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Production of Peptone from Enzymatic Hydrolysates of Chicken Feather Keratin and Use as an Alternative Source of Nitrogen in the Preparation of Some Medium

Yasmin.k.AL-rubaie[1], Elham.I.AL-Shamary[2]

[1,2]Dept. Food Sci. Coll. Agri.Eng.Sci., University of Baghdad
Email: Yasmin.kareem2202m@coagri.uobaghdad.edu.iq[1], elham.ismael@coagri.uobaghdad.edu.iq[2]

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ABSTRACT
The study aimed to use chicken feathers to reduce environmental pollution in the preparation of expensive peptone and use it as an alternative source of nitrogen in the preparation of some media for the growth of some microorganisms. The results showed that the chicken feather powder contained 4.12, 87.05, 5.12, 1.01 and 2.70% moisture, protein, ash, fat and carbohydrates. The results also showed that the keratin was decomposed by pepsin and chymotrypsin enzymes, and the best degree of decomposition obtained was 30% after 30 hours of decomposition using chymotrypsin enzyme. It was also found that the lowest solubility was obtained at pH (3, 4) and the best solubility was (30, 39, 50, 50.3 and 50.6) at pH numbers (5, 6, 7, 8 and 9). It was also noted that the highest water holding capacity was 3.50 at pH 7 for keratin hydrolysate using chymotrypsin enzyme after 30 hours of hydrolysis. The numbers of Bacillus subtilis and Staph aureus reached (141, 117) × 10^5 and (100, 89) × 10^5 and (135, 110) × 10^5 in the solid nutrient medium and the modified media with the addition of keratin hydrolysate after 24 and 30 hours of hydrolysis using chymotrypsin enzyme. However, the numbers of E.coli and Salmonella typhi reached (75, 54)× 10^5, (34, 14)× 10^5, (54, 35)× 10^5 respectively, in the commercial and modified MacConky agar medium.

Key words: peptone, enzymatic hydrolysates, chicken, keratin, mediu

INTRODUCTION

The media provide the nutritional, physical and chemical requirements that ensure the growth and reproduction of microorganisms. The requirements of microorganisms are different according to their types, physiology and the metabolic pathways and enzymes they possess. It is reflected in the great diversity in the components of the culture media used in the development of different types of them. The number of media increases with the increased discovery of different types of microorganisms, but despite their diversity. They contain a source of carbon and energy, a source of nitrogen and other requirements according to the needs of the microorganism (17,2).

The source of nitrogen varies according to the media, and peptone is the most important and expensive. Thus, there is an increasing demand for peptone, which is cheap and effective in the growth and reproduction of microorganisms, especially those used in the industrial production of various materials in various fields, including medicine, pharmaceuticals, industry and food. It represents the final product of protein degradation by various methods including enzymatic and acidic, which results in different peptides in the length of the chain and in their amino acid content. It affects the growth of microorganisms. Therefore, attention must be paid to choosing the appropriate source and the appropriate method for preparing peptone (30,8).

Annually, the amount of chicken consumed is constantly increasing compared to other types of meat. The increase in chicken production was certainly accompanied by an increase in the amount of waste produced, such as feathers, blood, internal organs, meat remains, and other inedible parts (32,3). Chicken feathers are a major source of poultry slaughterhouse waste. Millions of tons of feather biomass are produced each year worldwide (14).As a result of the increased demand for poultry meat; and the increase in the standard of living of the Iraqi individual(1).

Chicken feathers are usually disposed of either by burial, burning or dumping, which creates major problems affecting environmental pollution as well as human diseases such as cholorosis, fowl cholera (27).

Chicken feathers consist of stable structures due to the abundance of protein, which constitutes 5-7% of the weight of the chicken, and 90% of the protein keratin, which is a biopolymer with unique properties, including its high nitrogen content, which makes it a candidate for use in many fields. It is an environmentally friendly and economical method that reduces pollution and the burden on landfills (23,26). The waste generated as a

result of chicken consumption has increased around the world. For this reason, the costs of the poultry industry has become a real problem, and recently added value to inedible materials, and there are many researches on ways to use them safely and effectively (22). This standpoint and participate in the effective use of chicken waste and reduce its harmful impact on environmental pollution and global warming and achieve some sustainable development goals. Thus, study aimed to benefit from chicken feather keratin and use its enzymatic, acidic and basic decomposers in preparing cheap peptone as an alternative to expensive commercial peptone and using it in preparing different culture media at a low cost to grow some bacterial species.

