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Chicken feather hydrolysate as alternative peptone source for microbial cultivation [version 1; peer review: 1 approved with reservations, 1 not approved]

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V1 First published: 10 Dec 2018, 7:1918
<https://doi.org/10.12688/f1000research.17134.1>
 Second version: 30 Jan 2019, 7:1918
<https://doi.org/10.12688/f1000research.17134.2>
 Latest published: 07 Aug 2019, 7:1918
<https://doi.org/10.12688/f1000research.17134.3>

Abstract

Background: Commercially available conventional growth medium for the culture of microbes are expensive, hence the need for alternative cheaper sources. Poultry waste, in the form of feather and blood, are of value in biotechnology because of their high protein content. Hence the primary aim of this study was to produce a cheaper peptone alternative from chicken feather protein hydrolysate (CFPH) and blood meal (BM).

Methods: We monitored the growth of selected bacteria and fungi in different concentrations of medium produced from varying combination of peptone, CFPH and BM in order to determine the combination that produced maximum growth. Five different media, namely 100% peptone (control), 100% BM, 40% peptone + 60% CFPH, 40% BM + 60% CFPH and 20% peptone + 20% BM + 60% CFPH were prepared and used for the study. The different media were inoculated with 1 ml of each test organism (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida carpophila*, *Candida tropicalis* and *Pichia kudriavzevii*) and their growth monitored for 10 h.

Results: *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Staphylococcus aureus* grew best in the 100% peptone, *Klebsiella pneumoniae* grew best in 100 BM. The fungi species were observed to grow best in 100% peptone. The 60% CFPH + 40% peptone combination (CFPH obtained with precipitate of TCA, HCl, and HNO₃ gave the best growth of *E. coli*. The 60% CFPH + 40% peptone combination (CFPH obtained with precipitate of TCA) also gave the best growth of *C. tropicalis* and *Klebsiella pneumoniae* respectively.

Conclusions: Overall, the 60% CFPH + 40% peptone combination showed the most potential as an alternative to peptone, especially for

Open Peer Review

Approval Status

	1	2	3
version 3 (revision) 07 Aug 2019			
version 2 (revision) 30 Jan 2019	 view	 view	 view
version 1 10 Dec 2018	 view	 view	

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E. coli.

Keywords

Culture media, chicken feather, keratin, hydrolysate, bacteria, yeasts, growth rate, protein source

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Competing interests: No competing interests were disclosed.

Grant information: The author(s) declared that no grants were involved in supporting this work.

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How to cite this article: Akpor OB, Odesola DE, Thomas RE and Oluba OM. **Chicken feather hydrolysate as alternative peptone source for microbial cultivation [version 1; peer review: 1 approved with reservations, 1 not approved]** F1000Research 2018, 7:1918 <https://doi.org/10.12688/f1000research.17134.1>

First published: 10 Dec 2018, 7:1918 <https://doi.org/10.12688/f1000research.17134.1>

Introduction

Microbial culture medium is composed of different nutrients required by organisms for growth. The nutrient requirements of microorganisms differ from one to another as there are many types of microorganisms. Generally, the microbial growth composition includes carbon and energy sources, protein hydrolysates, otherwise known as peptones, extracts, buffers and sometimes gelling agents. Microorganisms will only be able to grow if they are provided with the appropriate nutrients for growth. Apart from carbon, microbes require a source of nitrogen. Amino acids, urea, ammonia and other compound may serve as the nitrogen source. Some organisms also possess the ability to metabolize peptides and more complex proteins (Sandle, 2016).

The protein source for microbial culture is derived from peptone, which is a good source of amino acids, peptides and proteins in growth medium. It is an excellent source of nitrogen. However, it is also a very expensive constituent of the microbial culture medium (Taskin & Kubanoglu, 2011). Different natural products, such as milk, animal tissues and plants, are being exploited for obtaining peptone in order to reduce the costs of production of the growth medium (Jayathilakan *et al.*, 2012; Ben Rebah & Miled, 2013).

It is estimated that about 20 million tons of chicken feathers are generated weekly worldwide and are considered menaces in terms of solid waste pollution (Egelyng *et al.*, 2018; Tesfaye *et al.*, 2018). In a bid to dispose of the large amounts waste chicken feathers generated worldwide, methods such as land-fill and burning have been used, which have taken a toll on the environment (Sharma *et al.*, 2017).

