Characterization and testing of Gelatin extracted from poultry waste

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Abstract- **In the present research article Gelatin was extracted from waste chicken feet using an alkaline and acid extraction procedure. The obtained gelatin was investigated and compared to commercially available gelatin in terms of the various properties and characteristics. The extracted gelatin had a higher moisture content of 95.917% and less protein and ash content. The peak in UV-visible spectroscopy was considerably lower than in commercial gelatin, while the peak in FTIR spectroscopy was remarkably similar in both gelatin spectrums. The influence of varying temperatures was also studied on the formation of gelatin films, as the resultant films casted from extracted gelation was highly brittle at moderate temperature. Crosslinking was accomplished via temperature variance that caused structural changes and improvement in mechanical strength and water resistance property of gelatin film. Bacterial decomposition was seen at room temperature. A perfect finished film layer was obtained after heating.**

Keywords- **gelatin, chicken feet, thermal crosslinking, biodegradable, biopolymer**

INTRODUCTION

Gelatin is a non-toxic, biodegradable, biocompatible polymer made up of denatured protein. It is obtained from the acid and alkaline treatment of collagen comprising a variety of amino acid residue combinations and proportions. It is a multifunctional protein and one of the most important biopolymers that has numerous uses in the pharmaceutical, cosmetic, health-care industries, as well as the food industry [1]. It doesn't occur naturally in nature [1,2,3]. It is available in granular powdered form or as sheets or films. Gelatin powder is tasteless, odorless, brittle transparent, slightly yellowish in color [4]. It has high water solubility and binding capabilities [4,5,6]. It is a heterogeneous collection of single and multi-stranded large molecular weight polypeptides with helix conformations and amino acids ranging from 50 to 1000 [7]. It can help with weight loss, osteoarthritis, rheumatoid arthritis, and brittle bones (osteoporosis).In the biomedical and pharmaceutical sectors, it is employed as hard and soft capsule microspheres, tablet binders, stabilizers in injections and wound dressing [1][4][2][5]. Gelatin is found in a variety of products, like paints, matches, and fertilizers, diapers, sanitary napkins as well as in contact lenses [5]. Wine, beer, and fruit juices have traditionally been clarified with gelatin in the past [6]. Many body lotions, shampoos, toothpastes, and other cosmetic items include a small amount of gelatin [7]. It is used in the food sector for gelling, clarifying, foaming, and as an additive in the manufacture of a variety of products, such as bakery, meat, and dairy to enhances the suppleness, consistency, and stability of a wide range of foods [8].

With a change in temperature, gelatin can form a physical hydrogel, however these hydrogels have poor chemical and mechanical stability, which can be enhanced by introducing crosslinkers such as glutaldehyde and epichlorohydrin. Gelatin, as a thermos reversible hydrocolloid, has a tiny temperature difference between melting and gelling temperatures. It sets at 20 °C and melts at 30 °C, forming thermos reversible gels [8]. Gelatin does not dissolve well in cold water, and the requisite solubility temperature is around 37°C, but it dissolves efficiently in hot water and gels on cooling. Because it is a fat-free, cholesterol-free, and purine-free, gelatin is frequently recommended in sports diets, such as bodybuilding, to increase protein levels [8].

Gelatin and its parent component, collagen, are chemically quite similar. Collagen is a natural fibrous protein present in the connective tissues or organs of many vertebrates and invertebrates, as well as in the

skin, bones, teeth, and tendons [1-4]. Tropocollagen is a basic structural unit of collagen that consists of three polypeptide chains joined by three amino acids per turn in a unique structural superhelix [4][9]. Following partial hydrolysis and denaturation of collagen, the triple helix structure is broken down, resulting in random gelatin coils [5][7]. Denaturation involves thermal treatment and hydrolytic breakdown of covalent and cross-linked bonds between collagen and polypeptide chains [3]. Extraction of gelatin from collagen can be done in a variety of ways. The major processes in the extraction of gelatin are to remove non-collagen contaminants and then transformation of water insoluble collagen to soluble gelatin. Finally, a series of refinement and recovery techniques are used to obtain highly purified dry gelatin after extraction. Gelatin can be extracted in a number of different ways. Recent studies have performed extraction in the presence of crude papain (1% w/w) for 12 hours at 37°C, by using food-grade acids (acetic, citric, and lactic acid) and with an acid and ultrasound-assisted method [5][6].

