



PRODUCTION OF PEPTONE FROM ALKALINE AND ACIDIC HYDROLYSIS OF CHICKEN FEATHER KERATIN AND USE IT AS AN ALTERNATIVE SOURCE OF NITROGEN TO PREPARE SOME CULTURE MEDIA

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ABSTRACT

The study aimed to reduce environmental pollution caused by chicken waste and exploit the high concentration of keratin in it, and analyze alkaline and acidic, and using it in preparing expensive peptone and using it as an alternative to nitrogen sources in some culture media to grow some microorganisms. The alkaline and acidic hydrolysis process of white chicken feather powder was applied using different concentrations of sodium hydroxide (NaOH) and hydrochloric acid (HCL). The results showed that the highest percentage of keratin was (81.2, 72)% when using the NaOH and HCL at a concentration of 4 molar. However, the highest degree of hydrolysis (35, 28)% for the NaOH and HCL prepared at a concentration of (2 and 3) molar respectively. The highest solubility was (48, 38)% and water holding capacity (3.1, 2.2) g/g respectively. The NaOH and HCL gave the highest solubility were used in preparing the modified Nutrient agar and MacConky agar media by replacing the nitrogen sources in them with these hydrolysates. The numbers of *Bacillus subtilis* (*B.subtilis*) reached (105, 87, 77) × 10⁵ C.F.U/ml. and *Staphylococcus aureus* (*S.aureus*). (95, 75, 64) × 10⁵ C.F.U/ml. using the commercial Nutrient agar and the modified media using the alkaline and acidic hydrolysates, while the numbers of *Escherichia coli* (*E.coli*) reached (69, 51, 42) × 10⁵ C.F.U/ml. and *Salmonella typhi* (*S.typhi*) (46, 36, 27) × 10⁵ C.F.U/ml. on the commercial MacConky agar modified with the alkaline and acidic hydrolysates.

Key words: Chicken waste, Environmental pollution, Microbiology development, White chicken.

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انتاج البيبتون من المتحللات القاعدية والحامضية لكيراتين ريش الدجاج واستعماله بديلا لمصدر النتروجين في تحضير بعض الأوساط الزرعية

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الخلاصة

هدفت الدراسة الحالية الى التقليل من التلوث البيئي المتسبب عن مخلفات الدجاج واستغلال التركيز العالي للكيراتين الموجود فيها وتحليله قاعديا وحامضيا للاستفادة من متحللاته في تحضير البيبتون غالي الثمن واستعماله بديلا لمصادر النتروجين في بعض الأوساط الزرعية لتنمية بعض الاحياء المجهرية. اجريت عملية التحلل القاعدي والحامضي لمسحوق ريش الدجاج الابيض باستعمال تراكيز مختلفة من هيدروكسيد الصوديوم وحامض الهيدروكلوريك، وظهرت النتائج ان اعلى نسبة منوية لحصيلة الكيراتين بلغت (81.2، 72) % عند استعمال القاعدة والحامض بتركيز 4 مولاري، في حين بلغت اعلى درجة تحلل (35، 28) % للمتحلل القاعدي والحامضي المحضران بتركيز (3 و 2) مولاري على التوالي واعلى ذائبية بلغت (48، 38) % وقابلية حمل الماء (3.1، 2.2) غم/غم على التوالي استعملت المتحللات القاعدية والحامضية التي اعطت اعلى ذائبية في تحضير وسطي Nutrient agar و MacConky agar المحورين باستبدال مصادر النتروجين فيها بهذه المتحللات بلغت اعداد بكتريا *Bacillus subtilis* وحدة مستعمرة/مل $10^5 \times (105، 87)$ و *Staphylococcus aureus* وحدة مستعمرة/مل $10^5 \times (75، 95، 64)$ باستعمال وسط Nutrient agar التجاري والأوساط المحورة باستعمال المتحلل القاعدي والحامضي، في حين بلغت اعداد بكتريا *Escherichia coli* وحدة مستعمرة/مل $10^5 \times (69، 51، 42)$ و *Salmonella typhi* وحدة مستعمرة/مل $10^5 \times (46، 36، 27)$ على وسط MacConky agar التجاري والمحور بالمتحلل القاعدي والحامضي.

الكلمات المفتاحية: مخلفات الدجاج، التلوث البيئي، تنمية الاحياء المجهرية، الدجاج الابيض.