MATERIALS AND METHODS

Preparation of feather powder

Collecting chicken feathers and Preparating its powder

White chicken feathers were collected from local markets in Baghdad city for the period from 10/7 to 22/8/2023. Chicken feathers were washed with water to remove dust residues, waste and other stuck dirt. The washing process was applied several times with water.After cleaning, the feathers were dried using Sonifer dryer at 65°C. The feathers were ground using a Panasonic meat grinder to obtain chicken feather powder, which was stored in clean bags until use (29).

Estimation of Moisture

The moisture percentage in chicken feather powder was estimated according to the method described in (7), and according to the equation below:

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

Estimation of Ash

The ash percentage in feather powder was estimated according to the method described (7) 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 (11) as follows:

The 0.5 g of the sample has been put in digestion flask, with an appropriate amount of Catalyst Kjedahl. Then, add 5 ml of sulfuric acid, 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, 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 0.1-molar 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 the percentage of fat

The percentage of fat in chicken feather powder was measured according to the method (7), and according to the following equation:

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

Estimation of the percentage of carbohydrates

The percentage of carbohydrates in chicken feather powder was estimated according to the method (FAO, 2003) from the following equation:

Carbohydrate % = 100 - (Moisture + Protein + Ash + Lipid)

Keratin extraction and purification

The 50 g of feather powder was added to a solution of sodium sulfide (0.5 M) at a 30 °C and a pH 10-13. Continuous stirring using a magnetic stirrer to dissolve the protein, the solution was filtered and centrifuged at a speed of 10,000 times the ground acceleration for 5 minutes.The protein was precipitated by the gradual addition of a solution of ammonium sulfate 70% at a ratio of 1:1. The centrifugation was carried out at a speed of 10000 times the ground acceleration for 5 minutes, the precipitate was collected and the subsequent steps

were completed.The 100 ml of distilled water was added to the precipitate with continuous stirring using a magnetic stirrer, then centrifuged at a speed of 10000 times the ground acceleration for 5 minutes and collected the precipitate, then dissolved again in 100 ml of 2 m sodium hydroxide solution, then centrifuged at a speed of 10000 times the ground acceleration for 5 minutes and collected the filtrate, and the dialysis process was carried out using a dialysis bag (8000-14000) Dalton, activated before use. The filtrate was placed inside the dialysis bag for 24 hours, taking into account changing the distilled water every 6 hours and drying the solution to obtain keratin powder(13).

Study of the chemical composition of keratin

The percentage of moisture, ash, protein, fat and carbohydrates was estimated according to the methods mentioned above.

Keratin hydrolysates using pepsin enzyme

Keratin was mixed at a ratio of 4:1 with distilled water in 50 ml flasks, using hydrochloric acid (2M) to adjust the pH to 2. Then, the flasks were placed in a water bath at 85°C for 15 minutes, cooled to 37°C, then pepsin enzyme 2500 units/mg was added, at a ratio of 1.5 g/100 g of keratin, and the flasks were placed in a shaking incubator at 37°C. The samples were withdrawn after (24 and 30) hours, and placed directly in a water bath at 85°C for 15 minutes to inhibit the activity of pepsin enzyme, and were dried, and stored until use.

Keratin hydrolysates using chymotrypsin enzyme

Keratin was mixed at a ratio of 4:1 with phosphate buffer solution (8pH) in 50 ml flasks, placed in a water bath at 85°C for 15 minutes, then cooled to 37°C, and the enzyme was added, at a ratio of 1.5 g/100 g of keratin. The flasks were placed in a shaking incubator at 37°C, the samples were withdrawn after (24 and 30) hours. Then, placed in a water bath at 85°C for 15 minutes to inhibit the enzyme activity, then dried and stored until use.

