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Proximate composition and antioxidant activity of some locally grown varieties of tomato (*Lycopersicum esculentum* L.) in Pakistan

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Abstract

Tomato (*Lycopersicum esculentum* L.) is known for its high carotenoid accumulation and has applications in nutritional products. The present experimental work was performed to estimate the fruit firmness, proximate analysis, vitamin C contents and antioxidant activity of five locally grown varieties of tomato. Antioxidant activity of tomato fruits extract was assessed by estimation of total phenolic contents (TPC), total flavonoids contents (TFC), DPPH radical scavenging assay, inhibition of linoleic acid peroxidation and reducing power. Observed tomato varities revealed high fruit firmness (1.16-1.93kg/cm^2) and high concentration of vitamin C (30.45-36.91 mg/100g). All the selected varieties of tomatoes showed high TPC (6.75-9.63mg/100g of dry weight GAE), TFC (0.76-2.69mg/100g of dry weight) and percent inhibition of linoleic acid peroxidation (53.74-87.22%). Moreover, excellent DPPH free radical scavenging activity (IC_{50} 16.74-46.43µg/mL) and reducing power potential were observed. Statistical analysis revealed significant variation in antioxidant potential among different fruit varieties.

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Introduction

Healthy food has ability to inhibit the free radical effects on the living organisms. Many experimental researches have revealed that continues consumption of fruits and vegetables can protect us against the free radicals (Lobo *et al.,* 2010). Nutritional valuable compounds such as certain vitamins, carotenoids, phenolic acids and flavonoids have defensive and protective ability against many harmful effects of reactive species (Wootton-Beard and Ryan, 2011; Harasym and Oledzki, 2014). These valuable compounds have high antioxidant potentials.

In recent years, researchers are kin to ascertain the level of antioxidant activity of some plant, in which tomato is the typical example (Lazarus *et al*., 2004). Tomato (*Lycopersicum esculentum* L.) is a basic "vegetable" that grown and consumed commercially in worldwide having nutritional importance. Tomatoes belong to the genus *Lycopersicum*, which is in the same family, *Solanaceae,* as potatoes (Gerszberg *et al.,* 2015). It is grown at large stage in cooler climate having typically red colour and edible property (Singh and Gu, 2010). According to botanically point of view tomato is a fruit but for culinary purposes considered as vegetable (George, 2009). The tomato fruits contain high amount of lycopene, which have many health beneficial effects (Hassimotto *et al*., 2005). Approximately about 170.8 million tons of tomatoes were produced in the world and 426.2 thousand tons in Pakistan in the year 2016 (FAO, 2017). Lycopene as an antioxidant, which is found in tomato in access exhibited significant beneficial effect, against many fetal diseases like prostate cancer, and perhaps other cancers (cervix, colon, pharynx, esophagus, stomach, pancreas, mouth and rectum) and prevent damage to DNA (Wang *et al.,* 1996). Studies showed that adequate levels of lycopene concentration in food may help to protect the eyes from damage also helpful to promote healthy eyesight (Prakash *et al*., 2001).

Tomato contains many other nutritional compounds other than lycopene such as flavonoids, phenolics acids and vitamin C having high antioxidant activity (Dewanto *et al.,* 2002). Nutritional profile of the tomato fruit mainly depends on many factors such genetic makeup, environmental factors (temperature, fertilizer concentration and water stress) and different stages during the ripening (Nour *et al*., 2013; Hallmann, 2012; George *et al*., 2004). Therefore the purpose of this experimental investigation was to check the fruit firmness, proximate composition, ascorbic acid and to check the antioxidant activity of five new hybrid tomato varieties grown in Pakistan.

Materials and methods

Sample Collection

Fruits of different varieties of tomato (*Lycopersicum esculentum* L.) were collected at mature stage from the Vegetable Research Department, Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan and packed in sealed polythene bags and stored in refrigerator at -4°C to preserve its nutrients. The tomato varities were further identified and authenticated by Dr Saeed Ahmad Shah Chishti, Vegetable Botanist hybrid seed production (HSP), Ayub Agricultural Research Institute Faisalabad.

Fruit Firmness

Fruit firmness of fresh tomatoes fruit was determined by randomly selects the fruit and then piercing with penetrometer (Rangana, 1986).

