009304X_{i1} + 0.000572X_{i2} - 0.007139X_{i3} - 0.005597X_{i1}^2 - 0.005597X_{i2}^2 \quad \text{(9)}
\]

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 °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 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:

$X_{11} = \text{temperature} = 64 \, ^\circ\text{C};$

$X_{12} = \text{time} = 5 \, \text{hr};$

$X_{13} = \text{pH} = 7.02$

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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:
\[ X_{21} = \text{Temperature} = 74^\circ C; \]
\[ X_{12} = \text{Time} = 3.72 \text{ hrs}; \]
Maximum yield = 16.22%.

Third Stage:
\[ X_{31} = \text{Temperature} = 84^\circ C; \]
\[ X_{32} = \text{Time} = 3.69 \text{ hrs}; \]
Maximum yield = 8.65%.

Fourth Stage:
\[ X_{41} = \text{Temperature} = 98^\circ C; \]
\[ X_{42} = \text{Time} = 3.83 \text{ hr}; \]
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 \degree 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\degree 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 (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\degree C,
Time = 5 hr, pH = 7

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

Third Stage: Temperature = 84 \degree C;
Time = 3.69 hr; pH = 7
References


Table 1: Overall Composition Of Calfskin

<table>
<thead>
<tr>
<th>Skin Constituents</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grease</td>
<td>1.0-10</td>
</tr>
<tr>
<td>Water</td>
<td>60-65</td>
</tr>
<tr>
<td>Protein</td>
<td>20-25</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>1.0</td>
</tr>
<tr>
<td>Mucopolysaccharide</td>
<td>0.5-1.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Protein Constituents</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen</td>
<td>90-95</td>
</tr>
<tr>
<td>Globular Protein</td>
<td>4.0-6.0</td>
</tr>
<tr>
<td>Epidermis</td>
<td>0.5-1.0</td>
</tr>
<tr>
<td>Elastin</td>
<td>Small</td>
</tr>
<tr>
<td>Retiction</td>
<td>Small</td>
</tr>
<tr>
<td>Muscle</td>
<td>Very Small</td>
</tr>
</tbody>
</table>

Table 2: Extraction Conditions.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Time, h</th>
<th>Temperature Range, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>1-6</td>
<td>55-65</td>
</tr>
<tr>
<td>Second</td>
<td>1-4</td>
<td>65-75</td>
</tr>
<tr>
<td>Third</td>
<td>1-4</td>
<td>75-85</td>
</tr>
<tr>
<td>Fourth</td>
<td>1-4</td>
<td>85-100</td>
</tr>
</tbody>
</table>

Table 3: Purification Conditions By Ion Exchange Resin.

<table>
<thead>
<tr>
<th>Column</th>
<th>Resin</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length cm</td>
<td>Diameter cm</td>
<td>Enter pH</td>
</tr>
<tr>
<td>80</td>
<td>3.0</td>
<td>A-20</td>
</tr>
<tr>
<td>80</td>
<td>3.0</td>
<td>C-113</td>
</tr>
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</table>
Table 4: Analysis Of Variance For Orthogonal Variable For The First Stage,

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Sum of squares</th>
<th>Degree of freedom</th>
<th>Mean square</th>
<th>Computed F</th>
<th>conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>X₁₁</td>
<td>60.872</td>
<td>1</td>
<td>60.872</td>
<td>5600</td>
<td>S</td>
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<tr>
<td>X₁₂</td>
<td>149.838</td>
<td>1</td>
<td>149.838</td>
<td>13784.045</td>
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</tr>
<tr>
<td>X₁₃</td>
<td>41.7926</td>
<td>1</td>
<td>41.7926</td>
<td>3844.76</td>
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</tr>
<tr>
<td>X²₁₁</td>
<td>2218.598</td>
<td>1</td>
<td>2218.598</td>
<td>204102.81</td>
<td>S</td>
</tr>
<tr>
<td>X²₁₂</td>
<td>1323.729</td>
<td>1</td>
<td>1323.729</td>
<td>121778.2</td>
<td>S</td>
</tr>
<tr>
<td>X²₁₃</td>
<td>1442.2898</td>
<td>1</td>
<td>1442.2898</td>
<td>137685.35</td>
<td>S</td>
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<tr>
<td>X₁₁X₁₂</td>
<td>2.02607</td>
<td>1</td>
<td>0.0202607</td>
<td>2.976</td>
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<tr>
<td>X₁₁X₁₃</td>
<td>0.0609005</td>
<td>1</td>
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<td>4.6026</td>
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<tr>
<td>X₁₂X₁₃</td>
<td>5.571122</td>
<td>1</td>
<td>0.0055712</td>
<td>0.522</td>
<td>NS</td>
</tr>
<tr>
<td>Error</td>
<td>0.1087</td>
<td>8</td>
<td>0.01087</td>
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<td></td>
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Table 5: Correlation Coefficients, Average Absolute Error And See For The Extraction Stages.

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

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

<table>
<thead>
<tr>
<th>Chemical Test</th>
<th>Parameter</th>
<th>Test Value</th>
</tr>
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<tbody>
<tr>
<td>Pb</td>
<td>Pb</td>
<td>1.2 ppm (N.V.not more than 1.5 ppm)</td>
</tr>
<tr>
<td>SO₂</td>
<td>SO₂</td>
<td>Nil</td>
</tr>
<tr>
<td>Ash</td>
<td>Ash</td>
<td>0.6%(N.V.Max.3.0%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Microbiological Test</th>
<th>Parameter</th>
<th>Test Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Bacterial Count</td>
<td>80 / gm</td>
<td></td>
</tr>
<tr>
<td>Colifroms</td>
<td>zero</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus auras</td>
<td>zero</td>
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<tr>
<td>Moulds and Yeasts</td>
<td>zero</td>
<td></td>
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</table>
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 oC
Fig. 5: Effect Of Ph On The Percentage Yield Of Gelatin At Different Times And At 64 °C.