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RESEARCH ARTICLE

IN VITRO STUDIES ON ALPHA AMYLASE AND ALPHA GLUCOSIDASE INHIBITORY ACTIVITIES OF TOMATO POWDER INCORPORATED RICE-BASED COLD EXTRUDATES

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ABSTRACT

Diabetes mellitus is a metabolic disorder and it is one of the free radical mediated diseases characterized by hyperglycemia. Among the various anti-diabetic therapeutic approaches one major strategy is reducing gastrointestinal absorption of glucose by inhibition of carbohydrate metabolizing enzymes α -amylase and α - glucosidase. The aim of this work was to evaluate the inhibitory activities of methanolic extracts of tomato powder incorporated rice based cold extrudates on α- amylase and α-glucosidase enzymes. The α- amylase inhibition assay showed that the methanolic extracts of control extrudates and TP extrudates were found to be 42.13 mg/ml and 40.47 mg /ml respectively exhibited 50% α- amylase inhibition activity at the mentioned concentrations. The α- glucosidase IC_{50} for the extrudate extracts of control extrudates and TP extrudates was found to be 52.50 mg/ml and 45.14 mg/ml respectively. The results of the work therefore clearly indicate the potential of these extracts to manage hyperglycemia.

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INTRODUCTION

Diabetes mellitus is a chronic endocrine disorder that affects the metabolism of macronutrients. It includes a group of metabolic diseases characterized by hyperglycemia, in which blood sugar levels are elevated either because the pancreas do not produce enough insulin or cells do not respond to the produced insulin. Therefore an important therapeutic approach to treat diabetes is to decrease postprandial hyperglycemia. This can be achieved by the inhibition of carbohydrate hydrolyzing enzymes like α-amylase and α- glucosidase. In the digestion of carbohydrates α-glucosidase and α- amylase play important enzymes role. Alpha Amylase is involved in the breakdown of long chain carbohydrates and α- glucosidase breaks down starch and disaccharides to glucose. They serve as the major digestive enzymes and help in intestinal absorption. Alpha amylase and glucosidase inhibitors are the potential targets in the development of pilot compounds for the treatment of diabetes and have long been targeted as potential avenues for better glycemic control in type 2 diabetic patients (Akkarachiyasit *et al*., 2010). The link between free-radicals and development of diabetes has been well established further free-radical damage to the pancreas has been implicated in the diabetogenic process. The tomatoes commonly used in diet almost all over the world, are a major source of antioxidants, and the consumption of fresh lycopersicon tomatoes has been reported to have health benefits such as cancer prevention and inhibition of lipid peroxidation (Ademosun *et al*., 2013). It is reported that regular consumption of tomato products has been associated with decreased risk of chronic degenerative diseases. Daily intake of lycopene at a dose of 90 mg/kg-/ bw could lower the level of serum free radical in streptozotocin (STZ) -induced hyperglycemic rats and inhibit platelet aggregation in type 2 diabetes (Zheng *et al*., 2013). Lycopene has been under considerable investigation for its antioxidant benefits in treating various chronic human diseases like cancer, cardiovascular diseases, osteoporosis and diabetes. As starchy foods are a main source of energy in the diets of Indian people, healthier starchy foods, providing beneficial functionalities for sustaining good health, should be recommended. The incorporation of dietary fiber in snacks has received increased attention in recent years due to its role in health and nutrition.

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Especially, researchers have focused on the use of fruits and vegetables for fiber supplementation and thereby evaluated them as a potential fiber source in the enrichment of foods (Altan *et al*., 2009). However, there is dearth of information on health benefits of tomato powder to justify its use as a substitute to the fresh tomatoes. Therefore, it is of interest to study the effect of incorporation of tomato powder in cold extrudates and inhibition of key enzymes linked to type-2 diabetes (α-amylase and α-glucosidase) to recommend as a substitute to common tomatoes in the management of many degenerative diseases like type-2 diabetes.

MATERIALS AND METHODS

Preparation of rice flour: The polished MTU 1001 rice was soaked in water for 4 hrs and drained, sun dried for 1.0 hr, milled to fine powder, sieved to particular size of 2 mm then packed in polythene bag.