MATERIALS AND METHODS

Sodium hydroxide, Acetic acid, distilled water, BSA (bovine serum albumin), cheese or muslin cloth, magnetic stirrer, filter papers, heating mantle, thermometer.

Extraction of gelatin: The alkaline and acid treatment extraction method was performed with slight modifications. An acid-alkaline treatment methodology was used to extract gelatin [5], which to weakens collagen structure, to solubilize non-collagen proteins, and to hydrolyze certain peptide linkages while keeping the collagen fiber consistency. Partially hydrolyzing and denaturing collagen results in the formation of random gelatin coils, which disrupts the triple helix structure. The denaturation process involves thermal treatment and the hydrolytic breakdown of covalent bonds.

Preparation of raw materials – 1 kg of chicken feet were purchased from the local market and thoroughly cleaned to remove all unwanted blood or contaminants. The nails were removed and cleansed to remove blood clots. It was then frozen for 20 hours at 4°C before being thawed. Skin and flesh were stripped of from bones and the deboned part is retained for future extraction, while the bones were discarded. The weight of the remaining deboned portion was calculated. The residual content was then immersed in a 0.2% (w/v) concentration of NaOH solution to remove non-collagenous material, and then the mixture was whirled with a magnetic stirrer for 40 minutes at room temperature. To soften the texture of the material and prepare it for the next step, the operation was repeated for three times. Cheese cloth was used to filter the solution. A solution of 0.2% (v/v) acetic acid was prepared, and the smooth mashed skin obtained from the alkaline treatment was soaked in it for 40 minutes. After 40 minutes the solution was poured into cheese cloth and rinsed repeatedly under running tap water until the pH was neutral. The amount of skin left in the cheese cloth was measured, and the final stage of extraction was completed by heating the leftover contents in distilled water at 70°C. The skin to distilled water ratio was 1:9 (w/v) with temperature not exceeding 70°C. The extract was then filtered through two layers of clean cheese cloth and refrigerated to cool and settle. Even the naked eye can detect a finely divided layer of translucent gel from distilled water. Filter paper was used to filter the end product. Gelatin gel is the resultant product on filter paper. For dry gelatin, the gel was placed in a Petri dish and kept at a low temperature in a hot air oven.

GELATIN PRESENCE IDENTIFICATION TEST

Distilled water was heated to a temperature of 40°C. 1 g of gelatin was added and thoroughly dissolved with further addition of 10 mL of 3 N HCl with continuous mixing. After that, 10 mL of 0.2 M $K_2Cr_2O_7$ was added to it, stirred for a while, and set aside.

CHARACTERIZATION

UV- VISIBLE SPECTROSCOPY

Gelatin was characterized by UV- visible at 200-400 nm. Extracted and commercial gelatin were dissolved in lukewarm distilled water and allowed to cool to room temperature. At 40°C, gelatin was dissolved in distilled water and the solution was allowed to cool. The UV-visible range was tuned to 200-400 nm, with distilled water serving as the standard.

FOURIER TRANSFORM INFRARED SPECTROSCOPY

This technique is effective for examining structural configurations of the sample. It is based on the principle that the fundamental components of a substance, such as chemical bonds, can be stimulated and absorb infrared light at specific frequencies. The IR absorption spectrum is a resultant fingerprint of the sample being analyzed. It has been used to determine the presence of functional groups and the secondary structure of gelatin.

pH

The pH was determined using a pH meter. 0.5 concentration of gelatin was dissolved in 100 mL of distilled water and pH was noted down.
Gelatin had a pH of 6.424.

MOISTURE CONTENT

METHOD: A petri dish with a lid was dried in oven until no moisture remained and then placed in a desiccator to cool. In the hot air oven, the temperature was maintained at 100°C. 6 g of sample was weighed and evenly distributed on the dish. The dish was removed after 3 hours and put to a partially covered desiccator to cool. The sample was dried and weighed.

