



RESEARCH ARTICLE

REVISED Chicken feather hydrolysate as alternative peptone source for microbial cultivation [version 3; peer review: 2 approved, 1 not approved]

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Abstract

Background: Commercially available conventional growth media for the culture of microbes are expensive, hence the need for alternative cheaper sources. Livestock 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: The growth of selected bacteria and fungi was monitored in different media prepared from varied concentrations 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 trichloroacetic acid (TCA), hydrochloric acid (HCl) and nitric acid (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*.

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

Keywords

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

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REVISED Amendments from Version 2

This revised version contains slight revision of the abstract. Also included was modification of the methods, particularly the source of the microbial species, incubation conditions and reference to preparation of blood meal. The second sentence in the first paragraph of the conclusion was also modified.

See referee reports

Introduction

Microbial culture media 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 media. It is an excellent source of nitrogen. However, it is also a very expensive constituent of the microbial culture media (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 media (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 media can be used as a measure of solving this problem. The chicken feather keratin can then be incorporated into the production of microbial culture media. 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, as described previously by Akpor *et al.* (2018a). Following disinfection, the feathers were sun-dried for one week and stored in baskets until when needed.

Feather hydrolysis

Hydrolysis of the feather carried out as reported by earlier investigators (Akpor *et al.*, 2018a). 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: hydrochloric acid (HCl), sulphuric acid (H₂SO₄), trichloroacetic acid (TCA) and nitric acid (HNO₃). This was carried out for 10 min at 25°C. The choice of the acids and concentration of NaOH was based on the findings during the method development stage of the study. During this stage several acids and different concentrations of the NaOH were tested, of which 1 M NaOH was ascertained to be the lowest concentration that gave maximum hydrolysis and while the acids gave the highest yield among the organic and inorganic acids that were used for precipitation.

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. Blood meal was prepared using a modification of the protocol

described by [Amu et al., 2018](#). The blood was immediately heated at 60°C for 30 min to coagulate. The coagulated blood was then placed in aluminum 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 media

For this study, different compositions of feather keratin, peptone (Oxoid, UK) and BM were used in the media formulations. Five different combinations were used to make the respective growth media, 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 media concentration of 5 g/l. Each media, 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*. All the isolates were part of the laboratory stocks in the Department of Microbiology, Landmark University, Nigeria.

Prior to use, stock microbial cultures of the isolates were first streaked on agar plates to ascertain their purity before subculturing into nutrient broth.

Microbial growth studies

For growth studies, the flasks containing the sterile media were inoculated with the test microbial species. Equal volumes of broth cultures of the pure isolates (0.5 ml) were used for inoculation. The bacteria and yeast cultures were incubated at 37±2°C and 25±2°C in shaking incubators at shaking speed of 100 rpm.

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. Post hoc test was carried out using the least-significance-difference (LSD). All experimental setups were in duplicates.

Results

Raw absorbance values for each microbe are available on figshare ([Akpore et al., 2018b](#)).

Growth rate of *Candida carpophila*

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

For the HCl feather hydrolysate media, the growth pattern observed followed the order: 100% peptone > 100% BM > 60% feather hydrolysate + 20% peptone + 20% BM > 60% feather hydrolysate + 40% peptone, while the 60% feather hydrolysate + 40% BM media showed the least growth. When H₂SO₄ feather hydrolysate media was used, the growth was in the order: 100% peptone > 100% BM > 60% feather hydrolysate + 40% BM > 60% feather hydrolysate + 20% peptone + 20% BM, while the 60% feather hydrolysate + 40% peptone was least. In the HNO₃ feather hydrolysate media, *C. carpophila* grew in the order 100% peptone > 100% BM > 60% feather hydrolysate + 20% peptone + 20% BM > 60% feather hydrolysate + 40% BM, 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 media composition for each of the acid feather hydrolysates used. However, the most frequent media 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 media was used, *C. tropicalis* showed no significant difference (p > 0.05) in growth with both 60% feather hydrolysate + 20% peptone + 20% BM 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% BM and the least growth was with 100% BM ([Figure 2](#)).

In the HCl feather hydrolysate media, the growth of *C. tropicalis* was highest with 100% peptone followed by 60% feather hydrolysate + 40% BM, 60% feather hydrolysate + 20% peptone + 20% BM, 60% feather hydrolysate + 40% peptone, with the lowest growth occurring in the 100% BM. For the H₂SO₄ feather hydrolysate media, the highest growth of *C. tropicalis* was observed with 100% peptone, followed by 100% BM, 60% feather hydrolysate + 40% BM, 60% feather hydrolysate + 20% peptone + 20% BM and the least growth was observed with 60% feather hydrolysate + 40% peptone. In the HNO₃ hydrolysate media, *C. tropicalis* had its highest growth with 100% peptone, followed by 100% BM, 60% feather hydrolysate + 40% peptone, 60% feather hydrolysate + 40% BM, and had its least growth with 60% feather hydrolysate + 20% peptone + 20% BM ([Figure 2](#)).

