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Curtailing environmental pollution by bioconversion of bio wastes into bioactive peptides

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Abstract
Poultry is one of the fastest growing segments of the agricultural sector in India.
The poultry sector in India has shown impressive growth, with poultry meat production increasing at an average annual rate of 80% between 2014 – 15 and 2021-22. The continuous growth of this industry had resulted in massive quantities of solid waste as feathers, viscera, bones, and dead on arrival. Under-utilization of these byproducts not only leads to loss of potential revenues but also to the added and increasing cost of disposal. Chicken intestine as a poultry industry byproduct is rich in protein, contains various endogenous enzymes and can be prepared as a hydrolysate via autolysis. Chemical, enzymatic and microbial methods can produce hydrolysed peptides from chicken intestines. In this study, enzymatic hydrolysis by using protease P (phycomycetes) fungal origin enzyme followed by ultrafiltration of chicken intestines proved to be more ecofriendly in the extraction of bioactive peptides.
The characteristics of the extracted bio active peptides were analysed by SDS PAGE and their bioactivity was assessed by DPPH and alpha amylase inhibition assays. The extracted bioactive peptides were dried in to powder and incorporated in canine pet food at levels of 5%, 6% and 7% of bioactive peptides. Hence, the study had opened up a way for the better utilization of chicken intestine in to bioactive peptides with multi-functional properties which in turn could be utilized in pet industry, which otherwise would have remained a waste posing a direct impact on the economy and environmental pollution.

Keywords: Chicken intestines, bio active peptides, SDS PAGE, DPPH & alpha amylase inhibition assay

Introduction
Poultry is one of the fastest growing segments of the agricultural sector in India.
The domestic poultry industry is the fast growing segment with a growth rate of 18%. Poultry intestines processing industry accounts for nearly 20-30% of the processing waste. The disposal of these enormous chicken wastes from slaughter houses poses a huge environmental concern. Among chicken by products, the chicken intestines are rich in protein but unfortunately not being utilized as protein source. The utilization of abundant chicken byproducts, including chicken intestine (37% of chicken weight) to produce value-added byproducts, positively impacts the economy. The intestinal parts from chicken have recently gained attention by producing value-added protein hydrolysate. They possess endogenous autolytic enzymes which hydrolyze the abundant proteins in the viscera, thus becoming protein hydrolysate.
Intestines have a potential for conversion in to useful products of higher value such as bioactive peptide which can be applied in animal feed as additives. Bioactive peptide from intestines are claimed to be rich in low molecular weight bioactive peptides with promising therapeutic, functional, and nutritional applications. Bioactive peptides are produced by methods such as enzymatic hydrolysis, microbial fermentation and chemical methods. However the use of enzymatic hydrolysis is preferred, especially in food and pharmaceutical industries because of the lack of residual organic solvents or toxic chemicals in the product.
By-products derived peptides have antioxidant, antimicrobial, antithrombotic, ACE-I inhibitory activity, immuno modulatory activity, lipid lowering activity and anti diabetic activity.

Bioactive peptide fits into the trends in the development of innovative functional products and nutraceuticals. Incorporation of these peptides in pet foods is gaining more importance owing to digestibility and palatability improvements.

Materials and Methods

The chicken intestinal samples were collected from retail outlets. Preparation of bioactive peptide was performed according to the method of Bhaskar *et al.* (2007) [1]. 500 gms of thoroughly cleansed and sterilized chicken intestines was minced in a Waring blender and centrifuged at 10,500 rpm for 30 minutes at 4 °C. Of the three phases, the protein rich segment was collected and hydrolysed with 1.0% fungal protease P (Phycomycetes) enzyme. The hydrolysate was again centrifuged at 11,000 rpm, for 20 minutes at 15 °C, to collect the supernatant. The bioactive peptide was filtered and separated into small molecular weight fractions by ultrafiltration at 4 °C using 10 kDa molecular weight cut-off and 3 kDa molecular weight cut-off to enrich specific hydrolysate fractions. Scavenging activity on DPPH free radicals by the samples were assessed according to the method reported by Sunitha *et al.* (2016) [8] with slight modifications. The alpha-amylase inhibitory assay was performed according to the method of Kim *et al.* (2004) [5].
Characterization of the bioactive peptides was done by SDS-PAGE according to the method of Laemmli (1970) [6] with slight modification. The extracted bioactive peptides was freeze dried and incorporated at different levels (5%, 6%, 7%) in the canine feed mash prepared by the department of Animal nutrition , MVC, Chennai-07 based on the nutrient specifications and recommendations given by the AAFCO (2014). The canine feed mash was extruded through BTPL twin screw extruder (Model – TSE 002, Kolkata, India) with the extruder temperature fixed at 124 °C and the prepared canine food was conveyed through pneumatic conveyer to the drier, where the product was dried at 80 ˚C for two hours thus forming pellets.

