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RESEARCH ARTICLE

ANALYSIS OF ANTIOXIDANT ACTIVITY IN PUSA RUBY TOMATO POWDER INCORPORATED COLD EXTRUDATES

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ABSTRACT

Tomato (*Solanum lycopersicum)* is known to be associated with a reduced risk of developing a certain chronic diseases and cancers due to the presence of lycopene, a bioactive carotenoid. The objective of this study was to determine the antioxidant activity in tomato powder and TP extrudate (Tomato powder incorporated extrudate). The results of present study showed that flavonoid content in tomato powder incorporated extrudate of IC_{50} value in control extrudate (22.28±0.01) was more when compare to the TP extrudates (15.51±0.01). The results of phytochemical screening showed that carbohydrates, alkaloids, proteins, amino acids, flavanoids, fixed oils and fats, terpenoids, cardiac glycosides, saponins and phenols are present in TP extrudates. Identification of carbohydrates, alkaloids, proteins, amino acids, flavanoids, cardiac glycosides, saponins, tannins and phenols were present in tomato powder. As the concentration increased the antioxidant activity increased in the extrudates. Lower IC_{50} value indicates the more antioxidant activity. IC_{50} value for control extrudates was 13.23±0.02, tomato powder 8.78±0.01 and TP extrudate 10.87±0.09.

INTRODUCTION

More recently, there has been renewed attention given to the antioxidant content of tomatoes because many epidemiological studies suggested that regular consumption of fruits and vegetables, including tomatoes, can play an important role in preventing cancer and cardiovascular problems. Tomato components like lycopene, phenolics, flavonoids and vitamins C and E are mainly responsible for the antioxidant capacity of raw tomatoes and processed tomato products. (Toor and savage, 2005). Antioxidants reduce oxidative damage to biomolecules by modulating the effects of reactive oxidants. Antioxidants may help the body to protect itself against damage caused by reactive oxygen species (ROS), as well as those of nitrogen and chlorine. In recent years, utilization of natural antioxidants to prevent quality deterioration of food products and preserve their nutritional value has been increasing. Tomatoes constitute an integral part of the human diet in worldwide (Masatcioglu *et al.,* 2013). The benefits of tomato and tomato products have been attributed mostly to their carotenoid content. In human diet, tomatoes and tomato products are the predominant sources of lycopene, which has been found to be available for antioxidant properties.

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Due to its stereochemical properties and ability to be efficient quencher of singlet oxygen and free radical lycopene is regarded as bio-antioxidant with high biological activity in the different tissues of human body (Abushitha *et al.,* 2000).

MATERIALS AND METHODS

Preliminary phytochemical screening of processed and prepared extrudates

The preliminary tests for carbohydrate, alkaloids, proteins, amino acids, flavanoids, fixed oils, terpenoids, cardiac glycosides, steroids, tannins, phlobatins, phenols and quinones were carried out as per the procedure given by Harbourne (1993).

Analysis of total flavonoid content

2.0 ml of the clear extract of each extrudate solution was mixed with 2 ml of 2% Aluminium trichloride ($AlCl_3$) in methanol. The mixture was incubated for 10 min at room temperature, and the absorbance was measured at 415 nm in spectrophotometer against blank samples. The total concentration of flavonoids in the extracts was determined as microgram of rutin equivalent (RE) according to the formula that was obtained from standard rutin graph (Meda *et al.,* 2005).

Absorbance= 0.0144×total flavonoid [µg rutin] + 0.0556

Free radical scavenging assay by DPPH method

The antioxidant activity was estimated by using 1-diphenyl-2-picrylhydrazil (DPPH) method as described by Dorman *et al.,* (2004).

Preparation of cold extrudate working solutions

The stock extrudate extracts were diluted to different concentrations viz. 4, 8, 12, 16, 20 and 24 mg / ml in methanol and used.

Preparation of standard stock solution of L-ascorbic acid

The stock solution of standard L-ascorbic acid was prepared freshly at concentration of 1.0 mg / ml in distilled water and used immediately. From the stock solution different concentration viz. 4, 8, 12, 16, 20 and 24 mg / ml were prepared in distilled water and used.

Principle

The scavenging reaction between DPPH and an antioxidant H-A can be written as

DPPH + H-A → DPPH-H + A
(Purple) (Yellow)

The antioxidant reacts with DPPH, which is a stable free radical that is reduced to the DPPHH and as consequence the absorbance decreased from the DPPH radical to the DPPH-H form. The degree of discoloration indicated the scavenging potential of the antioxidant compound present in extracts through hydrogen donating ability.