CALCULATION FORMULA:

Moisture content was calculated by formula-

$$Moisture\ (\%) = \frac{(W_1 - W_2)}{W_1} \times 100$$

Where, W_1 = weight (g) of sample before drying

W_2 = weight (g) of sample after drying

ASH CONTENT

METHOD: Placed crucible and lid in muffle furnace at 550°C set temperature to burn off all impurities from crucible's surface. The crucible was cooled in a desiccator for 30 minutes. 14 g gelatin was weighed on the crucible and left overnight in the furnace. After lifting the crucible from the furnace, immediately c it was covered with a lid to prevent the loss of fluffy ash and placed in a desiccator to cool down. When the sample was settled, ash was weighed. The ash content was determined using the following formula:

Ash content was calculated by formula:

$$Ash\ (\%) = \frac{Weight\ of\ ash}{Weight\ of\ sample} \times 100$$

PROTEIN CONTENT

METHOD: Lowry's technique was used to determine the protein content. With Folin- Ciocalteau reagent, which comprises of sodium tungstate molybdate and potassium, the phenolic group of tyrosine and theophany residues generates a blue purple color complex. BSA (bovine serum albumin) is utilized as a standard protein because of its low cost, high purity, and accessibility. The number of aromatic amino acids present determines the intensity of color, which varies for all proteins. The UV-visible wavelength was set to 660 nm.

1 mg/ml BSA stock solution and both commercial and extracted gelatin solutions were made. 10 mL of 1.56% $CuSO_4$ solution was coupled with 10 mL of 2.37% $C_4H_4Na_2O_6$ solution. 2 mL of solution was mixed with 100 mL of 0.1 N NaOH and 2% Na_2CO_3 solution to make analytical reagent. 2 mL of solution (a) was mixed with 100 mL of solution to make an analytical reagent (b). Folin—Ciocalteau reagent was made by combining equal amounts of Folin-reagent and distilled water. Stock solution and water were mixed in test tubes with 5mL final capacity to create multiple dilutions of BSA concentrations range between 0.5 mg/L to 1 mg/L. 2 mL protein solution was pipetted out from each test tube to fresh test tubes with labelled BSA concentrations and 2mL of analytical reagent was thoroughly mixed to each test tube. Covered test tubes were incubated at room temperature for 10 minutes. Incubate for next 30 minutes with further addition of 0.2 mL Folin reagent in each tube. A standard calibration curve for BSA stock solution samples was obtained by setting the UV visible at 600nm. Blank and gelatin samples were subjected to the same procedures. The absorption and concentration of extracted and commercial gelatin were displayed on a standard curve. [fig.5]

Table 1: Different dilutions of BSA solutions

BSA (mL)	Water (mL)	Concentration of sample (mg/mL)	Volume of sample (mL)	Analytical Reagent (mL)	Folin- reagent solution (mL)
0.25	4.75	0.05	0.2	2	0.2
0.5	4.5	0.1	0.2	2	0.2

1	4	0.2	0.2	2	0.2
2	3	0.4	0.2	2	0.2
3	2	0.6	0.2	2	0.2
4	1	0.8	0.2	2	0.2
5	0	1.0	0.2	2	0.2

Effect of temperature: Effect of temperature on gelatin films was studied at room temperature, 4°C and 80°C.

RESULT AND DISCUSSION

IDENTIFICATION TEST

The gelatin test was confirmed by presence of hydroxyproline in the extracted gel.

Yellow precipitate was observed after filtering which indicates the presence of hydroxyproline which is present in gelatin.

UV- VISIBLE SPECTROSCOPY

At an absorbance of 0.228, extracted gelatin had a concentration of 76.0550 mg/L. Separate and comparative plots of UV-visible spectroscopy of commercial gelatin and extracted gelatin were taken. The blue-coloured curve represents extracted gelatin. Commercial gelatin (red curve) showed a greater and sharper peak than extracted gelatin. This could be due to the presence of contaminants in the extracted gelatin. [Fig 1,2 & 3]