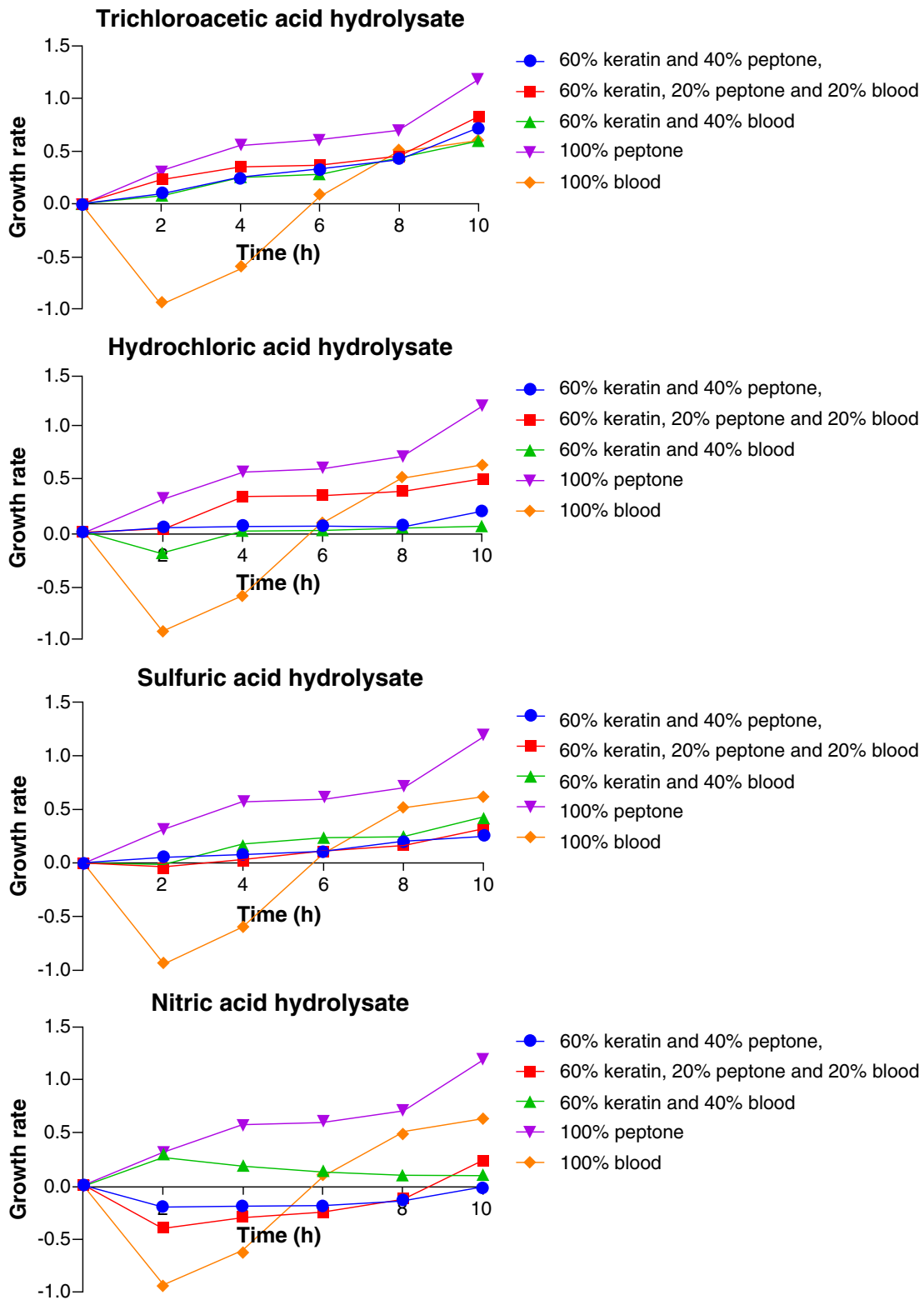


Figure 1. Growth rate of *Candida carphila* in media with the TCA, HCl, H₂SO₄ and HNO₃ hydrolysates.

