Journal of Engineering and Applied Sciences 14 (23): 8721-8726, 2019
ISSN: 1816-949X


# Chicken Feather Hydrolysate as Potential Peptone Source for Bacterial and Yeast Cultivation

[1]Oghenerobor B. Akpor, [2]Olarewaju M. Oluba, [3]Olayinka O. Alabi,
[3]Abigail G. Adeyonu and [3]Ayoola J. Shoyombo
[1]Department of Microbiology, College of Pure and Applied Sciences,
[2]Department of Biochemistry, College of Pure and Applied Sciences,
[3]Department of Agriculture, College of Agricultural Sciences,
Landmark University, Omu-Aran, Kwara State, Nigeria
akpor.oghenerobor@lmu.edu.ng

**Abstract:** To reduce the production cost of growth medium for microbial cultivation, a variety of natural products such as milk, animal tissues and plants have been exploited. The present study was aimed at generating Chicken Feather Protein Hydrolysate (CFPH) from alkaline hydrolysis of chicken feathers as a medium component in the cultivation of selected bacteria and yeasts. Alkaline hydrolysis of the raw chicken feather was carried out to obtain CFPH. The study was carried out using different combinations of CFPH: peptone ratios for the growth medium. Three bacteria *(Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumonia)* and two yeasts (*Candida carpophila* and *Candida tropicalis*) species were as the test microbial species used as test microorganisms. For growth rate studies, 0.5 mL of the broth cultures of the respective test isolates was inoculated in the growth medium. Immediately after inoculation and at every 2 h interval, for a 10 h duration, aliquot samples of the inoculated broth cultures were aseptically withdrawn from each flask for optical density measurement. The study revealed 60 % CFPH yield with the hydrolysis method employed. The results obtained demonstrated that growth performance of the test microorganisms varied for each of the CFPH: peptone combinations. Generally, growth rates of the yeast were observed to be significantly higher ($p<0.05$) in media with CFPH: peptone combinations of 4:6, 6:4 and 8:2 than media with peptone only. Although, in most of the bacteria species investigated, growth was better in the peptone only media, media containing 4:6, 6:4 and 8:2 CFPH: peptone combinations compared well in terms of growth rate. Data obtained in this study showed the potential of CFPH as an alternative growth substrate to peptone in microbial culturing. Thus, revealing the possibility of conversion of chicken feather waste to more valuable use.

**Key words:** Microbial cultivation, feather hydrolysate, growth rate, microorganisms, isolates, species

## INTRODUCTION

Chicken feathers are unwanted products of the poultry industry that most often has created a serious solid waste problem. Feathers are indicated to contain more than 90% protein, 1% lipids and 8% water (Poole *et al*., 2008). A primary source of keratin is found in the poultry industry which includes feathers from rendered chickens and turkey. The amino acid content of these materials is high enough to develop methods to hydrolyze keratin into digestible or other functional peptones (Atlas, 2005).

Media for microbial culture usually contains substances for nutrition and growth of microorganisms. Generally, the composition of media for microbial culture includes carbon and other energy sources, extracts, protein hydrolysates (peptones), extracts, buffers and gelling agents and growth factors (Latimer, 2019). Microbial growth media comprises of energy sources such as carbon. Carbon sources may be derived from carbon dioxide, carbohydrates and other organic compounds like acetate, lipids, proteins and hydrocarbons amongst many others. Microorganisms also require nitrogen sources which are most often incorporated in form of protein, peptones and amino acids (Toth *et al*., 2013).

Chicken feathers generated from poultry houses and poultry consumption contribute to the annual production of agricultural waste. They are composed of approximately 91% protein, 1% lipids and 8% water. The main physical

**Corresponding Author:** Oghenerobor B. Akpor, Department of Microbiology, College of Pure and Applied Sciences, Landmark University, Omu-Aran, Kwara State, Nigeria, akpor.oghenerobor@lmu.edu.ng

structure of chicken feathers is barbs and the main chemical composition of chicken feathers is a structural fibrous protein called keratin, usually the alpha-retains (Fan, 2008).