To reduce the burden of waste generated by disposed chicken feathers, a number of processes and operations involving the application of chicken feathers have been reported. Chicken feathers have been employed in the production of animal feed, textile production and paper production amongst many others (Chinta *et al.*, 2013; Moritz & Latshaw, 2001; Tesfaye *et al.*, 2017).

Chicken feathers contain more than 90% protein (keratin), 1% lipids and 8% water (Lasekan *et al.*, 2013). Keratin proteins are grouped into the alpha and beta keratins. Chicken feathers and feathers from most birds are composed majorly of the beta-keratin. Keratin contains all 20 amino acids linked together by peptide bonds, which include covalent disulphide bonds, ionic bonds, hydrogen bonds and hydrophobic bonds (Greenwold *et al.*, 2014). Keratins are bonded by a number of these bonds which make them naturally insoluble. These bonds require that they be broken in order to obtain the chicken feathers in usable forms for microorganisms. By hydrolysis, the bonds are broken, a soluble product is formed (hydrolysate) and the peptone can be obtained from the chicken feather keratin (Ayuthaya & Wootthikanokkhan, 2013). The conversion of such large amounts of chicken feathers into hydrolyzed forms for the manufacture of microbial culture medium can be used as a measure of solving this problem. The chicken feather keratin can then be

incorporated into the production of microbial culture medium. This study was therefore targeted at utilizing the keratin in the waste chicken feathers as a cheaper alternative to peptone and also a nitrogen source for microbial growth.

Methods

Pretreatment of chicken feathers

Chicken feathers were collected from the poultry house in Landmark University Commercial Farm in Omu-Aran, Kwara State, Nigeria. The feathers were first washed with water and laundry detergent before disinfecting with 5% hypochloride solution. Following disinfection, the feathers were sun-dried for one week and stored in baskets until when needed.

Feather hydrolysis

For hydrolysis, approximately 400 g of dried chicken feathers were placed in 10-l plastic container, after which 2000 ml 1 M NaOH was added. The feather-NaOH mixture was stirred vigorously and left to stand for 10 h. After hydrolysis, the mixture was filtered with a clean, dry muslin cloth and the unhydrolyzed fraction estimated. The quantification of the unhydrolyzed fraction was carried out after drying in an oven to constant weight and then weighed to ascertain the degree of hydrolysis.

Precipitation of feather keratin

Feather keratin was precipitated separately from the hydrolyzed feather solution, using 1 M solutions of the following acids: HCl, H₂SO₄, C₂HCl₃O₂ (TCA) and HNO₃. This was carried out for 10 min at 25°C.

The precipitated chicken feather hydrolysate was separated from the solution by filtration. The chicken feather hydrolysate of the respective acids was air-dried and quantified. The chicken feather hydrolysate of the respective acids (henceforth referred to as feather keratin or hydrolysate) were ground into fine powders using a laboratory blender.

Preparation of blood meal

Fresh cattle blood was obtained from the abattoir of Landmark University, Omu-Aran (Nigeria) Teaching and Research Farm. The blood was immediately heated at 60°C for 30 min to coagulate. The coagulated blood was then placed in aluminium foil and oven-dried at 50°C for 8 h. The dried coagulated blood was milled into powder, using a mechanical grinder. The powdered blood, referred to as blood meal (BM) was kept in air-tight container until required.

Chicken feather hydrolysate liquid growth medium

For this study, different compositions of feather keratin, peptone (Oxoid, UK) and BM were used in the medium formulations. Five different combinations were used to make the respective growth medium, as follows. Combination A: 60% keratin, 40% peptone, 0% BM; Combination B: 60% keratin, 20% peptone, 20% BM; Combination C: 60% keratin, 0% peptone, 40% BM; Combination D: 100% peptone; Combination E: 100% BM.

The respective components were weighed separately and dissolved in aliquot quantities of distilled water before combining

all the components together to make a final medium concentration of 5 g/l. Each medium, as composed, was dispensed in conical flask and sterilized in an autoclave (121°C for 15 min) at 15 psi.

