

# Antioxidant activity and in vitro protein digestibility of chicken feather protein hydrolysates

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## Research note

**Keywords:** Chicken feather waste, waste valorisation, protein digestibility, food deterioration

**Posted Date:** September 16th, 2019

**DOI:** <https://doi.org/10.21203/rs.2.14464/v1>

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# Abstract

**Objective** The hydrolysis of chicken feather waste was carried out using sodium hydroxide and the hydrolysed feather solution precipitated using different acids (nitric acid, sulfuric acid, hydrochloric acid and trichloroacetic acid). Hydrolysates of the different acids were evaluated for antioxidant activity via 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, ferric reducing power and metal chelating activities. In addition, the in vitro protein digestibility of the respective acid hydrolysates was carried out.

**Results:** Data obtained showed that nitric acid-precipitated hydrolysate had the highest DPPH scavenging activity while the hydrolysate precipitated with trichloroacetic acid exhibited the highest ferric ( $\text{Fe}^{3+}$ ) reduction potential. On the other hand, trichloroacetic acid-, sulfuric acid- and nitric acid-precipitated hydrolysates showed similar metal chelating potential compared to hydrochloric acid-precipitated hydrolysate with the least chelating potential. The in vitro protein digestibility of the different hydrolysates ranged from  $62.30 \pm 1.0\%$  (nitric acid) to  $73.10 \pm 1.3\%$  (trichloroacetic acid) and were significantly ( $p < 0.05$ ) higher compared to  $23.80 \pm 0.5\%$  (raw feather). These results indicate that alkaline-hydrolysed chicken feather hydrolysate may be useful as supplementary protein and antioxidants in animal feed formulations.

## Introduction

Feather wastes are often a source of environmental pollution. Thus, researches aimed at transforming these wastes into value-added products are warranted. The global annual contribution of solid waste in the form of feather is substantial. This is attributable to the rise in the global consumption of chickens [1,2]. Feathers are very high in protein (84%), but for its very low digestibility (5%) [3]. The principally protein in feather is beta keratin, which is recalcitrant to enzymatic breakdown by animal, plant and numerous microorganisms [4,5], hence often undergoes several chemical and microbial transformations in the environment if discarded without any treatment. These decomposition processes most often result in environmental pollution. Therefore, with the recent realities of the effects of climate change, and the call for more rigid regulations on refuse and waste disposal, new methods for handling feather wastes are required. In this context, the conversion of feather biomass into feather protein hydrolysates would be an interesting possibility.

The choice of method of for the hydrolysis of proteins most often is dependent on the source protein in question. Keratin from hair, horns, feathers, beaks or wool are most often hydrolyzed by treatment with acid, alkaline or microbial keratinases [6]. Therefore, the use of acids or alkalis in the hydrolysis of feather biomass is a very typical method used in biomass transformation process [7,8]. Unlike acids, alkaline hydrolysis of proteins is cost effective and produce a 100% tryptophan recovery rate [9]. Thus, chemical hydrolysis of chicken feather wastes using alkalis remains a viable option for the utilization of feather as feed stuff and food supplements.

Recently, there has been an increased interest in the search for natural antioxidants with less potential health hazard as an alternative to synthetic antioxidants. To this regard, research on the antioxidant property of agro-wastes has gained an increased interest. Antioxidants in foods in addition to their importance in animal health are also vital in the prevention of food deterioration [10]. Auto-oxidation process has been implicated in food deterioration [11]. The consumption of oxidized foods confers serious health challenges to the consumer and has been implicated in the pathogenesis of diseases such as ageing, cancer, diabetes, hypertension [12]. Bioactive peptides with high antioxidant activity have been extracted from enzymatically hydrolyzed feather keratin. Thus, antioxidant peptides of feather origin could find several applications as value-added products in the food industry. Information on the bioactivity of chemically hydrolyzed feather protein hydrolysate is scanty. Therefore, this study was designed to evaluate the *in vitro* antioxidant activity of alkaline-hydrolyzed chicken feather hydrolysate.

## Methods

### Chicken feather waste

White-colored chicken feather waste was collected from the slaughterhouse of the Landmark University Commercial Farm (Omu-Aran, Nigeria).