Preparation of tomato powder

Three selected varieties of tomatoes were screened for lycopene content and the variety Pusa Ruby having highest lycopene (Srivalli *et al* 2016) content among the three was selected and processed for further making of tomato powder (Nagamani 2014).

Preparation of cold extrudates

Two different types of pasta control extrudates Tagliatelle and Cavatelli were prepared in three different compositions 40:60, 50:50 and 60:40 of rice flour to refined wheat flour by the process of folding and passing through rollers of pasta presto making machine several times. Sheeted dough was extruded through a suitable die (width, 2.0 mm), cut to have desired size of extrudates and shade dried for 16 hrs. Now these standardized cold extrudates were steamed for 20 min at 102 – 105°C, spread over tray drier and dried for 1 hr at 60 °C. The dried products were boiled for 6 min and the sensory evaluation was conducted using 9 point hedonic scale by 15 semi trained panelists. Among the variations, the variation pasta Cavatelli control extrudate 40:60 rice flour and refined wheat flour got the highest sensory scores and best accepted by the panel members. Thus it was taken for further study by incorporated with tomato powder (TP) at 4 different levels i.e. 5, 10, 15 and 20% levels in which tomato powder at 10% were accepted by the panel members was selected for the further study.

Table 1. Standardized extrudates

S. No	Name of extrudate	Rice flour (%)	Refined wheat flour (%)	Tomato Powder (%)	Salt (g)	Water (ml)
1.	Control	40.00	60.00	-	0.5	50.0
2.	TP	36.00	54.00	10	0.5	50.0

Note: Values are expressed as mean of three determinations
Control: Rice flour + refined wheat flour
TP - Tomato powder incorporated extrudates

Inhibition of α-amylase enzyme activity (Singh and Jambunathan, 1982)

1, 4- α- D-glucagan-glucanohydrolase a diastase from fungi was purchased from Himedia. Various concentrations (10, 20, 40, 60, 80, 100 µg/ml) of the samples were dispersed in 1.0 ml of 2 M phosphate buffer (pH 6.9) with addition of enzyme buffer of 0.5 ml was added to the sample suspension and incubated at 37^{o}C for 2 hrs. After the incubation period, 2 ml of 3, 5- dinitrosalicylic acid (DNS) reagent was quickly added to all the mixture dispersions and heated for 5 min. After cooling, the solution was made up to 25.0 ml with distilled water and filtered. The α-amylase activity was determined at 540 nm using spectrophotometry to measure product absorbance (maltose) which reduces DNS. The produced absorbance was compared with blank. Percent inhibition was calculated using the following equation.

$$\text{Inhibition (\%)} = \frac{\text{(Enzyme activity of control - Enzyme activity of sample)}}{\text{Enzyme activity of sample}}$$

The IC_{50} values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by non linear regression analysis from the mean inhibitory values. Maltose standard was used as the reference alpha amylase inhibitor. All tests were performed in triplicate.

Calculation of IC_{50}: The IC_{50} was calculated using linear regression equation in which the concentration of the sample as the x-axis and percent inhibition as the y-axis. From the equation y = a+bx, IC_{50} values can be calculated using the following formula

$$IC_{50} = \frac{50 - a}{b}$$

Inhibition of α–glucosidase enzyme activity (Vishnu and Murugesan, 2013)

The inhibitory activity of α–glucosidase enzyme was determined by incubating 1.0 ml of starch substrate (2% w/v maltose or sucrose) with 0.2 ml Tris buffer (pH 8.0) and various concentrations of samples for 5 min at 37 ºC. The reaction was initiated by adding 1.0 ml of α - glucosidase enzyme (IU/ml) from Aspergillus niger (purchased from Sigma) to it, followed by incubation for 40 min at 35ºC. Then the reaction was terminated by the addition of 2.0 ml of 6 N Hydrochloric acid. The intensity of colour was measured at 540 nm and repeated thrice consecutively.

$$\text{\% Inhibition} = \frac{\text{(Enzyme activity of control - Enzyme activity of sample)}}{\text{Enzyme activity of sample}}$$

The IC 50 values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by non linear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha glucosidase inhibitor. All tests were performed in triplicate.