Procedure

The working cold extrudates of 4, 8, 12, 16, 20 and 24 mg / ml were prepared for various extrudate samples. A positive control of L-ascorbic acid was made at the same concentrations as the extrudate extracts. To each of the extrudate extracts and standard solutions were separately added with 5.0 ml of 0.04% DPPH. These solution mixtures were kept in dark for 30 min and read at absorbance of 517 nm in a spectrophotometer for decolorization that indicated the scavenging efficiency of the extracts. Lower absorbance of the reaction mixture indicted higher free radical scavenging activity. The scavenging activity against DPPH free radical concentration was calculated using the following formulae:

DPPH scavenging (%) = (A- B/A) X100

Where

A was the absorbance of the control (DPPH solution without the sample). B was the absorbance of DPPH solution in the presence of extrudate extract or standard ascorbic acid. The antioxidant activity was expressed as $IC_{50.}$ All the tests were performed in triplicate and the graph was plotted with the average of the three observations to obtain IC_{50}.

RESULT AND DISCUSSION

Antioxidant activity of processed and prepared samples
Phytochemical screening of extrudates

Phytochemicals also known as phyto nutrients are naturally occurring substances found in plants. These substances have been found to be beneficial to human health as well as possessing antioxidant activity (Praveena and Estherlydia, 2014). Plant derived compounds are well known for their therapeutic values since ancient times. Krishna *et al*. (2013) studied the identification of phytochemical in fruit paste of tomato. The fruit paste of tomato were extracted with methanol, ethanol, acetone, chloroform and ether and tested for phytochemical. Studies for identification of reducing sugar, pentoses, hexose, disaccharides, starch, glycogen-, proteins, amino acids, sterols, carotenoids, flavanoids and polyphenols were performed. Results showed that the various extracts posses both antibacterial and antifungal activity due to presence of phyto constituents mainly phenolic and sterol compounds.

Phytochemicals could act as an antioxidant and anti inflammatory. It plays vital role in detoxification of harmful and deleterious chemicals of the body. The phytochemical tests was carried out using standard methods of analysis of carbohydrates, alkaloids, proteins, amino acids, flavanoids, fixed oils and fats, terpenoids, cardiac glycosides, steroids, saponins, tannins, phlobatanins, phenols and quinines. The results of phytochemical screening are given in Table 1. Results of present study showed that carbohydrates, proteins, amino acids, flavanoids, fixed oils and fats, terpenoids, cardiac glycosides and saponins, are present in the control extrudate. Whereas carbohydrates, alkaloids, proteins, amino acids, flavanoids, fixed oils and fats, terpenoids, cardiac glycosides, saponins and phenols are present in TP extrudates. Identification of carbohydrates, alkaloids, proteins, amino acids, flavanoids, cardiac glycosides, saponins, tannins and phenols were present in tomato powder. Phytochemicals like saponin, flavonoids, glycosides and tannins from fruits and vegetables may play key roles in amelioration of diseases. Phytochemicals in fruits and vegetables can have complementary and overlapping mechanisms of oxidative agents, stimulation of the immune system, regulation of gene expression in cell proliferation and apoptosis, hormone metabolism and antibacterial and antiviral effects (Liu, 2003).

Total flavonoid content

The quantitative analysis of flavonoids was done in tomato powder incorporated extrudates and the result is given in Table 2 and Figure 1. Percent inhibition in control extrudate ranged from 14.83% to 67.82% and TP extrudates it was 18.17% to 82.47% (Figure 1). IC_{50} values of control extrudate (22.28±0.01) was more when compare to the TP extrudates (15.51±0.01) (Table 4.19) indicating high flavanoid content in TP extrudates due to addition of tomato powder incorporation.
The quantity of flavonoids present in tomato is influenced by genotype, environmental conditions, extraction procedure and cultural practices. Flavonoids are potent antioxidants *in vitro* and epidemiological studies suggest a direct correlation between high flavonoid intake and decrease risk of cardiovascular diseases, cancer and other age related diseases (Haddadin and Haddadin, 2015).

Table 1. Phytochemical screening of processed and prepared samples

S. No	Phytochemicals	Test	Control extrudate	TP extrudate	Tomato powder
1	Carbohydrate	Molisch test	+	+	+
2	Alkaloids	Mayer's test	_	_	_
		Wagner's test	_	+	_
		Hager's test	_	+	+
3	Proteins	Kjeldahl method	+	+	+
4	Amino acids	Ninhydrin test	+	+	+
5	Flavanoids	With ammonia solution	+	+	+
6	Fixed oils and fats	Foam test	+	+	_
7	Terpenoids	-	+	+	_
8	Cardiac glycosides	-	+	+	+
9	Steroids	Liebermann- Buchard test	_	_	-
10	Saponins	Foam test	+	+	+
11	Tannins	$FeCl_3$ test	_	_	+
12	Phlobatinins	With HCl	_	_	_
13	Phenols	Ferric chloride test	_	+	+
		Lieberann's test	_	+	+
14	Quinones	With conc HCl	_	_	_

Note: Values are mean of three determinations.
Control: Rice flour + refined wheat flour
TP - Tomato powder incorporated extrudate

Free radical scavenging assay by DPPH method

The intensity of the radical scavenging effect is measured by calculating half-inhibition concentration using IC_{50} which was the efficient concentration required for decreasing initial free radical concentration by 50 percent (Talukder *et al.,* 2013). IC_{50} was obtained by representing data at various concentrations. In the present study, the antioxidant activity of extracts was carried out by *in-vitro* antioxidant models in relation to ascorbic acid.

