www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(2): 1718-1721

www.thepharmajournal.com
Received: 01-12-2021
Accepted: 04-01-2022

E Vimalraj
Department of Livestock Products Technology (Meat Science), Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

R Ramani
Department of Livestock Products Technology (Meat Science), Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

V Appa Rao
Department of Livestock Products Technology (Meat Science), Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

M Parthiban
Department of Animal Biotechnology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

R Narendrababu
Department of Livestock Products Technology (Meat Science), Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

S Arulkumar
Division of Animal Nutrition, NDRI, Karnal, Haryana, India

Corresponding Author
E Vimalraj
Department of Livestock Products Technology (Meat Science), Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

Antidiabetic activity of peptides extracted from chicken intestine hydrolysate

E Vimalraj, R Ramani, V Appa Rao, M Parthiban, R Narendrababu and S Arulkumar

Abstract
Antidiabetic activity of peptides in Chicken intestine hydrolysate was assessed by α- amylase inhibitory activity assay. protease P food grade fungal enzyme was used to hydrolyse the chicken intestine. The protein hydrolysate solution was filtered and separated into small molecular weight fractions by ultrafiltration at 4 °C using 10kDa molecular weight cut-off and 3kDa molecular weight cut-off to enrich specific hydrolysate fractions. This permeate was defined as small peptides with molecular weight less than 10,000 Da and 3000 Da. The filtrate thus obtained was assessed for antioxidant invitro α- amylase inhibitory activity assay. Parameters like filterate, concentrate, and total recovery of hydrolysate was studied after ultrafilteration. The filtrate recovery of protein hydrolysate was 47.01 ± 0.2 per cent, concentrate recovery of protein hydrolysate was 5.12 ± 0.45 per cent, total recovery of protein hydrolysate was 52.14 ± 0.2 per cent. The IC_{50} value of protein hydrolysate was 273.04 ± 20 mg/ml, bioactive peptides (< 10 kDa) was 959 ± 8.1 mg/ml and bioactive peptides (<3kDa) was 362.86 ± 23mg/ml. Then it was compared with the standard Acarbose whose IC_{50} value was 158.42 ± 10 mg/ml. The test of significance revealed that the alpha amylase inhibitory activity between protein hydrolysate and bioactive peptides was highly significant (P<0.01). However, the bioactive peptides was having higher IC_{50} value than hydrolysate and standard Acarbose. It indicates lower potency of the extracted peptides when compared to the standard acarbose. But Peptides from chicken intestine hydrolysate have antidiabetic activity and offer a promising approach to prevent, control diabetes through regulated diet in animals.

Keywords: chicken intestine, enzymatic hydrolysis, protease p, bioactive peptides, antidiabetic peptides, α- amylase inhibitory assay

Introduction
Bioactive peptides are specific protein fragments that positively affect physiological functions and human health (Korhonen & Pihlanto, 2006) [9]. Peptides that inhibit carbohydrate digestion include a-amylase and a-glucosidase inhibitory peptides. The a-amylase secreted from salivary and pancreatic glands triggers the hydrolysis of polysaccharides such as starch, the main source of energy in human nutrition. This process releases oligosaccharides that can be further hydrolysed by a-glucosidases located on the gut wall (Caner *et al*., 2016) [5].
Alpha-amylase inhibitors can inhibit the activity of a-amylase in the saliva and gastrointestinal tract, reduce the absorption of starch and carbohydrates in food and thus lower blood glucose level (Yan *et al*., 2019) [14]. α-amylase cleaves the starch into smaller glucose unit as it is needed by brain. Excessive conversion can increase the blood sugar level in tissues, and due to overactivation of α-amylase and deficiency of insulin, hyperglycaemia condition can occur in some cases (Agarwal, 2016) [1].
α-Amylase is a well-known therapeutic target for the treatment and maintenance of postprandial hyperglycaemia (Kaur, N *et al*., 2021) [7]. Type 2 diabetes is defined as the type of diabetes where it gets resistant to insulin due to its insensitivity; therefore, insulin production decreases, which leads to failure of pancreatic beta-cell and glucose transport to muscle cells, liver and fat cells (Blair, 2016) [4]. Hence, an attempt is made to extract the antidiabetic bioactive peptides from chicken intestine hydrolysate.