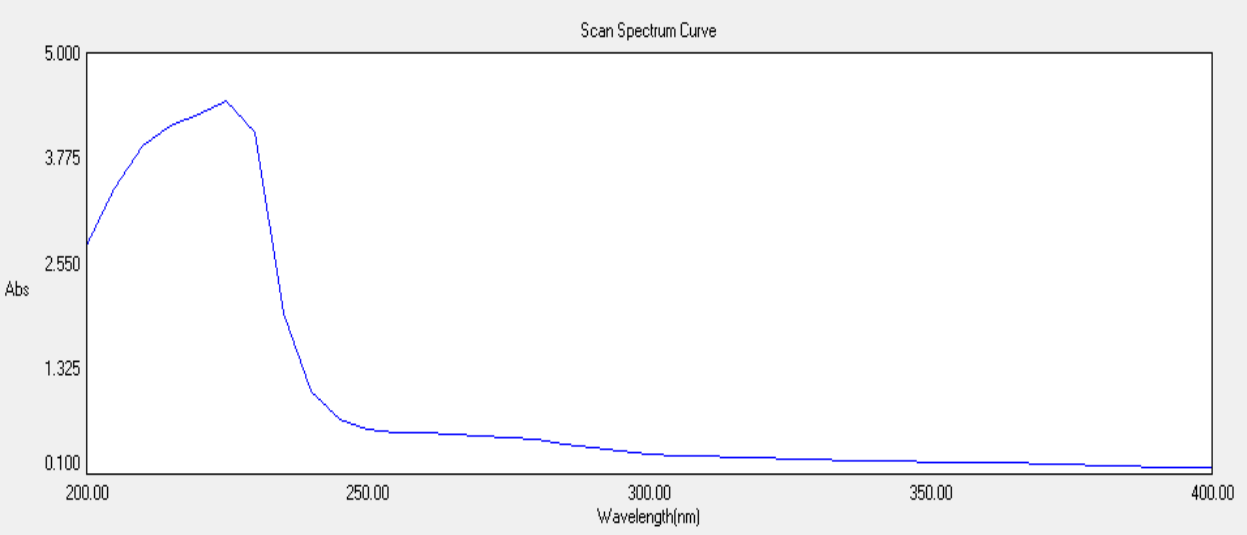

Fig.1: UV- Visible spectroscopy of extracted gelatin

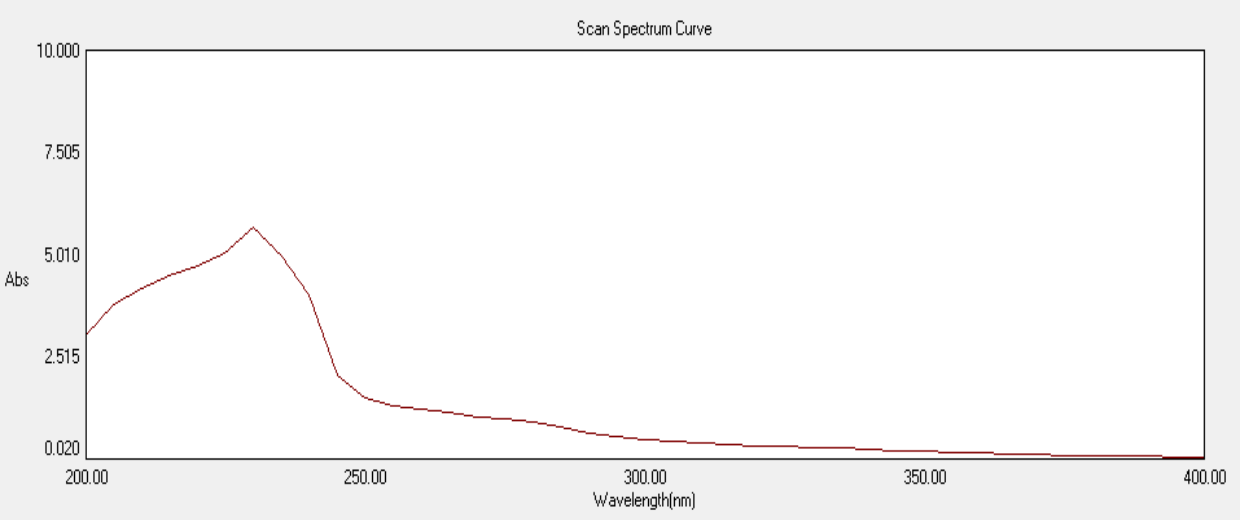

Fig.2: UV- Visible spectroscopy of commercial gelatin

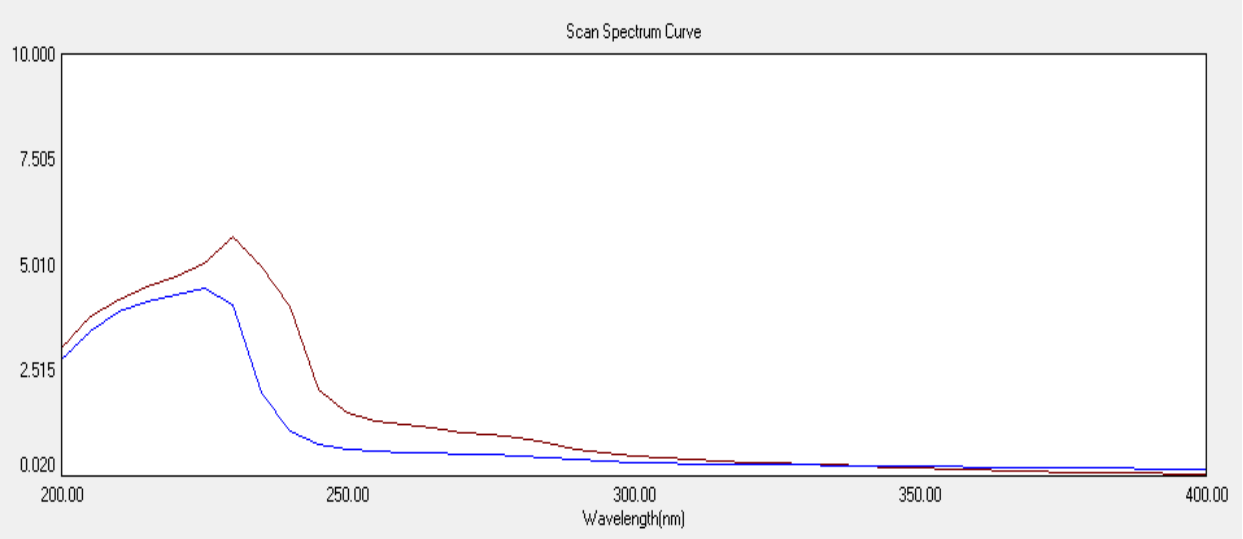

Fig.3: UV- Visible spectroscopy of commercial and extracted gelatin

FTIR (Fourier Transform Infrared Spectroscopy)

The following are the FTIR spectrums of gelatin: (3415 cm-1) amide-A and free water amide-I (1637 cm-1) amide-II (1539 cm-1) amide-III (1236 cm-1).

At 3415 cm-1 (O-H stretching) and at 1637 cm-1, there is a prominent IR absorbance band (H-O-H bending). Amide-I represents C=O stretching/hydrogen bonding pair with COO, Amide-II represents bending vibration of N-H groups and stretching vibrations of C-N groups, and Amide-III is connected to vibrations in plane of C-N and N-H groups of bound amides. The most sensitive spectral region to the protein secondary structure is the amide-I band (1700-1600 cm-1).

The FTIR spectroscopy of extracted gelatin is quite comparable to the FTIR spectroscopy of commercial gelatin. There is a strong IR absorption band at 3418 cm-1 (O-H stretching) and 1636 cm-1 (H-O-H bending). Amide-I represents the C=O stretching/hydrogen bonding pair with COO, Amide-II represents the bending vibration of N-H groups and the stretching vibrations of C-N groups, and Amide-III represents vibrations in the plane of C-N and N-H groups of bound amides. The amide-I band is the most sensitive spectral area to the protein secondary structure (1700-1600 cm-1). Peaks of conventional and extracted gelatin were surprisingly similar, and the results were identical.