Table 2. Flavonoid content of extrudates

S. No	Parameter	Control extrudate	TP extrudate
1.	IC_{50}	22.28±0.01	15.51±0.01
2.	Mean	40.879	53.229
3.	S.E	0.0669	0.3214
4.	C.D	0.1491	0.7161
5.	C.V (%)	0.200	0.740

Note: Values are expressed as mean ±standard deviation of three determinations.
Control: Rice flour + refined wheat flour
TP - Tomato powder incorporated extrudate

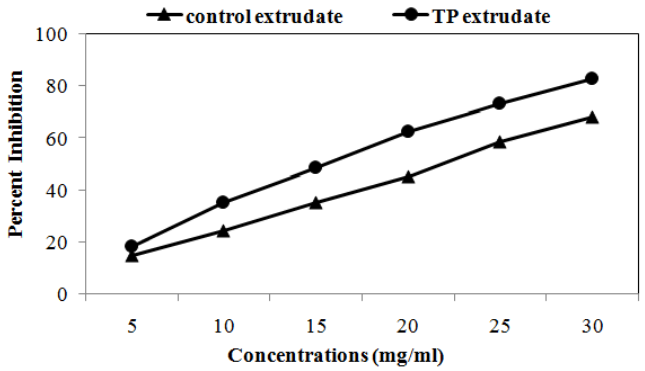

Figure 1. Flavonoid content of extrudates

DPPH radical scavenging activity

Antioxidants react with DPPH, which is a stable free radical and convert it to 1,1–diphenyl-2-picryl hydrazine. The degree of discoloration indicated the radical scavenging potential of the antioxidant components (Singh *et al.,* 2011).

As the concentration increased the antioxidant activity increased in the extrudates. Lower IC_{50} value indicates the more antioxidant activity. IC_{50} value for control extrudates was 13.23±0.02, tomato powder 8.78±0.01 and TP extrudate 10.87±0.09 (Table 3). The addition of the tomato powder extract to DPPH solution caused a rapid decrease in IC_{50} value as indication to its good scavenging capacity. Phytochemical analysis showed high total flavonoid contents in the TP extracts suggesting that the flavonoid compounds present in the extract could be responsible for the observed DPPH radical scavenging activity. Similar results were reported by Kim *et al.* (2013) in tomato powder incorporated pork patties. The total flavonoid content in TP extracts was 3.52 mg quercitin/ 100 g. The extract showed a potential antioxidant activity in the DPPH radical-scavenging assay (EC50=16.76 µg/ ml).

Table 3. Antioxidant activity of methanol extract of extrudates

S. No	Parameter	Control extrudate	TP extrudate	Tomato powder	Ascorbic acid
1.	IC_{50}	13.22±0.02	10.87±0.09	8.77±0.01	6.58±0.01
2.	Mean	37.97	50.55	57.87	60.92
3.	S.E	0.1856	0.0698	0.0223	0.0173
4.	C.D	0.4136	0.1555	0.0499	0.0385
5.	C.V (%)	0.59	0.16	0.04	0.03

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

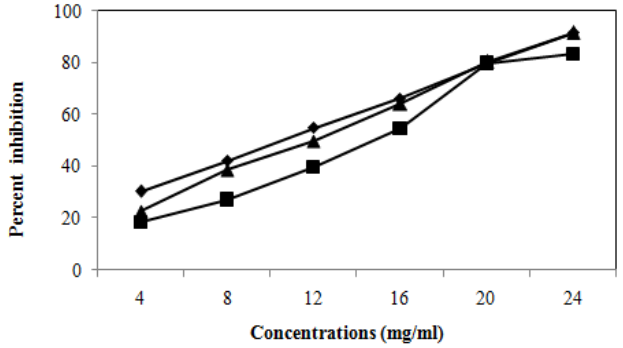

Figure 2. Free radical scavenging activity of extracts from tomato powder and TP extrudates measured using the DPPH assay

Haddadin and Haddadin (2015) studied on relation of lycopene pomace with antioxidant activity. The results of this research showed that percent inhibition of DPPH was ranged from 9.40 to 24.80 mg/ml. The DPPH test provided direct information about the reactivity of lycopene with a stable free radical, indicating that lycopene extract acted as a potent and direct free radical scavenger.

Conclusion

An experimental design to investigate the effect of antioxidant activity in addition of tomato powder. The results shows that the addition of tomato powder extract to DPPH solution caused a rapid decrease in IC_{50} values as indication to its good scavenging activity. The results indicated that tomato powder has a noticeable effect on scavenging free radicals. This could be attributed to its high content of lycopene and flavanoids. The flavanoid content of TP extrudates was 18.17% to 82.47%`. IC_{50} value of control extrudate was more than TP extrudates indicating high flavanoid content in TP extrudate.

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