### Preparation of chicken feather protein hydrolysate

Chicken feathers were washed with detergent and 5% hypochlorite solution, rinsed thoroughly with a copious amount of water, and sun-dried. The dried feathers were ground into powder using a mechanical grinder. Three hundred gram (300 g) of the powdered feathers was weighed and soaked in acetone for 6 h and then dried before being extracted with a 1 mol/L NaOH solution (wt/vol, 3:10) for 6 h at room temperature with constant stirring. Thereafter, the resulting mixture was filtered using a clean dry muslin cloth to remove unhydrolysed feathers. The hydrolyzed feather solution was divided into four portions. The pH of each of the hydrolyzed feather solution was adjusted to neutral separately with 10% trichloroacetic acid (CFPH<sub>TCA</sub>), 1 M H<sub>2</sub>SO<sub>4</sub> (as CFPH<sub>H2SO4</sub>), 1 M HNO<sub>3</sub> (as CFPH<sub>HNO3</sub>) and 1 M HCl (as CFPH<sub>HCl</sub>) respectively. The resulting mixture was centrifuged (3000 × *g*) at 4 °C for 10 min discarding the supernatant thereafter. The obtained CFPH was dialyzed with cellulose tubes immersed in distilled water for 72 h while changing the water 3 time within 24 h. The dialyzed feather hydrolysate was freeze-dried to obtain chicken feather protein hydrolysate powder which was stored in a dried airtight container and at 4 °C until it was required for further analysis. The procedures for the preparation of CFPH is shown in Figure 1.

### Compositional analysis

The unprocessed chicken feathers and the respective acid CFPs were analyzed for crude protein by the Kjeldahl method [13]. Similarly, amino acid profile for both raw chicken feather and the respective acid CFP was determined following hydrolysis with 6 M HCl (containing phenol) for 24 h at 115 °C in glass tubes sealed under vacuum according to the method of Ravindran et al. [14]. Each analysis was carried out in triplicates.

## Antioxidant assays

### 2.4.1. DPPH scavenging activity

The scavenging activity of the respective acid CFP against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was estimated following the method of Bersuder et al. [15] using butylated hydroxyanisole (BHA) as standard. The assay was carried out in triplicate. The DPPH radical scavenging activity was calculated in percentage according to the formula:

$$\text{Scavenging activity (\%)} = 1 - \frac{A_{\text{sample}} - A_{\text{sample control}}}{A_{\text{blank}}} \times 100$$

### 2.4.2. Fe<sup>3+</sup> reducing activity

The Fe<sup>3+</sup> reducing potential of the respective CFP was estimated according to the method Yindirir et al. [16]. Analysis for each sample was carried out in triplicates.

## Metal (Fe<sup>2+</sup>) chelating activity

The respective acid CFP were evaluated for iron chelating activity according to the methods described by Ebrahimzadeh et al. [17]. Analysis for each sample was done in triplicates. The iron chelating activity was calculated in percentage according to the formula:

$$\text{Iron chelating activity (\%)} = 1 - \frac{A_{\text{sample}} - A_{\text{sample control}}}{A_{\text{blank}}} \times 100$$

## Determination of in vitro protein digestibility

The in *vitro* protein digestibility of the respective CFP was evaluated using the multi-enzyme solution according to method described by Monjula and John [18] with little modifications. A known weight of the

respective CFPH containing 16 mg nitrogen was digested with 1 mg pepsin dissolved in 15 mL of HCl (0.1 M) at room temperature for 2 h. The reaction was inhibited by adding 15 mL TCA (10%). The resulting mixture was filtered using Whatman No. 1 filter paper. Thereafter the nitrogen content of the TCA-soluble fraction was determined using the micro-Kjeldahl method and the *in vitro* protein digestibility was estimated using the equation:

$$\text{In vitro protein digestibility (\%)} = \frac{\text{Content of protein released upon the digestion of 1 g of sample}}{\text{Content of 1 protein of 1 g of sample before digestion}}$$

## Statistical analyses

Results are presented as means  $\pm$  SD. The statistical analysis was by One-way analysis of variance (ANOVA) followed by Turkey's Multiple Comparison using SPSS version 20.  $P < 0.05$  was considered significant. All graphs were plotted using Graph Pad Prism.

