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Optimization of Gelatine Synthesis from Lime Fleshed Hide Trim Solid Waste

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Article

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ABSTRACT

This research takes a "waste to wealth" approach, generating useful products from solid tannery waste. It focuses on the production of gelatine from solid waste calcareous hide trimmings, which pollute the environment due to their high levels of lime, sulphide, fat, and protein. The lime trim contains the majority of the collagen so gelatine can be extracted from the cuttings using alkalis such as potassium hydroxide. Gelatine was extracted from waste using an alkaline and hydrothermal treatment method with potassium hydroxide. The most important factors in extracting gelatine from waste were found to be time, concentration, and temperature, with values of (1, 2, 3) h, (0.5, 0.75, 1) M, and (60, 70, 80) °C, respectively. The optimum conditions were 70 °C, 2 hours, and 0.75 M, as determined by optimization using Design-Expert software, and the optimum protein content was 87.25% using the Kjeldahl method for protein content analysis. After extraction, the gelatine has a viscosity of 240 cp, a gel strength of 245 g, a pH of 7.2, and a moisture content of 12.11%. This research can be fully utilized to reduce the cost of waste disposal and waste containers in the solid waste of leather tanneries while possibly generating additional revenue.

KEYWORDS

gelatine, fleshed waste, alkaline extraction, protein characterisation

INTRODUCTION

An Overview of leather industries

Despite being one of the most important industries the leather industry produces a massive amount of solid, gaseous, and liquid waste, polluting the environment and a sensitive environment (Figure 1) [1,2]. Leather processing produces 70 to 230 kg of solid waste per tonne of salted hides, which includes lime, sulphides, fats, and proteins, all of which are harmful to the environment and are among the most significant solid wastes [3-10]. Although these solid wastes contain hazardous chemicals, they also contain collagen, a structural protein used in leather production that has numerous applications such as cosmetics, photographic films, and scaffolding, among others, with alkaline, acidic, and

enzymatic treatments [11-13]. Because lime trim contains the majority of the collagen, gelatine can be extracted from the cuttings using alkaline treatments [14,15].

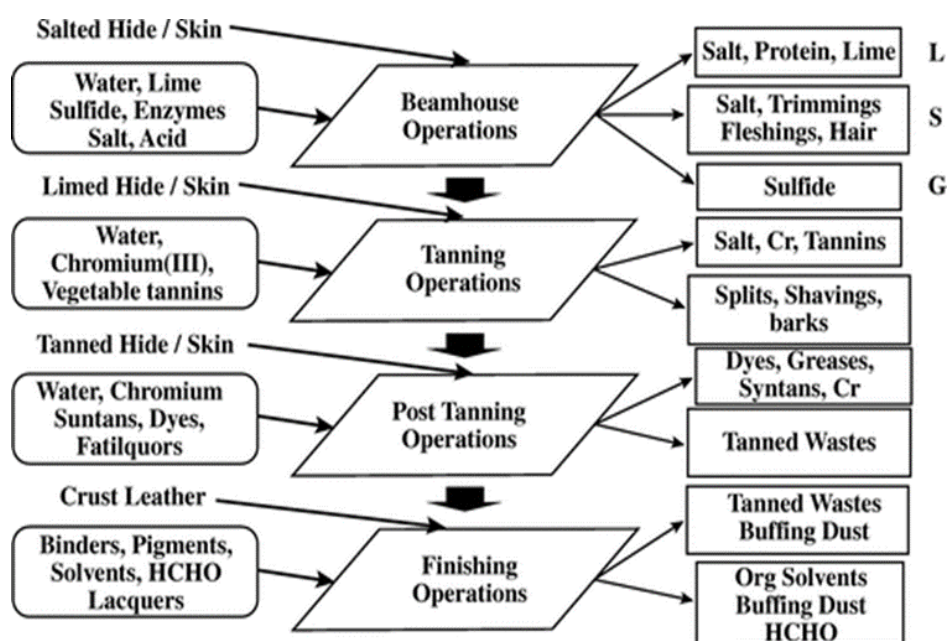


Figure 1. Tannery operations and their wastes [6]