Statistical analysis

Experimental results were Mean ± standard deviation of three parallel measurements. Linear regression analysis was used to calculate the IC_{50} value. Data were considered statistically significant only when p value < 0.05 (Snedecor, 1983).

RESULTS

In the present study, the *in vitro* carbohydrate inhibition activities of the tomato powder incorporated extrudate extracts were investigated using α- amylase enzyme, IC_{50} values were calculated and the results are presented in Table 2 and Figure 1.

Table 2. Inhibitory activity of methanol extract of extrudates against α – amylase

S. No.	Parameter	Control extrudate	TP extrudate	Maltose
1.	IC_{50}	42.13±0.03	40.47±0.08	16.25 ± 0.02
2.	Mean	51.82	55.37	56.93
3.	S.E	0.5767	0.1552	0.0342
4.	C.D	1.2850	0.3460	0.0762
5.	C.V (%)	1.36	0.34	0.07

Note: Values are expressed as mean ± standard deviation of three determinations. Means within the same column followed by a common letter do not differ significantly at p≤ 0.05.
Control: Rice flour + wheat flour
TP - Tomato powder incorporated extrudate

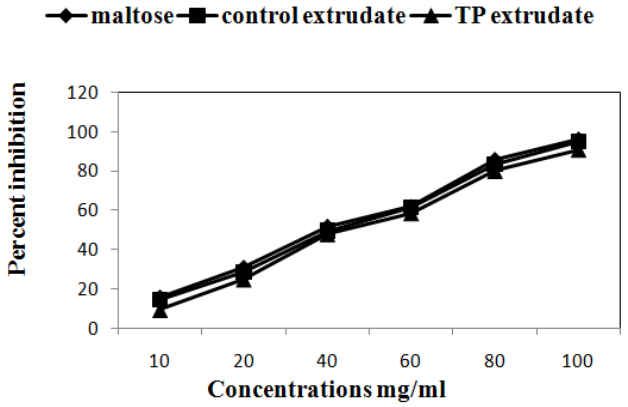

Figure 1. Percent inhibition of α amylase enzyme activity in *in-vitro* carbohydrate digestibility

Inhibition of α - amylase enzyme activity: In the present study the tomato powder incorporated extrudates were found to possess favourable α-amylase inhibitory effects on starch break down *in vitro*. The α-amylase inhibitor effectiveness of products was compared on the basis of their resulting IC_{50} value. TP extrudate inhibited the activity of α- amylase with an IC_{50} value of 40.47 mg /ml where as control extrudates with an IC_{50} value of 42.13 mg/ml. The maltose, standard positive control used in this study, inhibited the activity of α-amylase with an IC_{50} value of 16.25 mg/ml.

Table 2. Inhibitory activity of methanol extract of extrudates against α -glucosidase

S. No	Parameter	Control extrudate	TP extrudate
1.	IC_{50}	52.88[a]±0.04	45.14± 0.20
2.	Mean	52.453	47.57
3.	S.E	0.3892	0.3226
4.	C.D	0.8673	0.7188
5.	C.V (%)	1.00	0.75

Note: Values are expressed as mean ± standard deviation of three determinations.
Means within the same column followed by a common letter do not differ significantly at p≤ 0.05.
Control: Rice flour + wheat flour
TP - Tomato powder incorporated extrudate

Inhibition of α-glucosidase enzyme activity: In the current study, extracts of cold extrudates showed inhibitory activity against α-glucosidase enzyme activity. The percentage inhibitory activity for control extrudate ranged from 13.15% to 92.76% and TP extrudate was 11.69% to 86.36%. The α-glucosidase inhibitor effectiveness of extracts of TP extrudates were compared on the basis of their resulting IC_{50} value. TP extrudates inhibited the activity of α- glucosidase with an IC_{50} value 45.14 mg/ml and the control extrudate with IC_{50} value 52.50 mg/ml (Table 2 and Figure 2).