INTRODUCTION

Culture media are the environment that supports the growth of microorganisms or cells. It generally contains a suitable source of energy and compounds that regulate the cell cycle. A typical culture media consists of a combination of amino acid, vitamins, inorganic salts, glucose, hormones, and growth factors. The media also helps maintain pH. (Arora, 2013; Al-shamary et al., 2023). Microorganisms have different physiological properties and nutritional requirements and therefore cannot be grown in one type of culture media. The choice of culture media is very important and greatly affects the success of microorganism cell development experiments. The development of microorganisms is essential for diagnosing infectious diseases, obtaining antigens, genetic studies, and identifying microbial species. (Tankeshwar, 2021). Cultural media vary in their consistency, chemical composition, and purpose of use. Some are solid, liquid, and semi-solid. Containing limited amounts of nutrients or being rich in their nutrients. It is used to differentiate between different microorganisms or select specific microorganisms (Fatima, 2022). The world's population is increasing significantly, and due to this increase there is a continuous demand for food products especially meat, which is an important source of protein in the diet. Poultry production and consumption have increased as well (Ramalingum, 2021; Al-Sahoo & Al-Badri, 2016). Recent studies by the US Department of Agriculture showed that global poultry meat production for 2024 was estimated at about 103.3 million tons. The increase in production has led to the generation of waste from chicken slaughterhouses, including feathers, legs and heads, in addition to the blood resulting from the



slaughtering process All of these wastes affect the environment and cause pollution and health risks due to the spread of gases and diseases (Scanes *et al.*, 2024). and given the increasing demand for culture media to develop microorganisms with the emergence of microbial biotechnology. Since commercially available culture media For the cultivation of expensive microorganisms, peptone, which is the source of nitrogen, is the most expensive component in the culture media (Akporube *et al.*, 2023). Keratin has contributed to the development of microorganisms due to its high nitrogen content of up to 18% and various amino acid and its low cost in the development of different types of microorganisms such as *E.coli*, *S. aureus*, and others. In addition, to being an environmentally friendly method, keratin decomposers work as an alternative source of nitrogen in traditional media such as peptone to improve growth and metabolism and enhance cell adhesion and spread better than traditional substrates (Li, 2021; Al-Sa'ady & Aldulaimi *et al.*, 2023). In addition to its importance in the fields of agriculture, pharmaceutical industries, cosmetics, and leather industries (Feroz *et al.*, 2020), natural sources of keratin include chicken feathers, wool, hair, claws of animals, and horns (Donato & Mija, 2019). Keratin is utilized through chemical and enzymatic hydrolysis that requires breaking sulfur bonds to remove insoluble keratin while maintaining the covalent bonds of the primary protein chain (Shavandi *et al.*, 2017).

Based on that, the study aimed to reduce environmental pollution by employing chicken feathers and exploiting their high content of keratin in the preparation of alkaline and acidic hydrolysates and their use as a source of nitrogen in some culture media to grow some microorganisms instead of expensive peptone.

MATERIALS AND METHODS

Preparation of feather powder

Chicken feathers were collected from local markets form Baghdad city during the period from 10/7 to 22/8 of the year 2023. Then, the feathers were washed with water and laundry detergent to get rid of blood and dust residues, and feather powder. After cleaning, the feathers were dried using a Sonifer dryer at 65°C. The feathers were ground Use a Panasonic meat grinder to get chicken powder, then save the powder until use (Thanoon *et al.*, 2018).

Chemical composition of chicken feather powder

Estimation of Moisture

The moisture percentage in chicken feather powder was estimated according to the method described in (AOAC, 2010) and according to the equation below:

$$\text{Moisture \%} = \frac{\text{weight of sample before drying} - \text{weight after drying}}{\text{weight of sample before drying}} \times 100$$

Estimation of Ash

The ash percentage in feather powder was estimated according to the method described (AOAC, 2010) and according to the following equation:

$$\text{Ash \%} = \frac{\text{weight of residue} \times 100}{\text{weight of sample}}$$



Estimation of Protein

The protein percentage in chicken feather powder was estimated according to Kjeldahl method described (**Dijk, & Houba, 2000**) as follows: 0.5 g of the sample has been put in digestion flask, with an appropriate amount of Catalyst Kjeldahl. Then, add 5 mL of sulfuric acid (98%), prepare Blank from the above-mentioned materials without adding the sample, then the digestion process was applied by placing the digestion tubes in the heating unit and gradually raising the temperature until reaching 420 °C.

The sample turned to a light blue color, and then cooled to room temperature. The distillation process was applied in the distillation flask containing a 40% sodium hydroxide solution, then a 20% boric acid solution was added. It was placed under the condenser, making sure to immerse the condenser in the solution, then drops of the reagent solution were added. The titration process was applied with a 0.1M hydrochloric acid solution with 25 ml of distillation products until the color of the solution turned pink, and the percentage of protein was calculated from the following equation:

$$\text{Protein \%} = \frac{(b-a) \times 0.1 \times 14 \times 100 \times 6.25}{w \times 1000}$$

Estimation of fat

The percentage of fat in chicken feather powder was measured according to the method (**AOAC, 2010**), and according to the following equation:

$$\text{Lipid \%} = \frac{\text{weight of lipid} \times 100}{\text{weigh of sample}}$$

Estimation of carbohydrates

The percentage of carbohydrates in chicken feather powder was estimated according to the method (**FAO, 2020**) from the following equation:

$$\text{Carbohydrate \%} = 100 - (\text{Moisture} + \text{Protein} + \text{Ash} + \text{Lipid})$$

Preparation of the alkaline hydrolysates of chicken feather powder

The method described by (**FARAON *et al.*, 2023**) has been used. Twenty g of feather powder per experience was dissolved in 200 ml of sodium hydroxide solution (1,2,3,4) M at 90-95 °C for 60 min with continuous stirring. The pH was adjusted to 4.4 gradually using hydrochloric acid (1M) at 15 °C, then the centrifugation process was carried out at a speed of 8500 times the ground acceleration for 20 min, the washing process was carried out with water, the sample was then dried and preserved.