High quantity of nitrogen is required in the formulation of the media required for the cultivation of microorganisms in the laboratory. The nitrogen found in these media is generally, in the form of protein or amino acids. Growth substrate costs often make up the major part of the production costs of microbial cells and bioproducts from the fermentation industry and the nitrogen source tends to be the most expensive medium constituent (Atlas, 2005). Peptones as protein hydrolysate that are readily water soluble, not only serve as a source of organic nitrogen but also a source of amino acids or specific peptides. Different materials from animal and plant sources are used for the production of peptones, most of them are valuable and relatively expensive (Parrado *et al*., 1993; Dufosse *et al*., 2001; Vasileva-Tonkova *et al*., 2007).

Although, several studies on chicken feather waste have been reported, the majority of them were centred on their microbial degradation (Suneetha and Lakshmi, 2005; Deivasigamani and Alagappan, 2008; Han *et al*., 2012). The possible utilization of hydrolysate from chicken feather waste as growth substrate in microbial culture techniques could offer a sustainable solution to cutting down the associated cost of microbial culture medium as well as ameliorating the environmental concerns associated with its disposal. Therefore, the present research was performed to generate chicken feather keratin from alkaline hydrolysis of chicken feathers as medium component in cultivation of selected bacteria and yeasts.

## MATERIALS AND METHODS

**Chicken feather and test microorganisms:** This study was carried out between October 2018 and February 2019 in Landmark University, Omu-Aran, Kwara State, Nigeria. In the present study, feathers with white colour obtained from the chicken slaughterhouse of Landmark University Commercial Farm (Omu Aran, Nigeria) were used. A total of three bacteria *(Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumonia)* and two yeasts (*Candida carpophila* and *Candida tropicalis)* were used as test microorganisms.

**Production of chicken feather keratin:** Feathers were washed with deionized water and air dried. The dried feathers were cut into smaller pieces and then ground using a mechanical blender. CFPH was prepared following the procedure reported by Akpor *et al.* (2019) using 10% trichloroacetic acid as precipitant.

**Microbial growth studies:** To each respective media, 0.5 mL of the broth cultures of a test organism was inoculated and incubated in an orbital shaker with constant agitation (120 rpm) at 25°C±22°C for 24 h. Immediately after inoculation and at every 2 h interval, for a 24 h duration, aliquot samples of the inoculated broth cultures were aseptically withdrawn from each flask to measure the optical density at a wavelength of 750 nm using UV/vis-spectrophotometer (Sanyo, UK) at wavelength of 750 nm. Growth rate was calculated as:

$$\text{Growth rate (per day)} = \frac{\ln(C_1) - \ln(C_0)}{t_1 - t_0}$$

Where:

$C_1$ = The final absorbance
$C_0$ = The initial absorbance
$t_1$ and $t_0$ = The final and initial time, respectively

**Proximate composition analysis:** The unprocessed chicken feather and CFK were analyzed for moisture, ash, fibre and crude fat and protein, using the AOAC methods (Latimer, 2019). Phosphorus content was determined based on the procedures described by Twine and Williams (1971) while sodium and potassium were estimated using Atomic Absorption Spectrometry (AAS). Amino acids analysis of the samples was determined following hydrolysis with 6 mol/L HCl (containing phenol) for 24 h at 115°C in glass tubes sealed under vacuum.

**Statistical analysis:** Statistical analysis was carried out using the SPSS Statistical Software. Comparison of means was determined using the one-way Analysis of Variance (ANOVA) test at probability level of 0.05.

## RESULTS AND DISCUSSION

**Proximate composition:** The moisture content obtained for the CFPH (28.8%) was significantly higher compared with the raw feathers (11.5±0.5%). The CFPH was lower in fat (0.03%±0.0) and crude protein (61.63%±0.04) compared with the raw feather (0.93%±0.01 and 75.8%±0.1, respectively). The sodium and potassium levels were considerably higher in the CFPH (0.15%±0.01 and 0.65% ±0.01, respectively) compared with the raw feathers (0.05%±0.0 and 0.38%±0.04, respectively). There was a significant decrease in cysteine and tryptophan level in the CFPH compared to the raw chicken feather (Table 1).