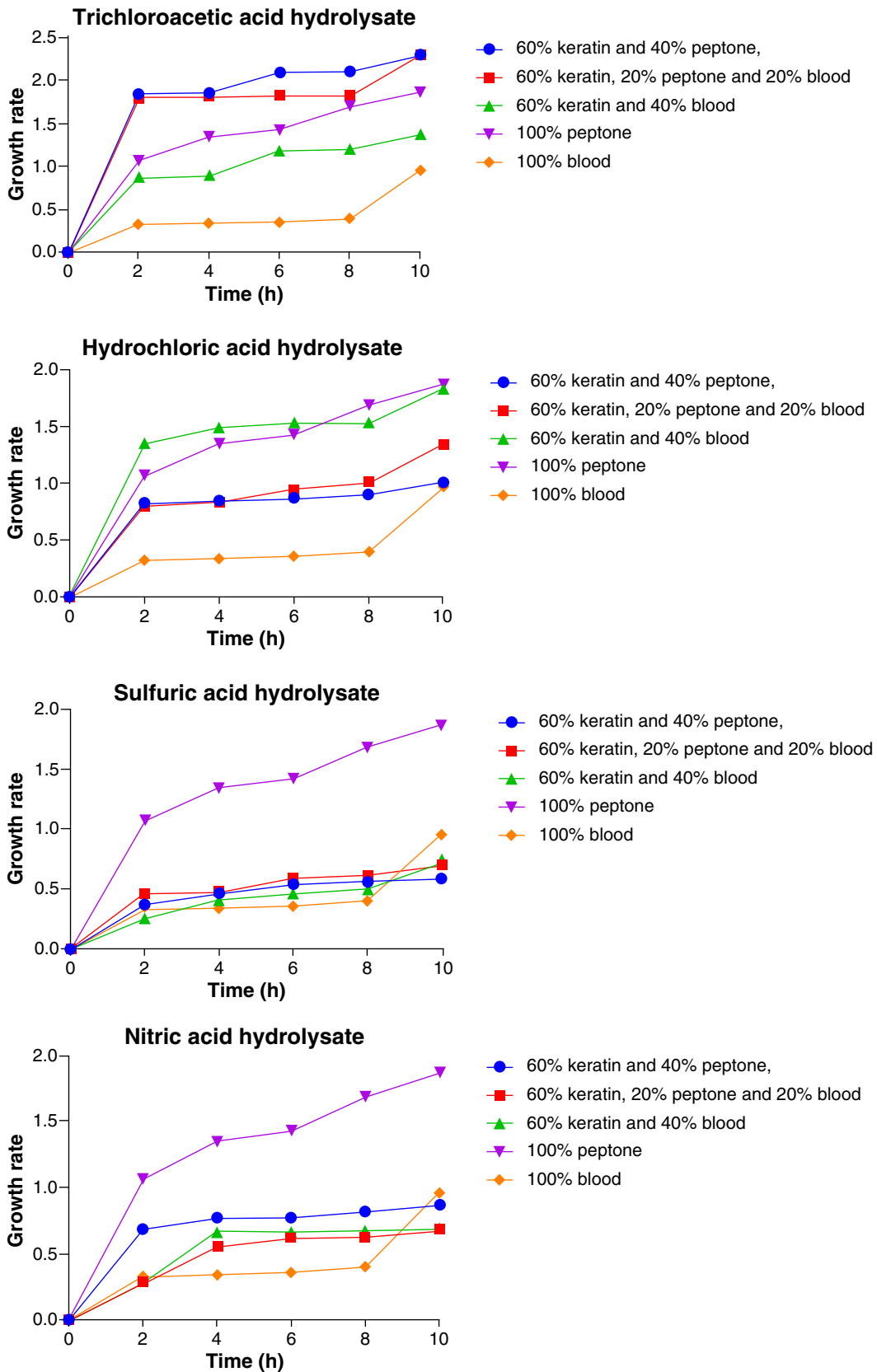


Figure 2. Growth rate of *Candida tropicalis* in media with the TCA, HCl, H₂SO₄ and HNO₃ hydrolysates.

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 media composition for all the acid feather hydrolysates. In the TCA feather hydrolysate media, 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% BM, 100% peptone while the least growth was observed with 100% BM and 60% feather hydrolysate + 40% BM. There was no significant difference ($p > 0.05$) in growth of the organism in both media compositions. When the HCl feather hydrolysate media was used, highest growth of the *E. coli* was with 100% peptone, followed by 60% feather hydrolysate + 40% peptone, 60% feather hydrolysate + 20% peptone + 20% BM and 60% feather hydrolysate + 40% BM while the least growth was with 100% BM ([Figure 3](#)).

For the H₂SO₄ feather hydrolysate media, *E. coli* had its highest growth with 100% peptone followed by 60% feather hydrolysate + 40% peptone, and then 60% feather hydrolysate + 40% BM and 60% feather hydrolysate + 20% peptone + 20% BM 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 HNO₃ feather hydrolysate media, 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% BM, 60% feather hydrolysate + 20% peptone + 20% BM and 100% BM, 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 media composition. In the trichloroacetic acid feather hydrolysate media, the highest growth of *Klebsiella pneumoniae* was with 100% BM, followed by 60% feather hydrolysate + 40% peptone, 60% feather hydrolysate + 40% BM, 100% peptone, and the least growth was with 60 feather hydrolysate + 20% peptone + 20% BM. In the HCl feather hydrolysate media, the *Klebsiella pneumoniae* had its highest growth with 100% BM, followed by 60% feather hydrolysate + 40% peptone, 60% feather hydrolysate + 40% BM, 60% feather hydrolysate + 20% peptone + 20% BM, and the least growth was with 100% peptone ([Figure 4](#)).

In the H₂SO₄ feather hydrolysate media, composition in which *Klebsiella pneumoniae* had its highest growth was 100% BM, followed by 60% feather hydrolysate + 40% BM, 60% feather hydrolysate + 40% peptone and then, 60% feather hydrolysate + 20% peptone + 20% BM, while the least was recorded in 100% peptone media. In the HNO₃ feather hydrolysate media, the highest growth of *Klebsiella* was observed in 100% BM, followed by 60% feather hydrolysate + 40% peptone, 60% feather hydrolysate + 40% BM, 60% feather hydrolysate + 20% peptone + 20% BM 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 media composition which yielded the highest growth of the organism was 100% peptone and the least growth was recorded with 100% BM. This was constant for all the acid hydrolysate media. In the TCA feather hydrolysate media, the media 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% BM, 60% feather hydrolysate + 40% BM, while the least growth of *Pseudomonas aeruginosa* was with 100% BM ([Figure 5](#)).

In the HCl feather hydrolysate media, after 100% peptone, *P. aeruginosa* had its highest growth with 60% feather hydrolysate + 40% BM, followed by 60% feather hydrolysate + 40% peptone, 60% feather hydrolysate + 20% peptone + 20% BM, while the organism had its least growth with 100% BM. In the H₂SO₄ feather hydrolysate media, 100% peptone remained the media composition in which the highest growth of *P. aeruginosa* was recorded, followed by 60% feather hydrolysate + 40% BM, 60% feather hydrolysate + 40% peptone and 60% feather hydrolysate + 20% peptone + 20% BM while the least growth was observed in 100% BM. In the HNO₃ feather hydrolysate media, following 100% peptone, the highest growth of *P. aeruginosa* was observed with 60% feather hydrolysate + 40% BM, followed by 60% feather hydrolysate + 40% peptone, 60% feather hydrolysate + 20% peptone + 20% BM. The least growth was observed with 100% BM ([Figure 5](#)).

Growth rate of the *Pichia kudriavzevii*

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

In the HCl feather hydrolysate media, the order of growth was: 100% peptone > 60% keratin + 40% peptone > 60% feather hydrolysate + 40% BM > 60% feather hydrolysate + 20% peptone + 20% BM > 100% BM. In the H₂SO₄ feather hydrolysate media, the growth of the organism followed the order: 100% peptone > 60% feather hydrolysate + 40% BM > 60% feather hydrolysate + 40% peptone > 60% feather hydrolysate + 20% peptone + 20% > 100% BM. In the HNO₃ feather hydrolysate media, the order of growth in the media compositions was: 100% peptone > 60% feather hydrolysate + 40% BM > 60% feather hydrolysate + 40% peptone > 60% feather hydrolysate + 20% peptone + 20% BM > 100% BM ([Figure 6](#)).