Statistical Analysis

The data was subjected to statistical analysis in SPSS (version 2.0) software with t-test and ANOVA.

Results

The Mean ± SE of yield, pH, instrumental colour and proximate composition of bioactive peptide is given in Table 1. The yield percent of bioactive peptide was 4.28±0.06 percent. The mean ± SE values of pH of bioactive peptide were 6.6±0.02. The mean ± SE values of Lightness (L*)**,** Redness (a*) and Yellowness (b*) of bioactive peptide from chicken intestine 37.53±0.87, 0.87±0.03, 12.22±0.43 respectively. The mean ± SE values of Hue and Chroma of bioactive peptide was 85.41±0.42 and 12.26±0.42 respectively.
The mean ± SE values in percent of moisture, protein, fat and ash of bioactive peptide from chicken intestine were 3.4±0.1, 63.63±1.09, 4.3±0.16, 9.2±0.5 and 19.38±0.57 respectively. The mean ± SE values for DPPH radical scavenging assay for antioxidant activity of bioactive peptides (< 10 kDa) was 0.15±00 mg/ml. Then it was compared with the standard butylated hydroxyl toluene whose IC_{50} value was 0.23±0.3 mg/ml. The mean ± SE values for alpha amylase assay for anti-diabetic activity of bioactive peptides was 158.42±10 mg/ml which was compared with the standard Acarbose whose IC_{50} value was 273.04±20 mg/ml. The bioactive peptide was having lower IC_{50} values than both the standards butylated hydroxyl toluene and standard Acarbose.
The SDS-PAGE showed that the bioactive peptides extracted from chicken intestine had molecular weight of between 9.4 kDa to 9.8 kDa. The mean ± SE values of IC_{50} values for DPPH Assay and Amylase acarbose assay of different levels (5%, 6% & 7%) of bioactive peptide incorporated pet food compared with the IC_{50} value of Canine feed mash prior to incorporation of bioactive peptides were as given in Table 2. The test of significance revealed that the IC_{50} values of canine food pellets with different incorporation levels of extracted bioactive peptides were highly significant (p<0.01).

Table 1: Mean ± SE of yield percent, pH, instrumental colour and proximate composition of bioactive peptide

S. No.	Parameters	Results
1.	Yield% (dry basis)	4.28±0.06
2.	pH	6.6±0.02
3.	Lightness (L*)	37.53±0.8
4.	Redness (a*)	0.87±0.03
5.	Yellowness (b*)	12.22±0.43
6.	Hue	85.41±0.42
7.	Chroma	12.26±0.42
8.	Moisture	3.4±0.1
9.	Crude protein	63.63±1.09
10.	Fat	4.3±0.16
11.	Ash	9.2±0.5
12.	Carbohydrate	19.38±0.57

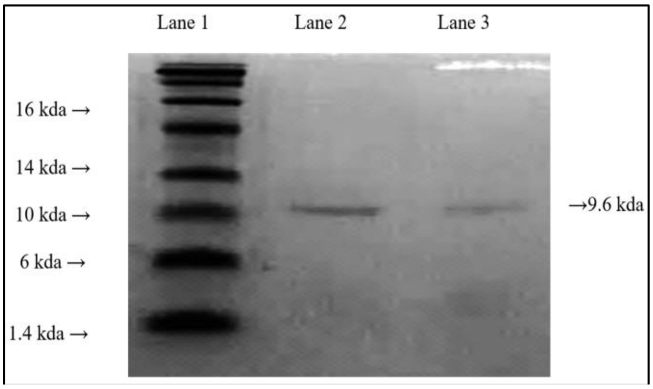
Table 2: Mean ± SE values of DPPH antioxidant activity with standard butylated hydroxyl toluene and α-amylase inhibitory anti-diabetic actikvity with standard acarbose in low molecular weight peptides