**Growth rates of the microbial species:** For the *Escherichia coli,* growth rate was observed to decrease with increasing proportion of CFPH in the media. Media

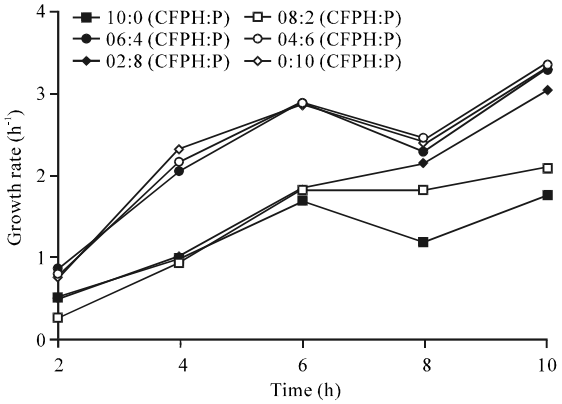


Fig. 1: Growth rate of the *Escherichia coli* at the different CFPH:peptone compositions

Table 1: Proximate composition analysis of raw feathers and chicken feather protein hydrolysate

| Parameters | Raw chicken feather | CFPH |
|---|---|---|
| **Proximate composition (weight %)** | | |
| Moisture | 11.5±0.5[a] | 28.8±0.02[b] |
| Ash | 1.33±0.03[a] | 1.4±0.34[a] |
| Crude fat | 0.93±0.01[a] | 0.03±0.00[a] |
| Crude protein | 75.8±0.1[a] | 61.63±0.04[b] |
| Crude fiber | 2.67±0.02[a] | 3.1±0.7[a] |
| **Elemental composition** | | |
| Phosphorous | 0.47±0.01[a] | 0.42±0.0[a] |
| Sodium | 0.05±0.0[a] | 0. 15±0.01[b] |
| Potassium | 0.38±0.04[a] | 0.65±0.01[b] |
| **Amino acids (g/100, protein g)** | | |
| Lysine | 0.98[a] | 0.93[a] |
| Threonine | 4.72[a] | 4.80[a] |
| Cysteine | 4.33[a] | 3.51[b] |
| Leucine | 8.05[a] | 7.80[a] |
| Isoleucine | 4.93[a] | 4.63[a] |
| Tryptophan | 2.38[a] | 0.65[b] |
| Methionine | 0.68[a] | 0.72[a] |
| Phenylalanine | 4.73[a] | 4.90[a] |
| Histidine | 0.45[a] | 0.48[a] |
| Valine | 8.50[a] | 8.63[a] |
| Arginine | 5.90[a] | 6.01[a] |
| Serine | 13.0[a] | 12.65[a] |
| Glycine | 9.52[a] | 9.55[a] |

Results are means±SD of triplicate determinations; [a, b]Values in the same row carrying different superscripts are significant ($p<0.05$); CFPH = Chicken Feather Protein Hydrolysate

containing peptone only showed significant ($p = 0.05$) growth pattern compared to media containing a combination of CFPH and peptone (Fig. 1).

Growth rate in *Staphylococcus aureus* was observed to decrease with increasing proportion of CFPH in the media. Generally, growth rate was observed to be significantly lower in mediums with CFPH: peptone of 0:10 and 8:2 than growth of other CFPH: peptone compositions ($p = 0.05$). However, growth rate of the *Staphylococcus aureus* did not show significant difference between medium with CFPH:peptone of 2:8 and 0:10, respectively (Fig. 2). The growth rate at

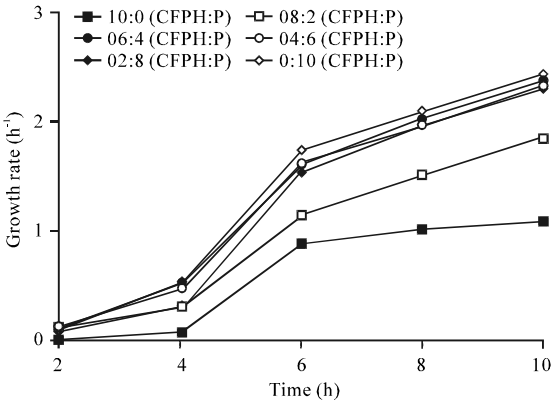


Fig. 2: Growth rate of the *Staphylococcus aureus* at the different CFPH: peptone compositions