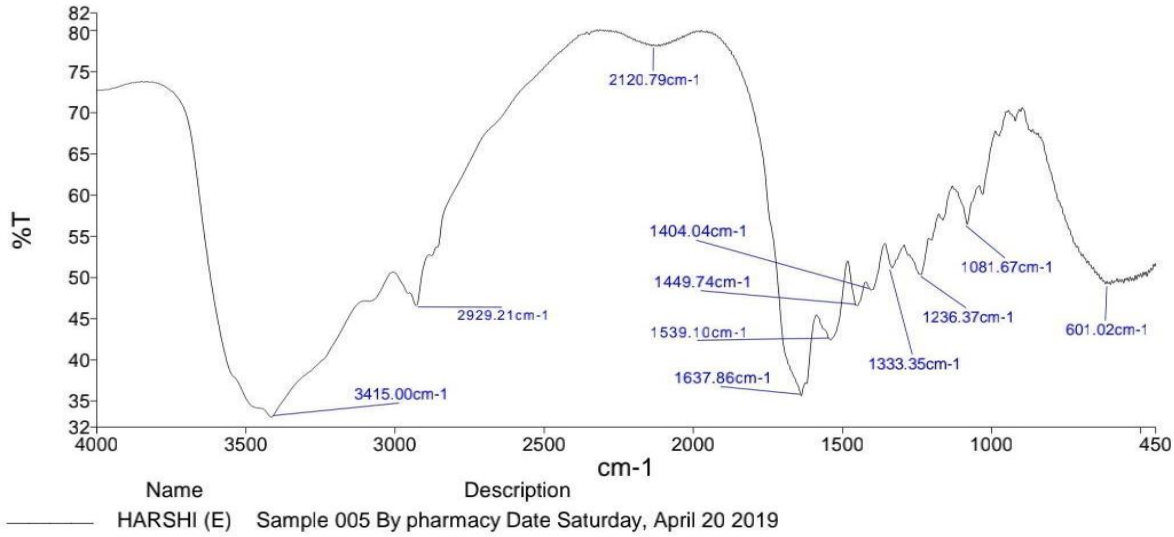

Fig.4 FTIR spectrums of extracted gelatin from poultry waste

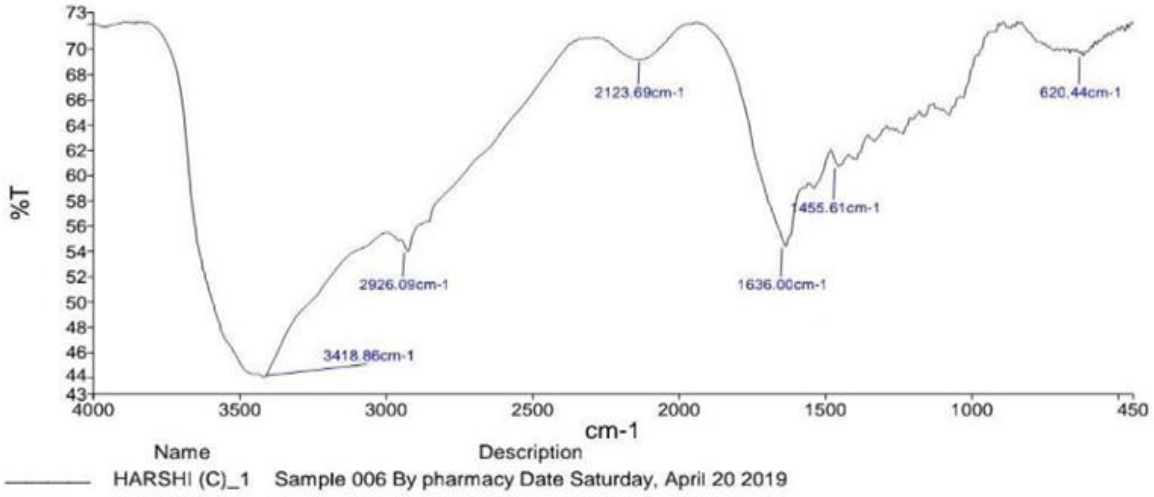

Fig.5 FTIR spectrums of commercial gelatin

FTIR spectrums of Extracted gelatin are as follows [Fig 4]: -

- amide-A and free water (3415 cm^{-1})
- amide-I (1637 cm^{-1})
- amide-II (1539 cm^{-1})
- amide-III (1236 cm^{-1})

The following are the FTIR spectrums of commercial gelatin [Fig 5]: -

- amide-A and free water (3418 cm^{-1})
- amide-I (1636 cm^{-1})
- amide-II (1455 cm^{-1})
- amide-III

pH

Commercial gelatin is a dark yellow granular product that is non-shiny, has no odour, and has a pH of 5.841. Extracted gelatin has an odorless, brittle pale-yellow color with a pH of 6.424, resembling small shattered glass fragments.