Preparation of the acidic hydrolysates of feather powder

The method described by (**FARAON *et al.*, 2023**) was followed, as 30 g of chicken feather powder per experience was dissolved in 200 mL of HCL (1,2,3,4)M at a temperature of 95°C for 60 min with continuous stirring. The resulting solution was filtered and the pH was adjusted to 4.4 gradually using sodium hydroxide solution (1M), then the centrifugation process was carried out at a speed of 8500 ×g for 20 min, and the washing process was applied with water. The sample was dried and stored until the protein concentration was estimated.

yield

The yield was calculated based on the method followed by **Maurya & Singh, (2024)** and according to the following equation:

$$\text{Percentage yield} = \text{weight of extracted keratin} / \text{total weight of chicken feathers} \times 100$$



Estimate the degree of hydrolysis

The method described by (Rahmi *et al.*, 2022) was used to estimate the degree of decomposition. 2 ml of the decomposed were mixed with 2 ml of TCA solution at a concentration of 20% (w/v) in 10 ml tubes, the mixture was left for 30 minutes, then a centrifugation process was carried out at a speed of 5000 times the earth's acceleration for 30 minutes. The Kjeldahl method was used to estimate the percentage of protein in the mixture and the floating fraction, and the degree of hydrolysis was calculated according to the following equation:

$$DH\% = (10\% \text{ TCA soluble nitrogen in the sample}) / (\text{Total nitrogen in the sample}) \times 100$$

Solubility

The method mentioned by (Jain & Anal, 2016) was adopted to estimate the solubility of the hydrolysates as follows:

The hydrochloric acid solution (0.1M) and the sodium hydroxide solution (0.1M) were used at pH (2, 4, 6, 7, 8, 10 and 12) to adjust the pH of the protein decomposer solution prepared by dissolving 200 mg of the decomposer powder, each separately, with an amount of distilled water, then the volume was completed to 20 ml. Then the solutions were mixed for 30 minutes at a speed of 150 rpm, and then the centrifugation process was carried out at a speed of 8000 ×g for 20 minutes. The supernatant was collected, and the percentage of protein was estimated using the Kjeldahl method, and the percentage of solubility was calculated from the following equation:

$$\text{Solubility \%} = (\text{protein content in the supernatant}) / (\text{total protein content in the sample}) \times 100$$

Water holding capacity:

The water holding capacity was estimated according to the method (Onsaard *et al.*, 2010) by dissolving 1 g of the hydrolysates powder with 20 ml of distilled water with mixing for 5 minutes, adjusting the pH to (4, 7, 10, 12) and leave it for 15 minutes at room temperature, and centrifuging was applied at a speed of 10,000 ×g for 10 minutes. Water holding capacity was defined as the weight of water that absorbs 1 g of protein hydrolysates.

Preparation of alternative culture media using feather chicken hydrolysates

The feather chicken hydrolysates prepared above were used as an alternative to the nitrogen source in preparing some culture media to grow some Gram-positive and Gram-negative bacterial species as follows:

Microorganisms: *B.subtilis*, *S.aureus*, *S.typhi*, and *E.coli* were obtained from the Biotechnology Laboratory, College of Agricultural Engineering Sciences, Baghdad University.

The culture media used in the study:

Nutrient agar, MacConky agar, and alternative media were used by replacing the nitrogen source with basic and acid hydrolysates of chicken feather powder. All culture media were sterilized by an autoclave, at 121°C and a pressure of 15 pounds/inch², for 15 minutes. Bacterial growth on commercial and alternative media: The pouring plate method described by (Ahmed & Al-Shamary, 2019) was used to growth different bacterial species, where 1 ml of each of the previously prepared decimal dilutions of different bacterial species was transferred to Petri dishes, and the commercial Nutrient agar and its alternative medium were poured into the dishes containing *B. subtilis* and *S.aureus* and mixed well and left to solidify, then the dishes were incubated at 37 °C for 48 hours. The commercial MacConky agar and its



alternative were poured into the dishes containing *S.typhi* and *E. coli* and mixed well and left to solidify, and then the dishes were incubated at 37 °C for 48 hours. Colonies were counted using a colony counting device.

RESULTS AND DISCUSSION

Chemical composition of chicken feather

The chemical composition of chicken feather powder showed that the percentage of moisture, protein, ash, fat and carbohydrates were (4.12, 87.05, 5.12, 1.01 and 2.70)% respectively (Table 1). It is noted from the results that the percentage of protein, which is mainly keratin, is high compared to other components of feathers, which encourages its exploitation and use as a source of protein and amino acid in various fields, including its use as an alternative, inexpensive source of nitrogen in the culture media used in the development of different types of microorganisms.

Table (1): Chemical composition of chicken feathers.