Test microbial species

A total of eight microbial species, consisting of five bacteria and three yeast species were used for the study. The bacteria species were *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The yeasts were *Candida carpophila*, *Candida tropicalis* and *Pichia kudriavzevii*.

Prior to use, stock microbial cultures of the isolates were first streaked on agar plates to ascertain their purity before subculturing into nutrient broth. Equal volumes of broth cultures of the pure isolates (0.5 ml) were used for inoculation. Bacteria and fungi were incubated at 24±2°C.

Microbial growth studies

For growth studies, the flasks containing the sterile medium were inoculated with the test microbial species. Following inoculation and every 2 h, for a 10 h duration, aliquot samples were withdrawn from each flask to monitor growth by measuring absorbance using a spectrophotometer at a wavelength of 750 nm, using sterile nutrient broth to normalize. Each experiment was carried out in duplicate.

Statistical analysis

Data analysis was carried out using the SPSS statistical software, version 13.0. Comparison of means was determined using the one-way ANOVA test at a significance level of $P < 0.05$. For the least-significant-difference post hoc multiple comparison test was used. All experimental setups were in duplicates.

Results

Raw absorbance values for each microbe are available on figshare (Akpor *et al.*, 2018).

Growth rate of *Candida carpophila*

As shown in Figure 1, the growth pattern of *Candida carpophila* in all the acid hydrolysate medium showed consistent increase with time. The highest growth was observed in the medium with 100% peptone. This trend was irrespective of the acid hydrolysate used. Besides, the 100% peptone medium, the ranking of the growth of the organism from the highest to the least in the other medium compositions varied for each acid feather hydrolysate. In the TCA feather hydrolysate medium, after 10 hours, the organism recorded the highest growth in the 100% peptone medium, closely followed by 60% feather hydrolysate + 20% peptone + 20% blood, 60% feather hydrolysate + 40% peptone, and 100% blood in that order, while the least growth was observed with 60% feather hydrolysate + 40% blood (Figure 1).

For the HCl feather hydrolysate medium, the growth pattern observed followed the order: 100% peptone > 100% blood > 60% feather hydrolysate + 20% peptone + 20% blood > 60%

feather hydrolysate + 40% peptone, while the 60% feather hydrolysate + 40% blood medium showed the least growth. When H_2SO_4 feather hydrolysate medium was used, the growth was in the order: 100% peptone > 100% blood > 60% feather hydrolysate + 40% blood > 60% feather hydrolysate + 20% peptone + 20% blood, while the 60% feather hydrolysate + 40% peptone was least. In the HNO_3 feather hydrolysate medium, *C. carpophila* grew in the order 100% peptone > 100% blood > 60% feather hydrolysate + 20% peptone + 20% blood > 60% feather hydrolysate + 40% blood, and the lowest growth was recorded in the 60% feather hydrolysate + 40% peptone (Figure 1).

Growth rate of the *Candida tropicalis*

As represented in Figure 2, the highest and the lowest growth of *Candida tropicalis* varied with medium composition for each of the acid feather hydrolysates used. However, the most frequent medium composition in which the highest growth of the organism was recorded was 100% peptone and the organism showed consistent increase in growth throughout the 10-h period with the highest growth at the 10th hour. When the trichloroacetic acid feather hydrolysate medium was used, *C. tropicalis* showed no significant difference ($p > 0.05$) in growth with both 60% feather hydrolysate + 20% peptone + 20% blood and 60% feather hydrolysate + 40% peptone and the organism's growth was observed to be highest with both compositions. This was followed by 100% peptone, 60% feather hydrolysate + 40% blood and the least growth was with 100% blood (Figure 2).

In the HCl feather hydrolysate medium, the growth of *C. tropicalis* was highest with 100% peptone followed by 60% feather hydrolysate + 40% blood, 60% feather hydrolysate + 20% peptone + 20% blood, 60% feather hydrolysate + 40% peptone, with the lowest growth occurring in the 100% blood medium. For the H_2SO_4 feather hydrolysate medium, the highest growth of *C. tropicalis* was observed with 100% peptone, followed by 100% blood, 60% feather hydrolysate + 40% blood, 60% feather hydrolysate + 20% peptone + 20% blood and the least growth was observed with 60% feather hydrolysate + 40% peptone. In the HNO_3 hydrolysate medium, *C. tropicalis* had its highest growth with 100% peptone, followed by 100% blood, 60% feather hydrolysate + 40% peptone, 60% feather hydrolysate + 40% blood, and had its least growth with 60% feather hydrolysate + 20% peptone + 20% blood (Figure 2).