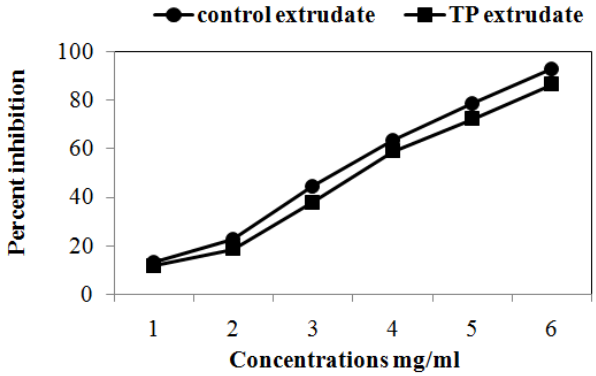

Figure 2. Percent inhibition of α- glucosidase enzyme activity in *in-vitro* starch digestibility

DISCUSSION

Management of blood glucose level is an essential approach in the control of diabetes complications. Inhibitors of carbohydrate analysing enzymes (α - amylase and α - glucosidase) have been helpful as oral hypoglycemic medicines for the control of hyperglycemia exclusively in the patients with type- 2- diabetis mellitus (Dastjerdi *et al*., 2015). Inhibition of these enzymes holds carbohydrates digestion and extend the total carbohydrate digestion time, leading to a decrease in the rate of glucose absorption and therefore reducing the post prandial plasma glucose rise (Wild *et al*., 2004). α-Amylase hydrolyzes starch to maltose, while α-glucosidase enzymes are responsible for the breakdown of oligo- and/or disaccharides to monosaccharides, and an inhibition of these enzymes therefore leads to a decrease in blood glucose level, and this is one of the therapeutic approaches for reducing postprandial blood glucose levels in diabetics. In α-amylase *In vitro* study, the extracts showed inhibition value between 16.25 mg/ml. to 42.13 mg/ml. During screening for phytochemicals it was found that the extrudates contain glycosides (results not reported). The glycosides present in the crude extracts acts as a substrate for the α-glucosidase enzyme and may be responsible for the inhibitory activity (Elya *et al*., 2015). Amylases degrade starch by cleaving glycosidic bonds. Glycoside was found from the sample has glycosidic bonds so it's changing the role of starch as a substrate. With this mechanism, the starch in the body was not changed to form a disaccharide. It could help the work of glucosidase which converts disaccharide into monosaccharides (glucose) and the level of glucose can be controlled. The result revealed that inhibition of α- glucosidase activity was more in TP extrudates than control extrudates. It may be due to increase in lycopene, fiber and presence of tannins (Srivalli *et al.,* 2016) due to incorporation of tomato powder in extrudates. In literature tannin has been reported as non-specific inhibitors for several hydrolytic enzymes such as lipases, α-glucosidases, α-amylases and invertase (Elya *et al*., 2015). Lycopene treatment significantly improved the blood glucose levels and body weight of diabetic rats (Haribabu *et al.,* 2013). The starch digestibility of fruit waste blend which contained hazel nut flour and tomato pomace was measured at 0.5, 5.0

concentrations obtained 28.5 and 69.2 mg maltose sample for extrusion cooking. Significantly ($p<0.05$) increased starch digestibility of extrudates was observed when compared to unextruded raw materials (Yagci and Gogus, 2010).

Conclusion

A stronger inhibition of α-glucosidase activity and α- amylase activities is desirable as this could address the main drawback of currently used α-glucosidase and α - amylase inhibitors drugs which is caused by the excessive inhibition of pancreatic α-amylase resulting in the abnormal bacterial fermentation of undigested carbohydrates in the colon. The results revealed that inhibition of α-glucosidase activity and α - amylase activity is more in TP extrudates. The combination of lycopene and other natural antioxidants present in the samples may be responsible for the potent inhibition of these enzymes. The results provide the basis for the development of staple foods with nutritional characteristics for today's functional food markets. The inhibition of key enzymes linked with type-2 diabetes (α-amylase and α-glucosidase) and antioxidative properties of the tomato powder used in this study could make them good dietary means for the management and/or prevention of type-2 diabetes.

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