Materials and Methods
Samples of chicken intestine were collected from local retail outlets. The chicken intestines were collected from retail outlets were ice packed and immediately brought to the Department of Livestock Products Technology (Meat Science), Madras Veterinary College, Chennai of

Commercially available food grade fungal enzyme, protease P "Amano"6 having not less than 60,000 u/g proteolytic activity, was procured from M/s.Amano Pharmaceutical Co. Ltd., Japan.

Methods

The protein hydrolysate was prepared according to the method of Bhaskar *et al.,* (2007) [3]. The chicken intestine of about 500 gms for 6 trails was used after proper cleaning of intestine in running tap water to remove the intestinal contents, dipped in boiling water for 5 minutes and then were cut in to small pieces. These small pieces were sterilized at 121 °C under 15 lbs pressure for 15 minutes. The sterilized chicken intestinal pieces were then cooled and minced in a Waring blender for 5 minutes, followed by centrifugation at 10,500 rpm for 30 minutes at 4 °C. After centrifugation, the contents were separated in to three phases in which the top layer contain fat, mostly of middle layer water and protein rich sediment at the bottom. Both the fat and water layers were discarded and only the protein rich sediment was collected and used for further processing.

Enzymatic hydrolysis of the protein rich sediment

The protein rich sediment was mixed with equal quantity of water (w/v), added with 1.0% fungal protease P (Phycomycetes enzyme) used for hydrolysis at 43±1 °C for 90 min in a hot water bath. After the period of time, hydrolysis was stopped by heating the mixture kept at 85 °C for 5 minutes. The hydrolysate was centrifuged at 11,000 rpm for 20 minutes at 15 °C and the supernatant was collected. The collected supernatant containing protein hydrolysate was used for further studies.

Ultrafiltration of the Protein Hydrolysate

The protein hydrolysate solution was filtered and separated into small molecular weight fractions by ultrafiltration at 4 °C using 10kDa molecular weight cut-off and 3kDa molecular weight cut-off to enrich specific hydrolysate fractions. This permeate was defined as small peptides with molecular weight less than 10,000 Da and 3000 Da. The filtrate thus obtained was assessed for bioactivity using In-vitro α-amylase inhibitory activity.

Anti-Diabetic Assay

Assay for α- amylase inhibitory activity

The α- amylase inhibitory assay was performed according to the method of Kim *et al.* (2004) [8] with slight modifications.

a. Preparation of reagents

1. Buffer solution (20 mM Sodium Phosphate with 6.7 mM Sodium Chloride, pH 6.9 at 20 °C) was prepared with solution containing 2.4 mg/ml of sodium phosphate, monobasic, and 0.39 mg/ml of sodium chloride, in distilled water and adjusted to pH 6.9 at 20 °C using 1 M NaOH/1 M HCl.
2. Starch solution [1.0% (w/v) Soluble Starch Solution] was prepared with concentration of 10 mg/ml solution using starch in Buffer: The solution was solubilized by boiling on a heating/stir plate for 15 minutes by mixing. The solution was allowed to cool at room temperature. Final volume was brought with addition of distilled water. The solution was mixed throughout the assay procedure.
3. Sodium hydroxide (NaOH) solution (2 M) was prepared with a concentration of 80 mg/ml using sodium hydroxide along with distilled water.
4. Potassium sodium tartrate tetrahydrate solution (5.3 M) was prepared with an concentration of 1,496 mg/ml in 2 M Sodium Hydroxide (NaOH) solution. The solids are dissolved by heating on a heating plate with mixing. Do not heat to a boil.
5. 3,5-Dinitrosalicylic acid solution (96 Mm) was prepared with a concentration of 21.9 mg/ml by using 3,5-Dinitrosalicylic acid in distilled water. The solids are dissolved by heating on a heating stir plate. Do not heat to a boil.
6. Colour Reagent solution was prepared to a volume of 100 ml by the addition 30 ml of warm (50–70 °C) distilled water to an appropriate size amber bottle.
   With mixing, slowly 20 ml of warm 5.3 M potassium sodium tartrate, tetrahydrate solution and 20 ml of warm 96 mM 3,5-Dinitrosalicylic acid solution was added. This solution is stable for 6 months at ambient temperature if protected from light.
7. α-Amylase Sample solution prepared by dissolving 0.3 grams of alpha amylase 10 units/ml in distilled water.