Estimation of the degree of decomposition

The method described by (24) was used to estimate the degree of decomposition, as 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 ground acceleration for 30 minutes. The method was used to estimate the percentage of protein in the mixture and the floating fraction, and the degree of decomposition was calculated according to the following equation:

DH% = (10% TCA soluble nitrogen in the sample)/(Total nitrogen in the sample) X 100

Solubility

The method mentioned by (15) was adopted to estimate the solubility of the decomposers as follows:

Hydrochloric acid solution (0.1M M) and sodium hydroxide solution (0.1M) were used to adjust the pH to (2, 3, 4, 5, 6, 7, 8 and 9) for 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. The solutions were mixed for 30 minutes at 150 rpm, then centrifuged at 8000 rpm the ground acceleration for 20 minutes. The supernatant was collected, and the percentage of protein was estimated. The percentage of solubility was calculated from the following equation:

Solubility % = (protein content in the supernatant)/(total protein content in the sample) X 100

Water holding capacity

Water holding capacity was estimated according to the method described by (20), which dissolving 1 g of the decomposable powder with 20 ml of distilled water with mixing for 5 minutes, and adjusting the pH to (5, 6, 7) and leaving it for 15 minutes at room temperature. The centrifugation process was carried out at a speed of 10,000 times the ground acceleration for 10 minutes. Water holding capacity was defined as the weight of water that absorbs 1 g of protein hydrolysates.

Preparation of alternative medium using protein hydrolysates

Protein hydrolysates of the chymotrypsin enzyme were used after 24 and 30 hours of decomposition time as an alternative to the nitrogen source in preparing some culture media to grow some Gram-positive and Gram-negative bacterial species as follows:

Microbial: Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, and Escherichia coli were obtained from the Biotechnology Laboratory for Postgraduate Studies, College of Agricultural Engineering Sciences.

The culture medium used in the study: Nutrient agar and MacConky agar (commercial medium)and alternative media were used by replacing the nitrogen source in them with chymotrypsin protein hydrolysates after 24 and 30 hours of degradation time and in the same proportions. All culture media were sterilized by an autoclave, at 121°C and a pressure of 15 pounds/inch^2 for 15 minutes.

Bacterial growth on commercial and alternative medium

The poured plate method described by (6) was used to growth different bacterial species. 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 Bacillus subilis and Staphylococcus aureus, mixed well and left to solidify.Then, the dishes were incubated in an incubator at 37 °C for 48 hours, and the commercial MacConky agar and its alternative medium were poured into the dishes containing Salmonella typhi and Escherichia coli, mixed well and left to solidify. The dishes were incubated in an incubator at 37 °C for 48 hours. Colonies were counted using a colony counting device.

RESULTS AND DISCUSSION

Chemical composition of chicken feather powder

The results showed that the chemical composition of chicken feather powder. The percentage of moisture, protein, ash, fat and carbohydrates reached (4.12, 87.05, 5.12, 1.01 and 2.70)% respectively (Table1). It is noted from the results that the percentage of protein. It is mainly represented by keratin is high compared to other components of feathers, which encourages its exploitation and use as a source of protein and amino acids 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 (%)
Moisture4.12 Protein87.05 Ash5.12 Fat1.01 Carbohydrate2.70

The reason for the difference in the chemical composition of chicken feathers is due to the diet of chickens (18) and the difference in chicken species and environmental conditions (28).

Estimation of the degree of keratin degradation using pepsin and chymotrypsin enzymes

The amino acid sequence of solid keratin, to which chicken feather keratin belongs, can be divided into three regions. It is worth noting that cysteine residues are not distributed equally over the three regions, as there are eight cysteine residues in the first region from the N-terminal side, which is represented by twenty-three amino acids and is the region richest in this acid (Table2). However, there is one cysteine residue molecule from the B-Sheet region and two cysteine residue molecules at the hydroxyl end of the C-terminal, and therefore it is necessary to isolate peptides rich in cysteine at the amino end using enzymatic, chemical, thermal or physical methods (25).

Table 2. Degree of enzymatic hydrolysis of keratin using chymotrypsin enzyme

Decomposition times Degree of decomposition%
12 hr.11.7 18 hr.18.6 24 hr.23.4 30 hr.30.0 36 hr.30.1 48 hr.30.2 L.S.D. value5.219 *
* ($P \leq 0.05$).