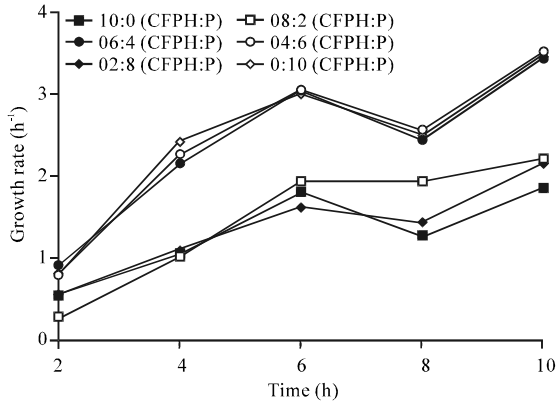


Fig. 3: Growth rate of the *Klebsiella pneumoniae* at the different CFPH:peptone compositions

CFPH: peptone of 2:8 and 0:10 showed significantly lower values than rates at CFPH:peptone compositions ($p = 0.05$).

Growth rate of the *Klebsiella pneumonia* in the different CFPH:peptone composition revealed consistent increases with time. After 24 h incubation, growth rate was observed to be 2.06, 2.36, 3.33 3.29, 2.02 and 3.42 $d^{-1}$. Growth rates at CFPH:peptone compositions of 4:6, 6:4 and 0:10 were observed to be significantly higher than compositions at other CFPH:peptone composition ($p = 0.05$) (Fig. 3).

The growth rate of the *Candida tropicalis* showed variation with the different CFPH: peptone compositions. In the different CFPH:peptone compositions, extended lag duration of 6 h was observed (Fig. 4). However, significantly higher growth rate was observed at CFPH: peptone composition of 6:4 compared to other CFPH: peptone compositions. In addition, CFPH: peptone composition at 0:10 was observed to be significantly lower than other compositions ($p = 0.05$).

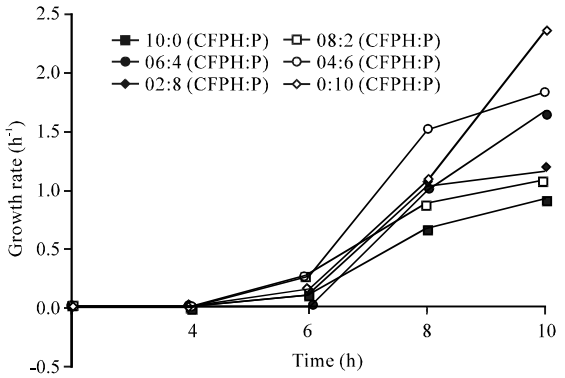


Fig. 4: Growth rate of the *Candida tropicalis* at the different CFPH:peptone compositions

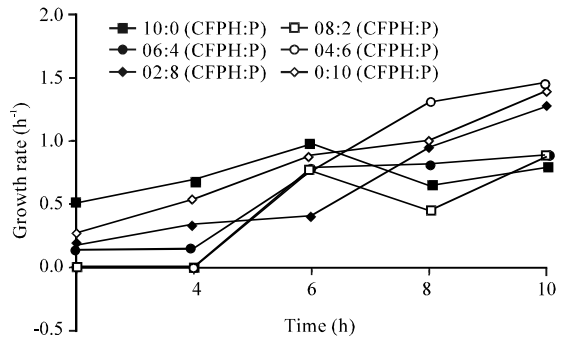


Fig. 5: Growth rate of the *Candida carpophilia* at the different CFPH:peptone compositions

The growth rate of the *Candida carpophilia* was observed to differ significantly in CFPH:peptone compositions of 4:6, 6:4 and 8:2 and 0:10 from compositions of 0:10, 2:8. Significantly higher growth rate was also observed in CFPH:peptone compositions of 4:6, 6:4 and 8:2 compared to 0:10 composition ($p = 0.05$). Although, growth was observed at the respective CFPH:peptone compositions, growth rate at the expiration of incubation was observed to be 0.90, 0.95, 3.51, 3.36, 3.62 and 1.89, in media with CFPH:peptone compositions of 10:1, 2:8, 4:6, 6:4, 8:2 and 0:10, respectively (Fig. 5).