Growth rate of the *Escherichia coli*

As illustrated in Figure 3, the *E. coli* showed consistent increase in growth with the highest growth at the 10th hour. The highest and lowest growths of the organism varied with medium composition for all the acid feather hydrolysates. In the TCA feather hydrolysate medium, the highest growth of *E. coli* at the end of the 10 h period was observed with 60% feather hydrolysate + 40% peptone, followed by 60% feather hydrolysate + 20% peptone + 20% blood, 100% peptone while the least growth was observed with 100% blood and 60% feather hydrolysate + 40% blood. There was no significant difference ($p > 0.05$) in growth of the organism in both medium compositions. When the HCl feather hydrolysate medium was used, highest growth of the *E. coli* was with 100% peptone, followed by 60% feather

Figure 1. Growth rate of *Candida carphophila* in medium with the TCA, HCl, H₂SO₄ and HNO₃ hydrolysates.

Figure 2. Growth rate of *Candida tropicalis* in medium with the TCA, HCl, H₂SO₄ and HNO₃ hydrolysates.

Figure 3. Growth rate of *E. coli* in medium with the TCA, HCl, H₂SO₄ and HNO₃ hydrolysates.

hydrolysate+ 40% peptone, 60% feather hydrolysate + 20% peptone + 20% blood and 60% feather hydrolysate + 40% blood while the least growth was with 100% blood (Figure 3).

For the H_2SO_4 feather hydrolysate medium, *E. coli* had its highest growth with 100% peptone followed by 60% feather hydrolysate + 40% peptone, and then 60% feather hydrolysate+ 40% blood and 60% feather hydrolysate + 20% peptone + 20% blood in which there was little or no significant difference in growth while the least growth of *E. coli* was recorded with 100% peptone. In the HNO_3 feather hydrolysate medium, the highest growth of *E. coli* recorded was with 60% feather hydrolysate+ 40% peptone, followed by 100% peptone, while the least growths were observed with 60% feather hydrolysate + 40% blood, 60% feather hydrolysate + 20% peptone + 20% blood and 100% blood, all showing no significant difference ($p > 0.05$) in growth (Figure 3).

Growth rate of the *Klebsiella pneumoniae*

The results in Figure 4 showed consistent increase in growth rate of *Klebsiella pneumoniae* throughout the 10 h period with highest growth at the 10th hour. The highest and lowest growths of the organism varied with the medium composition. In the trichloroacetic acid feather hydrolysate medium, the highest growth of *Klebsiella pneumoniae* was with 100% blood, followed by 60% feather hydrolysate + 40% peptone, 60% feather hydrolysate + 40% blood, 100% peptone, and the least growth was with 60 feather hydrolysate + 20% peptone + 20% blood. In the HCl feather hydrolysate medium, the *Klebsiella pneumoniae* had its highest growth with 100% blood, followed by 60% feather hydrolysate+ 40% peptone, 60% feather hydrolysate + 40% blood, 60% feather hydrolysate + 20% peptone + 20% blood, and the least growth was with 100% peptone (Figure 4).

In the H_2SO_4 feather hydrolysate medium, the medium composition in which *Klebsiella pneumoniae* had its highest growth was 100% blood, followed by 60% feather hydrolysate + 40% blood, 60% feather hydrolysate + 40% peptone and then, 60% feather hydrolysate + 20% peptone + 20% blood, while the least was recorded in 100% peptone medium. In the HNO_3 feather hydrolysate medium, the highest growth of *Klebsiella* was observed in 100% blood, followed by 60% feather hydrolysate + 40% peptone, 60% feather hydrolysate + 40% blood, 60% feather hydrolysate+ 20% peptone + 20% blood and the least growth of the organism was observed in 100% peptone (Figure 4).