## Results

### Proximate composition

The crude protein content of CFPH had significantly higher crude protein ( $88.6\% \pm 0.04$ ) compared with the raw feather ( $71.8\% \pm 0.1$ ). There was a significant decrease in methionine, lysine, cysteine and histidine level in the CFPH compared to the raw chicken feather (Table 1).

### Antioxidant property

The DPPH scavenging activity of the respective acid CFPH was observed to be concentration dependent. CFPH<sub>HNO<sub>3</sub></sub> exhibited the highest scavenging activity, followed by CFPH<sub>H<sub>2</sub>SO<sub>4</sub></sub> while CFPH<sub>HCl</sub> showed the least activity (Figure 2a). CFPH<sub>TCA</sub> showed significantly higher ferric reduction potential across all concentrations compared to CFPH of the other acids. No significant difference in ferric reduction activity was observed between CFPH<sub>H<sub>2</sub>SO<sub>4</sub></sub>, CFPH<sub>HNO<sub>3</sub></sub> and CFPH<sub>HCl</sub> (Figure 2b). The metal chelation activity of the respective acid CFPH was observed to be concentration dependent. No significant difference in iron chelating activity was observed between CFPH<sub>TCA</sub>, CFPH<sub>H<sub>2</sub>SO<sub>4</sub></sub> and CFPH<sub>HNO<sub>3</sub></sub> but the CFPH of the 3 acids exhibited significantly iron chelating activity compared to CFPH<sub>HCl</sub> (Figure 2c).

### In vitro digestibility

The *in vitro* protein digestibility recorded for the hydrolysates showed that CFPH<sub>HCl</sub> > CFPH<sub>HNO<sub>3</sub></sub> > CFPH<sub>TCA</sub> > CFPH<sub>H<sub>2</sub>SO<sub>4</sub></sub> with values 52.5%, 52.3%, 50.1% and 49.0% respectively. The differences in

digestibility across the different hydrolysates were not significant ( $p > 0.05$ ) but were significantly higher than that of the raw feather (Figure 2d).

## Discussion

In the present study, CFPH was demonstrated to show antioxidant activity *in vitro* through its scavenging action against DPPH,  $\text{Fe}^{3+}$  reduction potential and iron-chelating activity. These results agree with the report of a study Je et al. [19] in which protein hydrolysate obtained from bullfrog muscle was reported to demonstrate antioxidant activity using DPPH scavenging and ferrozine assays [19]. Similarly, Chan and Decker [20] showed that meat dipeptide carnosine antioxidant action was as a result of its chelation activity against prooxidant metals. In addition, hydrolysate obtained from porcine myofibrillar via enzymatic hydrolysis was reported to possess excellent DPPH scavenging and metal chelation activities [21]. The antioxidant activity of protein hydrolysate has been attributed to action of peptides [22]. Chemical or enzymatic hydrolysis disrupts protein tertiary structure thus enhancing the solvation properties of its amino acid residues and consequently its antioxidant activity. The resulting peptides protein hydrolysis have been demonstrated to show enhanced antioxidant activity compared to intact proteins. The excellent antioxidant potential of proteinaceous supplements has enabled their inclusion in foods to retard or inhibit the oxidation of foods. The antioxidant action of free of protein hydrolysates involve such mechanisms as deactivation of reactive oxygen species, reduction of hydroperoxides, chelation of prooxidant metallic ions, and changes in the physical properties of food systems [23,24].

The high amounts of sulfur containing amino acids, cysteine have been indicated to account for the antioxidant activity of feather keratin. For instance, in a study by Ohba et al. [25], enzymatic hydrolysate obtained from a mixture of horn, hoof and chicken feather was demonstrated to show enhanced antioxidant activity. In another related study, Fakhfakh et al. [26] reported that protein hydrolysate obtained from chicken feather fermented with the bacterium *Bacillus pumilus* A1 showed strong antioxidant activity.

Data from this study revealed that the use of alkalis in the hydrolysis of chicken feathers to obtain CFPH significantly improved the digestibility of feather *in vitro*. This is in agreement with the report of Steiner et al. [27] in which feathers treated with varying concentrations of NaOH or  $\text{H}_3\text{PO}_4$  showed improvement *in vitro* pepsin digestibility. In a related study by Papdopoulos [28] broiler feathers with various concentrations of NaOH or maxatase showed increased solubility and susceptibility to digestion by proteolytic enzymes. It could thus be argued that treatment with NaOH or enzyme weakened and exposed the disulfide linkages in feather keratin backbone thus increasing the solvation property of its amino acid residues culminating in increased solubility of CFPH and enhanced susceptibility to proteolytic digestive enzymes.