Gelatine and its Applications

Gelatine is a translucent, colourless, tasteless food fixative derived from the collagen of living beings' body parts, which is fragile when dry and rubbery when wet [16,17]. Furthermore, hydrolysed gelatine is also known as hydrolysed collagen, collagen hydrolysate, gelatine hydrolysate, hydrolysed gelatine, and collagen peptides [18-20]. Because gelatine is a collagen polymer hydrolysate, it has numerous applications as an environmentally friendly product formulation for a gelling agent in foods, soft drinks, medicines, drug or vitamin capsules, photographic films, papers, and cosmetic products [21,22]. Protein content in gelatine extract can be determined using a variety of methods, including the Kjeldahl method, the Dumas method, direct measurement methods using UV spectroscopy, and refractive index measurement. The Kjeldahl method is most commonly used for protein content analysis [23]. In this research, an attempt is made to recycle calcareous trim waste containing collagen, the main protein used in gelatine production, which increases the leather industry's sustainability and environmental protection by reducing pollution. Unlike other researchers, here potassium hydroxide was used to increase gelatine yield because potassium hydroxide has a smaller molecular size, allowing for greater penetration of the molecule into the collagen matrix and increased bond dissociation [24]. As a result, the yield of gelatine was maximized. Furthermore, using Design-Expert software, an optimisation process was carried out to determine the optimum levels of process parameters.

EXPERIMENTAL

Materials and Methods

Materials

Lime fleshed hide trim solid wastes were collected from the tannery in Bahir Dar, Ethiopia, where they were otherwise discarded as worthless waste after liming. These wastes contain collagen and were optimized to produce gelatine.

Chemicals

In the study, laboratory-grade ammonium salts such as ammonium sulphate (2%), mordanting enzyme, a universal mordanting enzyme (RS -200) (1%), water, and an alkali such as potassium hydroxide were used.

Machinery and Equipment

Machines in the tannery for processing, as well as test equipment for conducting experiments in the laboratory, were used. Test drums, a digital pH meter, a Bachfeld viscometer, a drying machine, and a gelometer are among them. In addition, an air oven, mini drums for deliming and pickling operations, and an automatic washing machine for heat energy extraction of collagen and adhesives are included. Other items used included a bottle, a stirrer, a polyester fabric, bikers, a sharp knife, a round bottom flask, an Erlenmeyer flask, a water bath, a shaker with a heating incubator, a refrigerator, a digital scale, a mixer.

Methods

The methodology used began with data collection methods and was carried out in three phases, which included waste purification and characterization, gelatine isolation, and characterization. A response surface method from Box Behnken Design by Design-Expert software was used to analyse the effect of the independent variables like temperature, time, and concentration on the dependent variables (% protein content) to optimize gelatine extraction production.

General Methodology of Research

The calcareous trim waste was collected from the local tannery as the first step. After collecting the waste, the material was further prepared for extraction by deliming and pickling. Following waste preparation, an alkaline extraction method was used to extract and optimize collagen from the waste, which was then followed by purification or protein enrichment via precipitation of the dissolved

protein. Concentration, time, and temperature were used as control variables in the alkaline extraction of gelatine, influencing the dependent variable (gelatine yield). Following precipitation, the physicochemical properties of the purified gelatine were characterized. Figure 2 depicts a schematic representation of the methodology.