Table(2) and (3) illustrate the degree of enzymatic degradation of keratin using pepsin and chymotrypsin enzymes at different degradation times. It is noted that the chymotrypsin enzyme is superior and obtains the highest degree of degradation of 30% after 30 hours of degradation time, noting an increase in the degree of degradation with increasing time and a stable degree of degradation approximately after 30 and 36 hours of the enzymatic reaction time for pepsin and chymotrypsin, respectively. However, it notes a clear decrease in the degree of degradation when using pepsin enzyme with an increase in degradation with increasing degradation time and the highest degree of degradation was obtained 9.3 after 24 hours of degradation time.

Prajapat et al., (21) indicated that the degree of degradation is affected by the type of enzyme used and environmental conditions such as temperature and acidity, as the optimal pH for the work of pepsin enzyme was

2 while chymotrypsin was 8. Also, indicated by Wang et al., (31). Lai et al., (16) indicated that enzymes differ according to their source, degree of purity, method of preservation, activity, and efficiency of the degradation process.Enzymes are considered biocatalysts that accelerate highly specialized reactions(4).

Table 3. Degree of enzymatic hydrolysis of keratin using pepsin enzyme

Decomposition times Degree of decomposition%
12 hr.4.8 18 hr.6.0 24 hr.9.3 30 hr.9.2 36 hr.9.2 48 hr. 9.1 L.S.D. value2.074 *
* (P≤0.05).

Solubility

The process of hydrolysis of keratin using different enzymes aims to break peptide bonds and reduce disulfide bonds to obtain keratin parts with low molecular weights. Thus, it is affecting its various properties, including solubility (12). The results shows the solubility of keratin hydrolysates using chymotrypsin and pepsin enzymes at different pH values ranging from (2-9), as it is noted that the lowest solubility was obtained at pH values (3, 4) close to the electrical isoelectric point of keratin and reached (20, 24)% (Table4).

Bragulla and Homberger (9) indicated that its electrical isoelectric point ranges from 5.4 -4.9, while Crisan et al. (10). The solubility of keratin is affected by different pH numbers, and is usually low at pH numbers ranging between (2, 4) and solubility increases towards the basic pH. Due to the breakdown of keratin's sulfur bonds in the basic environment, the difference in the electrical equivalence point may be due to the difference in the methods used to extract keratin. In addition, to the presence of a mixture of decomposers with different molecular weights, which is reflected the difference in the electrical equivalence point.

It is noted from the figures that solubility increases when moving away from pH numbers 3 and 4, and the best solubility was obtained from the neutral pH and in basic solutions, as solubility reached (31, 39, 50, 50.3 and 50.6) at pH numbers (5, 6, 7, 8 and 9) respectively. The decrease in solubility of pepsin enzyme hydrolysates at different pH levels compared to the solubility of chymotrypsin enzyme hydrolysates (Fig 5), and the highest solubility was (15.2, 15.3 and 15.5)% at pH levels (7, 8 and 9). It is noted from the results obtained that keratin hydrolysates after 30 hours of hydrolysis using chymotrypsin and pepsin enzymes have improved solubility properties compared to keratin that does not dissolve in water. The residues of positive and negative ions adsorbed on the outer surface of peptides and proteins cause electrostatic repulsion between protein molecules, which increases the force of repulsion between them and then their diffusion in the aqueous medium(19).

Table 4. Solubility of keratin hydrolysates by chymotrypsin at different pH values

PH No.Solubility%
PH: 230.0 PH: 320.0 PH: 424.0 PH: 531.0 PH: 639.0 PH: 750.0 PH: 850.3 PH: 950.6 L.S.D. value6.751 *
* (P≤0.05).

Table 5. Solubility of keratin hydrolysates by pepsin enzyme at different PH

PH No.Solubility%
PH: 29.0 PH: 34.0 PH: 46.0 PH: 58.0 PH: 610.0 PH: 715.0 PH: 815.0 PH: 915.0 L.S.D. value2.985 *
* (P≤0.05).

Water holding capacity

The results show that the water holding capacity of keratin hydrolysate after 30 hours of hydrplysis and at different pH numbers (5, 6 and 7). It is noted that the highest water holding capacity reached 3.50 g/g at pH 7, while it reached (1.6 and 2.7) g/g at pH numbers (5 and 6), (Table6)respectively, compared to keratin, which has no water holding capacity. This range of pH numbers was chosen to measure water holding capacity because it represents the range for most culture media used in growing microorganisms.