Proximate Analysis

The moisture contents of tomato fruits were determined as reported by Osborne and voogt, (1978) and by using the AOAC method, ash contents of tomato fruit was estimated (AOAC, 1980). Crude protein was estimated using micro-kjeldahl method (AOAC, 1980). Crude fibre was estimated as using reported method (AOAC, 1980). Estimation of total carbohydrate content was done by (Eyeson and Ankrah, 1975).

Estimation of Ascorbic Acid

Estimation of ascorbic acid in fresh tomato fruit was performed by previously described method (Baraket *et al.,* 1973).

Percentage Yield

Methanol was used as a solvent for the extraction of antioxidant compound from different tomato varieties. Tomato fruits were dried at room temperature for about two weeks. After grinding, it was soaked in 100% methanol and shake for 24 hours using Orbitrary Shaker (Gallen Kamp, England) at 140 rpm. Resulting mixture was then filter by using Whatman No. 1 filter paper and solvent used for evaporation evaporated with the help of vacuum rotary evaporator at 40°C under reduced pressure to get dry extracts. Dry extracts were then weighed by using analytical balance (AUY220-Shimadzu, Corporation, Japan) for the estimation of yield then extracts were stored at -4°C until further analysis (Sultana *et al*., 2007).

Antioxidant Activity

Estimation of TPC of tomato fruit extracts were performed by using Folin-Ciocalteu reagent as previously reported by (Apak *et al*., 2007). Estimation of TFC of tomato fruit extracts were performed spectrophotometrically by using previously reported method (Marinova *et al*., 2005). To check the free radical scavenging activity of tomato extract DPPH assay was performed as previously described (Marinova *et al*., 2005). The antioxidant activity of tomato dry fruit extract and pure compound in terms of % inhibition of linoleic acid peroxidation (Iqbal *et al.,* 2005). Reducing power of tomato extract was assessed by method reported before (Hussain *et al*., 2013) using double beam spectrophotometer (Hitachi U-2001p model 121-0032 Japan).

Statistical Analysis

Twelve samples of each tomato variety were collected and individually analyzed in triplicate. The data thus obtained were reported as mean ± SD. The analysis of variance (ANOVA) followed by using statistical analyses software, Minitab 18, significance level was $p < 0.05$.

Results and discussion

Proximate composition and Fruit Firmness

Proximate composition and fruit firmness data of different varities of tomato were described in Table 1. Fruits firmness in LITTH-559, LITTH-514, LITTH-539, LITTH-545 and SAHIL, were found to be 1.73, 1.51, 1.92, 1.23 and 1.16kg/cm² respectively. All tomato fruits start softens at the ripening stage but the softening not consistent among all the observed varities. LITTH-539 was most firm and LITTH-559, LITTH-514 were intermediate. Pervious literature revealed the average firmness (0.62 and 0.67kg/cm²) in eight tomato varieties (Gupta *et al*., 2011) which are remarkably different from our finding the variation may be due to variation in geographical and environmental factors. The firmness of the fruit is due to the pectic substance present in the cell wall of fruit and is found in different forms (Schwartz *et al.,* 2010). Protopectin is a form of pectic substances which is water insoluble. The transformation of protopectin into pectin occurred during the maturation of fruit. This reaction happens due to enzymatic action; pectin is first solubilized and then degraded completely as fruit is ripping, leading to a change in firmness (Toivonen and Brummell, 2008).

Moisture and Ash Contents

The moisture and ash contents of selected varieties were assimilated in Table 1. Moisture and ash content of LITTH-559, LITTH-514, LITTH-539, LITTH-545 and SAHIL varieties were found to be 94.81, 93.48, 94.92, 95.17, 92.28% and 7.27, 7.83, 7.15, 6.98, 8.56g/ 100g, respectively. Highest moisture content was found with LITTH-545 and highest ash contents for SAHIL. No significant ($p<0.05$) variations were recorded in moisture but significant ($p<0.05$) ash contents of investigated varieties were observed. Experimental data about the moisture and ash contents of these observable tomato varities were very close with results reported in literature (Abdullahi *et al.,* 2016).