The use of NaOH in the hydrolysis of feather suffers the disadvantage of loss of some amino acids thus lowering the quality of the resulting protein. As could be seen from the results obtained for the amino acid profile of the respective CFPH in this study. Methionine, lysine and histidine were significantly reduced in

CFPH when compared to raw feather. A recent study by Oluba et al. [29] on the partial or total substitution of fish meal with CFPH obtained through alkaline hydrolysis in rat diet showed that rats fed CFPH performed poorly compared to those fed fish meal diet. Methionine, lysine and histidine were also found to be limiting in the CFPH-based diets. Thus, chemically hydrolyzed CFPH may not be a good protein source in animal nutrition but could find better application as antioxidant in food packaging and storage as revealed in this study. Based on the results obtained from this study, the inclusion of CFPH in animal feed formulations could be advisable not only to preserve the integrity of the feedstuff but also contribute to enhancing the functional attributes of the feed.

## Limitations

Data presented in this study was obtained from the analysis of white-coloured feathers and so may not be all inclusive for feathers of diverse colourations.

## Abbreviations

CFPH: Chicken feather protein hydrolysate; DPPH:1,1-diphenyl–2-picrylhydrazyl; CFPH<sub>HNO<sub>3</sub></sub>: chicken feather protein hydrolysate precipitated with nitric acid; CFPH<sub>TCA</sub>: chicken feather protein hydrolysate precipitated with trichloroacetic acid; CFPH<sub>H<sub>2</sub>SO<sub>4</sub></sub>: chicken feather protein hydrolysate precipitated with sulfuric acid; CFPH<sub>HCl</sub>: chicken feather protein hydrolysate precipitated with hydrochloric acid.

## Declarations

## Acknowledgement

Authors wish to acknowledge the Management of Landmark University Commercial Farms for access to the slaughterhouse.

## Availability of data and materials

Not applicable

## Authors' Contributions

*OMO* conceived and designed the study, *OBA* interpreted the results and drafted the manuscript, *OOA*, *AJS*, and *FDA* carried out the experimental study, while *AGA* carried out statistical analysis. All authors read and approved the manuscript.

## Author's information

Not applicable

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable

## Competing interest

The authors wish to state that there are no competing interests associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

## Funding

Not applicable

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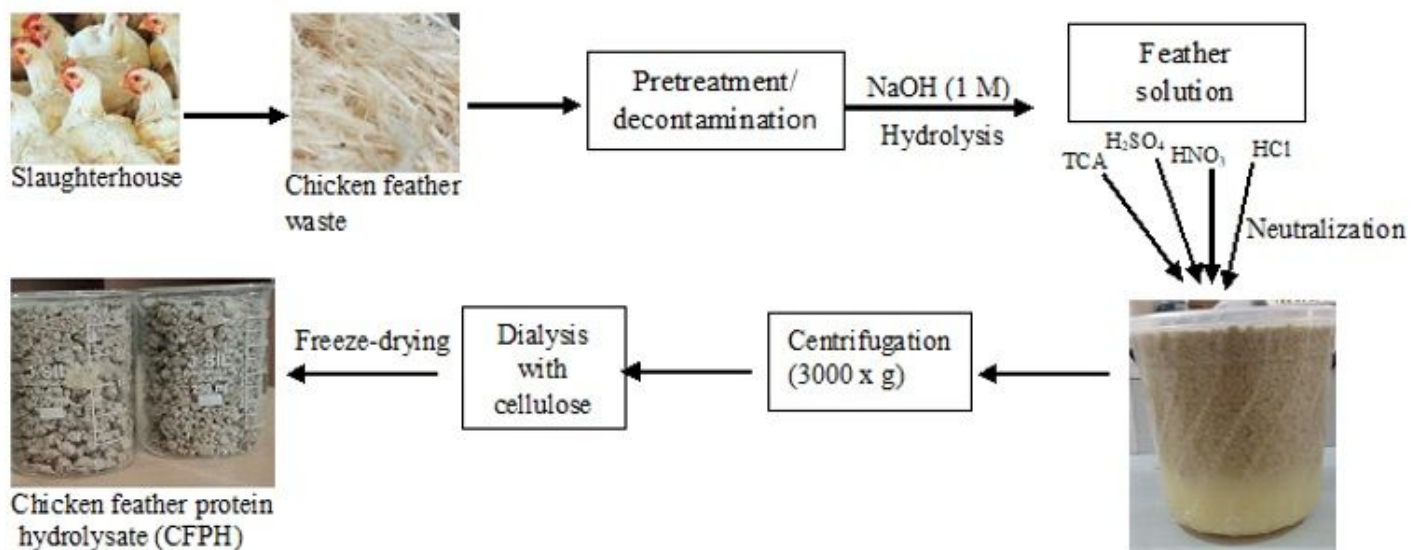
## Tables