In the present study, chicken feather protein hydrolysate was obtained from the raw chicken feather through alkaline hydrolysis at ambient temperature. The choice of alkaline hydrolysis instead of acid hydrolysis was deliberate. It is reported by earlier investigators higher protein hydrolysate yield and extraction efficiency is obtained with alkaline hydrolysis (Sharma *et al*., 2017; Akpor *et al*., 2019). Besides, alkaline hydrolysis is also indicated to more effective in the degradation of keratin and collagen containing wastes (Taskin *et al*., 2016). Most of the essential amino acids were detected in the CFPH used in this study. Although, hydrochloric and sulfuric acid are common acids that have been reported for the hydrolysis of proteins. Acid hydrolysis is reported to be destructive to essential amino acids such as tryptophan, methionine, cystine and cysteine (Pasupuleti and Braun, 2010).

Although, some investigators have reported higher protein hydrolysate yield at elevated temperatures of hydrolysis, it is however opined that hydrolysis at elevated temperature could lead to protein denaturation (Sinkiewicz *et al*., 2017). In a study on production of peptone from chicken feather, the findings revealed 0.06 g feathers/mL produced 50.6% yield when hydrolysis was carried out at 90°C. This was <60% yield obtained in this study, when 1 M NaOH was used for hydrolysis, at ambient temperature (Subosa *et al*., 2016). An improved protein hydrolysate yield of 94% has been reported when 2.5% NaOH was used for hydrolysis (Sinkiewicz *et al*., 2017).

Peptones are products of acid hydrolysis or enzymatic digestion of organic materials containing protein and serve as nitrogen sources or growth factors for microbial culture (Vasileva-Tonkova and Gesheva, 2007). Peptones composition has been reported to be a determinant of microbial growth dynamics. Earlier researchers have reported significant changes in generation time and microbial yields are significantly affected by the type and composition of peptone used in media (Kurbanoglu and Algur, 2004).

The results obtained in this study demonstrated that growth performance of the test microorganisms varied for each of the CFPH: peptone combinations. This is consistent with the report of Taskin *et al*. (2016) when protein hydrolysate from sheep wool was explored as peptone source for bacterial and fungal cultivation. In this study, growth rates of the yeast were observed to be significantly higher in media with CFPH: peptone combinations of 4:6, 6:4 and 8:2 than media with peptone only. Although, in most of the bacteria species investigated, growth rate was observed to be higher in peptone only media, media containing 4:6, 6:4 and 8:2 CFPH:peptone combinations compared well with the peptone only media. In a related study, growth of *E. coli* on laboratory-produced peptone was observed to be significantly higher than growth that was obtained on commercial peptone. The study further reported that growth of *Bacillus cereus* on commercial peptone and plate count agar showed significantly higher rate than growth that was obtained on laboratory-produced peptone (Gray *et al*., 2008).

## CONCLUSION

The findings of this study revealed that keratin from chicken feather waste could be effectively used as substitute growth factor in the cultivation of bacteria and yeast. Alkaline-hydrolyzed chicken feather could serve as peptone source for bacteria and yeast cultures. Further studies should, however be carried out on the application of the hydrolysates as animal feed supplements and as organic fertilizer in crop production which is the focus of our next investigation.

## SIGNIFICANT STATEMENT

This study discovered the optimum hydrolysis method for chicken feather to obtain feather keratin which could serve as peptone source, for microbial cultivation. This could be of benefit in waste management and reuse, since, chicken feathers which is mostly viewed as waste can be processed and as cheaper alternative to peptone and also a nitrogen source for microbial cultivation.

## ACKNOWLEDGEMENT

The researchers are grateful to the Landmark University Management for providing the facilities for the study. The cooperation of the Landmark University Commercial Farms, Omu-Aran is also appreciated.

## REFERENCES

Akpor, O.B., D.E. Odesola, R.E. Thomas and O.M. Oluba, 2019. Chicken feather hydrolysate as alternative peptone source for microbial cultivation. F1000Research,Vol.7.10.12688/f1000research.17134.1

Atlas, R.M., 2005. Handbook of Media for Environmental Microbiology. CRC Press, Roca Baton, FL.