MOISTURE CONTENT

The moisture content% of extracted gelatin was 95.917 %, and 84.333 % for commercial gelatin.

ASH CONTENT

The ash content was 0.125%. When compared to commercial gelatin, extracted gelatin had a lower ash level. Extract gelatin had a 0.125 % ash concentration, while commercial gelatin had a 0.25 % ash content.

PROTEIN CONTENT

Lowry's method of protein estimation was used to calculate the protein content.

BSA was used to generate a standard protein curve. Protein concentration in extracted gelatin was noted 76.0550mg/L at absorbance 0.228 and 134.412mg/L at absorbance 0.325 in commercial gelatin. [fig.5]

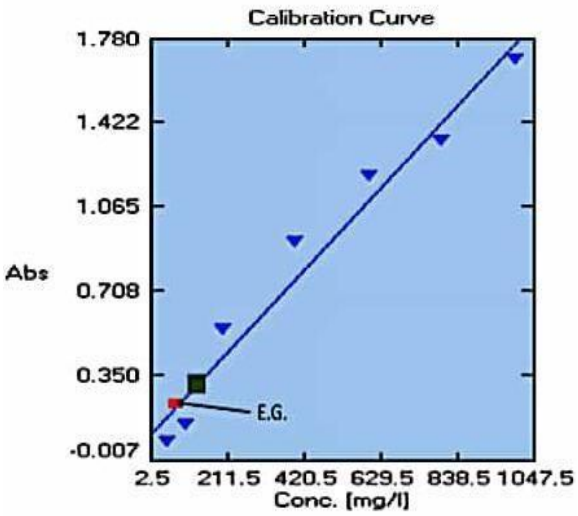

Fig.6: Graph showing conc. of (extracted gelatin) protein on standard curve of protein

EFFECT OF TEMPERATURE ON GELATIN FILM

Effect of temperature was studied on the behavior of the gelatin films. The following observations were noted table.2 and figures [7,8,9]

Table 2: Effect of temperature on gelatin film

at room temperature	at 4°C (cold) temperature	at 80ºC (heating)
● Gelatin kept in petri dish showed slightly decrease in amount with time. ● Formation of film was not noticed. White color layer started to form and bacterial growth was noted. Bacterial degradation occurred and had very foul and stinky smell ● Soluble	● Gelatin kept in petri dish showed decrease in amount was noted ● Film was obtained but it was not continuous and cracked. ● Soluble	● Gelatin was heated at 80°C temperature for about 5-6 hours till its quantity reduces to one-third and then left undisturbed to cool down. ● Proper, continuous film was formed. because of thermal crosslinking of gelatin. ● Insoluble

Fig.7: Film obtained at low temperature (4°C).

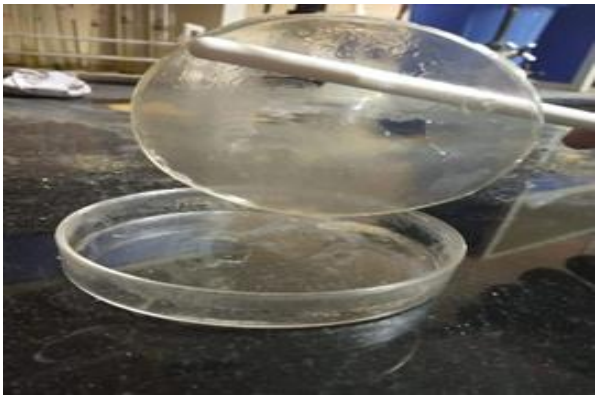

Fig.8: Film obtained after heat treatment (Thermal crosslinking)