Growth rate of the *Pseudomonas aeruginosa*

As represented in Figure 5, *Pseudomonas aeruginosa* showed consistent increase in growth for the 10 h period and the highest growth was recorded at the 10th hour. The medium composition which yielded the highest growth of the organism was 100% peptone and the least growth was recorded with 100% blood medium. This was constant for all the acid hydrolysate medium. In the TCA feather hydrolysate medium, the medium composition after 100% peptone that yielded the highest growth of *P. aeruginosa* was 60% feather hydrolysate+ 40% peptone, followed by 60% feather hydrolysate + 20% peptone + 20%

blood, 60% feather hydrolysate + 40% blood, while the least growth of *Pseudomonas aeruginosa* was with 100% blood (Figure 5).

In the HCl feather hydrolysate medium, after 100% peptone, *P. aeruginosa* had its highest growth with 60% feather hydrolysate + 40% blood, followed by 60% feather hydrolysate + 40% peptone, 60% feather hydrolysate + 20% peptone + 20% blood, while the organism had its least growth with 100% blood. In the H_2SO_4 feather hydrolysate medium, 100% peptone remained the medium composition in which the highest growth of *P. aeruginosa* was recorded, followed by 60% feather hydrolysate + 40% blood, 60% feather hydrolysate + 40% peptone and 60% feather hydrolysate + 20% peptone + 20% blood while the least growth was observed in 100% blood. In the HNO_3 feather hydrolysate medium, following 100% peptone, the highest growth of *P. aeruginosa* was observed with 60% feather hydrolysate+ 40% blood, followed by 60% feather hydrolysate + 40% peptone, 60% feather hydrolysate + 20% peptone + 20% blood. The least growth was observed with 100% blood medium (Figure 5).

Growth rate of the *Pichia kudriavzevii*

Figure 6 shows the growth pattern of *Pichia kudriavzevii* in the different medium. *Pichia kudriavzevii* showed consistent increase in growth with the highest growth at the 10th hour. The highest growth of the organism was 100% peptone medium, while the least growth was observed in 100% blood medium. In the TCA feather hydrolysate medium, the order of growth of *Pichia kudriavzevii* from the highest to the least in the medium compositions was 100% peptone > 60% feather hydrolysate + 40% peptone > 60% feather hydrolysate + 40% blood > 60% feather hydrolysate, + 20% peptone + 20% blood > 100% blood (Figure 6).

In the HCl feather hydrolysate medium, the order of growth was: 100% peptone > 60% keratin + 40% peptone > 60% feather hydrolysate + 40% blood > 60% feather hydrolysate + 20% peptone + 20% blood > 100% blood. In the H_2SO_4 feather hydrolysate medium, the growth of the organism followed the order: 100% peptone > 60% feather hydrolysate + 40% blood > 60% feather hydrolysate + 40% peptone > 60% feather hydrolysate + 20% peptone + 20% > 100% blood. In the HNO_3 feather hydrolysate medium, the order of growth in the medium compositions was: 100% peptone > 60% feather hydrolysate + 40% blood > 60% feather hydrolysate + 40% peptone > 60% feather hydrolysate + 20% peptone + 20% blood > 100% blood (Figure 6).

Growth rate of the *Proteus mirabilis*

The growth rate of the *Proteus mirabilis* in the different medium is shown in Figure 7. As shown in the Figure, the highest growth was recorded at the 10th hour for all the medium compositions against the respective acid hydrolysates.

In the TCA feather hydrolysate medium, besides 100% peptone, in which the highest growth was recorded, the second highest growth of *Proteus mirabilis* was with 100% blood medium,

Figure 4. Growth rate of *Klebsiella pneumoniae* in medium with the TCA, HCl, H_2SO_4 and HNO_3 hydrolysates.

Figure 5. Growth rate of *Pseudomonas aeruginosa* in medium with the TCA, HCl, H₂SO₄ and HNO₃ hydrolysates.

Figure 6. Growth rate of *Pichia kudriavzevii* in medium with TCA, HCl, H_2SO_4 and HNO_3 hydrolysates.