Chemical composition	Percentage (%)
Moisture	4.12
Protein	87.05
Ash	5.12
Fat	1.01
Carbohydrate	2.70

There are many studies that dealt with the chemical composition of chicken feather powder. **Tesfaye et al., (2017)** indicate that chicken feather powder consists of (5.4, 82.36, 1.49, 2.86 and 7.89)% which that moisture, protein, ash, fat and carbohydrates, respectively. **Oladele et al., (2024)** found that chicken feathers contain (11.5, 75.80, 1.33, 0.93 and 2.67)% moisture, protein, ash, fat and carbohydrates, respectively. Santos *et al.*, (2024) also indicated that the chemical composition of chicken feathers were (9.24, 85.92, 2.95, 2.01 and 0.89)% for moisture, protein, ash, fat and carbohydrates, respectively. The reason for the difference in the chemical composition of chicken feathers is due to the diet of chickens (**Munagala et al., 2022**) and the difference in chicken species and environmental conditions (**Tesfaye et al., 2017**).

yield

The results showed that the percentage of keratin yield obtained from alkaline and acidic hydrolysates of chicken feather powder, as an increase in the yield is observed with increasing concentration. The highest yield was obtained (81.2 and 72)% when using sodium hydroxide and hydrochloric acid with a molarity of 4 (Figure 2 and 3).

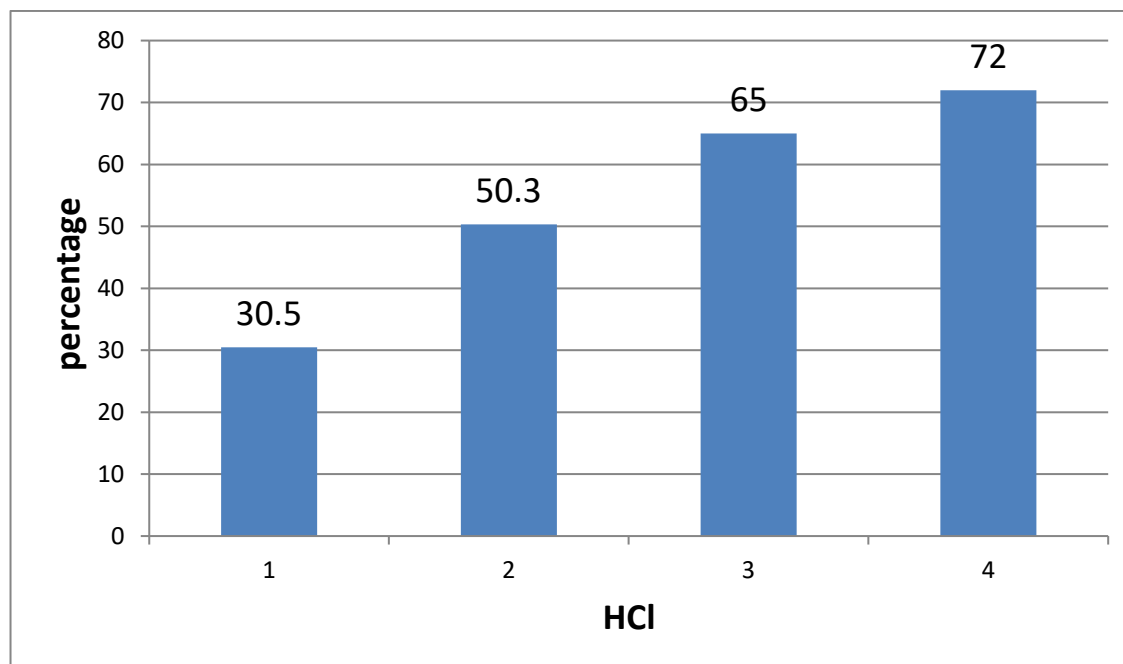


Figure (2): percentage yield using different concentrations of hydrochloric acid and at a temperature 90-95°C after 60 mins.

Faraon, et al. (2023) have recorded that a percentage of alkaline and acid hydrolysis of 75.5% and 64.6% when using sodium hydroxide and hydrochloric acid at a concentration of 1 and 4 M. It explained that the alkaline hydrolysis gave a precipitation percentage of up to 50% at the electrical neutrality point of keratin, while the acidic hydrolysis was 60%, indicating that the molecules resulting from acidic hydrolysis have a higher molecular weight compared to the bases decomposition. **Skergt et al (2023)** indicated that high temperatures with increased extraction time increase the extraction percentages and may reach 100%, but this leads to obtaining dark yellow or dark brown keratin.