Table 6. Water holding capacity of keratin hydrolysate using chymotrypsin enzyme after 30 hours at pH numbers (5, 6 and 7).

PH No.water holding capacity%
PH: 51.6 PH: 62.7 PH: 73.5 L.S.D. value0.863 *
* (P≤0.05).

(21) found that chicken feather protein hydrolysate has an excellent water holding capacity of 3.35 g/g, and some studies have indicated that polar groups such as CaOH and NH2 resulting from the enzymatic hydrolysis of keratin affect the amount of water absorbed(15). Zou et al., (33) indicated that the ability to retain water is due to the hydrophilic part of the protein.

Uses of enzymatic hydrolysates in the growth of microorganisms

Table 7. Numbers of B. subtilis and S. aureus bacteria on the commercial medium modified using chymotrypsin

Culture MediumNumber of bacteria and cfu/ml × 10^5		
S.aureus	B.Subtilis	
Commercial solid nutrient medium	141117	
Solid nutrient medium modified the addition of chicken keratin degrader after 24 hours of enzyme hydrolysis	100	89
Solid nutrient medium modified with the addition of chicken keratin degrader after 30 hours of enzyme hydrolysis	135110	
L.S.D. value17.503 *11.847 *		
* (P≤0.05).		

Table 8. Numbers of E.coli and S.Typhi on commercial solid MacConkey medium modified using a nitrogen source of chicken feather keratin degraders using chymotrypsin enzyme

Culture MediumNumber of bacteria and cfu/ml × 10^5		
S.aureus	B.Subtilis	
Commercial solid nutrient medium	75	54
Solid nutrient medium modified the addition of chicken keratin degrader	34	14

after 24 hours of enzyme hydrolysis Solid nutrient medium modified with the addition of chicken keratin degrader after 30 hours of enzyme hydrolysis L.S.D. value7.956 *6.229 *	52	35
* (P≤0.05).		

Tables (7 and 8) show the numbers of B.Subtilis and S.aureus on the nutrient medium agar and modified by replacing the nitrogen source with chicken feather keratin decomposer after 24 and 30 hours of hydrolysis using the enzyme chymotrypsin. It is noted that there is a great similarity in the numbers of bacteria in the alternative media compared to the commercial medium, as the numbers of B.Subtilis bacteria reached (141, 100 and 135)× 10^5 cfu/ml and the number of S.aureus bacteria (117, 89 and 110)× 10^5 cfu/ml in the commercial medium and alternative media by replacing the nitrogen source with chicken feather keratin degrader after 24 and 30 hours with the enzyme chymotrypsin, respectively.

However, the number of E.coli and S.typhi on the commercial MacConkey agar and alternative media reached (75, 34 and 52) and cfu/ml× 10^5 and (54, 14 and 35) cfu/ml× 105, respectively. It is noted from the results that the number increases with the increase in the decomposition time. The reason for this may be attributed to the fact that by increasing the decomposition time, short peptides with small molecular weights are obtained, which facilitates their entry into the bacterial cell, in addition to the possibility of the presence of free amino acids that the microorganism can benefit from directly without the need to expend energy in Synthesis.

Alamnie et al. (5) used chicken feather keratin hydrolyzate by Bacillus bacteria producing catanase enzyme instead of peptone in the growth medium of B. subtilis, Lactobacillus Paracasi, Saccharomyces cerevisiae and Kazachstania humilis yeasts and obtained promising results in this field as the numbers reached (7.8, 6.5, 7.3 and 6.9) × 10^9 cfu/ ml and (6.4, 6.1, 6.8 and 6) × 10^9 cfu/ ml for chicken feather peptone and commercial peptone respectively and confirmed that the hydrolyzates supported the growth of the microorganisms under study.

CONCLUSIONS

The study concludes that chicken feathers contain a high percentage of keratin protein. The possibility of preparing protein concentrates with high protein content from chicken feather powder. Enzymatic hydrolysates of keratin have high solubility at neutral and near basic pH numbers with the preference of enzymatic hydrolysates of chymotrypsin enzyme. The results proved that enzymatic hydrolysates of keratin using chymotrypsin enzyme are successful alternatives to the nitrogen source in the culture media used in the development of microorganisms.

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