Growth rate of the *Proteus mirabilis*

The growth rate of the *Proteus mirabilis* in the different media is shown in [Figure 7](#). As shown in the Figure, the highest growth

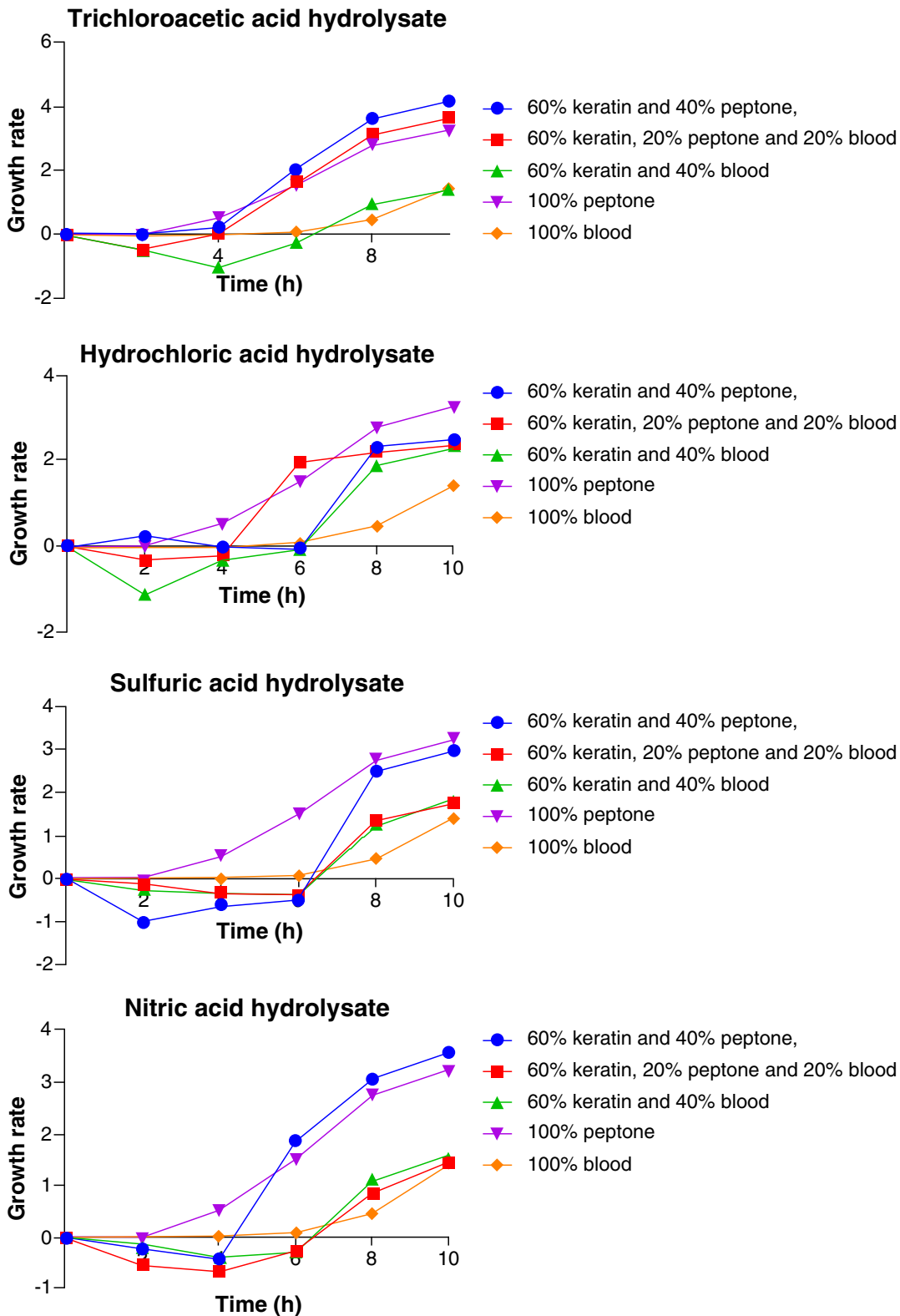


Figure 3. Growth rate of *E. coli* in media with the TCA, HCl, H₂SO₄ and HNO₃ hydrolysates.