Crude Protein

For the proper growth and maintenance of human body protein play key role and are, along with lipids and carbohydrates act as energy source. It also controlled the vital body function such as nutrients transport, enzymatic activity and other biological compound across the cell membrane (Wu *et al.,* 2014). The crude protein of LITTH-559, LITTH-514, LITTH-539, LITTH-545 and SAHIL tomato varieties were 14.83g/ 100g, 15.45g/ 100g, 14.97g/ 100g, 14.64g/ 100g and 16.25g/ 100g, respectively (on dry basis of tomato fruit) (Table 1). No significance difference ($p<0.05$) was observed in these local observable tomato varieties. Comparable result was observed about crude protein (4.2-4.8g/ 100g) in different varieties of tomato (Abdullahi *et al.,* 2016). Protein of 14.73g/ 100g and 15.62g/ 100g were reported in local grown tomato varieties dry extract (Gupta *et al.,* 2011).

Crude Fiber Contents

The results regarding the crude fiber contents were recorded in Table 1. Fibers are essential part of human diet because its effective fiber portion helps to increase the stool weight and decrease the gastrointestinal transit time (Jhonson and Marlett, 1986). Proximate composition analysis of tomato fruit of selected varieties revealed that fibre content of LITTH-559, LITTH-514, LITTH-539, LITTH-545 and SAHIL were 7.82, 7.75, 7.94, 8.12 and 8.37g/ 100g, respectively. These results were agreed with that of Gupta *et al.,* (2011) who reported the amount of fibre content (7.58-8.69g/ 100g) in tomatoes on fresh matter basis.

Total Carbohydrate and Crude Fat

The mean percentage of total carbohydrate and crude fat in five local tomato varieties were arranged in the following descending sequence: SAHIL > LITTH-539 > LITTH-514 > LITTH-559 > LITTH-539 and LITTH-514 > LITTH-539 > SAHIL > LITTH-545 > LITTH-559, respectively. There was no significant difference ($p<0.05$) in five observable local tomato varieties were observed. These finding about total carbohydrate were comparable to the previous finding by Pathak and Mahajan, (1978) and (Gupta *et al.,* 2011). Experimental results crude fat of the present research was in agreement to the previously finding by Gupta *et al.,* (2011), who reported the 1.56 to 1.61g/ 100g crude fat content in different tomato genotypes on dry matter basis. This variation in the total carbohydrate and crude fat was due to the varietal difference.

Ascorbic Acid

Ascorbic acid and dehydroascorbic acid are essential nutritional constituents of diet for its extraordinary biological function such as a co-factor in the biosynthesis of cholesterol, peptide hormones, L-carnitine and amino acid. Deficiency of ascorbic acid can cause many severe diseases such as scurvy and skin diseases (Grosso *et al.,* 2013). Data recording the ascorbic acid contents of five local tomato varities was assembled in Table 1. Highest concentration was recorded with SAHIL and lowest with LITTH-539. However, significant ($p<0.05$) differences were observed with ascorbic acid between different tomato varieties which are comparable with finding of Gupta *et al.,* (2010). Tomatoes are rich source of ascorbic acid with high quantity 230.91mg/ 100g (USDA database, 2007). Guil-Guerrero and Rebolloso-Fuentes, (2009) also revealed high range of ascorbic acid in tomato fruit with limit of 39-163mg/ 100g. These five different varieties of tomato showed different results of proximate analysis and ascorbic acid with reported literature due to the deference in geological environment, climate and ripening conditions.

Table 1. Fruit Firmness, Proximate Composition and Vitamin C contents of Tomatoes varieties.

	Tomato varities				
Proximate composition	LITTH-559	LITTH-514	LITTH-539	LITTH-545	SAHIL
Fruit firmness (kg/cm²)	1.73 ± 0.07 ^{c}	1.51 ± 0.05 ^{b}	1.92 ± 0.06 ^{d}	1.23 ± 0.09 ^{a}	1.16 ± 0.10 ^{a}
Moisture (g/100g)	94.81 ± 4.6 ^{a}	93.48 ± 4.8 ^{a}	94.92 ± 4.7 ^{a}	95.17 ± 4.7 ^{a}	92.28 ± 4.6 ^{a}
Ash (g/100g)	7.27 ± 0.36 ^{b}	7.83 ± 0.3 ^{bc}	7.15 ± 0.3 ^{ab}	6.98 ± 0.35 ^{a}	8.56 ± 0.42 ^{c}
Crude Protein (g/100g of dry weight)	14.83 ± 0.7 ^{a}	15.45 ± 0.5 ^{a}	14.97 ± 0.6 ^{a}	14.64 ± 0.7 ^{a}	16.25 ± 0.8 ^{a}
Crude Fibre (g/100g of dry weight)	7.82 ± 0.4 ^{a}	7.75 ± 0.39 ^{a}	7.94 ± 0.39 ^{a}	8.12 ± 0.41 ^{a}	8.37 ± 0.4 ^{a}
Total Carbohydrate (g/100g of dry weight)	65.2 ± 3.2 ^{a}	65.4 ± 3.27 ^{a}	64.9 ± 3.24 ^{a}	65.7 ± 3.29 ^{a}	67.49 ± 3.3 ^{a}
Total Fats (g/100g of dry weight)	1.25 ± 0.06 ^{a}	1.98 ± 0.09 ^{c}	1.83 ± 0.09 ^{c}	1.52 ± 0.07 ^{b}	1.56 ± 0.07 ^{b}
Ascorbic acid (mg/100g of fresh weight)	31.68 ± 1.5 ^{a}	32.11 ± 1.6 ^{a}	30.4 ± 1.52 ^{a}	34.2 ± 1.7 ^{ab}	36.91 ± 1.8 ^{b}