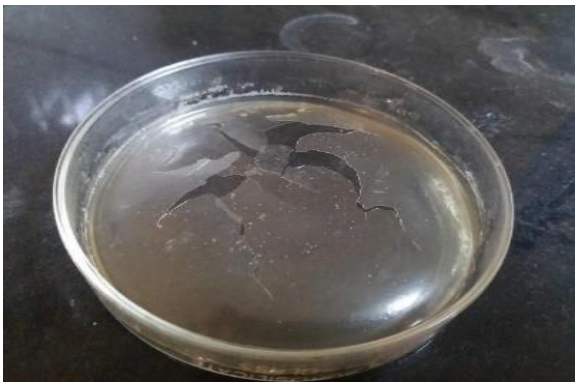

Fig.9: Comparative figure of films obtained

FTIR RESULT

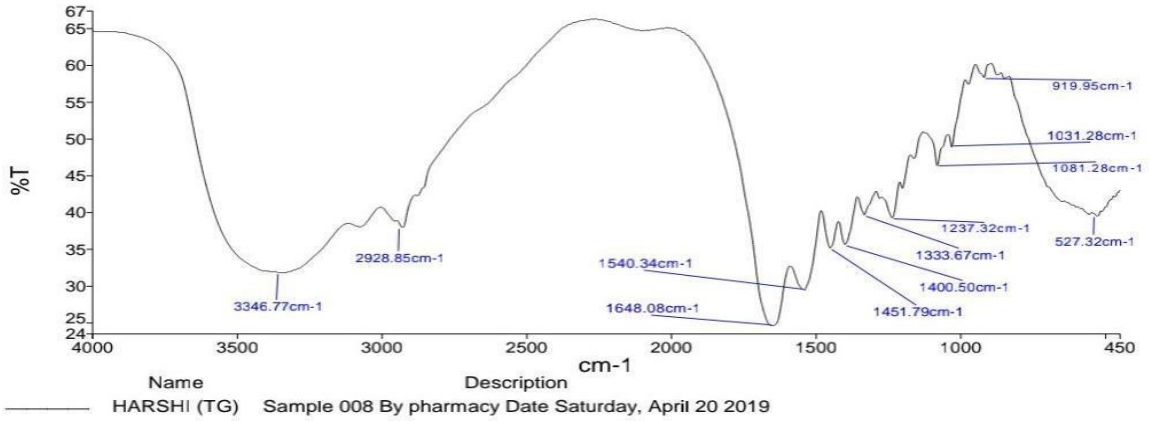

Fig.10: FTIR of film after thermal crosslinking

FTIR ANALYSIS

The IR spectra of the samples before heating consisted of the characteristic spectra at 1380-1460cm-I. There is a difference in the IR spectra of the heated gelatin from those of the unheated samples. When gelatin is heated above 140°C gelatin gradually changes into the insoluble state with the formation of a structure characterized by the presence of a large number of inter-chain cross-linkages.

CONCLUSION

Gelatin was extracted from poultry waste using the alkaline-acid treatment method, and its characteristics, identification, and properties were investigated. Positive results were obtained for the detection of gelatin in the extracted gel. Evaluation and comparison of the properties and characteristics of extracted gelatin with those of commercial gelatin. The majority of the physiochemical features and characteristics of extracted and commercial gelatin were identical. Commercial gelatin had a significantly lower peak in UV-visible spectroscopy, however both gelatin spectra's peaks in FTIR spectroscopy were strikingly similar. Investigation into the impact of varying and constant temperatures on the development of gelatin films revealed a pronounced effect of temperature on gelatin films. Thermal crosslinking carried via heating improved the mechanical property of the gelatin films and its response towards solubility. The research draws the conclusion that gelatin obtained from poultry waste can be deployed as a replacement for commercial gelatin in the food industry and is an effective substitute for mammalian gelatin. According to statistics, consumption of poultry meat in 2021 in India is 4107 thousand metric tons of poultry meat out of which 25–30 % is inedible waste generated from slaughtering. With the massive consumption and average increase in per capita consumption rate is 16% over last five years, the underutilized waste by-products such as gizzard, skin, viscera, skull, and feet could be a severe issue in terms of disposal and pollution. Chicken feet, on the other hand, are rich in collagen, which on degrading yields gelatin. Gelatin's extensive field of application can improve our country's economy and even help in waste management of by-products. Furthermore, India, as a secular and liberal country, has an immense diversity in religious beliefs and customs. Some gelatin sources, such as bovine for Hindu community and porcine for Muslims are forbidden meats associated with religious beliefs.

Gelatin's ability to create biocompatible materials is its most important feature. It is an excellent paradigm for developing cell-compatible, biodegradable polymers. Gelatin has several advantages over carbohydrate-based gelling agents. It offers a wide range of study topics to investigate and work on. Future research can concentrate on processing temperature-related changes in gelatin films as well as examining how gelatin changes at certain, fixed temperatures.

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