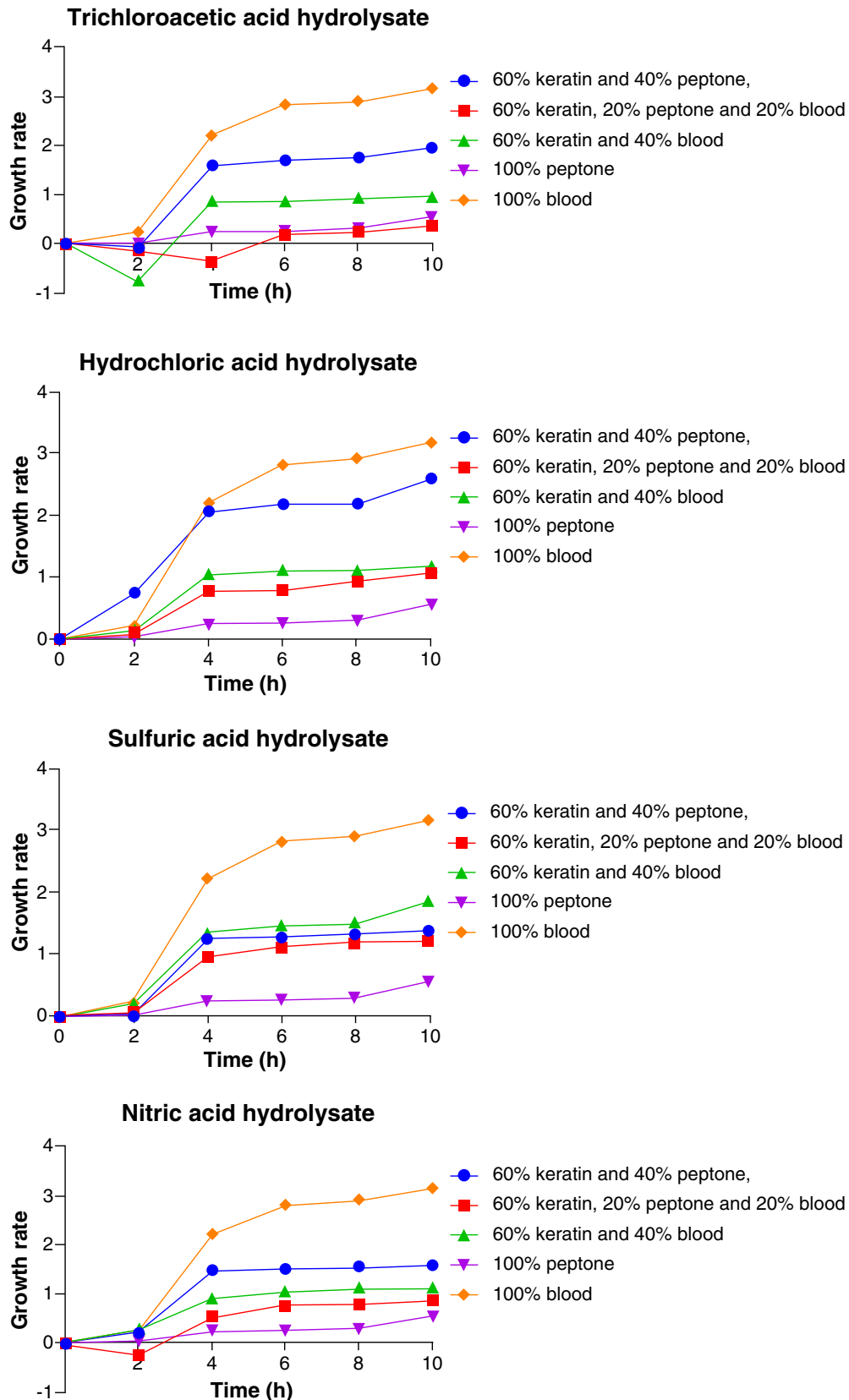


Figure 4. Growth rate of *Klebsiella pneumoniae* in media with the TCA, HCl, H₂SO₄ and HNO₃ hydrolysates.

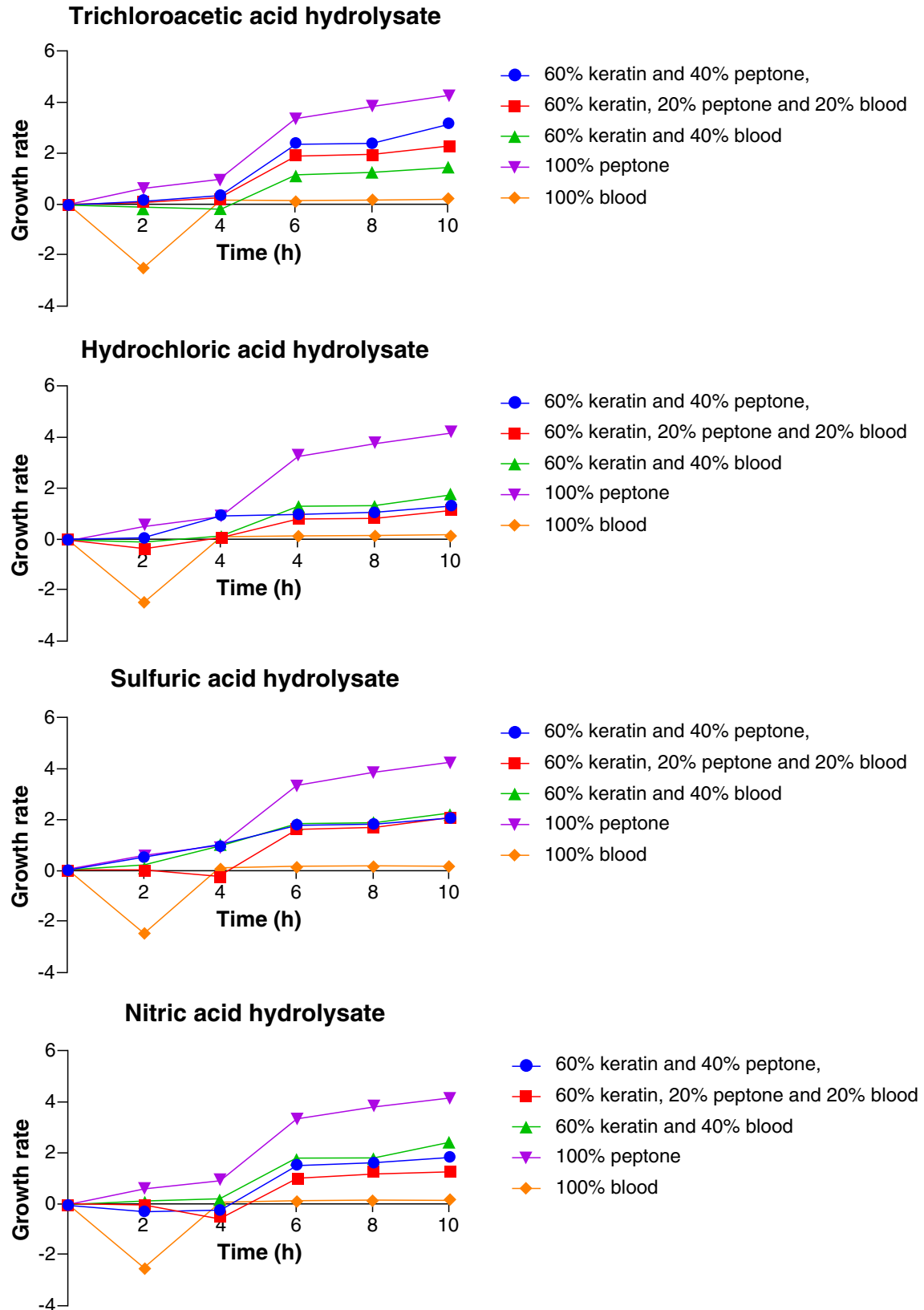


Figure 5. Growth rate of *Pseudomonas aeruginosa* in media with the TCA, HCl, H₂SO₄ and HNO₃ hydrolysates.