b. Protocal for alpha Amylase assay and IC_{50} (Inhibitory Concentration) value

1. 10µl of α- amylase solution was pre-mixed with 10µl of sample solution at different concentrations (in 10% DMSO).
2. Following incubation for 15 minutes, 500µl of 1% starch solution in sodium phosphate buffer (pH6.9) was added to start the reaction. The reaction was carried out at 37.5 °C for 5 minutes and terminated by addition of 600µl of the DNS reagent.
3. The reaction mixture was placed in a water bath at boiling point for 15 minutes and then cooled down to room temperature.
4. The α-amylase activity was determined at 540nm by a spectrophotometer.
5. Inhibitory activity was calculated by the following equation

$$\alpha\text{- amylase inhibitory activity (\%)} = \frac{A-B}{A} \times 100$$

Where: A was the optical density of reaction blank. The reaction blank mixture contained the same volume of the buffer solution instead of the sample; B was the optical density of the reaction in the presence of both α –amylase and peptide sample.

The effective concentration of sample required to inhibit α-amylase enzyme by 50% (IC_{50} value) was obtained by linear regression analysis of dose-response curve plotting between % inhibition and concentrations.

Statistical Analysis

The data was subjected to statistical analysis in SPSS (version 2.0) software with mean ± SE.

Results and Discussion

Ultrafilteration of the Hydrolysate

1. Filtrate Recovery

The mean ± SE values of filtrate recovery of protein hydrolysate was

47.01 ± 0.2 per cent and ranged from 46.57 to 47.93 per cent were presented in the table 1.

2. Concentrate Recovery

The mean ± SE values of concentrate recovery of protein hydrolysate was 5.12 ± 0.45 per cent and ranged from 3.37 to 6.7 per cent were presented in the table 1.

3. Total Recovery

The mean ± SE values of total recovery of protein hydrolysate was 52.14 ± 0.2 per cent and ranged from 51.3 to 53.05 per cent were presented in the table 1. Jamdar *et al.* (2005) had a total recovery of 66.6 per cent from autolysate of chicken intestine. while Picot *et al*. (2010) obtained recovery rate of 69% from north atlantic fish hydrolysate permeate for 4 kDa molecular weight cut off.

Table 1: Mean ± SE values of recovery rate of low molecular weight peptides by Ultrafiltration

Parameters	Recovery %
% Filtrate recovery	47.01 ± 0.2
% Concentrate recovery	5.12 ± 0.45
% Total recovery	52.14 ± 0.2

Alpha Amylase Assay For Anti-Diabetic Activity

The mean ± SE values of IC_{50} value of protein hydrolysate was 273.04 ± 20 mg/ml, bioactive peptides (< 10 kDa) was 959 ± 8.1 mg/ml and bioactive peptides (<3kDa) was 362.86 ± 23mg/ml. Then it was compared with the standard Acarbose whose IC_{50} value was 158.42 ± 10 mg/ml was presented in table 2. The test of significance revealed that the alpha amylase inhibitory activity between protein hydrolysate and bioactive peptides was highly significant (P<0.01). However, the bioactive peptides was having higher IC_{50} value than hydrolysate. So, higher IC_{50} value indicated lower potency of the extracted peptides when compared to the standard acarbose. Yu, Z *et al*. (2012) [15] studied the anti-diabetic activity of peptides from albumin against alpha amylase and had dissimilar results that peptides from albumin had alpha amylase inhibitory activity with an IC_{50} value of 120 ± 4 µg/ml. which revealed to be potential inhibitor of alpha amylase activity.

On contrary to our results Anindita *et al*. (2017) [2] had dissimilar results of the invitro anti - diabetic activities for tinosporasinensis and revealed that the extracted peptides were having an IC_{50} value of 0.75 µg/ml and Stoilova *et al*. (2017) [12] studied the invitro diabetic properties of rhamnacaece fruits and results were not in agreement that fruits exhibited an IC_{50} value of 660 µg/ml.

Unni krishnan *et al*. (2015) [13] studied the alpha amylase inhibition of marine green algae and its possible role in diabetes management and had dissimilar results that green algae was having IC_{50} value of 408.9 µg/ml. whereas green sea weeds had an IC_{50} value of 147.6 µg/ml. but, Mohamed, E. A. H *et al*. (2012) [10] investigated the alpha amylase inhibitory activity of orthosiphon stamineus and sinensetin plant extract and had dissimilar results that alpha amylase activity with an IC_{50} value of 36.70 mg/ml for stamineus and 1.13 mg/ml for sinensetin and concluded that inhibition of these enzymes provided a strong biochemical basis for the management of type 2 diabetes by controlling glucose absorption.