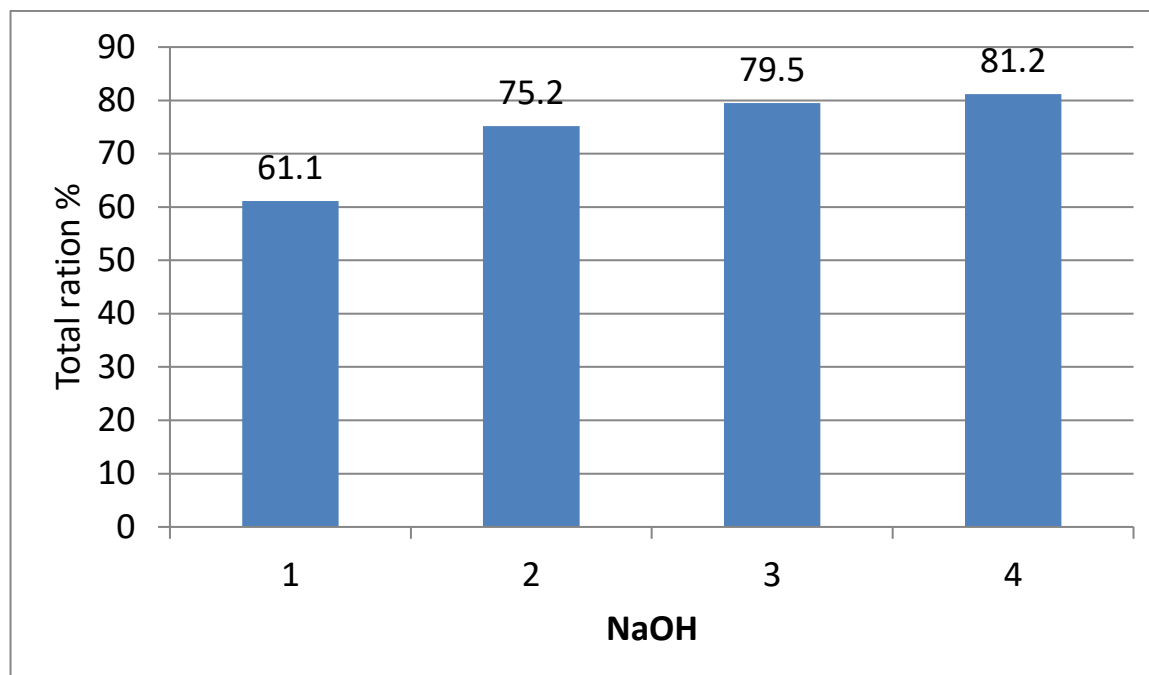


Figure (3): percentage of yield using different concentrations of sodium hydroxide and at a temperature 90-95°C after 60 mins.

Sinkiewicz *et al* (2017) confirmed that temperature helps to obtain a higher yield, but at the same time it leads to an increase in the destruction of amino acid. **Raydan *et al* (2024)** indicated the effect of the base concentration on the total. It was found that the concentration of sodium hydroxide 1% with a temperature of 64 °C was the most conservative of the keratin. The higher concentration of the base contributes to the deterioration of keratin and a decrease in its yield, in addition to that, rapid and equal precipitation of keratin occurs, while when using sodium hydroxide at a concentration of 5%. It showed slower and larger precipitation and darker color upon drying, which confirms that the high concentration of the base leads to the destruction of keratin into small peptides.

Degree of hydrolysis of alkaline and acidic

The percentage of the degree of alkaline and acidic hydrolysis of chicken feather powder keratin has studied. It is noted that the highest degree of hydrolysis is obtained at (35, 28)% respectively, with a decrease in the degree of hydrolysis being noted with an increase in the concentration of alkaline and acid despite the increase in the percentage (Figure 4 and 5).

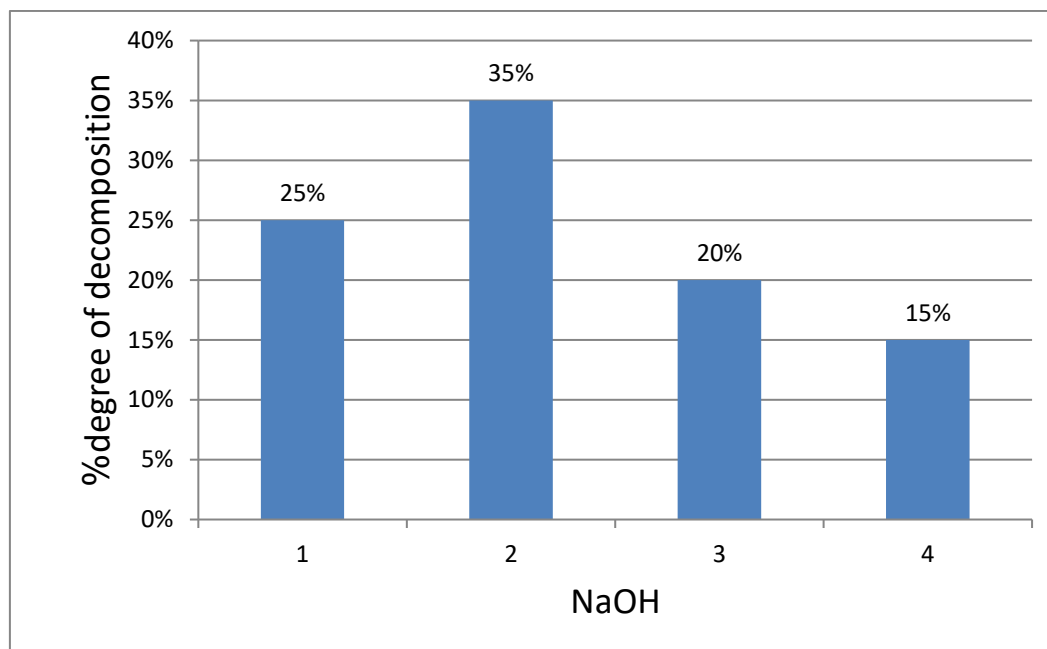


Figure (4): percentage of degradation of keratin extracted using different concentrations of sodium hydroxide