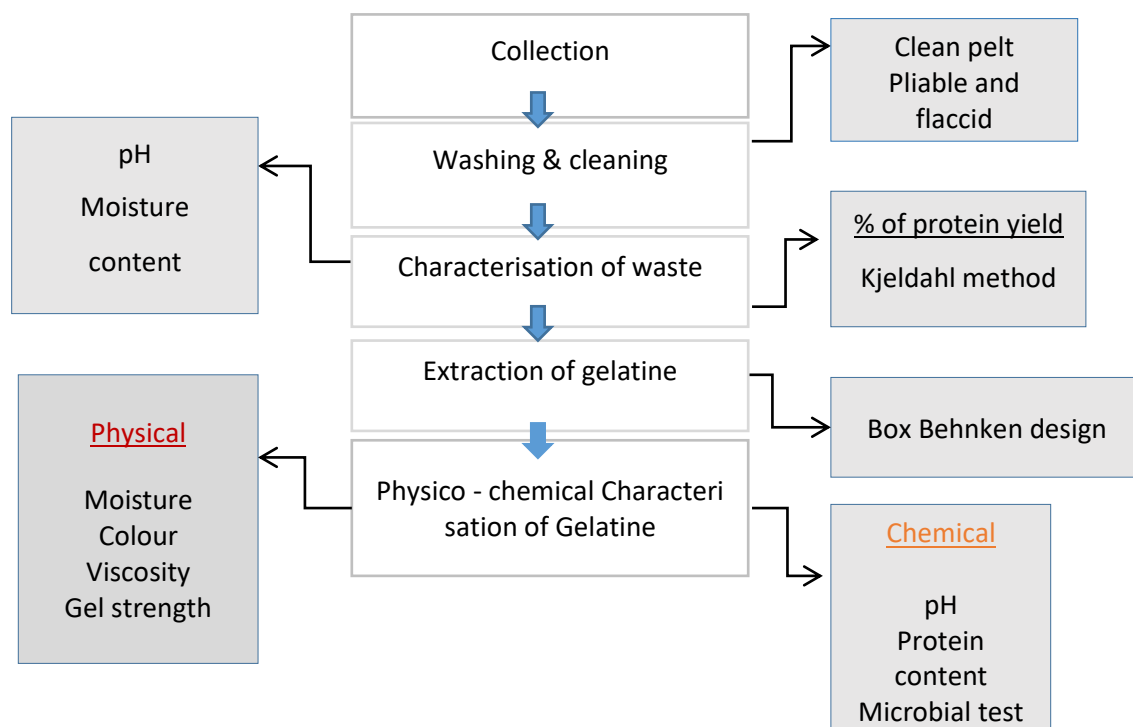


Figure 2. The general methodology of the work

Research Design

As previously stated, the extraction process has three independent variables, whereas protein yield has only one. A Box-Behnken design, which is a response surface methodology, was used to optimize the various parameters in the study. The Box-Behnken design is a response surface methodology (RSM) design with only three levels of experimentation.

Collection and Cleaning of wastes

After defleshing the hide, raw materials for lime waste were obtained from the Bahir Dar tannery. A 2 kg of hide waste was collected from the tannery and defleshed to remove the lime and elastin, which are not required for gelatine production. During deliming, the experimental and control bladders were incised with a knife. Following that, the incisions were examined with a phenolphthalein indicator to determine the lime streak in the pelts and to determine the depth of penetration of the deliming agents, i.e., the depth of deliming. Phenolphthalein was used to monitor the progress of deliming of

the pelt cross-section by observing the chemical's colours. Table 1 shows the formulations of deliming and bating operation recipes. The process of collection and cleaning is shown in Figure 3.

Table 1. Recipe for deliming and bating

Operation	Chemicals	Per cent (%)	Function	Time (min)	Remark
Deliming	Water	200	As media of transportation	3x15	Wash-drain
	(NH ₄) ₂ SO ₄	2	For lime extraction from the fibres	60	Check pH (8-8.5)
	Liquid NH ₃	0.5	For penetration		Check penetration
Bating	Bemanol RS-200	1	To remove hair roots and cleans off the scud left	60	Water Bubble

Bemanol RS-200 is a Stahl leather processing chemical supplier company product and is mostly used to remove scuds without affecting the environment.

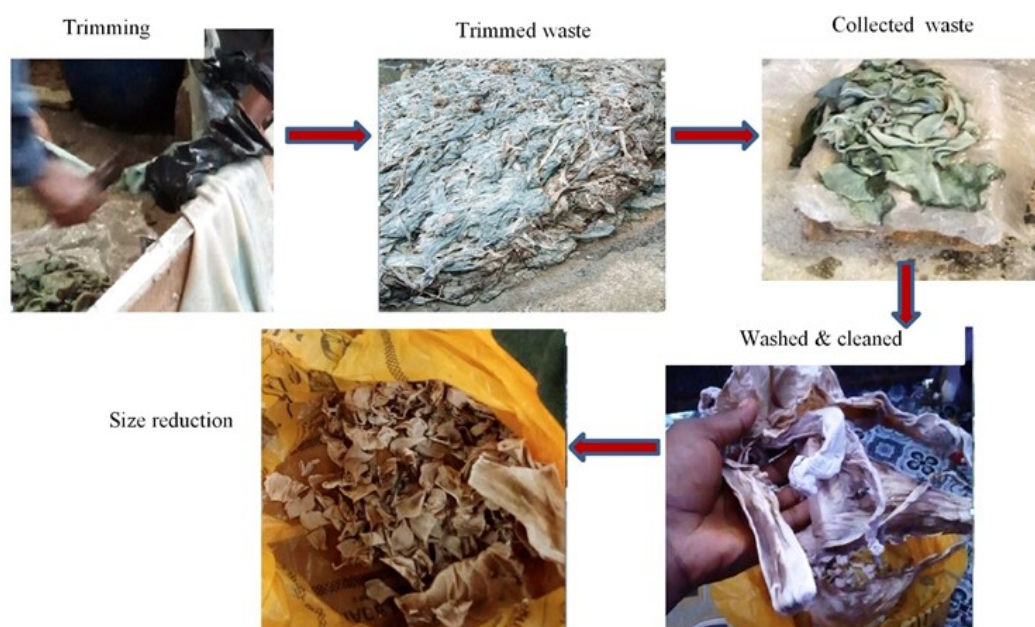


Figure 3. Collection and cleaning of the waste

The moisture content of pelt

The moisture content of the puff was determined using the design of the Association of Official Analytical Chemists (AOAC International). The sample was weighed before drying (m_1) and after drying (m_2) at 105 °C for 1 hour. After 1 hour, the moisture content was calculated using the formula below:

$$Mc (\%) = ((m_1 - m_2) / m_1) * 100 \quad (1)$$

where Mc is moisture content, m_1 and m_2 weights of the sample before and after drying respectively.