Figure 7. Growth rate of *Proteus mirabilis* in medium with TCA, HCl, H₂SO₄ and HNO₃ hydrolysates.

followed by 60% feather hydrolysate + 40% peptone, 60% feather hydrolysate + 40% blood and the least growth was with 60% feather hydrolysate + 20% peptone + 20% blood. In the HCl feather hydrolysate medium, after 100% peptone, the highest growth of *Proteus mirabilis* was observed with 100% blood, followed by 60% feather hydrolysate + 40% blood, 60% feather hydrolysate + 20% peptone + 20% blood, and the least growth was with 60% feather hydrolysate + 40% peptone (Figure 7).

In the H₂SO₄ feather hydrolysate medium, after 100% peptone, *Proteus mirabilis* growth pattern followed the order 100% peptone > 60% feather hydrolysate + 40% peptone > 60% feather hydrolysate + 40% blood > 60% feather hydrolysate + 20% peptone + 20% blood (Figure 7).

Growth rate of the *Staphylococcus aureus*

In presence of the different hydrolysates, *Staphylococcus aureus* showed consistent increase in growth throughout the period of incubation. The 100% peptone medium produced the best growth against all the acid hydrolysate medium, while the least growth of the organism varied with medium composition.

In the TCA feather hydrolysate medium, the 60% feather hydrolysate + 40% peptone medium was second to the 100% peptone medium in supporting the growth of *Staphylococcus aureus*, followed by 60% feather hydrolysate + 20% peptone + 20% blood, 100% blood and 60% feather hydrolysate + 40% blood medium in that order. In the HCl feather hydrolysate medium, the second highest growth of the *Staphylococcus aureus* was observed with 60% feather hydrolysate + 40% blood, followed by 100% blood, 60% feather hydrolysate + 40% peptone, and 60% feather hydrolysate + 20% peptone + 20% blood in that order (Figure 8).

In the H₂SO₄ feather hydrolysate medium, the 60% feather hydrolysate + 20% peptone + 20% blood medium ranked second to the 100% peptone medium in the observed growth pattern for *Staphylococcus aureus*, followed by 60% feather hydrolysate + 40% blood, 60% feather hydrolysate + 40% peptone, and 100% blood medium in that order. In the nitric acid feather hydrolysate medium, the growth pattern observed for *Staphylococcus aureus* followed the order 100% peptone > 60% feather hydrolysate + 40% blood > 60% feather hydrolysate + 40% peptone > 60% feather hydrolysate + 20% peptone + 20% blood (Figure 8).

Discussion

In this study, the chicken feather keratin peptone was obtained by alkaline hydrolysis and acid neutralization and precipitation delete. In most cases KOH, NaOH and Ca(OH)₂ are used for the hydrolysis. Taskin *et al.*, (2016) used the alkaline hydrolysis method with KOH in a study where peptone was obtained from sheep wool protein hydrolysate. From investigations, alkaline hydrolysis was reported to be able to produce a high yield of keratin and also enhance the keratin extraction effectiveness (Sinkiewicz *et al.*, 2017). Alkaline hydrolysis has also been studied and proven to be effective in the degradation of waste

containing keratin and collagen (Gousterova *et al.*, 2005). It is opined that the use of alkaline hydrolysis inactivates of pathogens and prions such as transmissible spongiform encephalopathy (TSE) and bovine spongiform encephalopathy (BSE). The use of alkaline hydrolysis yields a BSE and TSE free hydrolysate medium (Matthews & Cooke, 2003). Despite the fact that heat is employed in most chemical hydrolysis processes to improve yield, the alkaline hydrolysis in this study was carried out under room temperature for approximately 10 h. Chemical hydrolysis conducted at high temperatures is said to lead to the destruction of amino acids (Sinkiewicz *et al.*, 2017).

Although acid hydrolysis has also been used in some studies to obtain protein hydrolysates (Wisuthiphaet & Kongruang, 2015), it is argued that it could result in destruction of essential amino acids, such as methionine, cystine, cysteine and tryptophan, and conversion of glutamine and asparagine to glutamic and aspartic acid, respectively. It is indicated that during acid hydrolysis, the salts in hydrolysates can be injurious to the growth of the microorganisms (Bucci & Unlu, 2000).