Values are mean ± SD of multiple determinations. Different letters in superscript represent significant difference of ($p<0.05$) between different extracts.

Percent Yield of Extracts

The percent yield of extracts from five varieties of tomato using methanol as solvent is present in Table 2 and ranged from 35.55-45.56% (W/W). The highest amount was extracted from LITTH-545 (45.56% dry basis) and lowest was obtained from LITTH-559 (35.55%). For absolute methanol yield of extracts for Sahil was (41.11%), LITTH-439 (42.00%) and LITTH-514 (36.44%). Variation among extract yield of varieties was also found to be significant ($p<0.05$). Previously reported data revealed that polar solvents are used for the extraction polyphenols because of their polarity and compatibility (Siddhuraju and Becker, 2003).

Total Phenolics Content

Total phenolics content in extracts of tomato was determined by Folin-Ciocalteau method. Estimated TPC of local tomato varieties LITTH-559, LITTH-514, LITTH-539, LITTH-545 and SAHIL were 9.21, 9.63, 9.17, 6.75, 9.38mg/ 100g of dry tomato fruit extract, measure as gallic acid equivalent, respectively. The highest TPC concentration was observed in the extract of LITTH-539 and lowest was observed for LITTH-514 in methanolic extract. Significantly ($p<0.05$) variation of total phenolic contents among varieties was observed.

The results explained above were comparable with the reported total phenolic contents of tomatoes (Kahkonen *et al.*, 2009; (Pinela *et al.*, 2012). Different varieties of tomato showed different amounts of total phenolic contents depending upon nature, climate and ripening conditions. This shows that tomatoes are an excellent source of total phenolics in our daily diet.

Total Flavonoids Contents

The amount of total flavonoids contents (TFC) for different varieties of locally grown varieties of tomato are given in Table 2. The amount of TFC extracted from different varieties of tomato LITTH-559, LITTH-514, LITTH-539, LITTH-545 and SAHIL were 1.65, 1.26, 2.69, 1.71, 0.76mg/ 100g of dry plant material measured as catechin equivalents. The maximum TFC was found from the extract of line variety LITTH-539 while the minimum TFC was found from Sahil and LITTH-559, LITTH-514, LITTH-545 were intermediate. There were a significant ($p< 0.05$) differences in the TFC contents of extracts of different formation. These TFC values are much different from the TFC values 31.57-79.63mg /100mL reported by Hassimotto *et al.*, (2005).

Percentage Inhibition

Antioxidant activity of different varieties of tomato can be determined in linoleic acid system using thiocyanate method (Capanoglu *et al.*, 2003). All the extracts have shown valuable percentage inhibition ranging from 53.74-87.22%. A line variety LITTH-514 has minimum inhibition 53.74% and LITTH-539 has maximum inhibition 87.22%.

In other varieties, LITTH-559, LITTH-545 and Sahil have the % inhibition as 83.70%, 66.52% and 79.73%, respectively. These results showed that tomato is rich source of antioxidants suggesting the tomato as a viable source of antioxidant for nutraceutical and functional foods (Pinela *et al.*, 2012).

DPPH Radical Scavenging Activity

As the concentration of phenolics compounds or the degree of hydroxylation of the phenolics compounds increases DPPH radical scavenging activity decreases (Thai *et al.*, 2006). Free radical scavenging activity increases with increase extracts concentrations providing 50% inhibition (IC_{50}) are given in Table 2.