Gelatine extraction and characterisation (Phase 1)

Gelatine extraction and optimization

In this study, the studied independent variables were concentration, time, and temperature. The concentration was set to 0.5 M, 0.75 M, and 1 M; time was set to 1 h, 2 h, and 3 h; and the temperature was set to 60 °C, 70 °C, and 80 °C based on the literature, and [25-27]. As a result of the foregoing considerations, it was decided to optimize the extraction process using the response surface method (Box-Behnken design). Table 2 shows the number of runs and the experimental design.

Table 2. Number of runs and experimental design

Runs	Factors		
	Concentration	Time	Temperature
1	-1	-1	0
2	-1	1	0
3	1	-1	0
4	1	1	0
5	1	0	1
6	-1	0	1
7	-1	0	-1
8	0	-1	1
9	0	-1	-1
10	0	1	1
11	0	1	-1
12	1	0	-1
13	0	0	0

where, -1, 0, and 1 indicate (represents) the lower, middle (intermediate), and upper level of each factor for the design expert analysis and Runs means a number of experiments conducted in the laboratory.

As Table 2 shows, there were thirteen numbers of runs to extract gelatine based on each factor's level combination and the experimental design was obtained from the Design-Expert software, then the % of protein content was analysed by using the Kjeldahl method. So Table 2 is obtained from the Design-Expert software after putting the lower and upper limit (level) of each factor into the system and after Kjeldahl analysis, the response obtained and feed into the system to know whether the model is significant or not.

Application of the Kjeldahl method for protein content determination

The protein content was determined using the Kjeldahl method, with a sample (10 g) taken from each experiment. The digestion (breaking of nitrogen bonds in the sample and conversion to ammonium

ions) was accomplished by combining 10 g of sample, 20 ml of 98% concentrated H_2SO_4 , and 5 g of Missouri catalyst in a heating block and heating at 360 °C until white smoke was visible, after which the sample was allowed to cool to room temperature before being diluted with 100 ml of water and transferred to the distillation unit. The distillation (conversion of ammonium ions to ammonia) was performed using 50 ml of 50% sodium hydroxide mixed with the digested sample, 50 ml of 4% boric acid, and 6 drops of Tashiro's indicator, all of which were heated. The titration process used 0.25 mol/l HCl (determination of the amount of nitrogen in the form of ammonia as a function of the amount of HCl consumed). The Kjeldahl method steps were performed with sulphuric acid, boric acid, and hydrochloric acid, respectively.

$$\% \text{ Nitrogen (N)} = [(\text{ml HCl}) \times (14.007) \times (100)]/(\text{mg sample weight}) \quad (2)$$

where ml HCl is the volume of HCl in the titration, N HCl is the normality of HCl in the titration, and 14.007 is the atomic number of nitrogen.

$$\% \text{ Protein} = (\% \text{ nitrogen}) \times (\text{correction factor}) \quad (3)$$

ANOVA analysis

Analysis of variance (ANOVA) was used to validate the model's experimental response. The validity of the proposed experimental design was analysed by using the ANOVA method.

Characterisation of Gelatine

The physicochemical properties such as gel strength, moisture content, viscosity, colour, quality and pH of the extracted collagen were further characterised to confirm whether it was a gelatine standard or not.

Thermal analysis

Differential scanning calorimetry (DSC) is commonly used to determine protein thermal stability and thermodynamic parameters of protein denaturation. Perkin Elmer DSC 6000 was used in this study at Bahir Dar University's EITEX laboratory in Ethiopia. The thermal behaviour of extracted collagen was studied by weighing 12 mg of a sample and analysing it at temperatures ranging from 20 °C to 250 °C. The heat flux endo versus temperature was plotted, and parameters such as the peak, Delta H, and area were calculated.

Viscosity

The viscosity of the extracted gelatine was measured with a Brookfield digital viscometer by heating the extract to 32 °C in a water bath, then selecting spindle No (R-3) according to the solution and attaching the spindle to the viscometer. The lower tip of the spindle was then inserted into the solution and rotated in the solution at 60 rpm at room temperature. The viscometer displayed the viscosity in centipoise units directly on its screen.