Table 1: Chemical composition of raw chicken feather and the respective acid chicken feather protein hydrolysate (CFPH)

	Ratio of fish meal to chicken feather protein hydrolysate					
	100:0	80:20	60:40	40:60	20:80	0:100
<b>Proximate composition (%)</b>						
Moisture	7.3 ± 1.0 <sup>a</sup>	9.0 ± 2.0	9.0 ± 1.5	9.0 ± 1.3	8.0 ± 0.3	9.0 ± 0.7
Crude protein	24.42 ± 1.1 <sup>a</sup>	23.83 ± 0.2 <sup>a</sup>	21.12 ± 0.5	20.96 ± 1.2	22.16 ± 0.4	23.86 ± 0.0
Nitrogen-free extract <sup>1</sup>	55.88 ± 5.2 <sup>a</sup>	57.17 ± 3.8 <sup>a</sup>	61.08 ± 5.5 <sup>a</sup>	61.14 ± 2.1 <sup>a</sup>	60.34 ± 5.8 <sup>a</sup>	58.04 ± 4.9 <sup>a</sup>
Energy (kcal/100 g)	358.4 <sup>a</sup>	346.5 <sup>a</sup>	349.5 <sup>a</sup>	349.1 <sup>a</sup>	349.8 <sup>a</sup>	343.8 <sup>a</sup>
<b>Amino acid composition</b>						
Lysine	2.56 <sup>a</sup>	0.58 <sup>b</sup>	0.42 <sup>b,c</sup>	0.33 <sup>c</sup>	0.33 <sup>c</sup>	0.31 <sup>c</sup>
Threonine	4.50	4.52	4.38	4.35	4.35	4.33
Cysteine	1.02 <sup>a</sup>	2.85 <sup>a</sup>	3.27 <sup>b</sup>	3.33 <sup>b</sup>	3.50 <sup>b</sup>	3.30 <sup>b</sup>
Leucine	6.88	6.55	7.61	7.53	7.83	7.82
Isoleucine	4.58	4.55	4.38	4.53	4.52	4.50
Tryptophan	2.56 <sup>a</sup>	1.01 <sup>b</sup>	0.62 <sup>c</sup>	0.55 <sup>c</sup>	0.48 <sup>c</sup>	0.48 <sup>c</sup>
Methionine	2.79 <sup>a</sup>	1.53 <sup>b</sup>	0.88 <sup>c</sup>	0.85 <sup>c</sup>	0.81 <sup>c</sup>	0.77 <sup>c</sup>
Phenylalanine	5.53	5.50	5.33	5.35	5.30	5.22
Histidine	3.03 <sup>a</sup>	2.82 <sup>a</sup>	0.73 <sup>b</sup>	0.65 <sup>b</sup>	0.62 <sup>b</sup>	0.63 <sup>b</sup>
Valine	9.55	9.33	9.05	9.03	8.93	8.88
Arginine	4.83	4.85	4.55	4.33	4.55	4.52
Serine	12.58	12.80	12.85	13.20	13.20	13.05
Glycine	10.20	10.21	9.55	9.80	9.85	9.85