Ossai et al. (2022) ;Sommer et al (2024) indicated that the alkaline hydrolysis process leads to the decomposition of the disulfide bond (S-S) that undergoes oxidation and reduction reactions, resulting in low molecular weight peptides and amino acid. **Sinkiewicz et al (2017)** reported that the possibility of obtaining soluble keratin through basic, acid or enzymatic hydrolysis, through oxidation, reduction, thermal treatments, some organic solvents and some other methods. It explained that chemical hydrolysis leads to the destruction of the natural structure of keratin, which leads to feather hydrolysates becoming more soluble in water. It was indicated that the hydrolysis process depends on the pH, temperature and reaction time as well as the type and concentration of the base and acid used. It also explained the possibility of losing some amino acid during acid hydrolysis such as tryptophan, while basic hydrolysis is less loss of amino acid.

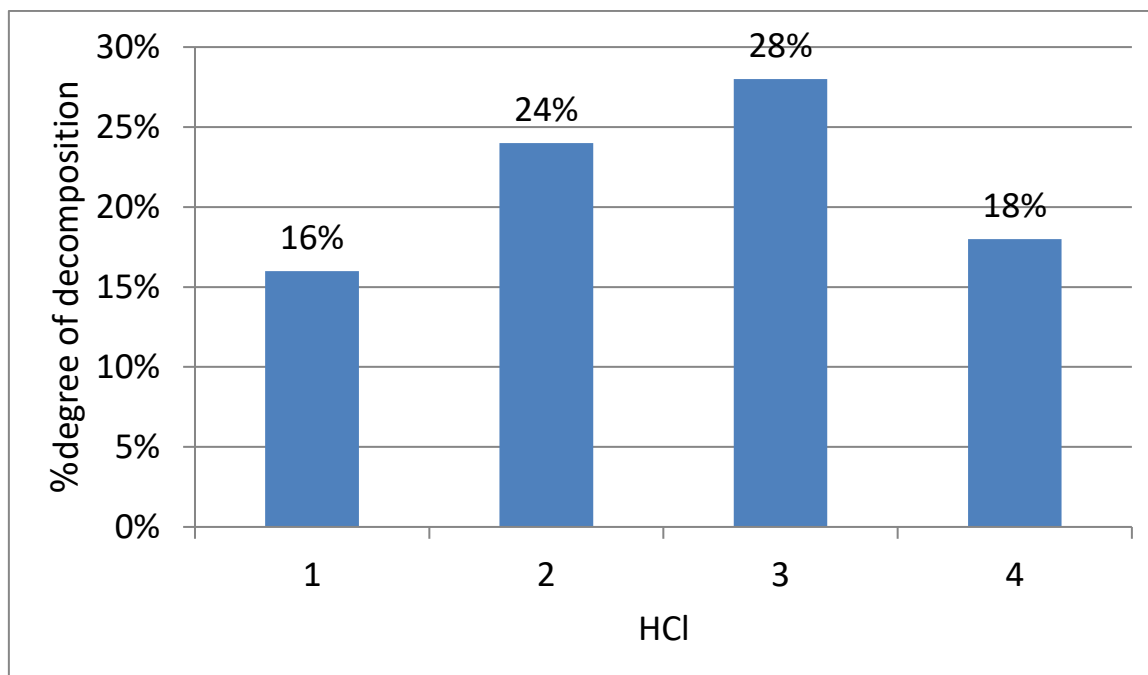


Figure (5): percentage of degradation of keratin obtained using different concentrations of hydrochloric acid.

Tursunova & Maksumova, (2024) found that the possibility of breaking down asparagine, glutamine and tryptophan during basic decomposition. **Raydan *et al.* (2024)** have explained that acid decomposition leads to the breakdown of essential amino acid such as Methionine, Cysteine and Tryptophan and converts glutamine and asparagine to glutamic acid and aspartic acid.

Solubility

Figures (5) and (6) show the percentage solubility of alkaline and acidic hydrolysates that obtained from different molarities of NaOH and HCl.