Gel Strength

The Brookfield texture analyser was used to measure the gel strength of extracted gelatine because it is designed to measure both viscosity and gel strength. Gel strength for gelatine is the force (in grams) required to indent the surface of a gelatine gel by 4 mm using a standard 0.5" diameter probe. To accomplish this, solutions containing extracted gelatine in solid form were melted in a 320 °C water bath for 15 minutes, after which the state changed from solid to liquid, and standard solutions of 112 ml were measured using a block bottle. The bottle was removed from the water bath and allowed to cool for 15 minutes before being placed in a cold water bath (circulating bath, Haake D3 version) at 10.0 ± 0.1 °C for 12 hours. The instrument was then configured according to the manufacturer's instructions, which included setting the distance to 4 mm, the speed to 0.5 mm/sec, and selecting the type of spindle. The Bloom bottle, with a diameter of 12.7 mm, was then placed centrally under the device's piston. The Bloom force was calculated using a 5 kg load cell and a crosshead speed of 0.5 mm/sec. When the probe penetrated the gel to a depth of 4 mm, the maximum force (g) was measured.

The moisture content of gelatine

The moisture content of the gelatine was determined using the oven drying method by measuring the initial weight before drying (m_1) and the final weight after drying (m_2) at 105 °C for 1 hour. The moisture content was calculated using the formula below:

$$Mc (\%) = ((m_1 - m_2) / m_1) * 100 \quad (4)$$

where Mc is moisture content, m_1 is the weight of the sample before drying, and m_2 is the weight of the sample after drying.

pH, Gel Color, and Gelatine Microbial Test

The pH of the gelatine was determined using a laboratory pH meter. Five experiments were carried out, the mean of which was expressed as the pH of the gelatine.

The extracted gelatine solution's colour was measured using a Hunter laboratory colourimeter (colour eye spectrophotometer), and the parameters were expressed as lightness (L^*), redness (a^*), and yellowness (b^*) [28].

Because the extracted collagen was prone to microbial infestation, it was necessary to see if the bacteria could grow in the solution. As a quantitative analysis of bacterial inhibition of collagen, the percentage bacterial reduction test was performed. Gram-negative *E. coli* (7.1×10^5 cfu/ml) and Gram-positive *S. aureus* (6.3×10^5 cfu/ml) bacteria were used. The American Type Culture Collection (ATCC) standard test method was used. The collagen-free control sample and the collagen-containing sample were placed in a Petri dish with 50 ml of physiological saline (0.85% NaCl) containing the above cell numbers of the test strains, and the mixtures were cultured for 24 hours at 37 °C in a shaking incubator. Following incubation, 1 ml of the bacteria-containing mixture was serially diluted, and 0.1 ml of each dilution was plated on MHA with 0.5% triphenyl tetrazolium chloride (TTC) solution. Viable bacteria were counted based on colony-forming units after 24 hours of incubation at 37 °C, and the mean value of cells in the lowest dilution was calculated. The sample's reduction rate (%) was calculated using the following equation:

$$R = ((A - B) / B) * 100 \quad (5)$$

where R is the per cent of reduction, A is the number of bacteria recovered from the inoculated untreated test, and B is the number of bacteria recovered from the inoculated treated test.

RESULTS AND DISCUSSION

Pre-treatment of the waste

Analysis of the penetration of decalcifiers

As stated in the methodology, the penetration of the deliming agent was evaluated using the phenolphthalein indicator, which shows less colour after neutralization of the lime in the pelt fibre. The pH (which is primarily determined by the availability of lime in the fibre cross-section) and time (duration of deliming) were determined in this analysis, yielding the neutralisation performance of the deliming agent. When ammonium sulphate was used to remove lime from the pelt fibres for 2 hours, the colour of the phenolphthalein became colourless.

Moisture content

The moisture content obtained was 19.5%. This indicates that the pelt contains a significant amount of moisture due to the opening of the pelt's fibre structure, which allows water molecules to enter

these cavities [29]. Hide in its raw state contains 64% water, which means it has a high-water storage capacity, creating favourable conditions for the development of microorganisms. A pelt with less than 5% moisture content is referred to as a dry pelt, which makes further processing difficult due to the hardening of the fibres. As a result, this result is less favourable for the development of microorganisms and avoids the problem of case hardening.