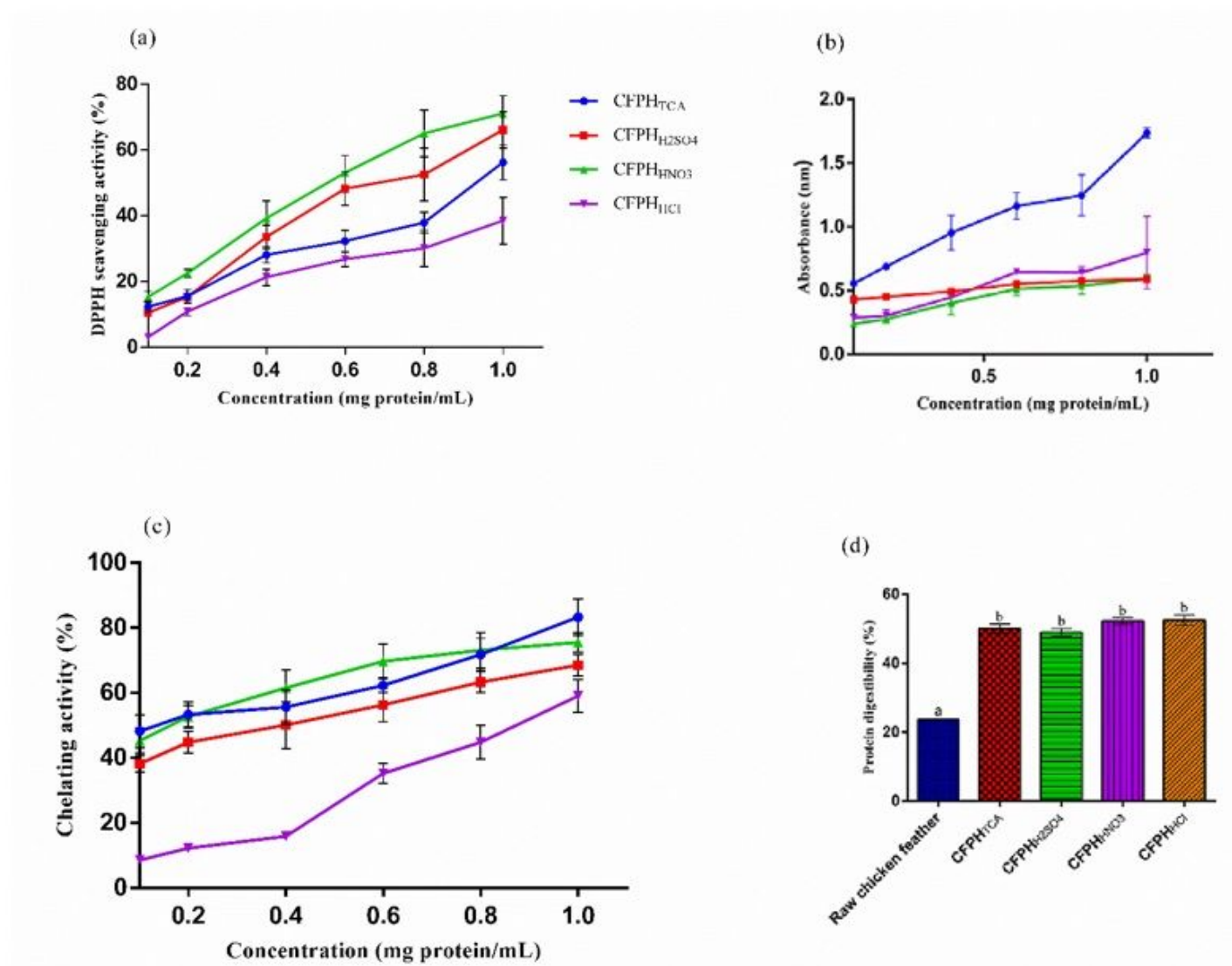
Values are given as mean ± SD of triplicate determinations. Values in the same row carrying different superscripts are significant ( $P < 0.05$ ).

## Figures



**Figure 1**

Flow chat for the extraction of chicken feather protein hydrolysate from waste feathers (CFPH)



**Figure 2**

(a) DPPH scavenging activity; (b) ferric reducing activity (c) metal chelating activity and (d) in vitro protein digestibility of sodium hydroxide-hydrolysed chicken feather protein hydrolysate precipitated with nitric acid (CFPH<sub>HNO3</sub>), sulfuric acid (CFPH<sub>H2SO4</sub>), hydrochloric acid (CFPH<sub>HCl</sub>) and trichloroacetic acid (CFPH<sub>TCA</sub>). Values are means  $\pm$  SD of three determinations. Note: CFPH, chicken feather protein hydrolysate