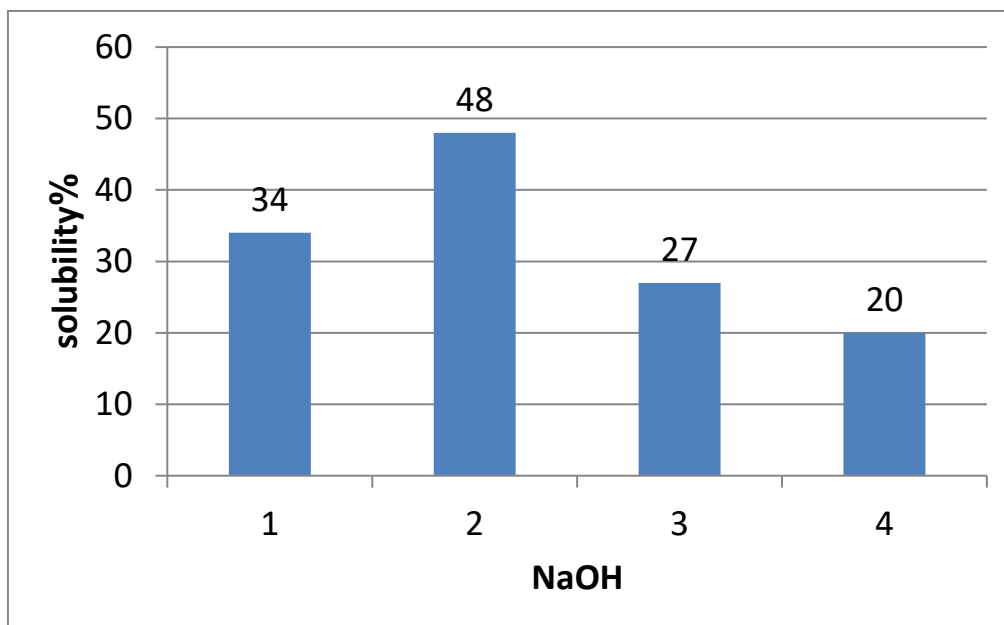


Figure (5): Solubility percentage of alkaline hydrolysis of chicken feather powder keratin.

It is noted that the percentage of solubility increases with increasing concentration of acid and base to reach the highest solubility (48, 38)% at concentrations of (2 and 3) molarity of NaOH and HCl respectively, then decreases again at concentrations higher than that. **Said et al. (2019)** indicated that basic solutions (NaOH) are compounds that break the bonds of protein compounds in feathers. These changes can increase solubility due to the increase in the content of NH_3^+ and COO^- and reduce the molecular weight. However, increasing the decomposition process due to the chemical reaction can lead to a decrease in the solubility of the protein. This also happens with amino acid.

Protein hydrolysis is the process of breaking down the peptide bonds of the protein into smaller compounds such as peptides and amino acid. This decomposition process will lead to denaturation of the protein, which leads to a change in shape, which leads to a change in solubility. This is because to changes in the nanostructure of the peptide chain, which is followed by a reduction in the molecular weight. The denigration process occurs due to the effect of chemical compounds such as acid and alkaline.

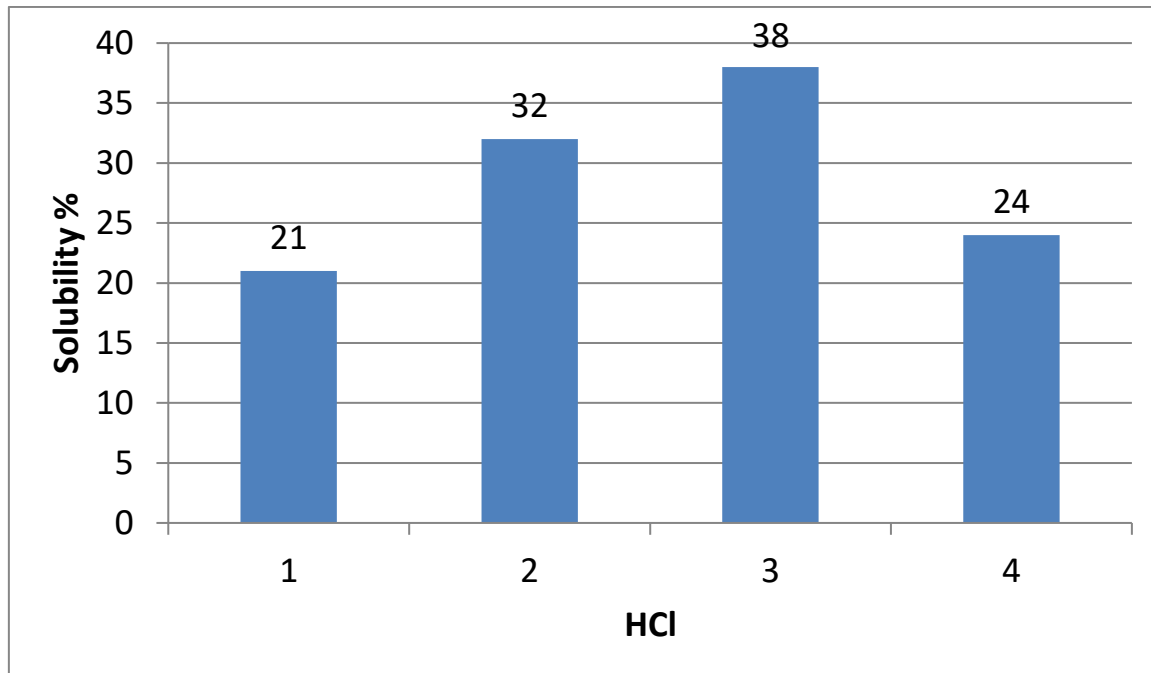


Figure (6): percentage of solubility of acid hydrolysis of chicken feather powder keratin.