S. No.	Sample	IC_{50} value
	Antioxidant activity	
1.	Standard Butylated hydroxyl toluene	0.23±0.3
2.	Bioactive peptides (<10 kDa)	0.15±0.0**
	Anti-diabetic activity	
3.	Standard Acarbose	273.04±2.0 **
4.	Bioactive peptides (<10 kDa)	158.42±1.0

NS-Not Significant *-Significant (p<0.05) difference **-Highly significant (p<0.01) difference
Means bearing different superscripts in the same row differ significantly

Table 3: Mean ± SE values of antioxidant and antidiabetic activity among different incorporation levels of extracted peptides in pet food

Antioxidant activity	
Levels	**IC_{50} value**
Standard Butylated hydroxyl toluene	1.84±0.4
5%	1.80^{c}±0.5
6%	1.41^{b}±0.6
7%	0.20^{a}±0.7
Antidaibetic activity	
Standard Acarbose	313.85±2.8
5%	264.28^{b}±1.3
6%	163.57^{c}±5.0
7%	59.26^{a}±4.4



Lane 1: Polypeptide protein marker
Lane 2: Extracted bioactive peptides
Lane 3: Extracted bioactive peptides

Plate 1: Molecular pattern of bioactive peptides on SDS-Poly Acrylamide Gel Electrophoresis (SDS-PAGE) – 15% separating gel

Discussion

Bhaskar *et al*. (2007) [1] prepared bioactive peptide from sheep intestine with a yield of 6% and Noman *et al*. (2018) [7] obtained an yield of 17% from chinese sturgeon muscles hydrolysate. The pH value of bioactive peptide obtained in this studdy was in accordance with Noman *et al*. (2018) [7] who studied the functional properties of the bioactive peptide of chinese sturgeon with a pH of 6. The lightness values were much lower and yellowness values higher in this study than the lightness value of 78.62 and yellowness value of 23.66 obtained from Pangasianodon hypophthalmus fish by Hassan *et al*. (2018) [3]. Haldar *et al.* (2018) [2] obtained a lower percent (42) of protein content in the hydrolysate of freshwater mussel and Noman *et al* (2018) [7] obtained a higher percent (79.69) of protein content in the hydrolysate from chinese sturgeon.

The molecular weights of extracted bioactive peptides obtained in this study were similar as obtained by Kim *et al.* (2009) [4] who characterized the antioxidant peptides from venison with molecular weights of 9.8 kda and 11 kda and also Thiansilakul *et al.* (2007) [9] who extracted anti-oxidant peptides from round scad muscle hydrolysate with a molecular mass of 9 kDa. Lower IC_{50} values indicated higher potency of the extracted peptides when compared to antioxidant activity of standard butylated hydroxyl toluene and anti-diabetic activity of standard acarbose. The canine food pellet with 7% of incorporated bioactive peptides was having lower IC_{50} value than other incorporation levels. This indicated that the extracted bioactive peptides had influenced the DPPH and Amylase acarbose inhibitory activity of canine food pellet at 7% level than the other levels. Thus, the study revealed that incorporation of bioactive peptides at 7% level in the canine feed mash had better antioxidant and antidiabetic activity than the other levels. At the same time, Zimmerman *et al*. (1996) [10] suggested an incorporation level of 6% bioactive peptides in post weaning diet of piglets.

Conclusion

Low value byproducts like chicken intestines can be effectively utilized for the extraction of bioactive peptides by enzymatic hydrolysis and ultrafiltration. This method proves to be more ecofriendly and economical. The extracted bioactive peptide (< 10 kDa) having lower IC_{50} values indicating high antioxidant and anti diabetic activity can be utilized as alternatives to synthetic antioxidants used in pet food, as a nutraceutical and as a therapeutic diet. The study had opened up way for the better utilization of chicken intestine in to bioactive peptides with multi-functional properties which otherwise would have been a waste. Conversion of poultry biowaste into biowealth, multi-functional bioactive peptides not only curtails environmental pollution but also brings multifaceted impacts and benefits, such as low cost and high environmental efficiency from the various technological pathways to process poultry waste, as well as considerable economic profits by their utility in canine pet feed industry.

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