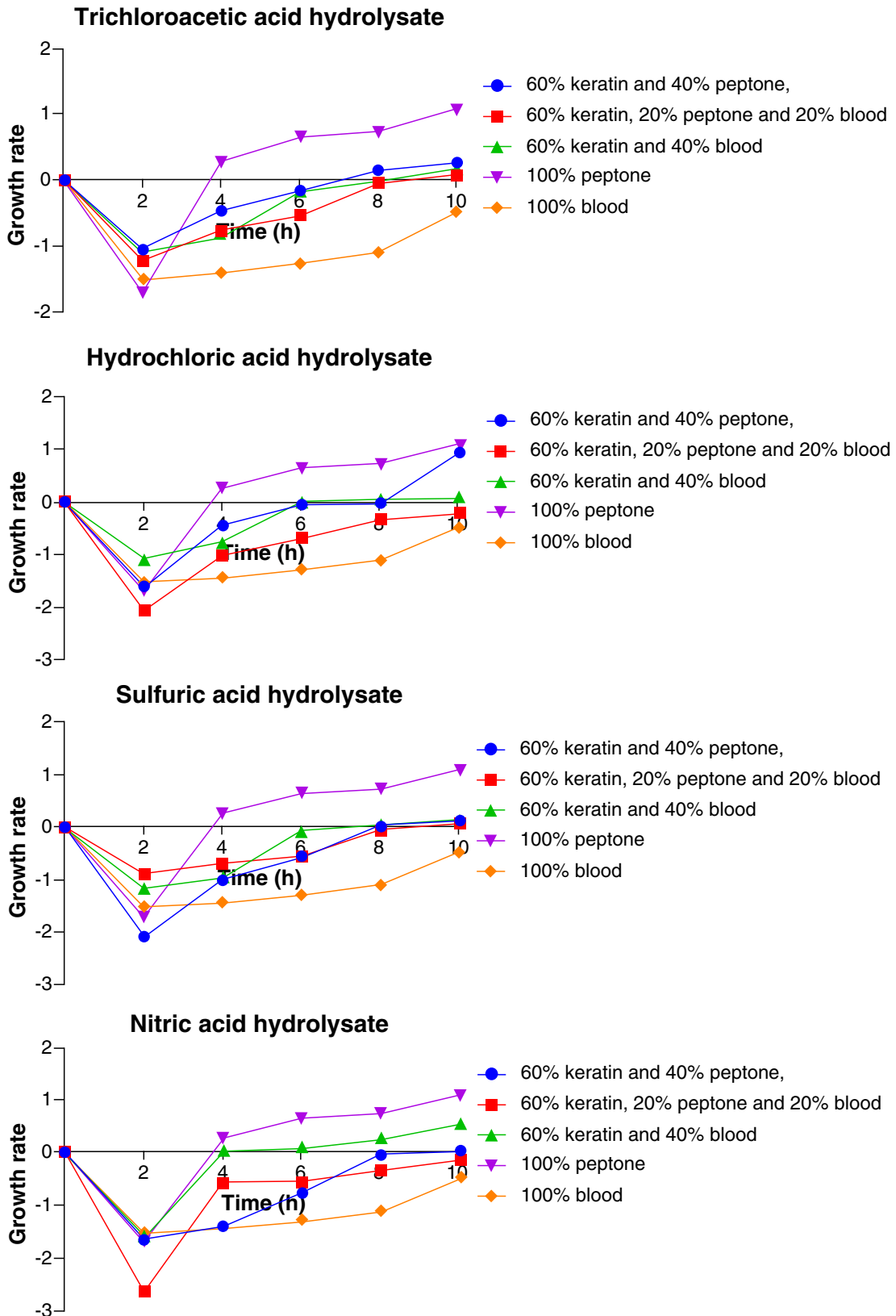


Figure 6. Growth rate of *Pichia kudriavzevii* in media with TCA, HCl, H₂SO₄ and HNO₃ hydrolysates.