Analysis of Gelatine Extraction from the dried wastes

Table 3 displays the results of gelatine extraction from dried wastes. According to the values in Table 3, the highest protein content was obtained with run number 7 (87.25%) where the temperature was at 70 °C, time at 2 h, and concentration at 0.75 M. When the temperature was raised from 60 °C to 70 °C, the protein content increased from 62.37% to 86.68%, indicating increased gelatine extraction up to 70 °C; however, the percentage decreased after this temperature [30]. It should be noted that protein degradation occurs at temperatures above 70 °C causing the protein to degrade and lose its protein content and character. Extreme values of variables such as temperature, time, and concentration damage and affect the protein extract [31]. At 70 °C, the yield increased as the time increased from 1 to 2 hours; however, at higher temperatures, the yield decreased as the time increased from 2 to 3 hours. Protein content decreases as time and temperature are increased beyond optimal values. As shown in the table, different concentrations affect protein content, but the effect is dependent on other variables, most notably temperature, indicating that concentration does not have a linear effect on protein content, but time and temperature do. The values in the table indicate that extreme values of temperature, time, and concentration reduce protein content as it degrades. Finally, the maximum protein content of 87.25% can be obtained at the optimum temperature of 70 °C, alkali concentration of 0.75 M, and time of 2 hours.

Table 3. Protein Content %

Std	Run	Factor 1 Temperature (°C)	Factor 2 Time (Hr)	Factor 3 Concentration (Molarity)	Response Protein content (%)
9	1	70	1	0.5	79.12
1	2	60	1	0.75	54.55
4	3	80	3	0.75	80.65
7	4	60	2	1	61.85
3	5	60	3	0.75	62.37
6	6	80	2	0.5	77.50
13	7	70	2	0.75	87.25
10	8	70	3	0.5	86.45

Std	Run	Factor 1	Factor 2	Factor 3	Response
		Temperature (°C)	Time (Hr)	Concentration (Molarity)	Protein content (%)
12	9	70	3	1	86.68
2	10	80	1	0.75	77.16
11	11	70	1	1	79.66
8	12	80	2	1	75.26
5	13	60	2	0.5	59.75

where Std stands for standard which is a randomised order of runs in the design expert software.

ANOVA analysis

The analysis of variance (ANOVA) results show that the proposed experimental model is significant, as shown in Table 4. According to the expert's design, any term in the model with a small P-value and a large F-value has a more significant effect on the relevant response variable. Table 4 shows that the ANOVA analysis of the extraction model is significant, indicating that the factors chosen in this experiment are appropriate for the process and dependent variable (response) of collagen extraction from waste. The ANOVA also shows that the independent variables temperature and time are more significant than concentration, indicating that the two facts have a large impact on the response (the dependent variable). Quadratic item A² is also significant, with a value of 0.0013, which is less than 0.05 and more reasonable than the other quadratic items.

Table 4. ANOVA analysis of the proposed model and the independent variables

Source	Sum of squares	Degree of freedom (df)	Mean Square	F-value	P-value	Significant
Model	1465.79	9	162.87	37.32	0.0063	*
A-Temperature	648.90	1	648.90	148.68	0.0012	**
B-Time	82.30	1	82.30	18.86	0.0225	*
C-Concentration	0.0496	1	0.0496	0.0114	0.9218	ns
AB	4.69	1	4.69	1.07	0.3762	ns
AC	4.71	1	4.71	1.08	0.3762	ns
BC	0.0240	1	0.0240	0.0055	0.9455	ns
A ²	620.59	1	620.59	142.20	0.0013	**
B ²	9.98	1	9.98	2.29	0.2276	ns
C ²	10.89	1	10.89	2.49	0.2124	ns
Residual	13.09	3	4.36			
Cr total	1478.88	12	123.24			

where ns is non-significant, * is significant, ** strongly significant, and Cr is composite reliability

The value of the source, the sum of squares, the Degree of freedom (df), the Mean Square, and the F-value are all taken from the Design-Expert software and each factor and their second-degree value has 1 degree of freedom as shown in Table 4 which is taken from the software.

Regression Analysis

The relationship between the dependent and independent variables was obtained using design expert software. The protein content (Pc) can be expressed as follows in the regression equation:

$$Pc = 87.25 + 9.01A + 3.21B + 0.0788C - 1.08AB - 1.08AC - 0.775BC - 16.48A^2 - 2.09B^2 - 2.18C^2 \quad (6)$$

where Pc is protein content, A², B², and C² are the quadratic (second-degree) values of temperature, time, and concentration, respectively, with A representing temperature, B representing time, and C representing concentration.

The coefficient estimate shows the predicted change in response per unit change in factor values when all other factors are held constant. The equation above clearly shows that each independent variable has a positive relationship with each dependent variable, implying that as the independent variables grow, so do the dependent variables in their linear expression. A coefficient represents the influence of a single component, whereas coefficients with multiple elements represent the interaction of multiple factors. The regression model also shows that time and temperature have a positive and high effect on the extraction process because they have a larger coefficient estimate than concentration, as demonstrated in Table 4.