According to results obtained, all the five tomato varieties (LITTH-545, LITTH-539, LITTH-559 and Sahil) exhibited significant radical scavenging activity at the test concentration ranging from 16.74-50.5µg/mL. LITTH-539 showed the highest DPPH radical scavenging activity with IC_{50} 16.74µg/mL, while LITTH-514 had the lowest activity with IC_{50} 50.05µg/mL. Obtained results showed marked deviation from the finding of (Guil-Guerrero and Rebolloso-Fuentes, 2009).

The significant differences in DPPH radical scavenging of different tomato varieties have shown that variation in varieties might have significant ($p<0.05$) influences on the antioxidant activity of tomato.

Table 2. Antioxidant activity and free radical scavenging capacity of methanol extract of different tomato varieties.

Name of Varieties	% age yield	TPC (mg/100g of dry plant material)	TFC (mg/100g of dry plant material)	% Inhibition	DPPH radical scavenging IC_{50} (µg/mL)
LITTH-545	45.56 ± 2.36[b]	9.21 ± 0.46[b]	1.65 ± 0.07[c]	66.52 ± 3.33[b]	33.7 ± 1.33[c]
LITTH-539	42.00 ± 1.68[b]	9.63 ± 0.19[b]	1.26 ± 0.06[b]	87.22 ± 1.74[d]	16.74 ± 0.83[b]
LITTH-559	35.55 ± 3.19[a]	9.17 ± 0.27[b]	2.69 ± .05[d]	83.70 ± 2.51[d]	31.66 ± 1.27[c]
LITTH-514	36.44 ± 1.82[a]	6.75 ± 0.61[a]	1.71 ± 0.05[c]	53.74 ± 4.84[a]	50.05 ± 1.00[e]
SAHIL	41.11 ± 2.47[ab]	9.38 ± 0.37[b]	0.76 ± 0.07[a]	79.73 ± 3.19[c]	46.43 ± 1.39[d]
BHT				91.71 ± 2.70[e]	8.96 ± 0.30[a]

Values are mean ± SD of triplicate determination. Different letters in superscript represent significant difference of (p<0.05) between different extract.

Reducing Power

Reducing power of different varieties of tomato is presented in Fig. 1. Reducing power of LITTH-545, LITTH-539, LITTH-559 and Sahil measured at concentration of 2.5-10mg/mL. Observed results revealed the increase of reducing power activity with the increase of concentration. All the extracts of analyzed varities expressed high activity at 10mg/mL as compared with the BHT. According to literature many bioactive compounds are responsible for antioxidant activity (Siddhuraju *et al.,* 2002). Measurement of reducing power can reflect some aspects of antioxidant activity in the sample. Observed results were comparable with finding about reducing power of (Chang *et al.,* 2006). Greater the intensity of the colour, greater will be the absorption; consequently, greater will be antioxidant activity (Wang *et al.*, 1996).

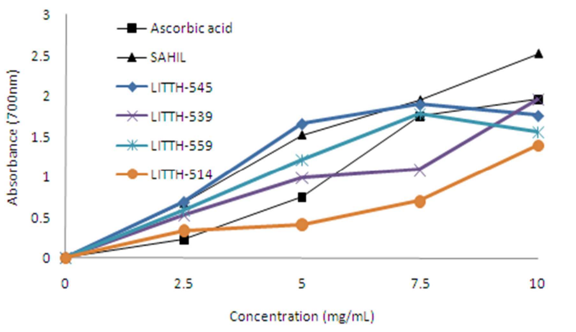

Fig. 1. Reducing Power of extracts of different tomato varieties.

Variation in the antioxidant activity of tomato varities are mainly depends on genotype, but many other factors such as cultivation practices (water stress, mineral availability), ripening stage and climatic environment (mostly light and temperature) (Dumas *et al.,* 2003). As far as we know, the antioxidant potential of the studied varieties was not previously reported.

Conclusion

Our findings in this study strongly demonstrated that all the observed new varieties of tomato expressed high firmness with increase in shelf life. All analyzed varieties of tomato showed high nutritional composition and can be considered as efficient source of nutrients for human diet. High antioxidant activity detected in all observed varieties can reduce the harmful effects of diseases. There informations can be used by nutritionalists and food technologists to improve the nutrition of local people and develop food products that would be beneficial to human health.

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