This study aimed to assess a cheaper source of protein for microbial culture than conventional nutrient medium. Chicken feathers were chosen as the material for research because of its abundance, cost-efficiency and high protein content. The results obtained in this study with reference to the performance of the organisms in the formulated medium compositions were viewed in comparison with the performance of the organisms in the commercially produced peptone. The comparison of the growth rate of the organisms in the different medium with that in peptone was deliberate. Studies have revealed peptone as an excellent nitrogen and protein source for growing microorganisms and manufacture of growth medium (Markings, 2018).

The results of the present experiment showed that in most cases, the organisms growth was highest with the 100% commercially produced peptone while in other cases, various medium compositions yielded higher growth of the organisms even better than in the 100% peptone.

The medium compositions that favored the highest growth of the organism differed with each acid hydrolysate for every organism. The best growth of *Candida carpophila* was recorded with the commercial peptone irrespective of the acid hydrolysate medium composition used. However, in the case of *Candida tropicalis*, the formulated medium compositions from the chicken feather hydrolysate were able to yield higher growths of *Candida tropicalis* even better than the commercial peptone but only in the TCA hydrolysate, while the commercial peptone yielded the highest growth of the organisms in the other acid hydrolysates. *Pseudomonas aeruginosa*, *Pichia kudriavzevii*, *Proteus mirabilis* and *Staphylococcus aureus* consistently had their highest growth with the commercial peptone irrespective of the acid hydrolysate used. In *Escherichia coli* and *Klebsiella pneumoniae*, variation of the highest growth with the hydrolysate medium compositions and growth yield of the organisms higher than in the commercial peptones persisted. The variations in the medium compositions that showed the

Figure 8. Growth rate of *Staphylococcus aureus* in medium with TCA, HCl, H₂SO₄ and HNO₃ hydrolysates.

best growth of the organisms were indications that the acids used in precipitating the keratin from the chicken feathers had significant impact on the efficacy of the medium compositions. The optimum concentration of chicken feather keratin concentration that yielded maximum microbial growth was 60% feather hydrolysate + 40% peptone in *Escherichia coli*.

The ideal chicken feather hydrolysate that yielded microbial growth was the TCA hydrolysate. In a study by Taskin *et al.* (2016), using sheep wool protein hydrolysate as a peptone source for microorganisms, *Staphylococcus aureus* was observed to show poor growth performance in the medium. However, growth performances of *Saccharomyces cerevisiae*, *Bacillus subtilis* and *Penicillium chrysogenum* were observed to be moderate in wool protein. Generally, the present study revealed that growth rate of the respective organisms varied from one medium composition to the other. This observation has been reported by earlier workers (Aspmo *et al.*, 2005; Jones & Fayerman, 1987).

Conclusion

Waste chicken feathers that were previously discarded and viewed as a burden to the environment can now be viewed as an important bio-resource and can be manipulated and explored widely as a biotechnological material. This study was able to characterize waste chicken feathers as a microbiological tool for microbial culture. Because of its high protein content, protein

essential for the growth of microorganisms can be synthesized from chicken feathers.

In the study, the potential of the chicken feather keratin to support the growth of both bacteria and fungi was established. Chicken feather hydrolysate was also proven to be an excellent substrate particularly for the growth of *Escherichia coli*. Using a suitable process of hydrolysis, peptone from chicken feather keratin can be re-modified, industrialized and produced in bulk on a commercial scale. This research work could serve as precursor to exploring many other waste materials of high protein content which could be of biotechnological value.

Data availability

The raw absorbance values from analysis of microbe growth using different growth media are available on figshare. DOI: <https://doi.org/10.6084/m9.figshare.7376564.v1> (Akpore *et al.*, 2018).

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

Grant information

The author(s) declared that no grants were involved in supporting this work.

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Version 1

Reviewer Report 29 January 2019

<https://doi.org/10.5256/f1000research.18732.r42593>

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Adriano Brandelli

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Poultry industry experienced a constant growth during the last decades, generating an increased amount of waste, in the form of feathers, viscera, skin, and dead-on-arrival (Brandelli et al. 2015¹). Alternatives for the correct management of such poultry waste have been evaluated. Conversion of feathers into hydrolysates is a possible alternative to produce ingredients for feed, fertilizers, and cultivation media formulations (Daroit and Brandelli 2014²). This topic merits investigation, since the correct management of such waste material has great environmental relevance. In addition, it could represent an added value to the industry.