Surface plots

Figure 4 depicts a three-dimensional graph that illustrates how numerous factors interact with one another. Utilizing response surface plots and associated contour plots in three dimensions, the impacts of the independent factors on extraction yield were shown. Two independent variables are used to model the plots as a function while the other variables are kept constant. As shown in Table 3, the yield of protein content increases from 54.55 to 62.37 when the temperature is maintained at 60 °C and the time is increased from 1-3 hours. The distance between each contour line is the same because, as the 3D plot demonstrates, temperature and time have no interaction effect on the protein yield. The amount of proteins extracted rises to some extent as time and temperature rise, but at 70 °C, the values of the response fall, as seen on the parabolic curve.

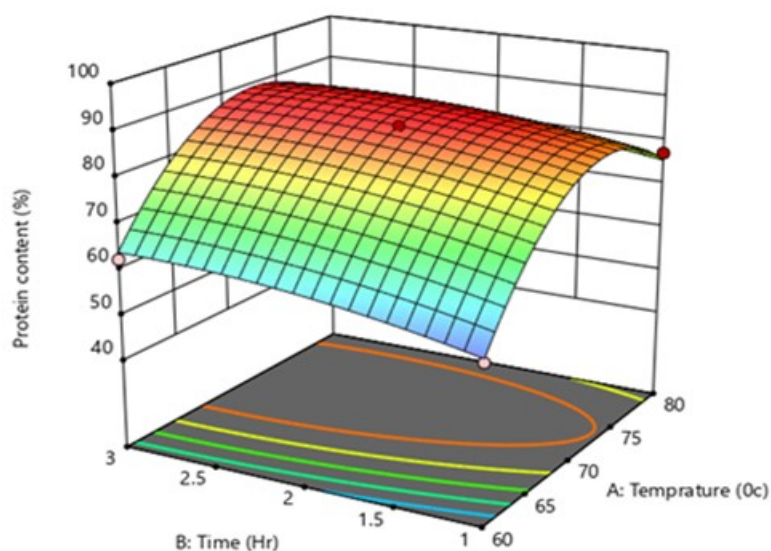


Figure 4. 3D plot of the response surface

Characterisation of extracted Gelatine

The following section describes the properties of extracted gelatine, including gel strength, viscosity, moisture content, pH, heat stability, and microbial test results. Table 5 shows the results. The characteristics of gelatine are obtained from the optimum levels, which means the expected response (dependent variable) from the experiment was protein content, so the experimental treatment that gives the maximum or optimum result (protein content) further investigated its characteristics rather than the entire experimental output. As a result, the gelatine characteristics analysis is based on the optimal values.

Gel strength

The value is reported as the "bloom" value, which also measures the strength and stiffness of the gelatine as well as the average molecular weight of its constituents [18, 32, and 33]. This value ranges from 30 to 300; less than 150 is considered low, between 150 and 220 is considered medium, and between 220 and 300 is considered high [34,35]. Table 5 compares the general characteristics of the resulting gelatine to the standard gelatine characteristics.

Table 5. Results of gelatine characterisation

Characteristics	Results	Standards
Gel strength	245 g	150-300 g
Viscosity	6.85 cp	6.37 to 7.28 cP
Moisture content	12.11%	8 to 13%
pH	7.2	6.5-8

According to the test results, a gel strength value of 245 g was obtained, indicating that the isolated protein has a large molecular weight and exhibits remarkably strong cohesive forces. Gelatine materials have increased adhesive strength due to higher cohesive forces in the material. KOH treatment typically yields better results with high bloom values, indicating that more gelatine was isolated from the waste of hide trimmings, giving the gelatine stiffer and higher gelation qualities.

Viscosity

The test results show a value of 0.00685 ps, or 6.85 cp, which is within the suggested range of 6.37 to 7.28 cP for hide protein extracts (gelatine) [36-38]. A viscosity of 6.85 cp indicates that there is more bonding among and between its molecular structures, making the final product impact resistant. According to the literature, hide gelatine is superior to other sources of gelatine because it has superior gel-making qualities (gel strength and viscosity) and strong film-forming properties [40]. As a result, the gelatine extracted from this work meets the standards and requirements for gelatine.