Table 2: Mean ± SE values of α - amylase inhibitory anti-diabetic assay in hydrolysate and low molecular weight peptides with standard acarbose

Anti diabetic activity	IC $_{50}$ value
Acarbose	158.42 ± 10.1
Protein hydrolysate	273.04 ± 20 **
Bioactive peptides (<10 kDa)	959 ± 8.1**
Bioactive peptides (<3 kDa)	362.86 ± 23**

NS -Not Significant

* - Significant (P<0.05) difference** - Highly significant (P<0.01) difference

Means bearing different superscripts in the same row differ significantly

Conclusion

From this study the IC_{50} value of α- amylase inhibitory assay for protein hydrolysate and bioactive peptides were higher than standard acarbose. But still they posses α- amylase inhibitory activity. Further studies should be done to isolate antidiabetic peptides from meat and meat byproducts. Bioactive peptides from chicken intestine hydrolysate fits into the trends in the development of innovative functional products and nutraceuticals for prevention and control of Postprandial glucose levels.

Acknowledgement

I am always indepted to AICRP for funding us, which was of great financial assistance in conducting this research work.

References

1. Agarwal P. Alpha-amylase inhibition can treat diabetes mellitus. Research and Reviews Journal of Medical and Health Sciences. 2016;5(4):1-8.
2. Anindita B, Maji B, Mukherjee S, Chaudhuri K, Seal T. *in vitro* Antidiabetic and Anti-oxidant Activities of Methanol Extract of Tinospora Sinensis. Journal of Applied Biology & Biotechnology. 2017;5(03):061-067.
3. Bhaskar N, Modi VK, Govindaraju K, Radha C, Lalitha RG. Utilization of meat industry by products: protein hydrolysate from sheep visceral mass. Bioresource technology. 2007;98(2):388-394.
4. Blair M. Diabetes mellitus review. Urologic nursing, 2016, 36(1).
5. Caner S, Zhang X, Jiang J *et al*.. Glucosyl epi-cyclophellitol allows mechanism-based inactivation and structural analysis of human pancreatic a-amylase. FEBS Lett. 2016;590:1143-1151.
6. Jamdar SN, Harikumar P. Autolytic degradation of chicken intestinal proteins. Bioresource technology. 2005;96(11):1276-1284.
7. Kaur N, Kumar V, Nayak SK, Wadhwa PK, Kaur P, Sahu SK. Alpha-amylase as molecular target for treatment of diabetes mellitus: A comprehensive review. Chemical Biology & Drug Design. 2021;98:539-560.
8. Kim YM, Wang MH, Rhee RH. A novel α-glucosidase inhibitor from pine bark. Carbohydrate research. 2004;339(3):715-717.
9. Korhonen H, Pihlanto A. Bioactive peptides: production

and functionality. International Dairy Journal. 2006;16:945-960.
10. Mohamed EAH, Siddiqui MJA, Ang LF, Sadikun A, Chan SH, Tan SC, *et al*. Potent α-glucosidase and α-amylase inhibitory activities of standardized 50% ethanolic extracts and sinensetin from Orthosiphon stamineus Benth as anti-diabetic mechanism. BMC complementary and alternative medicine. 2012;12(1):1-7.
11. Picot L, Ravallec R, Fouchereau-Péron M, Vandanjon L, Jaouen P, Chaplain-Derouiniot M *et al*, Impact of ultrafiltration and nanofiltration of an industrial fish protein hydrolysate on its bioactive properties. Journal of the Science of Food and Agriculture. 2010;90(11):1819-1826.
12. Stoilova I, Krastanov Albert, Trifonova D, Angelova GV. Phytochemical Constituents and *in vitro* Anti-Diabetic Properties of *Ziziphus jujuba* (Rhamnaceae) Fruits. International Journal of Pharmacognosy and Phytochemical Research. 2017;9(2):150-158
13. Unnikrishnan PS, Suthindhiran K, Jayasri MA. Alpha-amylase inhibition and antioxidant activity of marine green algae and its possible role in diabetes management. Pharmacognosy magazine. 2015;11(Suppl 4):S511.
14. Yan J, Zhao J, Yang R, Zhao W. Bioactive peptides with antidiabetic properties: a review. International Journal of Food Science & Technology. 2019;54(6):1909-1919.
15. Yu Z, Yin Y, Zhao W, Liu J, Chen F. Anti-diabetic activity peptides from albumin against α-glucosidase and α-amylase. Food chemistry. 2012;135(3):2078-2085.