Deivasigamani, B . and K.M. Alagappan, 2008. Industrial application of Keratinase and soluble proteins from feather keratins. J. Environ. Biol., 29: 933-936.

Dufosse, L., D. de la Broise and F. Guerard, 2001. Evaluation of nitrogenous substrates such as peptones from fish: A new method based on gompertz modeling of microbial growth. Curr. Microbiol., 42: 32-38.

Fan, X., 2008. Value-added products from chicken feather fibers and protein. Ph.D Thesis, Auburn University, Auburn, Alabama.

Gray, V.L., C.T. Muller, I.D. Watkins and D. Lloyd, 2008. Peptones from diverse sources: Pivotal determinants of bacterial growth dynamics. J. Appl. Microbial., 104: 554-565.

Han, M., W. Luo, Q. Gu and X. Yu, 2012. Isolation and characterization of a keratinolytic protease from a feather-degrading bacterium Pseudomonas aeruginosa C11. Afr. J. Microbiol. Res., 6: 2211-2221.

Kurbanoglu, E.B. and O.F. Algur, 2004. A new medium from ram horn hydrolysate for enumeration of aerobic bacteria. Turk. J. Vet. Anim. Sci., 28: 343-350.

Latimer, G.W., 2019. Official Methods of Analysis of AOAC International. 21st Edn., Association of Official Analytical Chemists, Gaithersburg, Maryland,.

Parrado, J., F. Millan, I. Hernandez-Pinzon, J. Bautista and A. Machado, 1993. Sunflower peptones: Use as Nitrogen source for the formulation of fermentation media. Process Biochem., 28: 109-113.

Pasupuleti, V.K. and S. Braun, 2010. State of the Art Manufacturing of Protein Hydrolysates. In: Protein Hydrolysates in Biotechnology, Pasupuleti, VK. and A.L. Demain (Eds.). Springer, Dordrecht, Netherlands, ISBN:978-1-4020-6673-3, pp: 11-32.

Poole, A.J., J.S. Church and M.G. Huson, 2008. Environmentally sustainable fibers from regenerated protein. Biomacromolecules, 10: 1-8.

Sharma, S., A. Gupta, S.M.S.B.T. Chik, C.Y.G. Kee and P.K. P oddar, 2017. Dissolution and characterization of biofunctional keratin particles extracted from chicken feathers. Mater. Sci. Eng., 191: 1-5.

Sinkiewicz, I., A. Sliwinska, H. Staroszczyk and I. Kolodziejska, 2017. Alternative methods of preparation of soluble keratin from chicken feathers. Waste Biomass Valorization, 8: 1043-1048.

Subosa, B.N.F., J.S. Mirando, A.P.V. Velasquez, V.L.Q. Alamar and G.G.L. Capinpin *et al*., 2016. Production of peptone from chicken feathers. Philippine Eng. J., 37: 63-78.

Suneetha, V. and V.V. Lakshmi, 2005. Optimisation of parameters for fermentative production of keratinase by feather degrading microorganisms. World J. Microbiol. Biotechnol., 7: 106-115.

Taskin, M., Y. Unver, A. Firat, S. Ortucu and M. Yildiz, 2016. Sheep wool protein hydrolysate: A new peptone source for microorganisms. J. Chem. Technol. Biotechnol., 91: 1675-1680.

Toth, E.M., A.K. Borsodi, T. Felfodi, B. Vajna and R. Sipos *et al*., 2013. Practical microbiology. Master Thesis, Department of Microbiology, Eotvos Lorand University, Budapest, Hungary.

Twine, J.R. and C.H. Williams, 1971. The determination of phosphorus in Kjeldahl digests of plant material by automatic analysis. Commun. Soil Sci. Plant Anal., 2: 485-489.

Vasileva-Tonkova, E. and V. Gesheva, 2007. Biosurfactant production by antarctic facultative anaerobe Pantoea sp. during growth on hydrocarbons. Curr. Microbiol., 54: 136-141.

Vasileva-Tonkova, E., M. Nustorova and A. Gushterova, 2007. New protein hydrolysates from collagen wastes used as peptone for bacterial growth. Curr. Microbial., 54: 54-57.