Water holding capacity

The figure (7) shows the water holding capacity of the acid and alkaline of chicken feather powder keratin prepared using sodium hydroxide and acid with molarity of 2 and 3, which were (3.1, 2.2) g/g, respectively.

Prajapati *et al.* (2021) showed that increasing the concentration of polar groups such as NH_2 and COOH resulting from the decomposition process has a significant effect on the water holding capacity of chicken feather keratin decomposition. Water holding capacity is an important property of proteins in food processing, as it directly affects the texture, color, appearance and storage life of the food material. It also has a significant effect on water binding in culture media prohibited for the growth of microorganisms.

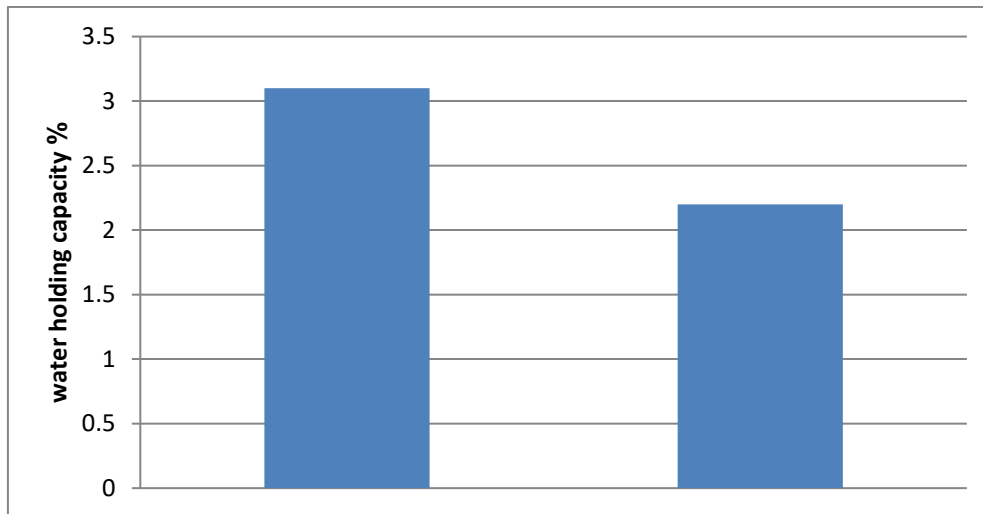


Figure (7): Water holding capacity of acidic and alkaline hydrolysates of chicken feather powder keratin.

Use of acidic and alkaline hydrolysates in preparing culture media for growing microorganisms

The numbers of *B.subtilis* and *S.aureus* on the solid nutrient media and the modified solid nutrient medium by replacing the nitrogen source with the alkaline and acid of chicken feather powder hydrolysates prepared using sodium hydroxide and hydrochloric acid at a concentration of 2 and 3 molar. The numbers of *B.subtilis* and *S.aureus* (105, 87 and 77) Conony and (95, 75 and 64) Conony on the commercial Nutrient agar media modified by adding the base and acid, respectively, while the numbers of *E.coli* and *S.typhi* reached (69, 51, 42) and (46, 36, 27) respectively. The results indicate the possibility of using bases and acid of chicken feather keratin as an alternative to peptone in the development of microorganisms in the study.

Table (1): shows the numbers of bacteria on Ready and modified Nutrient agar media

Media	No. of bacteria C.F.U/ ml×10 ⁵	
	<i>B. subtilis</i>	<i>S. aureus</i>
Nutrient agar	105	95
Nutrient agar modified by adding alkaline of chicken feather hydrolysates	87	75
Nutrient agar modified by adding acidic of chicken feather hydrolysates	77	64
L.S. D	*8.927	*7.661
(P≤0.05)		



Raydan *et al.* (2024) indicated that the salts formed during the acid decomposition of keratin may affect the growth of microorganisms. They also indicated that the alkaline hydrolysates of chicken feather powder, which was neutralized with different acid, gave growth rates close to the microorganisms under study including *S.aureus*, *E.coli*, *Proteus mirabilis*, *Pichia hudriavzevii*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Candida tropicalis*, and *Candida carpophila*, compared to the use of commercial peptone, and sometimes gave better results than commercial peptone.

Table (2): shows the numbers of bacteria in the standard and modified MacConkey medium.

Media	No. of bacteria C.F.U/ml×10 ⁵	
	<i>E.coli</i>	<i>S.typhi</i>
MacConky agar	69	46
MacConky agar modified by adding alkaline of chicken feather hydrolysates	51	36
MacConky agar modified by adding acidic of chicken feather hydrolysates	42	27
L.S.D	*7.485	*5.977
(P≤0.05)		

CONCLUSIONS

The study concludes that chicken feather waste is a polluting burden to the environment that can be converted into an important biological resource and used widely as a biotechnological material. The study focused on confirming the possibility of using alkaline and acidic hydrolysis of chicken feather keratin as an alternative source of nitrogen in some culture media to grow *B. subtilis*, *S. aureus*, *E. coli* and *S. typhi*. Therefore, the production process can be carried out on a commercial level and used as an alternative source of nitrogen in different culture media.

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