Moisture content, pH, Colour measurement, and Microbial test of gelatine

The weight before drying was 90.286 g (m₁), and after drying it was 78.35 g (m₂). The moisture content was calculated to be 12.11%, as required for a gelatine extract. Gelatine should have a moisture content of 8 to 13%, according to the literature, but a value above 13% is not recommended because it is easily destroyed by bacteria, reducing the product's shelf life [17,41]. The extract's measured pH was calculated using the average results of the five trials (6.9, 7.0, 7.2, 7.5, and 7.4); the mean pH was 7.2. At the isoelectric point, native collagen has a pH of 4.8, and adding alkali raises it to 7.2. According to the literature, gelatine's use is limited by its acidity and alkalinity, so the pH of gelatine should be in the 6.5-8 range [41-43]. As a result, the pH of the gelatine extracted for this study is normal, and it has a wide range of potential applications. Furthermore, the gelatine produced from the tannery's solid waste by KOH extraction meets the gelatine requirements, and thus this study can be fully utilized to reduce the cost of waste disposal and waste containers while also generating additional income.

The percentage of reduction of bacterial growth in the control and treated protein samples was calculated after selecting the gram-positive and gram-negative bacteria to analyze the bacterial growth and reduction using qualitative and quantitative methods. The per cent reduction observed for *S. aureus* and *E. coli* was 3.17% and 1.41%, respectively. This implies that the isolated protein has no antimicrobial activity in its liquid or undried forms. The L, a, and b values of the extracted gelatine were calculated. The isolated protein has transparent properties, as evidenced by the L, A, and b values of 78.94, 5.2, and 14.64 [27].

Thermal analysis of Gelatine

Because other gelatine properties, such as gel formation and melting temperature, are determined by the molecular weight and amino acid cross-linking and are influenced by the thermal energy used in the gelatine, thermal analysis of gelatine is required. The differential scanning calorimeter's investigation of the gelatine's thermal properties yielded peak, enthalpy, and region values of 88.20 °C delta H, 1896.3212 J/g, and 22755.8542mJ, respectively. Delta H denotes the amount of energy required to bring the system up to temperature T at constant pressure, where a positive value indicates that the process was endothermic, bringing energy into the system, and the peak denotes the temperature at which collagen melts. Gelatine has a melting point of 88.2 °C, as shown in Figure 5, and once melted, no ash is left behind. The first heating curve in the increment of the graph is distinguished by its maximal intense heat absorption, which causes the gel to melt and is superimposed on the almost linear temperature, and its reliance on the gelatine's heat capacity.

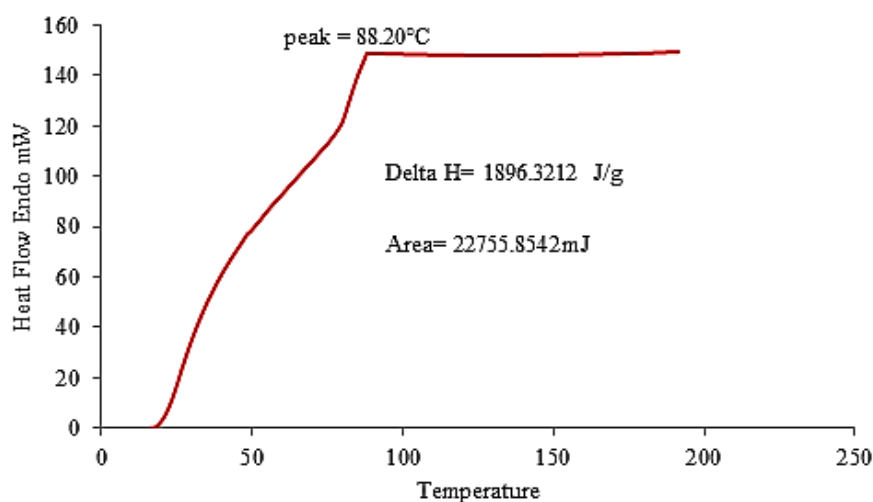


Figure 5. DSC curve of Gelatine

CONCLUSION

The hide-trimming waste, which is typically dumped into the environment after the liming operation, was used as raw material for gelatine production through an alkaline extraction method. The research found that the alkali content, extraction temperature, and time - all of which were treated as independent variables - all affect protein yield. At 70 °C for two hours, a 0.75 M alkali concentration could extract up to 87.25% of the protein. The use of potassium hydroxide in this study was intended to shorten the extraction time due to the chemical's lower molecular weight. This is supported by the fact that the extraction time was reduced from 3 hours to 2 hours, and the extracted gelatine had better properties than the standard ones, including 245 g, 6.85 cp, 12.11%, and 7.2 for gel strength, viscosity, moisture content, and pH. In general, this research advocates the use of waste in the

production of sustainable gelatine to benefit environmental, social, and economic factors. This is an effort to recycle calcareous trim waste containing collagen, the main protein used in gelatine production, which improves the sustainability and environmental protection of the leather industry by reducing pollution.

Conflicts of Interest

The authors declare no conflict of interest.

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