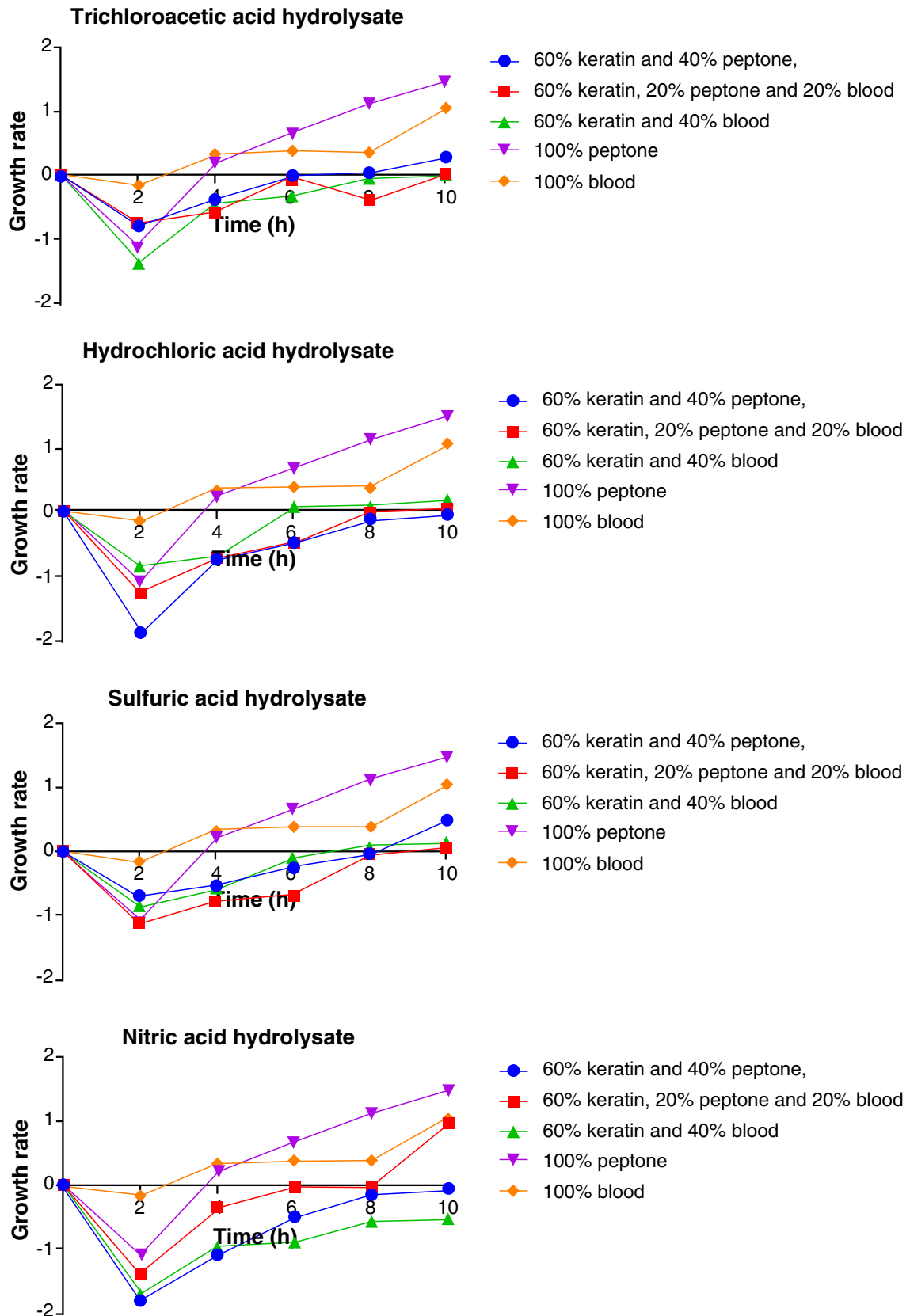


Figure 7. Growth rate of *Proteus mirabilis* in media with TCA, HCl, H₂SO₄ and HNO₃ hydrolysates.

was recorded at the 10th hour for all the media compositions against the respective acid hydrolysates.

In the TCA feather hydrolysate media, besides 100% peptone, in which the highest growth was recorded, the second highest growth of *Proteus mirabilis* was with 100% BM, followed by 60% feather hydrolysate + 40% peptone, 60% feather hydrolysate + 40% BM and the least growth was with 60% feather hydrolysate + 20% peptone + 20% BM. In the HCl feather hydrolysate media, after 100% peptone, the highest growth of *Proteus mirabilis* was observed with 100% BM, followed by 60% feather hydrolysate + 40% BM, 60% feather hydrolysate + 20% peptone + 20% BM, and the least growth was with 60% feather hydrolysate + 40% peptone (Figure 7).

In the H₂SO₄ feather hydrolysate media, after 100% peptone, *Proteus mirabilis* growth pattern followed the order 100% peptone > 60% feather hydrolysate + 40% peptone > 60% feather hydrolysate + 40% BM > 60% feather hydrolysate + 20% peptone + 20% BM (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 media produced the best growth against all the acid hydrolysate media, while the least growth of the organism varied with media compositions.

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

In the H₂SO₄ feather hydrolysate media, the 60% feather hydrolysate + 20% peptone + 20% BM ranked second to the 100% peptone media in the observed growth pattern for *Staphylococcus aureus*, followed by 60% feather hydrolysate + 40% BM, 60% feather hydrolysate + 40% peptone, and 100% BM in that order. In the nitric acid feather hydrolysate media, the growth pattern observed for *Staphylococcus aureus* followed the order 100% peptone > 60% feather hydrolysate + 40% BM > 60% feather hydrolysate + 40% peptone > 60% feather hydrolysate + 20% peptone + 20% BM (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 media (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 media. 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 media 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 media with that in peptone was deliberate. Studies have revealed peptone as an excellent nitrogen and protein source for growing microorganisms and manufacture of growth media (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 media compositions yielded higher growth of the organisms even better than in the 100% peptone.

The media 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 media composition used. However, in the case of *Candida tropicalis*, the formulated media 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*