In the current article, feathers were hydrolyzed by chemical methods and the resulting hydrolysates were tested as a peptone source for microbial growth. The utilization of byproducts obtained from abattoir as ingredients for culture media has been previously described. In particular, waste feathers from poultry processing industry have been converted as peptone source for bacterial growth (Taskin and Kurbanoglu, 2011³) and for development of culture media for production of mosquitocidal bacteria (Poopathi and Abidha, 2007⁴). More detailed studies include the use of feather peptone for microbial production of carotenoids (Taskin et al. 2011⁵) and protein hydrolysates (Kshetri, 2017⁶). Therefore, the results presented in this work are rather confirmatory, representing a restricted contribution to the field. Additional data should be included to improve this article. In this present form, the manuscript contains preliminary data and lacks sufficient novelty to be considered for indexing.

In addition, a limited number of microorganisms was tested. The selection criteria is not clearly described and should be justified. It seems difficult to take solid conclusions since the microbial species tested are limited and only a single strain of each species was tested. Considering that nutritional requirements can be very variable depending on the microorganism, the inclusion of fastidious microorganisms, lactic acid bacteria, and filamentous fungi should be considered.

Some points on Methods section need to be revised as follows:

- Feathers should be rinsed with abundant water to remove excess detergent before further

treatment. Residual detergent could be toxic for some microorganisms.

- The hydrolysates were air-dried. The reason for not using the oven is unclear since this method was used for blood meal. In this regard, blood meal is mentioned in the Abstract but was not considered in the Discussion. Why was cattle blood used instead of chicken blood?
- The origin of microbial species tested should be clearly described. Available collection strains should be used in this type of study.
- The evaluation was performed in liquid medium, supposedly to quantify microbial growth. However, only absorbance at 750 nm (not 600 nm?) was measured, and viable cell counts were not determined. The evaluation of growth on agar plates could give interesting results.

Finally, the Results section is redundant, since it essentially consists a repetitive description of growth curves. Presentation of results should be carefully reconsidered.

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Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Microbial biotechnology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 22 January 2019

<https://doi.org/10.5256/f1000research.18732.r42168>

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Swati Sharma

Faculty of Chemical Engineering and Natural Resources, Universiti Malaysia Pahang, Pahang, Malaysia

Abstract

1. Give full forms of TCA...
2.also gave the best growth of *C. tropicalis* and *Klebsiella pneumoniae* respectively. In this sentence in what respect is respectively used?
3. In the manuscript total eight microorganisms were considered and conclusion is all about one microorganism. Justify?
4. Specify from where blood meal was taken.

Introduction

1. Keratin proteins are grouped into the alpha and beta keratins. Chicken feathers and feathers from most birds are composed majorly of the beta keratin. Provide with suitable reference.

Methods

1. Cleaning method of feathers was obtained from previous research. Need to cite the reference for the same
2. What was the basis to choose 1M NaOH for the study. Why didn't you try different concentrations? Is it standardized in your laboratory or taken from previous research, if so cite the reference.
3. On what basis were acids chosen for precipitation?
4. Heated at 60°C for..... remove double C.
5. Naming should be same for the combination throughout the manuscript
6. Why didn't you try 100% feather hydrolysate?
7. Correct the mistake in sentence ed by 60% feather hydrolysate + 20% peptone + 20% blood, 60% feather hydrolysate + 40% peptone, and 100% blood in that order, while the least

growth was observed with 60% feather hydrolysate + 40% blood....it should be Blood meal not blood in whole manuscript and same thing apply for feather hydrolysate

8. Give names to all medium like a, b...

Results

1. Overall the result section is good.
2. Conclusion is well written.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: biomaterials, biopolymers, proteins, waste management

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 23 Jan 2019

Oghenerobor Akpor, Landmark University, Omu-Aran, Nigeria

The authors appreciate thoroughness of the reviewer and the comments. Almost all the points raised that required modification have been attended to in the revised version. With respect to the use 1 M NaOH and the acids for hydrolysis and precipitation, respectively, that was ascertained during method development and the pilot phase of the study.

Competing Interests: The authors have no competing interests

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