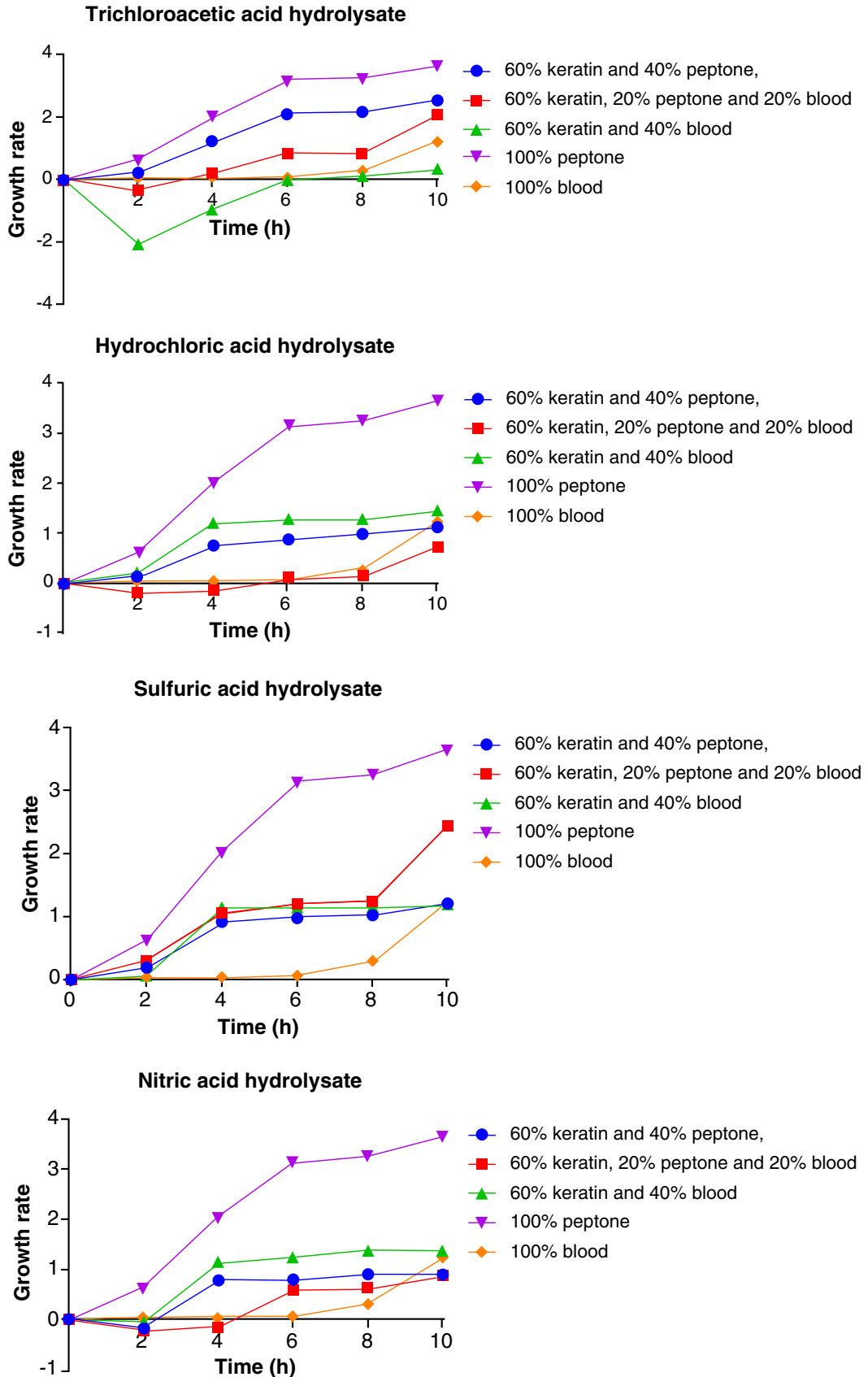


Figure 8. Growth rate of *Staphylococcus aureus* in media with TCA, HCl, H₂SO₄ and HNO₃ hydrolysates.

and *Klebsiella pneumoniae*, variation of the highest growth with the hydrolysate media compositions and growth yield of the organisms higher than in the commercial peptones persisted. The variations in the media compositions that showed the 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 media 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 media. 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 media 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. The study was able to utilize waste chicken feathers as a substitute for peptone in microbial culture.

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> (Akpor *et al.* 2018b).

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/) (CC-BY 4.0).

Grant information

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

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Version 3

Reviewer Report 16 August 2019

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Pius Okiki

Department of Biological Sciences, Afe Babalola University, Ado Ekiti, Nigeria

Competing Interests: No competing interests were disclosed.

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.

Version 2

Reviewer Report 01 July 2019

<https://doi.org/10.5256/f1000research.19748.r49287>

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Pius Okiki

Department of Biological Sciences, Afe Babalola University, Ado Ekiti, Nigeria

Abstract

The second sentence under background should read: "Livestock waste, in form of poultry feather and cattle blood, are of value ..."

The 1st sentence under subsection methods should read: "The growth of selected bacteria and fungi were monitored in different media prepared from varied concentrations of peptone, CFPH, ..."

Nitric acid was written as nitic acid throughout the manuscript. This should be corrected.

Methods

Reference citation should be provided for preparation of blood meal from cattle blood (one is provided below¹), preparation of feather keratin hydrolysate and the microbial growth studies.

The authors need to state where the bacteria and fungi used were obtained from; e.g. from the stock culture of the Microbiology Laboratory of their University, how the organisms were previously stored and if they were sub-cultured on agar plates before introduced into broth.

The optimal growth temperature for pathogenic microorganisms (bacteria and fungi) is 37°C and not 24°C. This should be corrected.

Statistical analysis: There was no evidence that LSD post hoc multiple comparison test was carried out. The growth performance of each microorganism tested in each formulated growth medium should be compared with that of standard peptone broth, and where there is no significant difference, such medium can easily replace peptone water.

Results

The results are well written, but statistical comparison of the growth rates of the microorganisms in the formulated media with those of peptone broth will make the results better presented.

In many places in the manuscript, medium was used in plural form as against media. This should be corrected.

Discussion

Should take into consideration the results obtained from statistical comparison of the growth rates of the microbes in the formulated media with that of standard peptone broth.

Conclusion

The 2nd sentence in the 1st paragraph could be written as: "The was able to utilize waste chicken feathers as a substitute for peptone in microbial culture". The 3rd sentence may not be necessary.

The second paragraph should be re-written. "Following a thorough statistical analysis, the chicken feather hydrolysate may show excellent growth performances for other microorganisms apart from *E. coli*".

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1. Amu W, Ayade G, Okon E: Evaluation of methionine-fortified blood meal as substitute for fish meal in poultry broiler diets. *Afr. J. Poult. Farming.* 2018; **6** (1): 216-224

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

Yes

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?

Partly

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

Partly

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: Antimicrobial and immunological studies of Nigerian plants. Drug resistant microbes in livestock and human, and development of new antimicrobial agents. Emerging/re-emerging diseases, especially zoonotic diseases.

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 01 Aug 2019

Oghenerobor Akpor, Landmark University, Omu-Aran, Nigeria

Abstract

All the corrections suggested in the abstract are accepted and have been effected in the manuscript.

Methods

All corrections as indicated by the reviewer were attended to except the one on statistical analysis. The authors disagree with the reviewer on the lack of evidence of LSD post hoc multiple comparison test. d.

Results

Corrections as indicated by the reviewer were effected except the ones on statistical analysis which the authors disagree with

Conclusion

All the indicated corrections were effected in the manuscript

Competing Interests: The authors have no competing interest

Reviewer Report 27 February 2019

<https://doi.org/10.5256/f1000research.19748.r43782>

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Adriano Brandelli 

Laboratório de Bioquímica e Microbiologia Aplicada, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

The manuscript was modified addressing only the comments from Reviewer #1, but none of the points raised in my review have been modified to date. Thus, I have no additional comments to make on the article until the authors provide a detailed response to the points raised in the initial review.

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?

Partly

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

Partly

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

Partly

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

Partly

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 06 February 2019

<https://doi.org/10.5256/f1000research.19748.r43783>

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Swati Sharma

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

The manuscript has been edited as per the suggestions.

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?

Partly

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

Partly

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

Partly

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

Partly

Are the conclusions drawn adequately supported by the results?

Partly

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.

Version 1

Reviewer Report 29 January 2019

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

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Adriano Brandelli 

Laboratório de Bioquímica e Microbiologia Aplicada, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

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 mosquitoicidal 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.

References

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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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